



WHITE SPOTS, AN ATYPICAL SYMPTOM OF OLIVE LEAF SPOT. G.E. Agosteo*, L. Scolaro. *Dipartimento di Agrochimica ed Agrobiologia, Università Mediterranea di Reggio Calabria, Piazza San Francesco di Sales 2, I-89061 Reggio Calabria, Italy. Fax: +39.0965.689049; e-mail: geagosteo@unirc.it

A rare symptom of 'Olive scab' or 'Olive leaf spot', disease caused by the fungus *Spilocaea oleagina* (Cast.) Hughes, first observed by Rambelli in Italy in 1958, appears on the upper blade of the leaves as white round spots, caused by the separation of the cuticle from the tissue underneath in consequence of the high mycelium growth of the pathogen and the interposition of air, when certain not well determined environmental conditions occur. Unlike the typical spots, 'White spots' don't show conidiphores and conidia of the pathogen. In the autumn 2001 the alteration was frequently observed on the new olive vegetation in some orchards in the Gioia Tauro plain in Calabria (southern Italy). The autumnal weather conditions, no rainfall, low RH and mild temperatures, inhibited sporulation and, conversely, stimulated mycelium growth of the pathogen. 'White spots' are distinct from a similar foliar symptom, known as 'Summer spots' in some Mediterranean countries, that occurs when the cuticle separates from the epidermal cells on old lesions that turn whitish after the release of conidia, if the leaf remains on the tree.

NEW SOURCE OF RESISTANCE TO *CLADOSPORIUM FULVUM* IN *LYCOPERSICON ESCULENTUM* VAR. *CERASIFORME*. A. Ambrico, O. Longo, D. Schiavone, F. Ciccarese*. *Department of Biology and Plant Pathology, University of Bari, Via G. Amendola 165/A I-70126 Bari, Italy. Fax: +39.080.5442906; e-mail: ficcare@agr.uniba.it

Use of resistant cultivars is the most effective control method of Leaf mold caused by *Cladosporium fulvum* on tomato. Many resistance genes, signed as Cf, were found in *Lycopersicon pimpinellifolium*, *L. hirsutum*, *L. peruvianum*, and *Solanum pennellii* but numerous races of *C. fulvum*, overcoming the used resistance genes, have rapidly evolved. Cf-5 and Cf-9 genes are extensively used for tomato breeding and conferred satisfactory levels of disease control. In this paper results of research on occurrence and inheritance of new resistance sources to *C. fulvum* are reported. Many accessions of wild tomato were tested. Cv Super Marmande as susceptible control was used. Tomato plants in a thermo-conditioned glasshouse at a temperature of $23 \pm 2^\circ\text{C}$ and 100% relative humidity were inoculated. In all tests an isolate of *C. fulvum* derived by naturally infected plants was used. Inoculations were carried out by spraying a conidia suspension at 8×10^5 CFU ml⁻¹ concentration on lower leaf surface. On susceptible and resistant plants the progress of infection was recorded using the 'celloidine streep' method. LA 1230 accession of *L. esculentum* var. *cerasiforme* resulted resistant to *C. fulvum* and the genetic analysis demonstrated that resistance is conferred by a single dominant gene. Chlorotic and necrotic reactions and lack of sporulation suggested that resistance mechanism is determined by a hypersensitive response. The resistance described above is the first record of a monogenic factor in *L. esculentum* var. *cerasiforme*.

EVALUATION OF SWEET PEPPER HYBRID VARIETIES FOR THE RESISTANCE TO *PHYTOPHTHORA CAPSICI*.

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Phytophthora blight (*Phytophthora capsici* Leonian) is the most important disease of sweet pepper in Calabria (southern Italy). In recent years the control of this disease has become more problematic due to the development of resistance to metalaxyl in field populations of the pathogen. In the spring 2001, in the Lamezia Terme plain, the F1 hybrid pepper rootstock Nunhems 9453, resistant to *P. capsici*, recently registered as 'Tresor' in the UE catalog, was evaluated in a soil naturally infested by *P. capsici*, in comparison with two experimental hybrid varieties of unknown susceptibility (PH 1904U e PH 2596B) and three susceptible commercial hybrid varieties (Isabelle F1, Laser F1 Clause, PS 840, the last one a 'topepo' type pepper). Pepper seedlings were transplanted in May, under tunnel, in single rows on polyethylene mulched bed. A randomized blocks experimental design was adopted, with six treatments and eight replicates. Five plants constituted each plot. In August, three months after transplant, both the number of dead or wilted plants (mortality) and the number of plants with symptoms of the disease (disease incidence) were determined. The hybrid Nunhems 9453 F1 showed the lowest values of both mortality (6%) and disease incidence (50%) compared with the other hybrids (53 and 65% mortality and 100 and 87% incidence for the two experimental hybrids, respectively; 100, 95 and 80% mortality and 100% disease incidence for the three commercial hybrids, respectively).

CLONING OF PLANT GENES ENCODING ABC TRANSPORTERS AND THEIR EXPRESSION IN RESPONSE TO BIOTIC AND ABIOTIC STRESSES. P. Ambrosino*, M. Ruocco, A. Bosco, F. Scala, G. Del Sorbo. *Department Ar.Bo.Pa.Ve., Section of Plant Pathology, University of Naples "Federico II", I-80055 Portici (Napoli), Italy. Fax: +39.081.2539339; e-mail: patambro@unina.it

ABC transporters are membrane permeases which utilise the energy deriving from ATP hydrolysis to drive the active transport of chemically unrelated compounds. Physiological functions of plant ABC transporters are largely unexplored. It has been hypothesised their involvement both in transport of plant defense factors (e.g. phytoalexins) and in protection of plant cells from pathogenicity factors (e.g. phytotoxins) secreted by pathogens. We have cloned four genes encoding ABC transporters from potato, which were named *StABC1* (*Solanum tuberosum* ABC transporter 1) *StABC2*, *StABC3* and *StABC4*, respectively. The expression of the mentioned genes was studied by RT-PCR either in plant organs and in *in vitro* cultivated potato cells after exposure to a number of endogenous and exogenous compounds. We found that *StABC2* is constitutively transcribed at low level but its expression is strongly up-regulated after treatment with some plant defense compounds (e.g. the terpenoid antifungal sclareol), pathogen-derived structures (e.g. partially purified *Botrytis cinerea* cell wall preparations), as well as herbicides (2,4-dichlorophenoxyacetic acid and sulfometuron methyl) and osmotic stress. *StABC2* expression is not induced by some phytotoxins (e.g. fusaric acid or 4,15-diacetoxyscirpenol) produced by species of *Fusarium* pathogenic to potato. These results indicate an involvement of *StABC2* in secretion of compounds responsible for defense to biotic and abiotic stresses.



EVALUATION OF SUSCEPTIBILITY OF APRICOT SELECTIONS TO PLUM POX VIRUS – M STRAINS T. Amenduni*, A. Bazzoni, M.R. Silletti, A. Minafra, B. Di Terlizzi, D. Bassi, R. Guerriero, V. Savino. *Dipartimento di Protezione delle Piante e Microbiologia Applicata, Università di Bari, Via G. Amendola 165/a, I-70126 Bari, Italy. Fax +39.080.5442911; e-mail: t.amenduni@agr.uniba.it

The actual risk of an epidemic spread of *Plum pox virus* (PPV) in the main apricot-growing areas of Italy has led to several studies for the identification of natural resistance sources to PPV. Results of trials for evaluating the behaviour of apricot selections, obtained from cultivars considered to be resistant are reported here. The accessions under trial (n. 223), selected by the University of Milano, Bologna and Pisa, were grafted on myrobalan rootstocks, graft-inoculated with PPV-M-0019-GR strain and grown under screen-house. Plants were subjected to visual inspections and tested by ELISA. Most of the selections (161) showed symptoms and were ELISA-positive, whereas 41 selections were symptomless but also ELISA-positive. All of the 21 selections that were symptomless and ELISA-negative proved to contain PPV where tested by RT-PCR.

CHARACTERIZATION OF *GANODERMA* ISOLATES FROM CENTRAL ITALY. T. Annesi, R. Coppola, E. Motta*. *Istituto Sperimentale per la Patologia Vegetale, Via C.G. Bertero 22, I-00156 Roma, Italy. Fax: +39.06.86802296; e-mail: e.motta@ispave.it

Ganoderma adpersum, *G. applanatum*, *G. lucidum*, *G. resinaceum* are important wood decaying species on ornamental and forest trees in central Italy. Isozyme analyses and observations on colony growth on malt agar were carried out on twenty isolates from sporocarps of these species, to develop a tool for specific identification of mycelia. Fourteen isozyme patterns were analyzed by polyacrylamide gel electrophoresis. Eight of them showed weak or no activity: alcohol dehydrogenase aminopeptidase, aspartate aminotransferase, formate dehydrogenase, glutamic dehydrogenase, lactate dehydrogenase, peroxidase, phospho-glucosyl isomerase, phospho-glucosyl mutase. Besides, acid phosphatase and shikimic dehydrogenase showed polymorphic patterns but lack of reproducibility among replicates. Malate dehydrogenase activity produced very similar profiles which did not allow differentiating individual species. Glucose-6-phosphate dehydrogenase profile presented a constant band in the four species (Rf = 0.85). Finally, esterase allowed distinguishing the isolates into two groups: *G. lucidum*-*G. resinaceum* and *G. adpersum*-*G. applanatum*, by the presence of a constant band for the *G. adpersum*-*G. applanatum* isolates only (Rf = 0.50). Also mycelial characteristics and colony morphology allowed separating the same two groups. The present study agrees with other isozyme studies on isolates from Australia and Southern America, and stresses the complex taxonomy of *Ganoderma* for the Italian studied species too. Further studies also by other techniques are needed.

COMPARISON AMONG DIFFERENT ISOLATES OF *GRAPEVINE LEAFROLL ASSOCIATED VIRUS 2* BY HMA. E. Angelini*, N. Bertazzon, M. Borgo. *Istituto Sperimentale per la Viticoltura, Viale XXVIII Aprile 26, I-31015 Conegliano (TV), Italy. Fax: +39.0438.64779; e-mail: isvbd@libero.it

Grapevine leafroll associated virus-2 (GLRaV-2) belongs to closterovirus genus. Several isolates were identified in grapevine worldwide. GLRaV-2 sources used in this study were 17 grapevine accessions from Italy, France, Greece and Brasil and 6 GLRaV-2 reference strains. Heteroduplex mobility assay (HMA) technique was applied in order to study genetic variability and to estimate phylogenetic distance. Comparison was made on ORF (Open Reading Frame) coding for viral coat protein, using primer pair GLR2CP1/2. Eight different HMA profiles were identified. Eleven grapevine isolates, including French, Greek and Brazilian accessions, showed the same HMA pattern as GLRaV-2 reference strains from cvs Semillon (USA) and Pinot noir 95 (France). Isolates recovered in Italian cvs Negro amaro and Vermentino were identical to reference strain from cv. Muscat de Samos (Greece). A third pattern was common to isolates amplified from 2 Italian cultivars; no reference strain shared this profile. Isolate from Italian cv. Montepulciano showed a unique HMA pattern. A peculiar HMA pattern was obtained from cv. Pollera, typical of Tuscany: in fact the presence of at least 2 diverse GLRaV-2 isolates was detected. None of the 17 samples exhibited a HMA profile similar to GLRaV-2 strain H4 (California) and to reference isolates from cv. Chasselas 8386 (Switzerland) and cv. Alphonse Lavalée 224 (France), all different among them. Estimated nucleotide homology between GLRaV-2 coat protein was in some cases as low as 83%.

A TEN-YEAR EXPERIENCE IN DIAGNOSIS OF CANKER STAIN OF PLANE TREE. T. Annesi, E. Motta*, M. Pilotti. *Istituto Sperimentale per la Patologia Vegetale, Via C.G. Bertero 22, I-00156 Roma, Italy. Fax: +39.06.86802296; e-mail: e.motta@ispave.it

Ceratocystis fimbriata f. sp. *platani* causes a destructive traheomycosis in *Platanus* spp. The main symptoms on diseased trees are: sparse chlorotic foliage or general wilting, necroses and cracks on the bark, the absence of any wood callus formation on the edges of the lesions and an internal bluish discoloration, very evident when bark is peeled off. Infected tissues of a dead plant contain the fungus and sawdust can disseminate the pathogen. Apart from phytosanitary measures (EC Plant Health Directive 77/93, 1976, and later amendments), no control method is available. An early and reliable detection of the disease focus is necessary for achieving its eradication. In a newly established focus, a single plant may be affected, but, as the fungus diffuses also through root anastomosis, in few years a group of dead plant will be visible. Sampling procedure is the basic step for the detection: a woody core 4-5 cm long is collected by a Pressler increment borer on the edge or inside of a discoloured area in a boundary plants, not yet dead but just partially infect. Hand-made sections are prepared, adjusting the core position for obtaining cross sections of the vessels, in order to observe inside them dark chlamydospores (200-400x) and identifying the fungus. A ten-year experience shows that a special attention has to be paid in studying possible new foci, as doubtful progressive death of consecutive trees can be due to other kinds of root damages, such as underground gas leaks, trenching near trees, and fire injuries.



EFFECTS OF ATMOSPHERIC CO₂ INCREASE ON FOREST PATHOGENS ATTACKS. N. Anselmi*, A. Mazzaglia, A. Vannini, R. Corvi, M. Nasini, T. Falessi. *Dipartimento di Protezione delle Piante, Università della Tuscia, Via S. Camillo De Lellis, I-01100 Viterbo, Italy. Fax: +39.0761.357473; e-mail: anselmi@unitus.it

The consequences of atmospheric CO₂ increase, main responsible of "greenhouse effect", on forestal ecosystems are more and more object of attentive researches. In this context, a series of experiments on the effects of atmospheric CO₂ increase on fungal pathogens attacks on forestal plants were carried out. This research was carried out in the province of Viterbo, Italy during 1998-2000, both in Open Top Chambers set up in a Mediterranean scrub area, near Montalto di Castro with *Quercus ilex*, *Phyllirea angustifolia* and *Pistacia lentiscus*, and in Rings set up in a *Populus* spp. plantation, near Tuscania. CO₂ level in control areas was maintained at natural level (350 ppm), whilst in the other areas it was increased at 750 and 500 ppm respectively. Starting from symptoms on monitored plants, the following diseases/pathogens were considered: a biotrophic leaf rust agent, *Melampsora allii-populina* on *Populus nigra*, two necrotrophic leaf agents *Phyllosticta ilicina* on *Quercus ilex* and *Marssonina* spp. on *Populus* spp., and an epiphytic disease, sooty mould on *Phyllirea angustifolia*, *Pistacia lentiscus* and *Populus nigra*. The increase of atmospheric CO₂, did not affect the disease level induced neither by *Melampsora*, nor by *P. ilicina*, nor by the three *Marssonina* species: *M. brunnea* on *P. x euramericana*, *M. populi-nigrae* on *P. nigra*, *M. castagnei* on *P. alba*. On the other side, an high level of atmospheric CO₂ seems to increase the sooty mould damages. It is probably related to the rise of phyllomyzous insects influencing its development.

INDUCTION OF SCOPARONE AND SCOPOLETIN IN ORANGE FRUITS BY A BIO-CONTROL AGENT (*RHODOTORULA GLUTINIS*). G. Arras*, Q. Migheli. *C.N.R. - Istituto di Scienze delle Produzioni Alimentari, Sez. Sassari, Via dei Mille 48, I-07100 Sassari, Italy. Fax: +39.079.232047; e-mail: g.arras@imfpp.ss.cnr.it

Biological control of *Penicillium digitatum* on orange fruits with an isolate of *Rhodotorula glutinis* (21A) obtained from tomato fruits in Sardinia is reported. Inhibition values of 88.4% in oranges and 96.2% in mandarins were obtained in artificially wounded fruits. Scanning electron microscope observations of the mode of action of the antagonist against the pathogen revealed rapid colonization of the fungal mycelium and the wounds, with lytic activity against the hyphae. With regard to the host-antagonist-pathogen interaction, it was observed that when the yeast was inoculated into the artificial wounds either alone or with the pathogen, it stimulated the fruit to produce phytoalexins (scoparone and scopoletin) in concentrations which varied significantly in relation to the time lag between inoculation with the antagonist and inoculation with the pathogen. The biosynthesis of scoparone in particular, four days after inoculation with the yeast only, was 69.0 µg g⁻¹ fresh weight of the fruit, 6.3 times higher than in the non-inoculated wound tissues (11.0 µg g⁻¹), while it decreased to 13.0 µg g⁻¹ when 21A was inoculated at the same time as *P. digitatum* and to 74.0 µg g⁻¹ when the pathogen was inoculated 24 h after the yeast.

DYNAMICS OF EPIPHYTIC AND ENDOPHYTIC POPULATIONS OF *PSEUDOMONAS SYRINGAE* PV. *TOMATO* IN NORMAL AND *GOX* TRANSGENIC VERSIONS OF TWO NEAR ISOGENIC TOMATO LINES (NILs) EXCEPT FOR THE *PTO* GENE. M. Antonelli*, E. Santangelo, V. Fonzo, G.P. Soressi, L. Varvaro. *Dipartimento di Protezione delle Piante, ²Dipartimento di Agrobiologia ed Agrochimica, Università della Tuscia, Via S. Camillo de Lellis, I-01100 Viterbo, Italy. Fax+39.0761.357473; e-mail: antonell@unitus.it

Pseudomonas syringae pv. *tomato*, causal agent of bacterial speck of tomato, can survive as an epiphyte on the leaf and infect the host when favorable conditions occur (high relative humidity and temperature > 18°C). Aim of the research was to study the ability of the pathogen to multiply on the phylloplane and into the mesophyll of four tomato NILs: 'Riogrande' (*pto/pto*) susceptible to *P. syringae* pv. *tomato*, 'Rimone' (*Pto/Pto*) resistant to this bacterium due to the presence of the *Pto* gene, and their corresponding *Gox* transgenic versions, 'RC 332' (*pto/pto*, *Gox/Gox*) and 'RC131' (*Pto/Pto*, *Gox/Gox*). The exogenous *Gox* gene is encoding for the glucose oxidase enzyme, which produces H₂O₂ involved in the plant specific, systemic resistance. The epiphytic and endophytic populations of *P. syringae* pv. *tomato*, after artificial inoculation, were quantitatively different on the four NILs. In fact, the bacterial population size on 'Riogrande' and 'RC332' genotypes was higher than on 'Rimone' and 'RC131'. As expected, the presence of the *Pto* gene in 'Rimone' and 'RC131', reduces their epiphytic and endophytic *P. syringae* pv. *tomato* populations. Moreover, in 'RC131', carrying both the *Gox* and *Pto* genes, a further decrease in bacterial population, probably due to an interaction between the *Pto* and *Gox* genes, was observed.

ANTAGONISTIC ACTIVITY OF THE BIOCONTROL YEAST *PICHIA GUILLERMONDII* 5A AGAINST *PENICILLIUM* SPP. ON ORANGE FRUIT IN COMMERCIAL PACKING-HOUSE. G. Arras, B. Scherm, Q. Migheli*. *Dipartimento di Protezione delle Piante, Università di Sassari, Via E. De Nicola 9, I-07100 Sassari, Italy. Fax: +39.079.229316; e-mail: migheli@uniss.it

Penicillium digitatum and *P. italicum* decays can cause heavy post-harvest losses in citrus fruits. The development of fungicide resistance by post-harvest citrus pathogens and an increasing environmental concern over pesticide residues in food, has prompted an urgent need for alternative control measures. Biological control of post-harvest diseases of fruit may be an effective alternative to chemical control. Three tests were conducted in 2000-2001 in two commercial packing-houses located in Sardinia, Italy, to evaluate the efficacy of biological, chemical and integrated treatments against *P. digitatum* and *P. italicum* on naturally inoculated orange fruits. Damage caused by the packing-house processing line was also assessed. Treating orange fruits with the yeast *Pichia guilliermondii* strain 5A generally led to a significant reduction of post-harvest decay compared to the processed control, while the commercial product Aspire®, based on *Candida oleophila*, was ineffective in inhibiting the pathogen when applied alone. The integrated application of thiabendazole or imazalil with the biocontrol agents significantly improved the control of fruit decay. Using thiabendazole at concentrations of 0.1 and 1.2 g l⁻¹, led to similar results in inhibiting fruit decay in two trials. Both yeasts were equally able to actively colonise the fruit during storage.



COMPARISON OF DIFFERENT TECHNIQUES TO ELIMINATE *ARTICHOKE LATENT VIRUS* (ARLV) FROM ARTICHOKE GERMPASM. G. Babes, V. Lumia, G. Pasquini, M. Barba*. *Istituto Sperimentale per la Patologia Vegetale, Via C.G. Bertero 22, I-00156 Rome, Italy. Fax: +39.06.86802296; e-mail: virologia@ispave.it

Vegetative propagation of globe artichoke (*Cynara scolymus* L.) by crown segments and offshoots facilitates the spread of systemic infections, in particular of viral origin, from one plant to another one. Sanitary status of artichoke crop is seriously compromised by viral infections which reduce yield and quality of production. For this reason European regulations for the commercialisation of propagative material foresees the absence of the most dangerous pathogens, including *Artichoke latent virus* (ARLV), from propagative material. In this paper we report the results obtained in eliminating ArLV from cv. Romanesco C3 using two techniques: *in vitro* thermotherapy and meristem shoot culture. Both techniques allowed obtaining healthy artichoke plantlets; the percentage ranged from 16% for *in vitro* thermotherapy to 28% for meristem shoot culture. ArLV-free plantlets have been transplanted and grown under controlled conditions. The absence of ArLV is routinely checked by molecular (RT-PCR and tissue printing molecular hybridization) and biological assays.

MOLECULAR CHARACTERIZATION OF *FUSARIUM OXYSPORUM* F.SP. *RADICIS-LYCOPERSICI* AND F.SP. *LYCOPERSICI* BY RAPD-PCR. V. Balmas, B. Scherm, F. Razu, A. Marcello, P. Di Primo, Q. Migheli*. *Dipartimento di Protezione delle Piante, Università di Sassari, Via E. De Nicola 9, I-07100 Sassari, Italy. Fax: +39.079.229316; e-mail: migheli@uniss.it

Fusarium oxysporum f.sp. *radicis-lycopersici* (FORL) causes Fusarium crown rot of tomato. The symptoms induced by the pathogen may be easily confused with those caused by the tomato vascular wilt pathogen, *F. oxysporum* f.sp. *lycopersici* (FOL). The accurate distinction between the two *formae speciales* has essential diagnostic implications. Aim of this work is to develop suitable markers to rapidly distinguish between the two pathogens by avoiding time consuming and expensive pathogenicity tests. A RAPD analysis was conducted on 60 FORL isolates from different geographic regions, representing all known vegetative compatibility groups (VCGs). Eight FOL isolates, and single representatives of several *formae speciales* of *F. oxysporum* were also included in the analysis. Thirty random primers were used to generate amplification profiles and 16 were selected as the most informative. The tested FORL isolates could be clustered within two major groups: the first one including representatives of VCGs 0090, 0091, 0092, 0093, and 0096; a second group including isolates belonging to VCGs 0094 and 0098 and FOL isolates.

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PRELIMINARY RESULTS ON THE PRESENCE OF VEGETATIVE COMPATIBILITY GROUPS IN *FUSARIUM CULMORUM*. V. Balmas*, Q. Migheli, A. Marcello, L. Corazza, T. Katan. *Dipartimento di Protezione delle Piante, Università di Sassari, Via E. De Nicola 9, I-07100 Sassari, Italy. Fax: +39.079.229316; e-mail: balmas@uniss.it

Fusarium culmorum (W.G. Smith) Sacc. is an ubiquitous phytopathogenic fungus able to infect various plants of agronomic importance. On wheat, *F. culmorum* is responsible for Crown and foot rot and Head blight. Infection by *F. culmorum* is associated with the presence of mycotoxins, which are extremely dangerous to humans and animals. To better understanding the genetic bases of the infection process by, and the *F. culmorum* population structure, we search for the presence of vegetative compatibility groups (VCGs) in this microorganism. A preliminary survey was carried out with 80 isolates of *F. culmorum* obtained in Italy from various crops, mainly cereals. Almost all the isolates gave rise to chlorate-resistant mutants after 10-40 d growth on substrates containing KClO₃ and KClO₃ + L-asparagine. Out of more than 200 chlorate-resistant mutants, 28 *nitM* mutants were obtained from 15 isolates, while *nit1* mutants and *nit3* mutants were more frequent. In nutritional complementation tests on Czapek-Dox medium, only the *nitM* mutants deriving from the same isolate developed a wild-type mycelium at the contact point. Further experiments will be devoted to examine whether different VCGs exist in this pathogen that may be linked to the geographic origin or to the parasitic specialisation of the isolates tested.

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GENETIC DIVERSITY BETWEEN *BOTRYTIS CINEREA* ISOLATES FROM ORCHARDS AND COLD STORAGE. E. Baraldi*, P. Bertolini, M. Pondrelli. *Dipartimento di Protezione e Valorizzazione Agroalimentare, Via Fanin 46, I-40127, Bologna, Italy. Fax +39.051.765049; e-mail: ebaraldi@agrsci.unibo.it

Ninety isolates of *Botrytis cinerea* collected from kiwifruit in orchards and in cold storage were phenotypically and genetically compared. The phenotypic characters evaluated were: conidia germination, germ tube length and mycelial growth at 0°C and 20°C. While at 20°C the two populations showed slight differences, when grown at 0°C, isolates from cold storage appeared to grow significantly faster than isolates from orchards. Isolates were then scored with six genetic markers with the aim to investigate population genetic structure and the mode of reproduction. We detected no genetic differentiation between isolates from orchard and isolates from cold storage. This suggests that low temperature adaptation of *B. cinerea* isolates does not have a genetic base. On the other hand we found great intrapopulation genetic diversity suggesting the occurrence of genic recombination which follows sexual reproduction or alternatively parasexuality.

FUNGAL FLORA AND OCHRATOXIN A PRODUCTION IN ITALIAN GRAPES IN 2001. P. Battilani*, P. Giorni, L. Languasco, A. Pietri, T. Bertuzzi. *Istituto di Entomologia e Patologia Vegetale, Università Cattolica S. Cuore, Via E. Parmense 84, I-29100 Piacenza, Italy. Fax +39.0523.599256; e-mail: paola.battilani@pc.unicatt.it

Ochratoxin A (OTA) occurrence in wine was firstly reported in 1996. During the following years several surveys confirmed its presence showing the potential risk of high intake for usual consumers of red wine or raisins. The EC, in Vth FP (QoL, KA 1, Food, Nutrition and Health), supported a project on 'Risk assessment and integrated ochratoxin A (OTA) management in grape and wine'. Twelve Partners are involved and an Italian Partner coordinates the group. The activity managed in Italy, according to a common protocol, consisted of sampling 16 vineyards, distributed on the territory, at 4 growth stages, between setting and ripening. Bunches sampled were used for fungal isolation and OTA quantification. Data on cropping system, grape phenology and meteorology were collected. From 2001 researches resulted that most of OTA-producing fungi isolated from grape were *Aspergilla* (88%), while *Penicillia* played a minor role. Among *Aspergilla*, section *Nigri* was dominant, with 86% of the isolates; 45% of these were uniseriates and 55% biseriates. *A. carbonarius* represented 50% of biseriates, with a high percentage of OTA-producing strains (78%), capable to synthesise relevant amounts of toxin. OTA-producing fungi were present in all the vineyards, while the toxin was detected only in traces on bunches. In previous years, some samples coming from these vineyards contained up to 13 ppb of OTA. The low levels of OTA detected in 2001 could be attributed to ecological conditions unsuitable for OTA production.

DETECTION OF GRAPEVINE LEAFROLL-ASSOCIATED VIRUS-2 (GLRaV-2) BY ELISA AND RT-PCR. N. Bertazzon, E. Angelini*, M. Borgo. *Istituto Sperimentale per la Viticoltura, Viale XXVIII Aprile 26, I-31015 Conegliano (TV), Italy. Fax: +39.043864779; e-mail: isvbd@libero.it

GLRaV-2 is one of the closterovirus associated with leafroll disease of grapevine. The aim of this study was the evaluation of 3 antisera and 4 primer pairs specific for detection of GLRaV-2. About 900 grapevine samples, rootstocks and scions, from Italy and other countries, were collected in 2001-2002. ELISA test showed that only some samples of Italian origin were infected (5.6%), while accessions from abroad of cv. 'Cabernet sauvignon' exhibited a high infection rate, in particular clone 191F (80% infection rate), 337F (55%) and 341 (25%). Comparison among Agritest, Sanofi and Bioreba antisera demonstrated the low reliability of Sanofi antiserum, due to high background values. Antisera furnished by Bioreba and Agritest displayed quite different results, even if antiserum obtained by Agritest was more sensitive. In particular, contrasting results concerned 34% of the samples belonging to the 3 clones of 'C. sauvignon'. RT-PCR were performed with primer pairs LR2-U2/L2, LRaV-2(1)/(2), GLR2CP1/2 and CP96f/r on 33 samples: 15 samples were negative with Bioreba and Agritest antisera, 6 positive with both serological tests and 12 showed contrasting results. Only 7 samples were negative with all primer pairs. The remaining 26 showed positive results with all primer pairs used. In order to evaluate sensitivity of primer pairs, other amplifications were performed on 7 positive samples, after dilution up to 1:10,000 of cDNA extracts. Primers performances were quite similar, even if GLR2CP1/CP2 pairs gave the best results at each dilution.

CERCOSPORA LEAF SPOT OF *FICUS CARICA*. A. Belisario*, M. Maccaroni, L. Corazza. *Istituto Sperimentale per la Patologia Vegetale, Sez. Malattie Crittogamiche, Via C.G. Bertero 22, I-00156 Roma, Italy. Fax: +39.06.86802296; e-mail: mal.crit@ispave.it

In 2001, a severe leaf drop was observed on plants of fig (*Ficus carica* L.) kept outdoors in a nursery in Rome. Reddish brown to dark brown angular spots tending to merge in brown dry large patches were evident on leaves, spots. Olivaceous mycelial mats were present on the abaxial leaf page containing the fructifications of *Cercospora bolleana* (Thum.) Speg. (teleomorph *Mycosphaerella bolleana* Higg.). The defoliation was observed in late spring, though the disease has been reported to occur in late summer-fall, causing consistent damage to the young plants. Among the four species of *Cercospora* reported on fig, *C. bolleana* represents the most common species in Europe. It has been reported in eastern Europe, Spain, Greece, and Italy. Olivaceous to dark brown slow-growing cultures were obtained on PDA both culturing small tissue fragments cut at the margin of leaf lesions and from single conidium cultures obtained by scrapping conidia directly from the lower surface of leaf lesions. Conidiophores were dark olivaceous brown, straight or geniculate, unbranched. Conidia were olivaceous, obclavate, straight or lightly curved, mostly 4 septate. The ITS-PCR sequence analysis exhibited 99% of identity of all 5 isolates analyzed. Their common ITS sequence was compared to the ITS sequences of several *Mycosphaerella* spp. available from GenBank showing high homology with *M. brassicicola* (Duby) Johanson ex Oudem and *M. fijiensis* Morelet.

EPIDEMIOLOGICAL AND BIOTECHNOLOGICAL ASPECTS OF BROOMRAPE BIOCONTROL IN SOME MEDITERRANEAN AREAS. A. Boari, A. Bottalico, J. Hereshorn, E. Dor, M. Vurro*. *Istituto di Scienze delle Produzioni Alimentari, CNR, Viale L. Einaudi 51, I-70125 Bari, Italy. Fax: +39.080.5486063; e-mail: a.boari@area.ba.cnr.it

Orobancha is a genus including many parasitic weed species, commonly called broomrapes, widely spread on almost all vegetables, grain legumes, sunflowers and many other plants in Europe, including the Balkans and Russia, around the Middle East and North Africa. *O. ramosa* L., whose distribution interests mainly the Mediterranean basin, North Africa and Asia, is one of the most damaging, together with *O. aegyptiaca*, infesting about 2.6 millions hectares of *Solanaceae*, above all tobacco, potato, tomato and eggplant. They cause both quantitative and qualitative damages through interfering with water and mineral intake. Difficulties in the control are due to the large amount of seeds produced (a well-developed *Orobancha* shoot can produce around 500,000 seeds) that can remain viable for decades, even in the absence of a host. The seeds germinate only if stimulated by host crop root exudates. Furthermore, plants have a long underground phase, so that when they emerge, most of the final damage has already been determined. Broomrapes are considered a good target for biological control because traditional methods proved to be ineffective. Research programs carried out in Italy and Israel have led to isolate some pathogenic fungi, mainly belonging to the genus *Fusarium*, resulted to have a great potential as soil mycoherbicides for broomrape control, being able to reduce shoot emergence as well as tubercles development. Studies on mass production, formulation and application strategies are in progress.

IDENTIFICATION AND PARTIAL CHARACTERIZATION OF A DNA LIGASE GENE FROM 16SrXII (STOLBUR) GROUP PHYTOPLASMA. A. Boarino*, C. Marzachi. *Istituto di Virologia Vegetale, CNR, Strada delle Cacce, 73, I-10135 Torino, Italy. Fax: +39.011.343809; e-mail: a.boarino@ifv.cnr.it

Phytoplasmas are wall-less phloematic prokaryotes inducing different syndromes in several plant species. They can not be maintained in axenic cultures, and this has hampered the study of their genomic organisation. To improve our knowledge on phytoplasma functional genes, databank (NCBI) bacterial gene sequences were used to construct degenerated primers for PCR amplification of partially homologous phytoplasmal sequences. One 340 bp phytoplasma specific amplicon has been obtained from 16SrXII-A subgroup phytoplasma (Stolbur) DNA, maintained in periwinkle. It has been cloned (pLig1) and sequenced. Sequence analysis with BlastN and BlastX revealed high similarity with bacterial NAD-dependent DNA ligase proteins (NAD-Lig). These are essential enzymes found in all bacteria that catalyse the formation of phosphodiester bonds at single-strand breaks between adjacent termini in double stranded DNA. Labelled pLig1 insert DNA was used to screen a Stolbur phytoplasma genomic library. One positive clone (pLig2) has been further characterised: it was 1816 bp long, partially overlapping to the 3' end of pLig1 and contained 1296 bp at the 3' end of the NAD-Lig gene. BlastX comparison of the total 1488 bp sequence confirmed the high similarity with bacterial NAD-Lig genes. RT-PCR on healthy and infected periwinkle total RNAs, with specific primers designed on Stolbur NAD-Lig specific sequence, confirmed the hypothesis of NAD-Lig gene expression in this phytoplasma.

SANITATION OF *PRUNUS SALICINA* SELECTIONS. G. Bottalico*, M.R. Silletti, A. Campanale, V. Savino. *Dipartimento di Protezione delle Piante e Microbiologia Applicata, Università di Bari, Via G. Amendola 165/A, I-70126 Bari, Italy. Fax: +39 080 5442911; e-mail: g.bottalico@agr.uniba.it

Some *Prunus salicina* selections, infected by *Prunus necrotic ringspot virus* (PNRSV) and *Plum pox virus* (PPV), were subjected to sanitation by *in vitro* culture of meristem shoot tips. To carry out sanitation trials it was first necessary to select culture media that allowed both stabilization of explants from meristem shoot tips and their fast development. Serological (ELISA) and molecular (RT-PCR) tests were performed to evaluate sanitation. Plantlets regenerated from apical meristems were assayed three times for virus presence by ELISA and the results showed that both viruses had been knocked out from most plantlets. Only a few still retain PPV, as determined by RT-PCR. The conclusion is that *in vitro* culture of meristem tips is a useful and effective technique for sanitation of *P. salicina* from PPV and PNRSV. Anyway, when analysing the results of the treatments, great attention must be paid to the reliability of diagnostic assays, mainly with young material coming from *in vitro* culture.

PRELIMINARY RESULTS OF SANITATION TRIALS OF VIRUSES-INFECTED OLIVE TREE. G. Bottalico*, M.E. Rodio, M. Saponari, V. Savino, G.P. Martelli. *Dipartimento di Protezione delle Piante e Microbiologia Applicata, Università di Bari, Via G. Amendola 165/A, I-70126 Bari, Italy. Fax: +39.080.5442911; e-mail: g.bottalico@agr.uniba.it

The use of molecular detection tools has shown that virus infections of olive are more frequent than as determined by bioassay. Sanitary selection and sanitation seem to be the only strategies for restraining virus dissemination and for producing propagative material complying with national and international quality requirements. Plants from 12 different cultivars infected either by *Olive yellowing-associated virus* (OLYaV) or *Cherry leafroll virus* (CLRV) were used for sanitation trials comparing meristem and shoot tip culture, chemo-therapy and heat-therapy. For meristem and shoot tip culture, explants were taken from *in vivo* and *in vitro* sources. Heat-therapy treatment was done *in vivo* (4 months – 1 year) or *in vitro* (10-30 days), at 32°C and 38°C respectively. The effectiveness of different concentration of ribavirin (1-6-12 mg l⁻¹) was also checked. RT-PCR was used with *in vivo* and *in vitro*-treated material to evaluate the results of the different sanitation treatments. Heat-therapy and shoot tip culture proved to be useful for the elimination of CLRV and OLYaV, respectively.

MORPHOLOGICAL AND MOLECULAR CHARACTERIZATION OF OCHRATOXIGENIC SPECIES OF *ASPERGILLUS* SECT. *NIGRI* ISOLATES FROM GRAPES. A. Bottalico, P. Battilani, G. Mulè, G. Perrone*, Z. Kozakiewicz, A. Logrieco. *Istituto di Scienze delle Produzioni Alimentari – CNR, Viale L. Einaudi 51, I-70125 Bari, Italy. Fax: +39.080.5486063; e-mail: g.perrone@area.ba.cnr.it

Potential ochratoxigenic *Aspergillus* strains isolated in 2001 from grapes collected from 16 vineyards all over Italy were classified according to their morphological features and characterized by sequencing of variable DNA regions (28S, ITS, calmodulin) and AFLP analysis. Among the 692 *Aspergillus* strains isolated from grapes, 87 % belonged to the *Aspergillus* section *nigri*, represented by the biseriata *A. carbonarius* (26.6%) and *A. niger* aggregate (28.8%), and by the uniseriate *A. japonicus/A. aculeatus* (44.6%). The sequence data were compared using DNAMAN and GCG package; whereas the AFLP data were elaborated with NTSYS program. The dendrogram obtained analyzing about 100 isolates showed two main cluster corresponding to *A. carbonarius* and *A. japonicus* isolates, while the *A. niger* isolates were grouped in more clusters. In particular for *A. carbonarius* were identified two specific peaks at 144 and 301 bp that could be useful to design specific molecular probes.

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THE ROLE OF ABC TRANSPORTERS OF *BOTRYOTINIA FUCKELIANA* (*BOTRYTIS CINEREA*) IN RESISTANCE TO ANTIBIOTICS PRODUCED BY FUNGAL BIOCONTROL AGENTS. B. Calò*, M. Lorito, F. Scala, A. Zoina, G. Del Sorbo. *Department Ar.Bo.Pa.Ve., Section of Plant Pathology, University of Naples "Federico II", I-80055 Portici (Napoli), Italy. Fax: +39.081.7755320; e-mail: barsacalo@yahoo.it

Trichoderma virens is a biocontrol agent producing antibiotics (e.g. glyotoxin) which are able to inhibit fungal growth and to synergize with lytic enzymes during mycoparasitism. The role of membrane permeases of the family of ABC transporters in protection from plant defence compounds as well as from natural and synthetic compounds has recently been elucidated in *Botrytis cinerea*. In the mentioned fungus, the ABC transporter encoded by the gene *BcatrB* confers resistance to resveratrol (a stilbene phytoalexin produced by grape) and to pyrrolnitrin and its synthetic derivatives (phenylpyrrole fungicides). We utilized mutants of *B. cinerea* targetedly disrupted either in *BcatrA* or *BcatrB* to demonstrate the involvement of the *BcatrB* gene product in protection from antibiotics produced by *T. viride* both *in vitro* and in biocontrol experiments of on gerbera petals. Furthermore, we found that exposure of germlings of a wild-type isolate of *B. cinerea* to *T. virens* culture filtrates or to its purified toxins determines a strong accumulation of *BcatrA* and *BcatrB* transcripts. Our results, taken together, indicate that ABC transporters can play an important role in the exchange of chemical signals between fungal phytopathogens and biological control agents, both during their direct interaction and in plant microenvironment.

BIOMOLECULAR CHARACTERIZATION OF *PHELLINUS TORULOSUS* POPULATION IN APULIA. G. Campanile*, S.L. Giove, N. Luisi. *Dipartimento di Biologia e Patologia Vegetale, Università di Bari, Via G. Amendola 165/A, I-70126 Bari, Italy. Fax: +39.080.5442906; e-mail: luisin@uniba.it

Phellinus torulosus (Pers.) Bourd. et Galz. is a dangerous agent of white alveolar decay, that infects especially the roots of old trees and shrubs of many species. The aim this work was to study, through the use of molecular markers (RAPD), the genetic variability of *P. torulosus* population, in order to compare the existence of different morphotypes with the maternal origin and the geographical origin of the isolates. The knowledge of the genetic variability of the pathogen is an essential prerequisite to get ready efficient systems of defence. In this work 144 isolates of the fungus coming from different host species occurring in 13 oakwoods of Apulia have been studied. The random amplification (RAPD-PCR) of DNA of single isolates has been performed using a set of 20 different primers, 16 of them have induced amplification products of different size, separable on agarose gel. Through statistical analysis (band distance and coefficient of genetic similarity and following UPGMA elaboration), dendrograms have been obtained that gave the possibility of displaying the genetic relationship among the isolates. The distribution of the samples as to the individualised morphotypes doesn't show any relationship that, on the contrary, is fairly good with geographical origin and host species.

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EFFECT OF CRUDE EXTRACTS FROM DIFFERENT *ASTER* SPP., ON GROWTH OF PHYTOPATHOGENIC FUNGI *IN VITRO*. M. Cammareri, M. Zaccardelli*, A. Errico, C. Conicella. *Istituto Sperimentale per le Colture Industriali, MiPAF, Strada Statale 18 156, I-84091 Battipaglia (SA), Italy. Fax: +39.0828.340169; e-mail: iscibattipaglia@tiscalinet.it

The recent alternative uses of plants for production of substances for pharmaceutical and phytotherapeutic applications, is of great interest. Among these substances, saponins are actively studied for their antitumor, antifungal, antibacterial and antiviral effects. In plants, saponins play a role in the complex defense mechanisms against phytopathogenic micro-organisms and insects. Species belonging to *Asteraceae* family are very important for saponin production and, particularly in *Aster* genus twenty-one saponins were extracted some of them showing good inhibitory activity against leukemia. In this work, crude leaf extracts from eight different *Aster* species were evaluated for their activity to inhibit the growth *in vitro* of seven different fungi strains, six of them phytopathogenic. The collection of leaves occurred at post-flowering stage. The growth of *Alternaria* spp. and *Sclerotinia* spp. was stimulated by extracts of all *Aster* species whereas, the growth of *Rhizoctonia* spp. and *Trichoderma viride* was neither stimulated nor inhibited. Only *Aster caucasicus* was able to inhibit the growth of *Fusarium solani* and, drastically, the growth of *Botrytis cinerea* (growth reduction of 72%). To use formulations containing extracts of *A. caucasicus* to control *B. cinerea*, it is necessary to ascertain the absence of toxicity of the saponins for man and animals.

EFFECT OF CaCl₂ AND TWEEN 80 ON THE BIOCONTROL ACTIVITY OF YEASTS. A. Campisano*, L. Strano, V. Coco, V. Grimaldi, G. Cirvilleri. *DISTeF, Università di Catania, Via Valdisavoia, 5, I-95123, Catania, Italy. Fax: +39.953.50043; e-mail: acampis@tin.it

Postharvest application of CaCl₂ to biocontrol yeasts may enhance their efficacy against *Penicillium digitatum* diseases on stored fruits. Influence of 1% CaCl₂ and 0.1% Tween80 on the efficacy of four biocontrol yeasts (*Debaryomyces hansenii* DB-VPG 4025, *Pichia guilliermondii* NRRL Y18314, *Saccharomyces cerevisiae* P1.6 and *Pichia anomala* J121) was assayed on oranges. The results carried out on Tarocco oranges in small scale experiments were confirmed on Valencia late oranges. *Pichia guilliermondii* showed the highest inhibition. The application of 1% CaCl₂ e 0.1% Tween 80 reduced severity of infection by more than 50%. The efficacy of *D. hansenii* was not modified by the application of CaCl₂ and that of *P. anomala* was slightly reduced. Further trials on Valencia late oranges were carried out by injecting the antagonists and the pathogen directly inside the wound to determine the growth of microorganisms and their efficacy in mould biocontrol. The yeasts *S. cerevisiae*, *D. hansenii* and *P. anomala* showed similar efficacy when applied alone or with CaCl₂. *P. guilliermondii* showed the highest inhibition (90%) when applied with CaCl₂. The application of CaCl₂ didn't influence fruit epicarp colonisation by *P. guilliermondii* e *S. cerevisiae*, while it reduced the population of *D. hansenii* (from 10⁶ to 5x10⁵ cfu cm⁻²) and *P. anomala* (from 8x10⁶ to 2x10⁵ cfu cm⁻²). These findings may lead to the development of a control strategy involving CaCl₂ and the appropriate yeasts, selected accordingly to the purpose.



OCCURRENCE AND DETECTION OF STRAWBERRY VIRUSES AND VIRUSLIKE DISEASES IN ITALY. M. Cardoni*, A.R. Babini, R. Bissani. *C.A.V. Centro Attività Vivaistiche, Via Tebano 144, I-48018 Faenza (RA), Italy. Fax: +39.0546.47189; e-mail: cav@mbox.dinamica.it

Currently strawberry cultivation is very important in Italy. The most important strawberry growing areas are: Campania, Emilia-Romagna, Veneto, Basilicata and Piedmont. Minor strawberry production areas, interesting for their out-of-season crops, include Trentino and Sicily. Many strawberry virus and virus-like diseases are present in Italy. Many of these do not induce distinct symptoms in commercial cultivars; often the only indications of infection are loss of vigour, lower yields and a general "running out" of cultivars. Viruses rarely occur singly in strawberry; frequently several viruses are present in combination. There are four major aphid-borne viruses (*Strawberry mottle virus*, *Strawberry crinkle virus*, *Strawberry mild yellow-edge virus* and *Strawberry vein banding virus*) affecting strawberry in Italy that causing plant decline and five nepoviruses (*Arabis mosaic virus*, *Strawberry latent ring spot virus*, *Raspberry ring spot virus*, *Tomato black ring virus* and *Tomato ring spot virus*) that cause diseases in the Italian fields but their importance has been minimised with nematode control by fumigation. To obtain virus-free strawberry plants, in Italy a national genetic-sanitary certification program was set up in 1984. This certification scheme operates on a voluntary basis. Under this program, about 100 source plants of 20 different varieties and new candidate varieties are kept under screen-house conditions, in accordance with the indications of the EPPO.

GENETIC VARIABILITY OF GRSPaV. P. Casati, A. Minfra*, P.A. Bianco. *Istituto di Virologia Vegetale, (CNR), Sez. di Bari, Via G. Amendola 165/A, I-70126 Bari. Fax: +39.080.5442911

Grapevine Rugose Wood is a syndrome formed by four different diseases, distinguishable on the basis on their specific symptomatology expressed on selected indicator grapevine species and varieties. Among these diseases, Rupestris stem pitting (RSP) has been commonly found associated with Grapevine Rupestris Stem Pitting associated Virus (GRSPaV), a member of the genus *Foveavirus*. The present work has been carried out in order to search for a possible genetic variability of GRSPaV: RSP affected plants of different origin have been examined by RT-PCR. Three different primer pairs, able to distinguish three GRSPaV sequence variant groups in the coat protein region sequences, were used in the RT-PCR tests. Then, the amplified cDNA was employed for the subsequent RFLP and sequencing analyses. The results obtained, clearly identified the presence of all the three above cited groups (tentatively indicated as 1°, 2° and 3°). Moreover the comparison, at the nucleotide level, among the isolates of the groups 3°, revealed values of similarity from 89% to 95% within the same group: lower levels of similarity were found when these sequences were compared with the GRSPaV isolate from California (USA).

ANTAGONISTIC ACTIVITY OF *BACILLUS SUBTILIS* STRAINS AGAINST *MONILINIA LAXA*. STUDY OF A FORMULATE FOR POST-HARVEST APPLICATION. L. Casalini*, M. Mari, G.C. Pratella. *C.R.I.O.F., University of Bologna, Via Fanin 46, I-40127 Bologna, Italy. Fax: +39.051.765049

Brown rot, caused by *Monilinia laxa* (Aderh. & Ruhl.) Honey is one of the most important diseases of stone fruit. In Italy, since postharvest chemical treatments on stone fruits are not authorised, finding alternative methods to control the disease is an essential requisite. In our laboratory, a 10⁸ vegetative cells suspension of two endophytic *Bacillus* strains are able to control the pathogen *M. laxa* on stone fruits. At this concentration the strains reduce the incidence of disease from 80-100%. Competition for nutrients seems to be one of the mechanisms involved in the biocontrol. In a 5% nectarine juice, the percentage of germinated pathogen conidia as well as the germ tube length are reduced by 50-70% in the presence of the biocontrol agent whit respect to control. The cell free filtrates were not effective in controlling the growth of the pathogen when inoculated on fruits proving that the *Bacillus* strains probably do not produce any inhibitory components. Considering the antagonistic capability of the two strains we have analysed a spores based formulate. Spores are highly resistant and easy to store but are less effective in controlling the pathogen compared with vegetative cells; in fact when applied directly on fruit wounds spores have not the capability to break dormancy. To allow the spores to break dormancy we have applied germinants prior to start the formulate treatment.

REDUCTION OF PATULIN CONCENTRATION IN BIOCONTROL YEAST-TREATED APPLES. R. Castoria*, L. Caputo, V. Morena, F. De Curtis, V. De Cicco. *Dip.to di SAVA - Università del Molise, Via De Sanctis, I-86100 Campobasso, Italy. Fax: +39.087.4404678; e-mail: castoria@unimol.it

The mycotoxin Patulin is produced by the fungal pathogen *Penicillium expansum* in pome fruits infected during storage, thus determining contamination of juices. Biocontrol agents (BCA) are currently used as valuable alternatives/integrations of fungicides for preventing postharvest rots. We tested 3 efficient BCA (RG, *Rhodotorula glutinis*, CL, *Cryptococcus laurentii* and AP, *Aureobasidium pullulans*) for their capability of resisting to and metabolizing Patulin *in vitro*. Optical density determinations of growth and HPLC analyses showed that RG and CL were both able to grow in the presence of Patulin and to metabolize the mycotoxin, being RG more efficient in Patulin metabolism. AP neither resisted to nor significantly metabolized the mycotoxin. Treatment with BCA or fungicides determines a strong reduction of infections by *P. expansum*, but a low percentage of fruits is infected by the fungus and represents the source of Patulin contamination of commercialized juices. Therefore, we assayed RG also for its ability to affect Patulin accumulation *in vivo*, i.e. in the fraction of apples in which treatment with the BCA was not successful in preventing fungal infection. Patulin concentration in RG-treated infected apples was about 50% lower than in untreated infected fruits, indicating that residual biocontrol yeast cells present in infection sites could metabolize Patulin or somehow negatively affect its accumulation.

EPIDEMIOLOGICAL STUDIES ON *PSEUDOMONAS CORRUGATA* AND ITS POSSIBLE TRANSMISSION TO TOMATO FRUITS AND SEEDS. G. Cirvilleri*, P. Bella, A. Pacetto, V. Catara, R. La Rosa. *DISTEF – Patologia Vegetale, Università di Catania, Via Valdisavoia 5, I-95123 Catania, Italy. Fax: +39.095.350043; e-mail: cirville@mbox.fagr.unict.it*

A bioluminescent strain of *Pseudomonas corrugata*, causal agent of tomato pit necrosis, was used to investigate epidemiology and population dynamics of the bacterium inoculated in tomato flowers and fruits. Peptone wash water and homogenates of fruit pulp were serially diluted and plated on NAG+Rif¹⁰⁰ TC¹⁰ before and after enrichment. Identity of *P. corrugata* colonies was confirmed by morphology on NAG, growth on selective media, bioluminescence, and PCR assay. Tomato fruits at the mature stage showed bacteria in the surface, pulp and seeds. Population of the pathogen in the surface, in the pulp and in the seeds was affected by the type of treatment. All tomatoes produced from *P. corrugata* inoculated flowers contained the pathogen on the surface of fruits (100% in enriched samples) as well as in the pulp homogenates (100% before and after enrichment). All tomatoes produced from inoculated green tomatoes contained *P. corrugata* on the surface as well as in the pulp homogenates (100% after enrichment). *P. corrugata* was recovered from the surface and from the pulp of tomato fruits after 20 days of storage at 4°C, and from the seeds of tomato fruits produced from inoculated flowers and green tomatoes. Results suggest that *P. corrugata* 4.3t strain can be transmitted from flowers to fruits and seeds, thus surviving during fruit development and ripening.

PHYLOGENETIC ANALYSIS OF POTATO VIRUS Y PEPPER VENIAL NECROSIS STRAIN (PVY-*pvn*). A. Crescenzi*, A. Fanigliulo, S. Comes, R. Pacella, P. Piazzolla. **Dipartimento di Biologia, Difesa e Biotecnologie Agro-Forestali. Università degli Studi della Basilicata, Campus Macchia Romana 3A310, I-85100 Potenza, Italy. Fax: +39.0971.205503; e-mail: crescenzi@unibas.it*

A Potato virus y (PVY) isolate recovered from *Capsicum annuum* L. and named PVY-*pvn* has been recently sequenced, highlighting a remarkable divergence from 'potato' isolates belonging to PVY^N and PVY^O groups (Crescenzi *et al.*, 2002. XIIth International Congress of Virology, Paris). The phylogenetic analysis (distance matrix) performed on PVY species is reported, aiming at studying the evolutive distance between PVY-*pvn*, PVY^N, PVY^O and those isolates defined *non potato* (Blanco-Urgoiti *et al.*, 1996. *Archives of Virology* 141, 2425-2442). The analysis performed on the whole polyprotein and on each functional product highlights that PVY^N and PVY^O groups are always monophyletic. The polyprotein tree seems to suggest that the *non potato* isolates cluster together. However the trees of all separate proteins do not confirm this finding. The different trees obtained do not suggest recombination events giving origin to the PVY-*pvn* isolate. In fact, different tree topology may be cause of accidental changes in the proteins because they are not so clearly different. The analysis performed on P1 protein suggests that PVY-*pvn* could be a separate group from the PVY^N and PVY^O ones and from *non potato* isolates too. The present study shows the distinctiveness of PVY-*pvn* isolate within PVY species and the need to deepen the knowledge relative to other pepper and further 'non potato' isolates.

CHERRY CHLOROTIC RUSTY SPOT DISEASE IS ASSOCIATED TO DOUBLE-STRANDED RNAs SIMILAR TO THOSE OF MYCOVIRUSES. L. Covelli*, F. Di Serio, M. Malfitano, C. Hernández, A. Ragazzino, R. Flores. **Istituto de Biología Molecular y Celular de Plantas (UPV-CSIC), Universidad Politécnica de Valencia, Avenida de los Naranjos s/n, E-46022, Valencia, Spain. Fax: +34.96.3877859; e-mail: lcovelli@ibmcp.upv.es*

Cherry chlorotic rusty spot disease (CCRS), described in Italy, is associated with 12 double-stranded RNAs (dsRNAs) and 2 small circular RNAs. It appears different from other cherry diseases except the 'Amasya disease' observed in Turkey. In this work we have characterized two of the dsRNAs isolated from CCRS-affected cherry trees. The aminoacid sequence deduced from the dsRNA 3 shows a single ORF without similarities with other sequences deposited in databases. In contrast, the single ORF deduced from the dsRNA 6 presents the 8 motifs conserved in the RNA dependent-RNA polymerases (RdRp) characteristic of fungal viruses with a dsRNA genome. Fungal hyphae have been consistently observed in the symptomatic areas of the infected organs, although no phytopathogenic fungus has been isolated so far. These results suggest that CCRS disease may have a fungal aetiology. In line with this hypothesis, attempts to transmit the pathogenic agent to indicator plants by graft and mechanical inoculations or to observe virus-like particles by electron microscopy have been unsuccessful. Consequently, the dsRNAs are most likely the components of one micovirus with a multipartite genome or of several micoviruses.

EXPERIMENTAL RESULTS ON ANTIFUNGAL COMPOUNDS FROM ORNAMENTAL PLANTS. P. Curir*, C. Pasini, M. Sacco, F. D'Aquila. **Istituto Sperimentale per la Floricoltura di Sanremo, Corso Inglese 508, I-18038 Sanremo, Italy. Fax: +39.0184.695072; e-mail: difesa@istflori.it*

During several years of investigation at ISF, Sanremo, Italy, many antifungal compounds have been detected within the tissues of some ornamentals. Their fungitoxic activity has been mainly evaluated through *in vitro* experiments, during which the inhibitory effect of each molecule was tested against different pathogenic fungi. Antifungal molecules were extracted from carnation (*Dianthus caryophyllus*), *Genista monosperma*, *Khaya senegalensis*, *Aesculus pavia*. The most active compounds proved to be flavonols, with variable degrees of glycosylation, isoflavonols and coumarin derivatives. All the isolated compounds showed an appreciable inhibitory activity at concentrations ranging from 100 to 300 micromolar, depending on the fungal species. The pathogens tested in the inhibition trials were various *formae speciales* of *Fusarium oxysporum*, such as the f.sp. *dianthi*, *cyclaminis*, *lilii*, *asparagi*, and *Pythium* sp., *Phytophthora cryptogea*, *Armillaria mellea*. Flavonol glycosides were particularly effective against *F. oxysporum* f.sp. *dianthi*, but the effectiveness for a given concentration was variable depending on the tested *forma specialis*. Coumarin derivatives inhibited *Pythium* sp., while isoflavones were particularly active against *A. mellea*. Further studies are in progress, with the aim of proposing the utilization of such plant new molecules as natural pesticides with a reduced ecological impact.

RELIABILITY OF FIRE BLIGHT PREDICTION SYSTEMS MARYBLYT AND BIS ON APPLE. B. Cvjetković*, E. Halupecki. *Department of Plant Pathology, Faculty of Agriculture, Sve-tošimunska 25, 10000 Zagreb, Croatia. Fax: +385.1.2393786; e-mail: bcvjetkovic@agr.hr

Fire blight caused by bacteria *Erwinia amylovora* occurred on apples and pears in eastern parts of Croatia in 1995. Following its appearance all quarantine measures were undertaken but the disease was not eradicated. Since then it has spread but it has not yet been observed neither in western parts of the country nor in the coastal region. Correct decision-making in the application of chemical control has major impact in the integrated control. In order to achieve correct decision making monitoring and a meteorological station network in apple orchards was established. The most interesting location was Osijek where natural infections have been occurring since 1996. The forecasting models, Maryblyt and BIS, were introduced to determine their comparability and to estimate their usefulness in the region where we checked them from 1997 until 2001. The possibility for infection through flowers was calculated from meteorological data. Based on observations in orchards and predictions by both forecasting models, the years 1998 and 2001 were not favorable either for infections through flowers or for disease development. Flower infections were observed in 1997, 1999 and 2001 in accordance with predictions of both models. In 2001 infection occurred after flowering and there were shoot blight. In that year the Maryblyt model was more useful because it correctly predicted the occurrence of shoot blight.

ANTAGONISM OF *SCOPULARIOPSIS CANDIDA* AGAINST *PHYTOPHTHORA NICOTIANAE*: ULTRASTRUCTURAL EVIDENCE. M. De Stefano*, R. Nicoletti, F. Raimo. *Zoological Station A. Dohrn, Villa Comunale, I-80131 Napoli, Italy. Fax: +39.081.7641355; e-mail: destefa@alpha.szn.it

A number of fungal antagonists of *Phytophthora nicotianae* were isolated from the rhizosphere of tobacco plants and their interactions with the pathogen were inspected in dual cultures *in vitro*. Unlike other isolates, isolate SP1 belonging to the species *Scopulariopsis candida*, which is a new record as an antagonist of *P. nicotianae*, did not completely inhibit growth of the latter. However, isolate SP1 induced aberrant mycelial growth and malformation of sporangia, which were evident even when its concentrated culture filtrate (20% v/v) was added to the growth medium of *P. nicotianae*. Alterations occurring in both dual cultures and cultures added with concentrated culture filtrate were further inspected by means of electron microscopy. Scanning electron microscopy evidenced remarkable alterations of the hyphae of *P. nicotianae*, which appeared to be twisted and flattened along the growth axis. Transmission electron microscopy revealed that such alterations are related to the loss of the ultrastructural integrity of the hyphae. These evidences confirm that *S. candida* can act as an ecological antagonist of *P. nicotianae*, and that toxic metabolites may play a role in such antagonistic behaviour.

ANTAGONISTIC ACTIVITY OF THE ENTOMOPATHOGENOUS FUNGUS *BEAUVERIA BASSIANA* AGAINST GRAPEVINE PATHOGENS: PERSPECTIVE OF COMBINED USE AGAINST INSECTS AND FUNGI. F. De Luca, A. Vecchione*, I. Pertot. *Istituto Agrario San Michele all'Adige, Via E. Mach 1, I-38010 San Michele a/a (TN), Italy. Fax: +39.0461.650872; e-mail: antonella.vecchione@ismaa.it

Some fungi of the *Beauveria* genus are well known for their pathogenic activity against several species of insects and they can be used in organic and integrated pest management of several crops. A partial antagonistic activity has been reported also against some micro-organisms. A combined activity against insects and fungi could be an additional tool for biological pest control. The *in vitro* inhibitory activity of two organisms (B1 and B3) isolated from dead insects because of *Beauveria bassiana* infection has been evaluated against plant pathogens: *Armillaria mellea* and *Penicillium digitatum*. Dual culture test with the isolates and the pathogens has been used. In this test the pathogen and the biocontrol agent grown at different temperatures have been evaluated with the software *Image Pro Plus*. Both the isolates have antagonistic activity against the two pathogens. B1 strain is faster growing than B3 and shows also a partial hyperparasitism against *A. mellea*. *P. digitatum* is less inhibited than *A. mellea* and it also shows a light antagonistic activity against B1 e B3.

IRON INVOLVEMENT IN CELLULOSE DEGRADATION BY *PHAEOMONIELLA CHLAMYDOSPORA* AND *PHAEACREMONIUM ALEOPHILUM*, PATHOGENS ASSOCIATED TO ESCA DISEASE. S. Di Marco*, F. Osti, A. Cesari. *IBIMET, CNR, Via Gobetti 101, I-40129 Bologna, Italy. Fax: +39.051.6399024; e-mail: sdimarco@agrsci.unibo.it

The involvement of iron in cellulose degradation by *Phaeomoniella chlamydospora* (*Pch*) and *Phaeacremonium aleophilum* (*Pal*), pathogens associated with Esca of grapevine and kiwifruit decay, was investigated under laboratory conditions. *Pch* and, to a lesser extent, *Pal*, submitted to chrome azurol S test, were found to produce compounds with capability in chelating and reducing iron (siderophores). Hydroxyl radical formation, detected by deoxyribose assay, in *Pch* and, to a lesser extent, in *Pal*, was significantly dependent on ferric iron concentration in the substrate. For both fungi, chromatographic assays showed the production of crystalline cellulose degradation compounds, in the presence of iron in liquid culture. Moreover, the fungal degradation of filter paper (remazol brilliant blue dye-assay) was significantly influenced by the presence of iron. Degradation of carboxymethylcellulose sodium salt was assessed for *Pal* and, to a lesser extent, for *Pch*, by viscosimetric assays. This cellulolytic process, probably due to enzymatic activity, was not affected by iron. Results suggested that iron reduced by fungal siderophores can react with oxidants, following a Fenton type reaction, to generate hydroxyl radicals. These reactive compounds could be associated with cellulose degradation in the wood brown rot process, in addition to enzymes.



PEACH LATENT MOSAIC VIROID: NEW VARIANTS WITH CHARACTERISTIC INSERTIONS ISOLATED FROM PEACH TREES AFFECTED BY CALICO. F. Di Serio*, T. Amenduni, C. Hernández, A. Myrta, V. Savino, R. Flores. *Dipartimento di Protezione delle Piante e Microbiologia Applicata, Università degli Studi and Istituto di Virologia Vegetale del CNR, Sezione di Bari, Via G. Amendola 165/A, I-70126 Bari, Italy. Fax: +39 080 5442911; e-mail: csvvfd12@area.ba.cnr.it

The involvement of *Peach latent mosaic viroid* (PLMVd) in the aetiology of peach calico (PC), an extensive white-cream bleaching of leaves, stems and fruits, has been recently proven. It has been also shown that the pathogenicity determinant of PC is contained in a sequence fragment of 12-13 nucleotides always located between the first and the last nucleotide of the PLMVd reference sequence (Malfitano, Di Serio, Covelli, Ragozzino, Hernández and Flores. XII International Congress of Virology, Paris, 2002). This insertion has been found in some PLMVd variants from the peach cv. Bellella di Melito affected by PC. In 2001, peach trees cv. Sprincrest showing typical PC symptoms were found in Apulia. Molecular characterization of this PC isolate showed new PLMVd variants containing insertions of 12-13 nt located in the same position and with sequences partially or completely identical to those of the PLMVd-PC variants previously described. Graft inoculations of GF305 peach seedlings and molecular characterization of the transmitted PLMVd populations showed that bleached branches of the field plants displaying PC were infected by a PLMVd population at least in part different from that invading non symptomatic branches of the same plants. These results provide interesting data to further explore the role of the insertion and its polymorphism in PC pathogenicity.

IDENTIFICATION OF CITRUS INFECTIOUS VARIATION VIRUS BY MOLECULAR HYBRIDIZATION. M.T. Fatone*, G. Loconsole, L. Barbarossa, M. Castellano, V. Savino. *Dipartimento di Protezione delle Piante e Microbiologia Applicata, Università di Bari, Via G. Amendola 165/A, I-70126 Bari, Italy. Fax: +39 080.5442911; e-mail: mt.fatone@agr.uniba.it

Citrus infectious variegation is a virus disease found in all grown citrus species in several citrus-growing areas, causing problems for production especially in some susceptible varieties of lemon and mandarin. The causal agent is the Ilarivirus *Citrus variegation virus* (CVV), that has been almost totally detected until now by indexing and serological assays, but in the second case not always with reliable results. In this context, the main aim of the work was to make available a sensitive molecular diagnostic system to facilitate citrus sanitary controls of infections associated to CVV. A RNA fragment, complementary to a part of CVV capsidic protein, was transcribed and labelled by digoxigenin (DIG) and used as a non-radioactive RNA probe for the detection of the virus associated to the citrus infectious variegation. The results of dot-blot hybridization showed that the probe was effective for the identification of CVV either in infected citrus trees held under controlled conditions (greenhouses) or in orchards. The dot-blot hybridization seems to be a more sensitive method for the diagnosis of CVV than the TAS-ELISA assay by using the now available commercial kit.

DETECTION AND CHARACTERISATION OF GRAPEVINE LEAFROLL ASSOCIATED VIRUS-4 AND 5. M.A. Dridi, C. Turturo, P. Saldarelli, A. De Stradis, M. Digiaro, D. Boscia*. *CNR-Istituto di Virologia Vegetale, Sez. di Bari, Via G. Amendola 165/A, I-70126 Bari, Italy. Fax: +39.080.5442911; e-mail: csvvdb08@area.ba.cnr.it

Two polyclonal antisera were produced against a Mediterranean GLRaV-4 isolate (Koudsi Y252) and a GLRaV-5 isolate (cv. Emperor, source from the USA). The antiserum raised to GLRaV-5 (As-LR5/BA) may be used for immune electron microscopy (IEM), whereas it is not reliable for routine ELISA. When the antiserum to GLRaV-4 (As-LR4/BA) was compared with an American antiserum (As-CA4), the two reacted in a different manner: isolate LR106 (GLRaV-4 type strain) was not recognised by As-LR4/BA and Koudsi Y252 was not identified by As-CA4. These results were partially confirmed by IEM. RT-PCR results showed that Koudsi Y252 was amplified by degenerate primers (able to amplify GLRaV-4 and GLRaV-5) but not by specific primers. The sequence of RT-PCR products showed 88% of identity between Koudsi Y252 and LR106. The variability observed between Koudsi Y252 and LR106 (sequence, RT-PCR with specific primers, ELISA, IEM) may indicate that the Mediterranean isolate of GLRaV-4 is a new strain of the virus, even though this needs to be substantiated by sequencing of the coat protein gene. A survey of 320 vines of several geographic origins showed that the incidence of each virus (GLRaV-4 and GLRaV-5) was approximately 6%.

USE OF POLICLONAL ANTIBODIES IN THE DIAGNOSIS OF PHYTOPHTHORA CINNAMOMI. L. Ferraris, P. Roggero, F. Cardinale, D. Valentino, G. Tamietti*. *University of Turin, DiVaPRA-Plant Pathology, Via Leonardo da Vinci 44, I-10095 Grugliasco (TO) Italy. Fax: +39.011.670854; e-mail: giacomo.tamietti@unito.it

A polyclonal antiserum against water soluble proteins from *Phytophthora cinnamomi* mycelium was produced in rabbit. Bleedings were carried out after 25, 65, and 180 days. The obtained antisera gave positive reactions in western-blot analyses against 9 species of *Phytophthora* and *Pythium* sp., but not against *Rhizoctonia solani*, a binucleate *Rhizoctonia*, *Verticillium dahliae*, *Fusarium oxysporum* and *Cryphonectria parasitica*. The antiserum obtained 25 days after the injection was less avid, but more specific than the one from the 180 days bleeding. All *Phytophthora* species showed common epitopes on proteins of a molecular mass of 77, 66, 51, and 48 kD. On the other hand, a species specific protein of 55 kD was immunodecorated only in *P. cinnamomi* samples. When used in ELISA assays, the 1:10,000 diluted antiserum revealed only the *Phytophthora* isolates, giving the strongest reaction with *P. cinnamomi* protein extracts (150-175% higher absorbance than with other *Phytophthora* species). The antiserum is active at dilutions ranging from 1:10,000 to 1:40,000 with a sensitivity threshold of 2 µg of total fungal proteins. In western-blot, the antiserum positively identified *P. cinnamomi* in infected chestnut, blueberry and azalea tissues.

STRAWBERRY LATENT RINGSPOT VIRUS (SLRSV) CAUSE OF DIFFERENTIATION AMONG RAGGIOLA AND FRANTOIO OLIVE CULTIVARS. L. Ferretti*, F. Faggioli, G. Pasquini, R. Sciarroni, G. Pannelli, L. Baldoni, M. Barba. *Istituto Sperimentale per la Patologia Vegetale Via C.G. Bertero 22, I-00156 Roma, Italy. Fax: +39.06.86802296; e-mail: virologia@ispave.it

The olive cultivars Raggiola and Frantoio are considered very different from the morphological and agronomical point of view. On the contrary, genetic analysis (AFLP) showed a high homology among these two cultivars that can be considered similar or identical from the genetic point of view. For this reason, we focused our attention on the possible cause(s) of the phenological differentiation. Since the morphology of 'Raggiola' fruits and leaves resembled the symptoms caused by SLRSV (Marte *et al.*, 1986), an investigation on the presence of this virus and the other viruses found in olive (CLRV, ArMV, CMV, OLYaV, OLV-1, OLV-2) has been done. Results showed a perfect association among 'Raggiola' samples and the presence of SLRSV. This strict correlation was pointed out also in an olive tree showing either shoots with 'Raggiola' or 'Frantoio' phenotype. In fact, molecular analysis (RT-PCR) confirmed that the presence of SLRSV is always associated with samples of 'Raggiola' phenotype. All these data suggest that SLRSV can be the cause of the morphological and agronomical differentiation among Raggiola and Frantoio olive cultivars.

IDENTIFICATION AND CHARACTERIZATION OF *RHIZOPYCNIS VAGUM* ISOLATES OF DIFFERENT ORIGIN. S. Ghignone, C. Mariani, G. Tamietti, A. Infantino, N. Pucci, C. Montuschi, M. Girlanda*. *Dip. Biologia Vegetale - IPP Sez. Torino, V.le Mattioli 25, I-10125 Torino, Italy. Fax +39.011.6707459; e-mail: mariangela.girlanda@unito.it

Rhizopycnis vagum D.F. Farr is a recently described coelomycete known to contribute to Vine decline of cucurbits. The fungus was first reported from cantaloupe in Texas in 1991, then from melon and watermelon in California, Arizona, Guatemala, Honduras, Costa Rica, and cucurbits in Spain and Italy. However, *R. vagum* appears to be associated with other hosts in Italy. In 2001 it was isolated from tomato roots showing corky root symptoms. In 1989 a sterile fungus was isolated from surface-sterilized mycorrhizal roots of healthy-looking *Pinus halepensis* and *Rosmarinus officinalis* plants in a Mediterranean forest. This fungus was then regularly obtained from asymptomatic roots of both hosts over an 11-year period, and it was identified as *R. vagum* based on ITS sequences. *R. vagum* isolates representative of the different geographical/host origins were characterized as a first step to understand their ecological potential. Endophytic isolates from *P. halepensis* and *R. officinalis* were assayed in greenhouse for pathogenicity on cantaloupe, as compared to American isolates from melon and the two other Vine decline and Root rot pathogens, *Monasporascus cannonballus* and *Acremonium cucurbitacearum*. Microsatellite markers were analysed to assess genetic variation among and within *R. vagum* populations. ITS specific primers were also designed and tested for sensitivity, selectivity against a range of taxonomically- and ecologically-related fungi, and detection capability in plant material.

PHYTOPATHOLOGICAL PROBLEMS AND POSSIBLE SOLUTIONS FOR SOILLESS FLORICULTURAL CROPS. A. Garibaldi*, A. Minuto, V. Grasso, M.L. Gullino. Dipartimento di Valorizzazione e Protezione delle Risorse Agroforestali, Patologia Vegetale, Università di Torino, Via Leonardo Da Vinci 44, I-10095 Grugliasco (TO), Italy. Fax: + 39 011 6708541; e-mail: manaresi@rettorato.unito.it

The adoption of soilless cultivation is an alternative to soil disinfection practices carried out in order to prevent pest, disease and weeds spread. The inclusion of methyl bromide, a widely used soil fumigant, among the list of ozone depletion substances and its phasing-out by 12-31-2004, could increase the interest for soilless cultivation, firstly for cut flower production. The advantages are an increase of productivity, an easier crop management and the reduction of conventional soil-borne pathogens. The environmental impact of open systems should be limited to reduce the release in the environment of fertilizers. The closed systems seem to be the best solution, even if the need of disinfecting the recirculating nutrient solutions increases the cost of investments. Moreover, the establishment of new diseases seems a risk that needs to be taken into account as well as the appearance of diseases already known, but not yet present in soilless system. To avoid the disease spread risks, a research was carried out to evaluate the adoption of active [chemical (fungicides), physical (U.V. radiation)] and passive disinfection systems (slow sand filtration). The encouraging results obtained using U.V. and, particularly, slow sand filtration seem to permit a non chemical management of recycled solutions, even if the effect on their chemical parameters (*i.e.*, pH, O₂, E.C., etc.) should be considered.

SOIL SURVIVAL OF PSEUDOMONADS CAUSAL AGENTS OF 'TOMATO PITH NECROSIS'. S. Greco, P. Bella, G. Polizzi, G. Cirvilleri, V. Catara*. *Dipartimento di Scienze e Tecnologie Fitosanitarie, Sez. Patologia Vegetale, Università di Catania, Via Valdisavoia 5, I-95123 Catania, Italy. Fax: +39.095.350043; e-mail: vittoria@mbox.fagr.unict.it

In this study different trials were set up to investigate soil survival of *Pseudomonas corrugata* and *P. mediterranea* sp. nov. strains, in pots with and without tomato plants. Bacterial population density of spontaneous Rif^r strains was assessed by dilution-plating. In a first experiment, a strain per each species was inoculated into pots with different types of soil and incubated in a growth chamber at 25°C. Concentration of both strains declined more drastically in sandy soil than in sandy-loam soil; in sandy-loam soil strain CFBP 5449 (*P. corrugata*) was detected up to ten weeks after inoculation whereas strain CFBP 5447 (*P. mediterranea*) was detected up to the sixth week. After eighteen weeks, both strains were detected only in sandy-loam soil. In the second experiment, tomato seedlings cv. Arletta were transplanted in sterile sandy soil inoculated with both strains, alone or in mixture. Bacterial concentration of both strains was stable over 5-weeks period of sampling; the same concentration was obtained in a sampling performed after 19 weeks. Both strains were able to colonize endophytically tomato roots. Eleven weeks after inoculation both strains were detected in the pith of tomato plants and population density of individually inoculated strains was significantly higher than observed in bacterial mix.



CONTROL OF SOILBORNE PATHOGENS WITHOUT METHYL BROMIDE: RESULTS AND PERSPECTIVES. M.L. Gullino*, A. Minuto, G. Gilardi, A. Camponogara, A. Garibaldi. *Dipartimento di Valorizzazione e Protezione delle Risorse Agroforestali, Patologia Vegetale, Università di Torino, Via Leonardo Da Vinci 44, I-10095 Grugliasco (TO), Italy. Fax: +39.011.6708541; e-mail: gullino@agraria.unito.it

Based on a survey, recently carried out in Italy, tomato, pepper, eggplant, strawberry and melon still rely on methyl bromide (MB) for soil disinfestation. Since MB production and marketing will be completely forbidden by 12-31-2004 (European regulation CE 2037/2000), the Italian growers need a quick and effective transfer of feasible and useful alternative strategies. Aimed at this purpose, several solutions were tested in order to solve some of the major problems that affect floriculture and vegetable crops. Chemical (methylisothiocyanate generators, others thiocyanates, chloropicrin alone or combined with 1,3 dichloropropene, iodomethane, not fumigant compounds, physical (steam, solarization), biological alternatives (*Fusaria* and *Trichoderma* strains, *Streptomyces griseoviridis*, *Coniothyrium minitans*), others strategies (grafting) and new technologies (soilless crop) were evaluated in the horticultural sector. None of them should be considered as the only solution for soil disinfestation, while their combination could often provide a reliable solution. Moreover, the experience gained in more than 20 years in this crucial field suggests a continuous monitoring where new alternatives are adopted in order to avoid the adaptability of old pathogens to the new environment or the spread of new potential biotic and/or abiotic problems.

FUNGI ASSOCIATED WITH VINE DECLINE AND ROOT ROTS OF CUCURBITS IN ITALY. A. Infantino*, G. Ciuffreda, C. Montuschi, A. Carlucci, N. Pucci, A. Savino, S. Frisullo. *Istituto Sperimentale per la Patologia Vegetale, Via C.G. Bertero 22, I-00156 Roma, Italy. Fax: +39.06.86802296; e-mail: epid&resist@ispave.it

Soil-borne diseases are important yield-limiting factors in many cucurbit growing areas. In addition to the well documented *Fusarium* wilt and *Verticillium* wilt, Root rot and Vine decline have been recently observed in Italy in several cultivated fields of melon, watermelon and cucumber. Symptoms on the aerial part start with yellowing of the crown leaves followed by collapse of the vines just prior to harvest. Belowground symptoms include necroses and brownish lesions on the taproot at the junction of secondary and tertiary roots, often associated to root rot and to reddish and corky lesions. The fungal species more frequently associated to symptomatic roots were *Monosporascus cannonballus*, *Acremonium cucurbitacearum*, *Plectosporium tabacinum* and *Rhizopycnis vagum*, with a frequency varying with host species, geographic origin and symptomology. Pathogenicity of the isolates of these species was tested in the greenhouse by artificial inoculation on watermelon, melon (*R. vagum* was tested only on melon). This is the first report of *A. cucurbitacearum* and *P. tabacinum* on melon, watermelon and cucumber and of *R. vagum* on melon in Italy. Vine decline causes severe damages in many cultivated cucurbit areas worldwide and is of complex aetiology. The contribution of the different species to the disease must be assessed for the choice of the most appropriate control strategies.

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DETOXIFICATION OF THE TOXIN PRODUCED BY PYRENOPHORA GRAMINEA IN GUSA LINES OF BARLEY. A. Haegi*, P.A. Lazzeri, A. Porta-Puglia. *Istituto Sperimentale per la Patologia Vegetale, Via C.G. Bertero 23, I-00156 Roma, Italy. Fax: +39.06.86802296; e-mail: epid&resist@ispave.it

Pyrenophora graminea, the causal agent of barley leaf stripe, produces the toxin Pg which is able to reproduce the typical symptoms of the disease upon infiltration into barley leaves. This toxin is composed by glycidic and proteic moieties; the toxic activity resides in the glycidic component and is inactivated by the enzyme β -glucuronidase. This feature suggested that barley plants transformed with the gene codifying for β -glucuronidase (*gusA*) could be resistant to the toxin Pg and presumably be able to interfere with the necrotrophic phase of fungal invasion during which sporulation occurs. Therefore, three 'Golden Promise' barley lines transformed with *gusA*: 19, id and 2a, were studied for the reaction to the Pg toxin. Each plant has been analysed for β -glucuronidase activity using the quantitative method based on 4-methylumbelliferyl β -D-glucuronide (MUG) and for reaction to the Pg toxin by infiltration into barley leaves. Results show that expression of β -glucuronidase in transformed barley plants confers resistance to the toxin Pg. In line 19 all plants that have a β -glucuronidase activity higher than 2.4 nmoles MU min⁻¹ mg⁻¹ of protein do not develop stripes after toxin infiltration and there is a good correlation between β -glucuronidase activity and resistance to the toxin. In line id this correlation is less clear, suggesting that the insertion site and the kind of expression of the inserted gene are relevant for resistance to the toxin. Line 2a resulted unreliable for this study due to the instability of the transgene.

REACTION OF NICOTIANA TABACUM, TRANSGENIC FOR A STEROID RECEPTOR, AGAINST ROOT-KNOT NEMATODES ATTACK. T. Irdani*, S. Caroppo. *Istituto Sperimentale per la Zoologia Agraria, Via di Lanciola 12/A, Cascine del Riccio, I-50125 Firenze, Italy. Fax: +39.055.2492255; e-mail: carosal@interfree.it

Tobacco plants, transgenic for a steroid receptor such those for glucocorticoids (GR), have been constructed with recombinant DNA-techniques and successively assayed for their reaction to the root-knot nematode attack. Transgenic plants expressing GR constitutively were employed in infection experiments in climate chamber because their altered morpho-physiological development, accounting for the overexpression of this heterologous protein, carried out a modification of the endogenous hormone equilibrium. Since the relationship between the plant hormonal status and the resistance to pathogens is proved to be strictly linked, quantification of the nematode reproduction was assayed comparing the reactions of control and transgenic plants inoculated with a population of *Meloidogyne incognita*. Even if the root galling index did not differ between the two classes of plants, marked differences were noticed in the reproduction rate of the nematode which resulted significantly lower for the transgenic plants.



RESISTANCE ACTIVATORS AND ATMOSPHERIC POLLUTANTS: INTERACTION BETWEEN BTH AND OZONE. M. Iriti, G. Rabotti, F. Faoro*. *CNR, Istituto di Virologia Vegetale, Sezione di Milano e Dipartimento di Produzione Vegetale, Università di Milano, Via Celoria 2, I-20133 Milano, Italy. Fax +39.02.50316781, e-mail: franco.faoro@unimi.it

Among atmospheric pollutants, ozone (O₃) is the most abundant during the growing season, causing widespread biological and economic damages to plants. O₃ decomposes spontaneously in aqueous solutions to form H₂O₂ and other active oxygen species that cause severe damages on membrane lipids and proteins. Benzothiadiazole (BTH) is a resistance activator that can elicit a long-lasting, non-specific systemic resistance against a broad spectrum of pathogens. BTH is particularly efficient as it inhibits catalase and ascorbate peroxidase, leading to H₂O₂ accumulation in the tissues as O₃ does. To study the combined effect of O₃ and BTH we have fumigated with O₃ bean plants at different intervals after BTH spray and carried out biochemical, cytochemical and ultrastructural studies on leaf tissues. These investigations showed that, when BTH was applied up to 3 days before fumigation, bean susceptibility to O₃ improved significantly, both in terms of symptoms and cell damages. On the contrary, when fumigation was carried out 7 days after BTH spray, plants were more tolerant to O₃ than untreated controls, showing no apparent symptoms and a few deranged cells. The localization of H₂O₂ allowed correlating the increased susceptibility to the accumulation of high level of this compound in the tissues up to 24-48 h from BTH treatment. The increment of ascorbate and guaiacol peroxidase activity are instead involved in the increased tolerance observed in longer induction times.

CLONING OF ABC TRANSPORTER-ENCODING GENES IN TRICHODERMA SPP., TO DETERMINE THEIR INVOLVEMENT IN BIOCONTROL. S. Lanzuise, M. Ruocco, V. Scala, S.L. Woo, F. Scala, F. Vinale, G. Del Sorbo, M. Lorito*. *Department of Ar.Bo.Pa.Ve., Section of Plant Pathology, University of Naples Federico II and CNR Institute for Plant Protection, Via Università 100, I-80055 Portici (NA), Italy. Fax: +39.081.2539339; e-mail: lorito@unina.it

ATP-binding cassette (ABC) transporters are ATP-dependent permeases which mediate transport of a number of different substrates over biological membranes. The mechanism of resistance conferred by over-expression of ABC transporter genes relies on increased energy-dependent efflux, which, in turn, causes decreased intracellular accumulation of toxicants. We considered that ABC transporters of *Trichoderma* spp. have an important role in a number of processes such as resistance to environmental toxicants produced either by soil microflora or introduced by human activity (e.g. fungicides, heavy metal pollutants), secretion of factors (mycotoxins and cell-wall degrading enzymes) necessary for the establishment of a compatible interaction with a host fungus or for the creation of a favourable microenvironment. We have cloned four different ABC transporter sequences of *Trichoderma atroviride* strain P1, named TABC1, TABC2, TABC3 and TABC4. Results of a RT-PCR analysis performed with primers designed on TABC2 sequence indicated that this gene is activated when *T. atroviride* is treated with culture filtrate of *Botrytis cinerea*, *Rhizoctonia solani* or *Pythium ultimum*, or is grown in the presence of *B. cinerea* or *R. solani* mycelia.

EFFICACY OF A *PENICILLIUM OXALICUM* ISOLATE IN THE BIOLOGICAL CONTROL OF *PYRENOCHAETA LYCOPERSICI* (CORKY ROOT) ON TOMATO. E. Lahoz*, A. Infantino, A. Carella, A. Porta-Puglia. *Istituto Sperimentale per il Tabacco, Via P. Vitiello 66, I-84018 Scafati (SA), Italy. Fax: +39.081.8506206; e-mail: e.lahoz@uniplan.it

Biological control is one of the most promising alternative to pesticides in the management of diseases caused by soil-borne pathogens. The efficacy of a *P. oxalicum* isolate (PCA) in controlling corky-root caused by *Pyrenochaeta lycopersici* was assayed by *in vitro* and *in vivo* experiments. PCA showed high aspecific endochitinase activity (reduction of 62% of turbidity). Dual cultures on PDA and cultures amended with concentrated culture filtrate (20% v/v) reduced the mycelial growth of two *P. lycopersici* isolates respectively of 80% and 85%. Disease severity was significantly reduced in the treatments with the antagonist. Root and plant dry matter weight were significantly higher in treatments with PCA plus *P. lycopersici* than in those with *P. lycopersici* alone and with PCA alone compared to untreated control. Height of the plants was lower in the plots with the pathogen, while no differences were observed among the other treatments. The control of several above-ground diseases associated to its ability to grow in a wide range of environmental conditions make *P. oxalicum* a good candidate as biological control agent (BCA). The mechanisms of action of PCA and its possible use on large scale are under study.

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SANITARY STATUS OF STONE FRUITS AND GRAPEVINE IN MARCHE REGION. FIRST CONTRIBUTION. P. La Notte, G. Romanazzi*, T. Amenduni, F. Capocasa, S. Virgili, B. Mezzetti, B.M. Branzanti, O. Silvestroni, V. Savino. *Dipartimento di Biotecnologie Agrarie ed Ambientali, Università di Ancona, Via Breccie Bianche, I-60131 Ancona, Italy. Fax: +39.071.2204858; e-mail: romanazzi@unian.it

The current work regards the first investigation on the sanitary status of stone fruits and grapevine in Marche Region, central Italy. Surveys were carried out in the main stone fruit and grapevine areas, with serological detection of the main viruses for the species. The investigation aimed to detect ACLSV, ApMV, PDV, PNRSV, and PPV on 252 stone fruit samples, and of GVA, GFLV, GFkV, GLRaV-1, GLRaV-2, GLRaV-3, and GLRaV-7 on 258 grapevines. The most common stone fruits virus was PNRSV (13.1% on the whole, 21.3% on peach), followed by ACLSV (6.3%), and PDV (2.8% on the whole, 24.1% on sweet cherry). Apricot had the highest number of infected samples (32.6%), followed by sweet cherry (31.0%). The 8 almond plants were negative in ELISA tests (virus free). It is comforting the absence of PPV in commercial orchards, although the position of the region and the almost lack of nurseries requires a constant attention. The most common grapevine viruses were GVA and GLRaV-1 with an incidence of 48-50% each, followed by GFkV with 21% and GLRaV-3 with 14%. GFLV and GLRaV-2 were found in 11.2 and 7.7% of plants, respectively. The presence of GVB and of GLRaV-7 is negligible: it was detected in 3 and 2 plants respectively. On the whole, 21.7% of grapevines were virus free in ELISA; this indicate a rather worrying sanitary status, also considering that most plants were subjected to clonal and sanitary selection and did not show any disease symptom in field.

ACTIVITY OF ANTAGONISTS AND NATURAL COMPOUNDS AGAINST POWDERY MILDEW OF CUCURBITS: LABORATORY AND FIELD TRIALS. G. Lima*, F. De Curtis, D. Piedimonte, A.M. Spina, V. De Cicco. *Dipartimento di Scienze Animali, Vegetali e dell'Ambiente, Sez. Patologia Vegetale, Università del Molise, Via De Sanctis, I-86100 Campobasso, Italy. Fax: +39.0874.404678; e-mail: lima@unimol.it

The effectiveness of antagonists and natural compounds against Powdery mildew of cucurbits (*Sphaerotheca fusca*) was tested both under controlled conditions and in the field, in the years 2000, 2001 and 2002. Weekly treatments were applied on leaves; the fungicide Topas®, at intervals of 14 days, and the bio-fungicide AQ10®, also applied weekly, were included as controls. Among the antagonists, the yeasts *Rhodotorula glutinis* (Rg), *Cryptococcus laurentii* (Cl) and *Aureobasidium pullulans* (Ap), applied alone or combined with additives (mineral oil or gum xanthan) significantly reduced the disease severity on melon leaves. Their activity generally resulted comparable to that of Topas® and was similar or higher than that expressed by AQ10. All the antagonists survived on the leaves in the field at high levels of population even if the climate was hot and dry. Among the natural compounds, tested on melon leaves grown in plastic pots or on cucumber grown in the field in an organic farm, mineral oil (1%), potassium bicarbonate (0.5%), sodium bicarbonate (0.5%), calcium chloride (1%) and, particularly, powder of milk (10%) significantly reduced the severity of *S. fusca* on leaves as much as the wettable sulphur (control). This study pointed out that antagonists and natural substances are very promising for use in organic and integrated agriculture in alternative to synthetic fungicides.

OCCURRENCE OF CITRUS PSOROSIS VIRUS IN APULIA. G. Loconsole*, M.T. Fatone, A. Minafra, O. Potere, M. Castellano, V. Savino. *Dipartimento di Protezione delle Piante e Microbiologia Applicata, Università di Bari, via Amendola 165/a, I-70126 Bari, Italy. Fax +39.080.5442911; e-mail: giuliana.loconsole@agr.uniba.it

Field surveys and laboratory tests were carried out to evaluate the incidence and the association with specific symptoms of *Citrus Psorosis Virus* (CPsV) and to detect latent infections, using DAS-ELISA and molecular hybridization with a digoxigenin-labelled riboprobe. The probe was synthesized on a 600 bp cloned fragment of the coat protein gene from a local CPsV isolate (101ps). The survey, carried out in April-May 2002, regarded commercial groves of different citrus species (sour orange, sweet orange, Clementine, Satsuma Myiagawa) and cultivars ('Navelina' ISA315, common 'Navelina', 'Navelina' old clone, Clementine 'Comune' and 'Spinoso'). Laboratory tests showed a slight difference in infection rates detected by DAS-ELISA (8%) and molecular hybridization (9%). No relationship was observed between the presence of CPsV and specific symptoms such as bark scaling, flecking of leaves, oak leaf pattern, leaf crinkling and dieback.

SANITARY STATUS OF FRUIT TREES GERMPASM IN SICHUAN PROVINCE, CHINA. X. Liu, D.E. Sallustio, I. Adò, M. Barba*. *Istituto Sperimentale per la Patologia Vegetale, Via C.G. Bertero 22, I-00156 Rome, Italy. Fax: +39.06.86802296; e-mail: virologia@ispave.it

During a field survey, carried out in spring 2002, different experimental and commercial fruit-tree fields were visited in Sichuan province of south-western China. Within the framework of the investigation different plants, showing symptoms of probable viral aetiology, were identified. Symptoms consisted in bark split, chlorotic ring spots and leafroll in plum trees; chlorosis and leaf roll in peach and apricot trees; gums and splits of cherry epidermis; dark green and yellowing in apple leaves. Forty leaf samples of different fruit trees were collected and analysed by serological (DAS and DASI-ELISA) and molecular tests (RT-PCR, IC-RT-PCR, molecular hybridization technique). In particular, diagnostic analysis was performed to check the presence of *Apple mosaic virus* (ApMV), *Plum dwarf virus* (PDV), *Plum necrotic ring spot virus* (PNRSV), *Apple chlorotic leaf spot virus* (ACLSV), *Plum pox virus* (PPV) and *Peach latent mosaic viroid* (PLMVd). Obtained results showed that ACLSV is the most spread virus in stone fruit samples (22.5%), followed by PDV (12.5%); PNRSV was detected in the 5% of the collected samples. Otherwise ApMV resulted widespread (20%) in apple trees. None of the samples was found infected by PPV. Molecular hybridization analysis revealed an high diffusion of PLMVd (46.6%) in peach orchards of Sichuan province.

CHLOROPHYLL *a* FLUORESCENCE AND PHOTOSYNTHETIC GAS EXCHANGE AS TOOLS FOR PHYTOPATHOLOGICAL DIAGNOSTICS. G. Lorenzini, C. Pucciariello, C. Nali*. *Dipartimento di Coltivazione e Difesa delle Specie Legnose "Giovanni Scaramuzzi", Università di Pisa, Via del Borghetto 80, I-56124 Pisa, Italy. Fax: +39.050.960622; e-mail: cristina.nali@agr.unipi.it

Early recognition of biotic and abiotic stress factors that may ultimately result in the impairment of plant performances is a crucial topic: the first symptom to appear is usually a decrease in photosynthesis. Chlorophyll fluorescence analysis is a powerful tool for rapid and noninvasive evaluation of photosynthesis. At ambient temperature, a small amount (about 1%) of photosynthetically active radiation (400-700 nm) absorbed by chlorophyll is re-emitted as fluorescence in the near infra-red band (> 690 nm). This fluorescence arises principally from PSII, the primary site of charge of separation and O₂ evolution in photosynthesis, and the quenching of this fluorescence is a reliable indicator of photochemical (useful) and non-photochemical (wasteful) fates of the absorbed photons. The functioning of the multi-component PSII is sensitive to many stress factors and, as a consequence, fluorescence monitoring has found widespread application in physiological plant pathology. Although this method is effective as non-destructive assay of photosynthetic injuries, it is affected by the plant materials and operational conditions: therefore, it is necessary to use it for accurate assays twinned with measurements of CO₂ uptake and biochemical analysis. Examples of the diagnosis of photosynthetic dysfunction of plants caused by ozone stress and wilt fungal diseases are discussed.



CHARACTERIZATION OF *PSEUDOMONS FLUORESCENS* STRAINS CAUSAL AGENTS OF STEM AND PITH NECROSIS OF TOMATO IN APULIA. P. Lo Cantore, N.S. Iacobellis*. *Dipartimento di Biologia, Difesa e Biotecnologie Agro Forestali, Università della Basilicata, C/da Macchia Romana, I-85100, Potenza, Italy. Fax: +39.0971.205702; e-mail: iacobellis@unibas.it

Recent investigations have ascertained that different fluorescent pseudomonads are responsible for stem and pith necrosis of tomato in Apulia. These bacteria produced green, blue and/or orange fluorescent pigments, and showed the LOPAT profile of the group V_b of the fluorescent pseudomonads. The nutritional analysis of nine virulent isolates with the computerised system Biolog lead to the identification of four of them as strains of *Pseudomonas fluorescens*, three as strains of *P. fluorescens* biotype F and two as strains of *P. chlororaphis* (*P. fluorescens* biotype D) and *Pseudomonas* spp., respectively. The virulence of the above bacteria was generally lower than strains of *P. corrugata* used for comparison though different results may be obtained in relation to the type of pathogenicity assay and the inoculation method adopted. Nevertheless, in the different pathogenicity assays all the strains induced the root proliferation. Antagonism assays showed that all strains of *P. fluorescens* produced substances capable to inhibit the growth of *Bacillus megaterium*, *Escherichia coli* K12 and *Rhodotorula pilimanae*. Some of the above strains produced indoles and/or formed white precipitates in agar when grown near to the type strain of *P. tolaasii*. All the bacterial strains were apparently resistant to copper (200 µg l⁻¹ copper sulphate).

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A PRELIMINARY SURVEY OF CITRUS VIROIDS IN CAMPANIA. M. Malfitano*, M. Barone, D. Alioto, N. Duran-Vila, A. Ragozzino. *Dipartimento di Arboricoltura, Botanica e Patologia Vegetale, Università di Napoli, Facoltà di Agraria, Via Università 100, I-80055 Portici, Napoli Italy; Fax: +39.081.7755114; e-mail malfitan@unina.it

Until today, five viroid species (CEVd, CVd-I, CVd-II, CVd-III, CVd-IV) have been identified in citrus. CEVd and CVd-II, a specific variant of HSVd, are respectively the causal agents of Exocortis and Cachexia, two economically important diseases of citrus susceptible species. Non pathogenic HSVd variants, CVd-I, CVd-III, CVd-IV do not appear associated to specific symptoms in commercial species and varieties. CVd-I and specially CVd-III cause reductions in size of trees grafted on *Poncirus trifoliata* (Duran-Vila, 2000. Monografia de la SEF n.2, 87-91.). Ninety-five sources of several citrus species and cultivars from different areas of Campania were collected during summer 2001 and evaluated for the presence and distribution of citrus viroids, by sequential polyacrylamide gel electrophoresis (sPAGE) and molecular hybridisation with specific probes. CEVd, CVd-II, CVd-III and CVd-IV were diagnosed and mixed infections were frequently detected. Highest infection rates were recorded for CVd-II and CVd-III (84.2 and 81.0%). Data collected revealed a wide distribution of viroids, independently of age, species and cultivars analysed. None of the examined trees were infected by CVd-I, indicating that this viroid may probably not be present in Italy (La Rosa *et al.*, 1988. Proc. 6th Int. Citrus Congress, 903-907). Further investigations are necessary to establish association between viroid species and field symptomatology.

DETECTION OF *SPHAEROPSIS SAPINEA* IN SYMPTOMLESS PINE TREES BY REAL TIME PCR. N. Luchi, P. Capretti*, M. Pazzagli, P. Pinzani. *Dipartimento di Biotecnologie Agrarie, Sez. Patologia Vegetale, Piazzale delle Cascine 28, I-50144 Firenze, Italy. Fax: +39.055.3288273; e-mail: paolo.capretti@unifi.it

Sphaeropsis sapinea is a pathogenic fungus diffused in the Mediterranean area. Three groups, present in Europe, N. America and S. Africa, differentiated on the basis of their morphological characteristics and specific DNA markers, have been described. The incidence of damage by this parasite in Italy (group A), on different hosts (*Pinus halepensis*, *P. pinea*, *P. pinaster* and *P. nigra*), is probably related with climatic change. In order to be able to adopt control methods, it seems useful to determine the presence of the fungus on trees in an asymptomatic phase, before the disease outbreak. Recently, an original procedure has been proposed for the quantitative detection of specific PCR products, based on the use of a fluorogenic probe, designed to hybridise within the target sequence. During the past years, real-time quantitative PCR has mainly been used in clinical research and only few reports deal with the application of fluorescent PCR technology in plant pathology. The application of this technique on pine colonised by *S. sapinea* in symptomless phase seems a new and useful tool in diagnostic of plant pathogens, especially to detect, identify and quantify latent pathogens and endophytes in symptomless plants.

COLONY MORPHOLOGY AND FRACTAL GROWTH OF *PSEUDOMONAS SAVASTANOI*. G. Marchi*, L. Gagnani, G. Surico. *Dipartimento di Biotecnologie Agrarie, Sez. Patologia Vegetale, Università di Firenze, P.le delle Cascine 28, I-50144 Firenze, Italy; Fax: +39.055.3288273; e-mail: guido.marchi@unifi.it

Bacterial colonies are normally grown on substrates with adequate nutrient levels and an intermediate agar concentration. Under such favourable conditions, the colonies are usually simple, compact and with a smooth envelope. By contrast, under adverse conditions (very low level of nutrients, or high concentrations of agar, or both), the most noticeable character of the bacterial response is the formation of complex growth patterns during colony development. *Bacillus subtilis*, *Proteus mirabilis* and *Escherichia coli* are bacteria showing a capacity for fractal growth. When *Pseudomonas savastanoi* was grown on various concentrations of agar and peptone, it also sometimes formed colonies whose shapes had points of similarity with colony shapes from one or more of the first three bacteria. In particular, the colony shapes of levan-negative *P. savastanoi* strains were similar to those of *P. mirabilis* and *E. coli* grown in the same or nearly the same conditions. By contrast, levan-positive strains of *P. savastanoi* on substrates with low-agar (5 and 7.5 g l⁻¹) and peptone concentrations of 5 and 10 g l⁻¹ presented colony shapes of the Diffusion Limited Aggregation type similar to that of *B. subtilis* colonies, with a fractal dimension between 1.69 and 1.78. However, with *B. subtilis* the same fractal size of the colonies was achieved with lower peptone levels and higher agar levels. The capacity to adapt to an adverse environment may indicate a multicellular organization of the bacterial population.

EXPRESSION OF FUNGAL GENES ENCODING ABC TRANSPORTERS IN POTATO AND *ARABIDOPSIS* FOR THE STUDY OF RESISTANCE TO BIOTIC AND ABIOTIC STRESSES. R. Marra*, M. Ruocco, M. De Palma, G. Del Sorbo, F. Scala. *Dept. ArBoPaVe Sect. Plant Pathology, University of Naples Federico II, Via Università 100, I-80055 Portici (NA), Italy. Fax: +39.081.7755320; e-mail: robertamarra@tin.it

Overexpression of some genes encoding ABC transporters determines simultaneous resistance to a number of chemically unrelated compounds, a phenomenon known as multidrug resistance (= MDR). Genes determining MDR have been isolated both from saprophytic (e.g. *atrB* from *Aspergillus nidulans*) and phytopathogenic (e.g. *BcatrB* from *Botrytis cinerea*) fungi. *atrB* confers resistance towards many metabolic inhibitors (e.g. 4-nitroquinoline-N-oxide, cycloheximide), including several classes of agricultural fungicides (phenylpyrroles, anilino-pyrimidines, benzimidazoles, triazoles). *BcatrB* confers resistance towards resveratrol (a stilbene grape phytoalexin) and the phenylpyrrole fungicides fenpiclonil and fludioxonil. We hypothesized that the mentioned genes constitute an useful tool to elucidate the mechanisms underlying resistance of plants to environmental stresses. We are studying the effect of transgenic expression of the fungal genes *atrB* or *BcatrB* in potato and *Arabidopsis thaliana* plants. We obtained eight potato lines expressing *BcatrB* and 26 lines expressing *atrB*, respectively. Five *Arabidopsis* plants expressing *atrB* and five expressing *BcatrB* have also been obtained. The expression of the transgenes has been verified by RT-PCR and their effect on plant resistance towards a number of different xenobiotic compounds, including environmental pollutants and pathogen-derived phytotoxins, is being tested.

TOMATO PLANT PHYTOVIRUS INFECTIONS RELATED TO DIFFERENT PRODUCTION SYSTEMS. M.G. Morano, A. Petrozza, G. Lacertosa, P.D. Grieco*, F. Cellini. *Metapontum Agrobios, S.S. Jonica 106 km 448.2, I-75010 Metaponto (MT), Italy. Fax: +39.0835.740204; e-mail: pdgrieco@agrobios.it

Production systems can influence plant characteristics, as well as qualitative and quantitative aspects of the fruit product. Moreover high nitrogen fertilisation and water stress can modify plant physiology, making the host more susceptible to phytopathogenic attacks. This research was conducted in Metaponto (MT), at the Metapontum Agrobios experimental fields. A complete randomised scheme, with three replications, was used for each production systems (organic, integrated and conventional). In total, 2800 plants of tomato (six varieties) were monitored. Immunodiagnostic and molecular assays were performed on symptomatic and asymptomatic plant samples caused by phyto virus infections (at three stages of plant growth). Seven different phyto viruses were identified: CMV, TSWV, PVX, PVY, AMV, TAV, and PZSV. Field monitoring results point out that phyto virus infection was highest for organic system, followed by conventional and integrated one, indicating a relevant role of the production systems. The type of nitrogen fertilisation applied in organic system and the even greater level in conventional one could have caused a vegetative conditions more conducive to a higher phyto virus infection level.

MOLECULAR APPROACH IN STUDIES ON BIOLOGY OF THE WEAK PATHOGEN *BISCOGNIAUXIA MEDITERRANEA* INVOLVED IN OAK DECLINE. A. Mazzaglia, I. Librandi, A. Vannini, N. Anselmi*. *Dipartimento di Protezione delle Piante, Università della Toscana, Via S. Camillo De Lellis, I-01100 Viterbo, Italy. Fax: +39.0761.357473; e-mail: anselmi@unitus.it

The xylariaceous fungus *Biscogniauxia mediterranea* (De Not.) O. Kuntze is an important weak pathogen involved in oak decline of Mediterranean environments. Research with traditional approach, even if intense, got into difficulties to clarify some aspects connected to the long endophytic phase of its life cycle. Analysis of rDNA sequences (ITS) on species belonging to different genera of *Xylariaceae*, besides discriminating among them, permitted us to define two highly specific primers for *B. mediterranea*. They are useful for the identification of the pathogen even in asymptomatic tissues, without the need of *in vitro* isolation. RAPDs profiles obtained with a pool of selected random primers from the whole fungal genome were compared in order to evaluate genetic variability of isolates from various provenances and *Quercus* species. Isolates from various plant tissues and from plant either asymptomatic or declining were also compared. Results showed an increasing trend considering progressively a single plant, plants from the same site and, moreover, from different geographic areas. The genotypes, influenced by the isolation time and not by plant tissue, seem to be subjected to some selective pressure during the infection phase. Our research, besides evidencing the high adaptative strategy of *B. mediterranea*, confirmed the efficacy of molecular techniques in studying phytopathogens with complex life cycle like this.

EPIDEMIOLOGICAL OBSERVATIONS ON TOXIGENIC SPECIES OF *FUSARIUM*. A. Moretti*, G. Mulè, G. Tocco, A. Logrieco, P. Nicholson, A. Bottalico. Istituto di Scienze delle Produzioni Alimentari, Viale Einaudi 51, I-70125, Bari, Italy. Fax: +39.080.5486063; e-mail: moretti@area.ba.cnr.it

Fusarium head blight of wheat is a disease of increasing importance in Italy both for yield losses and health risk since the causal fungal species can produce mycotoxins that can occur in meals and cereal based food. This disease, reported for soft wheat, has also been recorded on durum wheat, especially in central-northern localities of Italy, where the climatic conditions are conducive for fusariosis. Among the species involved, are *Fusarium avenaceum*, *F. culmorum*, *F. graminearum*, and *F. poae*. *Microdochium nivale* is also often associated with this disease. The results of the epidemiological and toxigenic surveys carried out in two years on fungal species occurring in experimental fields of durum wheat in Marche, Lazio, Puglia, Basilicata, and Sicilia, and soft wheat in Emilia-Romagna and Umbria, indicated that the mycotoxin profile in grain was related to the particular *Fusarium* species present. Differences in the species detected at each location indicates that each species have specific ecological requirements to initiate disease and produce toxins. Mycological observations using specific DNA molecular markers and competitive PCR on ear samples, collected at random at three growth plant stages (blossoming, milk and full kernel maturations) were related together with meteorological data recorded. The results are being used to develop a preliminary provisional model for the disease and mycotoxin risk assessment.

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IN VITRO STUDY OF THE INTERACTION OF VINE TISSUE CULTURES WITH *PHAEOMONIELLA CHLAMYDOSPORA*. L. Mugnai*, C. Amalfitano, M. Pasi, A. Arrigo.

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The increase in resveratrol and ϵ -viniferin levels was studied in vine calli inoculated with *Phaeomonniella chlamydospora*. Resveratrol occurs normally in vine tissues, but with e.g. *Plasmopara viticola* and *Botrytis cinerea* concentrations increase after fungal infection. Some viniferins however form only after infection has taken place. Endogenous resveratrol levels were thought to indicate the resistance of the vine to downy mildew. The cvs Roussanne and Colorino (which have shown a certain degree of resistance to esca in the field), Moscato (medium resistant), and Semillon (very susceptible) were tested. In non-inoculated calli endogenous resveratrol levels varied among cultivars and were not closely related to cultivar susceptibility to esca (although the lowest concentration was found in cv. Semillon). By contrast, in inoculated calli resveratrol levels increased more rapidly and reached higher concentrations in the more resistant cultivars than in the medium-resistant and susceptible cultivars. Concentrations of ϵ -viniferin increased much more slowly and to lower levels in the susceptible cultivar after inoculation with *P. chlamydospora* than they did in the more resistant cultivars. The data obtained suggest a role of resveratrol and its transformation products (viniferins) in the interaction between *P. chlamydospora* and grapevines.

VERTICILLIUM PSALLIOTAE, A NEW ANTAGONIST OF *RHIZOCTONIA SOLANI*. R. Nicoletti*, F. Raimo, A. Carella.

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Verticillium isolates were recovered on agar medium precolonized by *Rhizoctonia solani* from soil samples collected in tobacco farms located near Lecce, southern Italy. The isolates did not match morphological features of *Verticillium biguttatum* W.Gams, a well known mycoparasite which was repeatedly isolated under the same circumstances. In fact they were found to belong to *V. psalliotae* Treschow, a species classified in a different section (*Prostrata* instead of *Albo-erecta*). Since its taxonomic characterization (1941) the species is known to be pathogenic toward cultivated mushrooms, and it is cited on several species of both higher Basidiomycetes and rusts; moreover it is reported as a parasite of Insects and Nematodes. Interactions between *V. psalliotae* and *R. solani* were studied *in vitro* both in dual cultures on 2% water-agar and by adding concentrated culture filtrates in *R. solani* cultures on the same substrate. Results provided experimental evidence of an antagonistic aptitude toward *R. solani*; therefore, *V. psalliotae* should be added to the known list of fungal antagonists of this species. Further studies are currently in progress concerning characterization of fungitoxic metabolites, as well as chitinase and β -glucosidase activities from culture filtrates.

FIRST REPORT OF *AMERICAN PLUM LINE PATTERN VIRUS* IN ALBANIA, ITALY, AND TUNISIA. A. Myrta*, H. Abbadi, M. Al Rwahnih, M.C. Herranz, B. Di Terlizzi, A. Minafra, V. Pallás.

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American plum line pattern virus (APLPV) is included in the EPPO Quarantine A1 list of plant pathogens. This virus was recorded for the first time in the Mediterranean from Palestine in 2001. This prompted a more extensive investigation whereby 13 commercial plum orchards, three varietal collections and the virus-infected stone fruit collection of IAM. Bari was surveyed in Italy, and one varietal collection in Spain. A total of 701 samples were collected and tested by ELISA. Only ELISA-positive samples were re-tested by molecular hybridisation. All samples from commercial orchards (516) and varietal collections (170) were APLPV-negative. By contrast, out of 15 samples tested, APLPV was detected in four Japanese plums grown in the virus-infected collection, originating from Italy (one each from Apulia and Sicily), Albania, and Tunisia. All APLPV-infected plants were collected as symptomatic samples during previous surveys but had not been tested for the presence of this virus. This is the first report of APLPV from Albania, Italy and Tunisia. More extensive surveys are underway for assessing the incidence of APLPV in the Mediterranean.

NEW HOST-PLANTS OF *PHYTOPHTHORA* SPECIES IN ITALY. A. Pane*, R. Buffa, S.O. Cacciola, G. Magnano di San Lio, G. Perrotta.

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Recent results of a survey aimed at identifying *Phytophthora* species infecting nursery and landscape shrubs are described. Isolates, obtained from infected tissues on selective media, have been identified and characterised by morphological, biochemical and molecular characters (ITS-RFLP, nested-PCR). *P. palmivora*, isolated from new hosts, such as myrtle and different species of *Grevillea*, *Coronilla* and *Lavandula*, resulted to be very common in nurseries of ornamental plants. All the isolates showed identical electrophoretic phenotypes and the same restriction profiles, suggesting that they are clonal populations, probably originated from a strain accidentally introduced from abroad. It has been confirmed that *P. drechsleri* and *P. nicotianae* are frequently isolated from rosemary that has also been found as a new host of *P. cryptogea*. Sage and various species of *Cistus* have been identified as new hosts of *P. cryptogea* in Italy. Furthermore, a non-papillate species referred to *P. gonapodyides* has been isolated from *Cistus*. A non-papillate new species has been isolated from *Cytisus*, it appears to be distinct from all the others of Waterhouse's groups IV, V and VI as well as from *Phytophthora* sp. 'group O' recently described. By nested-PCR with species-specific primers, it was possible to directly diagnose in root tissues the presence of mixed infections caused by different species of *Phytophthora* or the presence of species, such as *P. cactorum*, inhibited by selective media.



REPLICATION OF CIRV DI RNA IN *SACCHAROMYCES CEREVISIAE*. V. Pantaleo, L. Rubino*, M. Russo. *Dipartimento di Protezione delle Piante e Microbiologia Applicata, Università degli Studi, e Istituto di Virologia Vegetale del CNR, Via G. Amendola 165/A, I-70126 Bari, Italy. Fax: +39.080.544291; e-mail: csvvlr02@area.ba.cnr.it

Carnation Italian ringspot virus (CIRV) is a species of the genus *Tombusvirus* in the family *Tombusviridae*. Replication in planta requires the expression of two amino-coterminal viral proteins of 36 kDa and 95 kDa, which constitute the viral replication complex. Two plasmids were constructed from which the sequences encoding these two proteins could be transcribed *in vivo* in *Saccharomyces cerevisiae*. The two proteins were correctly translated and integrated into membranes of yeast cells. An additional plasmid was introduced in yeasts expressing the viral replicase from which a defective interfering (DI) RNA could be transcribed. The viral sequence was terminated by the *Tobacco ringspot virus* satellite ribozyme which cleaved 19 nucleotides downstream of the 3' end of DI RNA. The DI RNA transcripts were amplified by the viral replicase because: (1) accumulation of DI-7 RNA took place in the absence of DNA-mediated transcription, (2) a negative strand was synthesized, (3) head-to-tails dimers were synthesized from monomers and monomers from dimers, (4) the authentic 3' end was restored, (5) a specific *cis*-acting signal is required at the 3' end (6) BrUTP was incorporated into nascent RNA.

CHARACTERIZATION OF A ToMV STRAIN ISOLATED FROM A RESISTANT TOMATO LINES. G. Parrella*, C. Vovlas. *Istituto di Virologia Vegetale del CNR, Sezione di Bari, Via G. Amendola 165/A, I-70126, Italy. Fax: +390805442911; e-mail: csvvvp06@area.ba.cnr.it

A new resistance breaking strain of *Tomato mosaic virus* (ToMV-MK) able to overcome the *Tm-2^a* gene is described. ToMV-MK, isolated during 2001 in Apulia (southern Italy) from the tomato variety Naxos, was able to cause severe systemic symptoms in plants carrying *Tm-2^a* with Moneymaker genetic background. On the contrary, plants with Ailsa Craig or Vendor genetic backgrounds, carrying *Tm-2^a* gene, were not affected. *Tm-1* gene controlled efficiently ToMV-MK in plants with the three genetic backgrounds considered. The sequence of the 30-kDa movement protein of ToMV-MK was determined and compared with those from other resistance-breaking strains but no substitutions in common were found. Three out of five substitutions found in ToMV-MK movement protein occurred in the carboxy-terminal domain of the protein. One of these substitutions, Asp→Tyr²⁴⁰, was apparently associated with changes of the 30-kDa secondary structure in a surface-accessible area including the last 30 aminoacids of the C-terminus domain. The results suggest that changes in the C-terminus secondary structure of the 30-kDa movement protein might alter the recognition event between the 30-kDa and the *Tm-2^a* gene product, conferring to ToMV-MK the ability to overcome the resistance. In *Tm-2^a* tomato lines with Vendor and Ailsa Craig genetic backgrounds, probably the presence of other factors, alone or in addition to *Tm-2^a*, increase the efficiency of resistance to ToMV-MK.

MOLECULAR CHARACTERIZATION OF ABUTILON MOSAIC VIRUS (AbMV) STRAIN FROM APULIA. G. Parrella*, C. Vovlas. *Istituto di Virologia Vegetale del CNR, Sezione di Bari, Via G. Amendola 165/A, I-70126 Bari, Italy. Fax: +39805442911; e-mail: csvvvp06@area.ba.cnr.it

Abutilon mosaic virus (AbMV – genus *Begomovirus*, family *Geminiviridae*) represent historically one of the first viruses studied by plant virologists. It is transmitted in a persistent manner by *Bemisia tabaci*. The present study concerns the molecular characterization of the AV1 gene, coding the coat protein, of an AbMV strain (named AbMV-PG) identified on symptomatic plants of *Abutilon sellovianum* var. *marmorata*, growing in some nurseries of Apulia (southern Italy). The whole amino acid sequence and the nucleotide sequence corresponding to the *core region* of 575 nt of the AV1 gene of AbMV-PG, have been used for phylogenetic analysis with those comparable sequences from other *Begomovirus* available to EMBL and SWISSPROT. The phylogenetic analysis showed that the Apulian isolate belongs to the western isolate of *Begomovirus* and is more related to the English and to the Indian strains of AbMV than to the Hawaiian strain. Moreover, amino acid changes in the coat protein, involved in the lost of vector transmissibility of the AbMV Indian strain, were identified in the CP sequence of AbMV-PG. For this reason, transmissibility of AbMV-PG by *Bemisia tabaci* is considered improbable.

EVALUATION OF SOME PRODUCTS FOR THE CONTROL OF LIMONIUM RUST (*UROMYCES LIMONII*). C. Pasini*, P. Curir, M. Sacco, F. D'Aquila. *Istituto Sperimentale per la Floricoltura di Sanremo, Corso Inglesi 508, I-18038 Sanremo, Italy. Fax: +39.0184.695072; e-mail: difesa@istflori.it

Rust, caused by *Uromyces limonii*, is a common disease in most Limonium cultivations. The pathogen is spread mainly on *L. sinuatum*, *L. latifolium*, and on susceptible varieties obtained from these two species. Rust infections occur on leaves, and even on stems of *L. sinuatum*, usually during spring and fall, when the humidity is higher. Information on chemicals are not available for this minor crop, and then growers are using fungicides that seem to have activity against rusts of other ornamentals. In 2001-2002 three experimental trials were carried out in open field to evaluate the effectiveness of some chemicals and one microorganism against the Limonium Rust. The following compounds were sprayed: acibenzolar-S-methyl (Bion), azoxystrobin (Ortiva), bitertanol (Baycor or Proclaim), kresoxim-methyl (Stroby), myclobutanil (Systane), penconazole (Topas), trifloxystrobin (Flint), triforine (Saprol), and an isolate of the fungus *Aphanocladium album*. Under conditions favourable for Rust development, the new strobilurin fungicides, in particular azoxystrobin and kresoxim-methyl, had a satisfactory protectant effect, but these chemicals are not registered till now on ornamentals in Italy. The ergosterol biosynthesis inhibiting (EBI) fungicides achieved similar effect in reducing damage. The plant activator acibenzolar-S-methyl and the isolate of *A. album* partially controlled the disease. No phytotoxicity symptoms were observed in these experiments.

VIRULENCE IN *STEMPHYLIUM VESICARIUM* POPULATIONS ISOLATED FROM PEAR. E. Patteri, V. Rossi*, R. Bugiani, S. Giosué. *Istituto di Entomologia e Patologia Vegetale, Università Cattolica del Sacro Cuore, Via E. Parmense 84, I-29100 Piacenza, Italy; Fax: +39.0523.599256; e-mail: vittorio.rossi@pc.unicatt.it

Brown spot, caused by *Stemphylium vesicarium* (Wallr.) Simons, is the most important pear disease in Italy and Spain. Recently, it caused severe epidemics also in northern Europe. The disease is caused by fungal strains producing a host-specific toxin which is responsible for the disease symptoms on some pear varieties. Preliminary studies on virulence variability between and within *S. vesicarium* populations were carried out. 28 monosporic isolates obtained from affected pear tissues collected in as many orchards of the Emilia-Romagna region (PI, pear isolates) were used in comparison with 5 isolates from onion, pea and asparagus (OI, other isolates). *In vitro* virulence assays were carried out on three pear varieties showing decreasing susceptibility to the disease under orchard conditions: drops of a conidial suspension were applied on the leaves and disease symptoms were observed for a 30-day incubation period. Fungal isolates showed differences in disease incidence and in time of symptom appearance, and significantly interacted with host varieties. Isolates were grouped in four statistically homogeneous clusters: PI causing very severe symptoms on all the varieties; PI causing severe disease on 'Abate' and 'Conference', not on 'William'; PI causing light symptoms on 'Abate' only; OI causing sporadic symptoms on any variety. Similar results were found by comparing many monosporic isolates obtained within a *S. vesicarium* population collected in an orchard.

AIRBORNE SPORES IN A RICE FIELD: *PYRICULARIA GRISEA* AND *BIPOLARIS ORYZAE* DETECTION. A.M. Picco*, M.A. Brandolini, M. Rodolfi, E. Lorenzi, D. Rodino. *Dipartimento di Ecologia del Territorio e degli Ambienti Terrestri, Sez. Micologia, Università di Pavia, Via S. Epifanio 14, I-27100 Pavia, Italy. Fax: +39.0382.34240; e-mail: apicco@et.unipv.it

The knowledge of the temporal pattern of rice pathogenic fungi dispersal, together with a correct examination of crops, can contribute to our understanding of disease distribution and management. Concentrations of *Pyricularia grisea* (Cooke) Sacc. and *Bipolaris oryzae* Shoem. airspores in a rice field located around Pavia (northern Italy) were estimated by using an automatic volumetric spore trap (VPPS Lanzoni). Measurements of their concentration were made between June and October over two years (1996 and 2001). Temperature, humidity, rainfall and fitness of the rice plants were monitored during the vegetative seasons. Peaks of *P. grisea* and *B. oryzae* airspores were always detected just 6-7 days before the onset of the typical symptoms of blast and brown spot. A significantly different spread of the two pathogens was detected as *P. grisea* spores always appeared later than *B. oryzae* ones. Peaks of *B. oryzae* spores were detected after a short rainy period, while the highest *P. grisea* spores concentrations were noticed when daytime temperature was about 24-27° for 4-5 days at least. *P. grisea* airspores concentrations/RH and *P. grisea* airspores concentrations/leaf wetness are correlations currently under analysis. Results confirm that there might be a correlation between spore release pattern and environmental conditions; we propose the aeromycological monitoring as an useful diagnostic method to forecast rice diseases.

DETECTION OF PATHOGENIC AGROBACTERIA BY PCR IN DIFFERENT SOILS. R. Peluso, A. Raio, A. Zoina*. *Dipartimento di Arboricoltura, Botanica e Patologia Vegetale, Università di Napoli "Federico II", Via Università 100, I-80055 Portici (NA), Italy. Fax: +39.081.7755320; e-mail: zoina@unina.it

Agrobacteria are able to live as saprophytes in natural and cultivated soils. Isolation of non pathogenic agrobacteria is easily performed by dilution plating method while this technique is not adequate to detect pathogenic forms. Survival and persistence of pathogenic agrobacteria in soil is poorly documented since a reliable detection procedure is not available so far. A rapid and specific procedure to detect pathogenic agrobacteria in soil was used in this study. A high incidence of crown gall disease on stone fruit rootstocks was observed in a nursery field not far from Naples, Italy. After plant uprooting, *Eruca sativa*, *Zea mays* and GF677 rootstocks were transplanted in that field in order to study the survival and the dynamic of natural pathogenic agrobacteria in rhizospheric and nonrhizospheric soil of host and non-host plants. Soil samples were repeatedly collected during the cultural cycle of the three plant species and were processed both by dilution plating method and by PCR after a rapid DNA extraction procedure. A total of 3000 agrobacterium-like colonies were isolated with selective media from rhizosphere and non rhizosphere soils. All of them were inoculated into tomato plants and only two resulted pathogenic. However, all soil samples were positively amplified by PCR. These results show that the procedure based on PCR may be very useful to detect the presence of virulent agrobacteria potentially dangerous in the soil. PCR analysis was successful and sensitive both in sandy and clay soils.

A PCR-BASED DIAGNOSTIC METHOD FOR THE DETECTION OF *PYRICULARIA GRISEA* IN RICE AND ITS EVALUATION ON A LARGE FUNGAL COLLECTION. E. Piotti*, M.M. Rigano, D. Rodino, S. Castiglione, M. Rodolfi, A.M. Picco, F. Sala. *Dipartimento di Biologia, Università di Milano, Via Celoria 26, 20133 Milano, Italy. Fax: +39.02.50314764; e-mail: francesco.sala@unimi.it

Rice blast, caused by the fungal pathogen *Pyricularia grisea* (Cooke) Sacc., is a disease of worldwide occurrence. It is responsible for considerable damages in rice crops in Italy as well as in other parts of the world. *P. grisea* is highly variable with respect to its infectivity. Therefore, to define the Italian *P. grisea* population, 45 DNA samples were extracted from different isolates and fingerprinted by using 'Pot 2' repetitive-element-based polymerase chain reaction. Statistical analysis clustered isolates into 9 discrete lineages. Diagnostic methods are currently based on the detection of typical symptoms although frequently mistaken with those of other rice fungal diseases. A rapid and specific method to detect *P. grisea* is still lacking. In this study, a method based on the *P. grisea* specific *Pwl 2* gene has been developed. A specific PCR primer pair, designed on the basis of *Pwl 2* sequence, amplifies the DNA of all members of the 9 *P. grisea* lineages and of infected rice samples collected in the field, but not of other rice fungal pathogens or of rice DNA. Detection limit of the PCR was evaluated using serial dilution. In order to increase the sensitivity threshold a third internal PCR primer was used in a hemi-nested PCR. These results are the bases for the constitution of a rapid diagnostic test.



MOLECULAR IDENTIFICATION AND CHARACTERIZATION OF *PHAEACREMONIUM* SPP. ISOLATED FROM KIWIFRUIT. A. Prodi, S. Sandalo, P. Nipoti*, R. Credi. *Dipartimento di Scienze e Tecnologie Agroambientali, Università di Bologna, Via Filippo Re 8, 40126 Bologna, Italy. Fax: +39.051.2091445; e-mail: pnipoti@agrsci.unibo.it

The role of *Phaeoacremonium* spp. in the aetiology of grapevine Esca disease was demonstrated. Recently, this fungus has also been isolated from a wood decay of kiwifruit. During field investigations of kiwifruit orchards in Emilia-Romagna and Lazio, we observed plants affected by an unusual wood disease. The main symptom was an abnormal hypertrophy of the trunks. For this characteristic, as disease denomination, we proposed the term "Elephantiasis". The most frequent fungi isolated from trunk cross sections of affected plants were: *Fusarium* spp., *Phaeoacremonium* spp., *Acremonium* spp. and *Phialophora* spp. As the microscopic morphological features of these last three genera are similar, a molecular study for their precise identification was performed. The universal oligonucleotide primer sets ITS4 and ITS5 were used to amplify the ITS regions of the nuclear ribosomal DNA containing ITS1, ITS2, the 5.8 gene and small portions of 18S and 28S rDNA. The electrophoretic patterns of eighteen isolates were compared with seven *Phaeoacremonium* species (*P. aleophilum*, *P. angustius*, *P. chlamydosporum*, *P. inflatipes*, *P. parasiticum*, *P. rubrigenum* and *P. viticola*). From the PCR assays, seventeen of our isolates belong to the genus *Phaeoacremonium* for the presence of the specific 620 bp DNA band. By means of RFLP analysis using *RsaI*, *HhaI* and *TaqI* restriction enzymes, *P. aleophilum* was found to be the most frequent species. Other isolates showing different RFLP patterns are currently under investigation.

PRELIMINARY STUDIES ON POSSIBLE VIRUS DISEASES OF THREE WOODY SPECIES IN BASILICATA, ITALY. G.L. Rana*, I. Camele. *Dipartimento di Biologia, Difesa e Biotecnologie Agro-Forestali, Università della Basilicata, C.da Macchia Romana, I-85100 Potenza, Italy. Fax: 0973/205503; e-mail: rana@unibas.it

Plants of *Fraxinus ornus* L. and *Alnus cordata* (Loisel.) Desf. (= *A. cordifolia* Ten.) showing chlorotic mottle and *Cornus sanguinea* L. with vein clearing, yellow ringspot and line pattern, and extended yellowing of their leaves were observed in some woods of Basilicata region in southern Italy in the last three years. Results of mechanical inoculation tests of sap, extracted from symptomatic leaves of the above single tree species in presence of 0.1 M phosphate buffer pH 7.2 containing 1% Na-ascorbate or nicotine traces, to herbaceous species commonly used as plant virus hosts were repeatedly negative. Nevertheless, electron microscopic observations carried out on crude juice obtained from small symptomatic leaf pieces of *F. ornus* and *C. sanguinea*, evidenced that a few virus-like particles of elongated-flexuous and isometric type were present in the former and in the last, respectively. No viral presence was ascertained in symptomatic *A. cordifolia*. *C. sanguinea* with similar symptoms on its foliage was reported to harbour mixed infections by tomato bushy stunt *Tombusvirus* (TB-SV) and cherry leaf roll *Nepovirus* (CLRV). The scarce concentration of isometric virus-like particles visualized at electron microscopy points out that CLRV could be the virus infecting *C. sanguinea* in Basilicata. This hypothesis, if adequately confirmed by graft, pollen and seed transmission trials and, overall, serological tests, would constitute the first report of a virus infection of such forestal species in Italy.

DISTRIBUTION AND INCIDENCE ON SOYBEAN SEEDS OF *DIAPORTHE PHASEOLORUM* VAR. *SOJAE* ISOLATES WITH DIFFERENT MOLECULAR PROFILES. N. Pucci, G. Conca, L. Riccioni*. *ISPaVe, Via C.G. Bertero 22, I-00156 Roma, Italy; Fax: +39.06.86802296; e-mail: luca.riccioni@ispave.it

Previous research showed a very high morphological and molecular variability among isolates of *D. phaseolorum* var. *sojae* (DPS) and the phylogenetic study, based on the DNA sequence of ITS region of 11 isolates, resolved four separated clades. As they were supposed to belong to different taxa, we were interested to evaluate the difference of morphological characters of the isolates belonging to each clade, and their diffusion in Italy. Polymerase chain reaction restriction fragment length polymorphisms (PCR-RFLP) were used to analyse 29 infected seed samples, with infection levels of *Phomopsis/Diaporthe* spp. ranging from 1 to 27 % and coming from different growing areas (kindly supplied by Ente Nazionale Sementi Elette, Milan, Italy). The DNA of 295 isolates, obtained after incubation of seeds on PDA plates, was amplified with universal primers ITS4 and ITS5, and the amplification product was digested with *AluI*, *RseI*, *HhaI* and *MseI* restriction enzymes for species identification and grouping of DPS isolates, on the base of the different molecular profile. Morphological confirmation was also carried out on 25-30 day-old colonies. Twenty-one % of the isolates were identified as DPS and, in particular, a molecular profile was more frequent (52%). Their morphological characteristics, as the presence of the perithecia, suggest that they are the 'true' *D. phaseolorum* var. *sojae*. The distribution in the different growing areas was also analyzed.

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DEVELOPMENT OF A REAL-TIME PCR ASSAY TO INVESTIGATE THE RESISTANCE OF WHEAT VARIETIES TO SOIL-BORNE WHEAT MOSAIC VIRUS. C. Ratti*, G.R.G. Clover, L.I. Ward, C. Rubies-Autonell, G.E. Budge, C.M. Henry. *DiSTA, Via Filippo Re 8, I-40126-Bologna, Italy. Fax: +39.051.2091439; e-mail: crubies@agrsci.unibo.it

Soil-borne wheat mosaic virus (SBWMV) was first described in the USA in 1919 and first reported in Italy in 1960. SBWMV causes serious disease of wheat, reducing yield by up to 70%. Growing resistant varieties represents the only economical means of controlling SBWMV. Real-time reverse transcription-polymerase chain reaction assays, based on TaqMan chemistry, were developed at Central Science Laboratory, York (UK), for the detection and quantification of both SBWMV and its vector, *Polymyxa graminis*, in plant tissue. The assay incorporates a RNA and DNA specific internal control to increase accuracy. DNA/RNA from an infected plant was diluted in DNA/RNA from a healthy plant to produce a standard curve. This standard curve was used to quantify the amount of SBWMV and *P. graminis* in samples. This method enabled sensitive, reproducible and specific detection of both virus and vector. In the 2000/2001 season, root and leaf samples of 6 English and 7 Italian varieties were collected from a trial field infected by SBWMV. Samples were collected 5 times during the period 01-17-01 / 03-21-01 and tested using the new detection assays. The results indicate that virus and vector concentration differ between varieties. However, the concentration of SBWMV in roots showed a positive correlation with concentration of *Polymyxa* for the majority of the varieties tested. The concentration of virus and vector in varieties from the same field will be studied in greater detail in the future.

STUDY OF POLYMORPHISMS OF MITOCHONDRIAL DNA OF THE PHYTOPATHOGENIC FUNGUS *DIAPORTHE HELIANTHI*. C. Reggio*, F. Vinale, A. Zoina, F. Scala, G. Del Sorbo. * Dept. ArBoPaVe Sect. Plant Pathology, University of Naples "Federico II", I-80055 Portici (NA), Italy. Fax: +39.081.7755320; e-mail: carmenreggio@inwind.it

Diaporthe helianthi (anamorph: *Phomopsis helianthi*) is a phytopathogenic fungus causing stem canker, foliar necrosis and wilt symptoms on sunflower (*Helianthus annuus*). Previous studies revealed a great variability in virulence among isolates of the fungus. In order to reveal the possible occurrence of correlations between virulence and molecular traits of the fungus we studied the variability of its mitochondrial genome in a collection of 16 isolates of *D. helianthi* differing in their level of virulence and geographic origin. The variability of mitochondrial DNA was assessed by analysing: (a) the restriction fragments length polymorphisms (RFLP) of mitochondrial DNA using a homologous 558 bp probe derived from the coding sequence of the *atp6* gene, encoding the subunit 6 of the mitochondrial ATP synthase; (b) the polymorphisms occurring in the sequence of a relevant portion of the coding sequence of the *atp6* gene, which was PCR-amplified from all isolates of our collection by using appropriate primers. RFLP analysis was conducted with several restriction endonucleases and revealed the occurrence of a broad variability among all the isolates of the collection. However, we found that all isolates characterized by a high level of virulence, all originating from France and ex-Yugoslavia, displayed identical restriction patterns. The sequences of the *atp6* gene displayed a very high level of homology, ranging between 95.5% and 99.8%, thus indicating lack of significant polymorphisms.

POLYGALACTURONASE INHIBITING PROTEIN (PGIP) IN CEREALS. S. Roberti, D. Pontiggia, C. Capodicasa, M. Gerunzi, F. Favaron, G. De Lorenzo, F. Cervone, R. D'Ovidio*. *Dipartimento di Agrobiologia e Agrochimica, Università della Toscana, Via San Camillo De Lellis s.n.c., I-01100 Viterbo, Italy. Fax: +39.0761.357238; e-mail: dovidio@unitus.it

Polygalacturonase-inhibiting proteins (PGIPs) are plant cell wall protein that modulate the activity of fungal endopolygalacturonases (PG) favouring the formation of oligogalacturonides active as elicitors of plant defense responses. A number of pgip genes and of their encoded products have been characterized from dicotyledonous species, whereas only a few data are available on PGIPs from monocots and none corresponding genes have been characterized so far. In this work we report the structural and functional characteristics of PGIPs and of their encoding genes from two of the most important crop species: wheat and rice. Deduced amino acid sequences of PGIP from both species show the typical LRR modular organization and a conserved distribution of cysteine residues. Nevertheless the overall sequence similarity with PGIPs from dicots can reach a value as low as 35.5% between wheat and soybean PGIPs. To test their recognition specificities, wheat and rice pgip genes have been expressed in a heterologous host and tested against a number of polygalacturonases from fungal pathogens. Also native PGIPs have been tested against PGs. Both wheat and rice PGIPs are very active against many PGs, including *Fusarium graminearum*, but have no effect against PGs from two different strains of *F. moniliforme*.

ABIOTIC STRESS IN POTATO TUBER: ANALYSIS OF SOME FACTORS RELATED TO OXIDATIVE STRESS. M. Reverberi, A.A. Fabbri, S. Zjalic, S. Briganti, C. Fanelli*. *Dipartimento di Biologia Vegetale, Università di Roma "La Sapienza", Largo Cristina di Svezia 24, I-00165 Roma, Italy. Fax: +39.06.6833878; e-mail: corrado.fanelli@uniroma1.it

In previous studies about different kinds of stress (wounding and cold storage) below bud potato tuber (*Solanum tuberosum* L. cv. Kennebec), a relationship between lipoperoxides (LOOH) and indole-3-acetic acid (IAA) has been shown (Fabbri *et al.*, 2000; *J. Exp. Bot.* 51: 1267-75; Reverberi *et al.*, *Free Rad. Res.* 2001, 35: 833-841). Endogenous LOOH, originated from lipoxygenase (LOX) action and exogenously added, increased the formation of IAA at different times after wounding (20 min) and after cold storage (from 90 to 180 days). Furthermore LOX inhibitors (salicylhydroxamic acid, SHAM, 1mM and nordihydroguaiaretic acid NDGA, 1 mM) inhibited this effect confirming the correlation between LOOH and IAA in potato tubers. It is known that also the biosynthesis of jasmonic acid (JA) and its derivative methyljasmonate (MeJA) is related with the formation of LOOH. In this work a possible correlation between MeJA and IAA in potato tubers sliced below bud has been studied. MeJA (10 µM) added on potato tuber slices enhanced, after 24 h the production of IAA and IAA, added at the same concentration, increased the formation of JA and MeJA 20-30 min after wounding. These results, obtained by GC/MS analyses, showed a possible direct or indirect correlation between MeJA and IAA.

COMPATIBILITY OF DIFFERENT STRAINS OF *TRICHODERMA* AND *GLIOCLADIUM* WITH FUNGICIDES AND HERBICIDES. R. Roberti*, F. Badiali, A. Pisi, G. Filippini, A. Cesari. *DIPROVAL, Sez. Fitoiatria, Università di Bologna, Via Fanin 46, I-40127 Bologna, Italy. Fax: +39.051.2091364; e-mail: rroberti@agrsci.unibo.it

The compatibility of some antagonistic fungi, *Gliocladium roseum* 47, *Trichoderma atroviride* 59, 312, *T. harzianum* 24, 144, *T. longibrachiatum* 9 and *T. viride* 15, towards five fungicides, carboxin, guazatine, prochloraz, thiram and triticonazole, and four herbicides, chloresulfuron, chlortoluron, flufenacet and pendimetalin, has been tested *in vitro*. These antagonists are active against *Fusarium* foot rot, the fungicides are those commonly used for wheat seed dressing and the herbicides are the ones applied for soil treatments. Fungal colony growth was evaluated at five different concentrations of active ingredients. The ultrastructural features of the hyphae were observed at scanning electron microscope (SEM). All the antagonists showed compatibility with carboxin and thiram, while they were inhibited by prochloraz; the carboxin and thiram EC₅₀ values were > 90 µg ml⁻¹ and prochloraz ≤ 0.2 µg ml⁻¹. *G. roseum* 47 showed also compatibility with guazatine and triticonazole: EC₅₀ > 53 and > 30 µg ml⁻¹ respectively. The *Trichoderma* isolates exhibited various degrees of sensitivity to triticonazole from 1.1 to > 30 µg ml⁻¹. *T. atroviride* 312, *T. harzianum* 144 and *T. longibrachiatum* 9 showed compatibility with all the herbicides, tested at field dosages, while *G. roseum* 47 was inhibited by flufenacet and chlortoluron. At SEM the antagonist mycelia, more sensitive to fungicides, revealed convoluted and highly branched hyphae and extruded material outside their walls.

LETTUCE BIG-VEIN: NEW INSIGHTS ON AN OLD DISEASE. P. Roggero. *Istituto di Virologia Vegetale, CNR, Strada delle Cacce 73, I-10135 Torino, Italy. Fax: +39.011.343809; e-mail: p.roggero@ifa.to.cnr.it*

The description of Big-vein disease of lettuce dated to 1934. Characteristic symptoms are chlorosis along veins (the so called enlargement of veins), thickening of the whole leaves and crinkling. Plants are stunted, with heads of reduced size, of diminished quality and value. The disease becomes evident mainly during cooler seasons and is soil-transmitted by the fungus *Ospidium brassicae*. Although the viral nature of the disease had been suspected for long time, only in 1983 the Varicosavirus *Lettuce big-vein virus* (LBVV) and in 2000 the Ophiovirus *Mirafiori lettuce virus* (MiLV) were isolated from diseased plants. It was found later that MiLV and LBVV are usually present in mixed infection in lettuce, both are transmitted by *O. brassicae* but only MiLV induce big-vein whereas LBVV causes only a latent infection. Temperatures above 20°C affect symptom development but not the presence of the viruses whereas over 28°C transmission by *O. brassicae* is inhibited. The sequence and genome organization of both viruses have just recently been determined. These recent findings allow new studies on this aetiological complex disease.

ENZYMES PRODUCED BY ANTAGONIST FUNGI CAN BE EFFECTIVELY APPLIED AS NEW BIOPESTICIDES. M. Ruocco*, S.L. Woo, S. Lanzuise, V. Scala, F. Vinale, F. Scala, M. Lorito.**CNR Institute for Plant Protection, Via Università 100, Portici I-80055 (NA), Italy. Fax: +39.081.2539339; e-mail: lori-to@umina.it.*

Antagonistic fungi of the genera *Trichoderma* and *Gliocladium* secrete complex enzyme mixtures (chitinases, glucanases, cellulases and proteases) capable of lysing the cell wall of plant pathogenic fungi and showing a strong antifungal activity. *In vitro* experiments had previously shown that these synergistic mixtures can also increase the toxic effect on phytopathogens of chemical fungicides, thus eventually reducing their application dose. In this work, performed within the framework of the EU project 'Substitution of methyl bromide fumigation and disease management in strawberry crops by IPM strategies', different *Trichoderma* strains and culture conditions have been tested, and an efficient system to produce potent combinations of fungicidal enzymes has been developed. The dialyzed culture filtrates, characterized by the presence of the lytic enzymes, have been applied directly *in vivo* to control *Botrytis cinerea* and *Colletotrichum acutatum* on strawberry, both on fruit in post harvest and on plants growing in greenhouse. In addition, *in vivo* synergistic interactions with several chemical fungicides have been identified, and protocols for joint application and formulation of enzymes and fungicides have been developed.

EXPRESSION OF HETEROLOGOUS GENES IN *TRICHODERMA* SPP. AS A NEW TOOL TO DELIVER PROTECTIVE MOLECULES IN PLANTS: TRANSFER OF AN AVIRULENCE GENE FROM *CLADOSPORIUM FULVUM*. M. Ruocco, S. Lanzuise, S.L. Woo, F. Scala, P.J.G.M. de Wit, M.H.A.J. Joosten, M. Lorito*. **Department of Ar.Bo.Pa.Ve., Plant Pathology Section, University of Naples "Federico II", Via Università 100, Portici I-80055 (NA), Italy. Fax: +39.081.7755320; e-mail: lorito@umina.it*

The avirulence gene *Avr4* of *Cladosporium fulvum* encodes a race-specific elicitor that induces a hypersensitive response in tomato plants carrying the resistance gene *Cf4*. *Trichoderma atroviride* strain P1 is a mycoparasite that has been studied extensively for its biocontrol ability against numerous phytopathogens. Its hydrolytic enzymes and the encoding genes have been utilized successfully to improve plant disease resistance. In addition, *Trichoderma* promoters responding to the presence of various pathogenic fungi or activated during the interaction with the plant have been isolated and characterized, and a reliable transformation system for expression of foreign genes has been developed. The avirulence gene *Avr4* from *C. fulvum* was transferred to *T. atroviride* by using various regulatory regions, including a promoter cloned from *Aspergillus nidulans*. Several mutants have been obtained and analysed by PCR, Southern and Northern. Further molecular and physiological characterisation of the *Trichoderma* transformants is underway, in order to determine whether they are able to induce resistance to *C. fulvum* when applied on susceptible tomato lines.

A COMPARISON OF LATERAL FLOW AND ELISA FOR THE DETECTION OF *TOMATO MOSAIC VIRUS* IN TOMATO. A. Salomone, C. Bruzzone, G. Minuto, A. Minuto, P. Roggero*. **Istituto di Virologia Vegetale, CNR, Strada delle Cacce 73, I-10135 Torino, Italy. Fax: +39.011.343809; e-mail: p.roggero@ifa.to.cnr.it*

The tobamovirus *Tomato mosaic virus* (ToMV) represents one of the main virological problems for the cultivation of tomato varieties not resistance to this virus. Since this virus is highly infective and easily transmitted during plants handling and by seeds, early diagnosis is important to reduce damage. Identification based on symptoms expression on leaves and fruits is unreliable. ELISA is the laboratory diagnostic technique currently used but results are usually obtained within days. Lateral flow (LF) is a new fast field diagnostic technique, allowing detection of viruses within minutes and without the use of any equipment. We have developed LF tests for ToMV based on polyclonal antibodies conjugated with colloidal gold and activated nitrocellulose membranes, then we have compared the results with those obtained with ELISA. The LF system allows virus detection on leaves, fruits and tomato seeds and has a detection limit slightly higher than ELISA. During 2 years, a total of 180 tomato samples have been analysed directly in the field by LF and later in the laboratory by ELISA; 66 were positive and 109 negative with both techniques. Only 5 samples were positive by ELISA and negative by LF. Thus lateral flow seems a reliable field technique for the diagnosis of ToMV in tomato, providing results similar to ELISA, but allowing an immediate decision for plants handling.

CLONAL AND SANITARY SELECTION OF OLIVE IN ABRUZZO AND APULIA. M. Saponari, S. Murolo, T. El Beaino, V. Savino*, G.P. Martelli. *Dipartimento di Protezione delle Piante e Microbiologia Applicata, Università di Bari and Istituto di Virologia Vegetale, CNR Sez. di Bari, Via G. Amendola 165/A, I-70126 Bari, Italy. Fax: +39.080.5442911; e-mail: savino@agr.uniba.it

Olive is the host of several viruses, most of which cause latent infections. Some viruses are well-characterised and ubiquitous able to infect many crops, whereas others are apparently restricted to olive. The impact of virus infections on olive production is unknown, but yield losses may not be negligible. The implementation of preventive measures, sanitary selection at first, seems to be the only useful strategy to restrain spreading of olive viruses. Sanitary improvement programs were applied in different Italian regions to produce virus-tested and virus-free olive propagative material, according to the national bylaws (DM 06/16/1993, DM 04/14/1997). In the framework of a sanitary improvement programme of the main olive cultivars grown in Abruzzo and Apulia (central and south-east Italy) the following activities were carried out: identification of typed areas of cultivation; identification of the grooves object of selection; pomological and sanitary selection of individual trees; laboratory testing. Due to the widespread occurrence of latent viral infections, visual inspections are unreliable, thus field observations need to be complemented by sensitive and reliable laboratory tests (dsRNA analysis, RT-PCR, molecular hybridisation). The sanitary status of very few of the selected plants proved to be conform to legislative provisions.

IDENTIFICATION OF AFLATOXIN-PRODUCING ISOLATES OF *ASPERGILLUS FLAVUS* AND *ASPERGILLUS PARASITICUS* BY REVERSE TRANSCRIPTION-POLYMERASE CHAIN REACTION (RT-PCR). B. Scherm, V. Balmas, M.L. Murgia, M. Palomba, Q. Migheli*. *Dipartimento di Protezione delle Piante, Università di Sassari, Via E. De Nicola 9, I-07100 Sassari, Italy. Fax: +39.079.229316; e-mail: migheli@uniss.it

One secondary metabolic pathway in filamentous fungi that has been studied extensively by several groups is the pathway for the mycotoxin aflatoxin, produced by *A. flavus* and *A. parasiticus*. We are developing the RT-PCR (reverse transcription-polymerase chain reaction) technique to monitor the toxigenic activity of *Aspergillus* spp. isolated from different matrices of plant origin. The preliminary phase of this project is based on 16 isolates of *A. parasiticus* and *A. flavus* characterised by a different level of toxicity. Total RNAs of the isolates grown under inducing and non-inducing conditions were extracted and subjected to RT-PCR analysis by using specific primers based on the conserved regions of 9 genes of the aflatoxin B1 biosynthetic pathway and of two regulatory genes. The level of transcription was evaluated by measuring the expression of *tub1*, coding β -tubulin. The transcription was correlated to the actual production of aflatoxin by HPLC/mass spectrometry. The first results allowed to identify some key genes whose expression appears essential in the biosynthetic process of aflatoxin by all the tested strains of *Aspergillus*, opening the perspective to adopt RT-PCR and microarray techniques to rapidly identify the toxigenic isolates.

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SELECTION OF ANTAGONISTIC *TRICHODERMA* SPP. BY ANALYSIS OF THE EXPRESSION OF GENES INVOLVED IN THE BIOCONTROL ACTIVITY. B. Scherm, V. Balmas, A. Marcello, C.A. Cozzolino, Q. Migheli*. *Dipartimento di Protezione delle Piante, Università di Sassari, Via E. De Nicola 9, I-07100 Sassari, Italy; Fax: +39.079.229316; e-mail: migheli@uniss.it

Different mechanisms may coexist in the interaction between antagonist, pathogen, plant tissue, and other components of the natural microflora. As a consequence, it is not clear which direction should be taken in the selection of new antagonists or in the improvement of those already known. A possible approach which can be applied in the case of *Trichoderma* spp. is based on the functional characterisation of genes encoding for extracellular lytic enzymes. Indeed, the secretion of such enzymes (chitinases, endoglucanases, cellulases, proteinases) confers a high antagonistic capability to many biocontrol agents through pathogen cell wall degradation. This project is aimed at the early identification of *Trichoderma* spp. isolates with high lytic potential based on the RT-PCR (reverse transcription polymerase chain reaction) technique by using specific primers matching conserved sequences of genes encoding for extracellular lytic enzymes. The first results indicate that, among the isolates tested, the level of transcription of some key genes implicated in the antagonistic potential against *Rhizoctonia solani* correlates with the biocontrol capability as tested *in planta*.

Research supported by the Italian Ministry for Agricultural and Forest Policies – “Protezione delle piante mediante l’uso di marcatori molecolari (PROMAR)”.

BIOCONTROL ACTIVITY OF NEW ANTAGONISTIC YEASTS AGAINST *PENICILLIUM EXPANSUM* ON APPLE. B. Scherm, A. Muzzu, M. Budroni, Q. Migheli*. *Dipartimento di Protezione delle Piante, Università di Sassari, Via E. De Nicola 9, I-07100 Sassari, Italy. Fax: +39.079.229316; e-mail: migheli@uniss.it

Penicillium expansum causes severe rots on apple fruit during storage and shelf life. Aiming at the development of new antagonistic yeast active in controlling different postharvest pathogens of fruit, several isolates were obtained from fig, cactus pear (*Opuntia ficus-indica*), apple and lemon fruit grown in untreated orchards. The selection procedure included two different approaches: by the first one fruits were dipped in sterile buffer and dilution directly plated on nutrient medium. By the second approach, dilution was used to treat artificially-wounded apple fruits, which were subsequently inoculated with *P. expansum*. The wounded areas remaining healthy after one week incubation at 25°C were removed for isolation of the candidate antagonists on nutrient medium. This methodology allowed us to select two yeast strains (*Pichia membranifaciens*, from fig, and *Candida guilliermondii*, from cactus pear) with remarkable antagonistic properties against *P. expansum*. In over 15 trials carried out on the cv. Golden delicious and Fuji, the two yeasts reduced apple rot with up to 100% efficacy. Killed yeast cells and culture filtrates hadn’t biocontrol activity either *in vitro* or *in vivo*. Further experiments will be aimed at the molecular characterisation of the antagonistic yeasts and at the elucidation of their mechanism of action against the pathogen.

Research supported by the Italian Ministry for the Agricultural and Forest Policies - “Protezione delle piante mediante l’uso di marcatori molecolari (PROMAR)”.



CLONING AND EXPRESSION OF THE *cp* GENE, ENCODING CERATO-PLATANIN, A PROTEIN INVOLVED IN THE PLANE CANKER STAIN. A. Sereni, B. Pantera, S. Tegli, C. Comparini, L. Carresi, L. Pazzagli, G. Cappugi, G. Del Sorbo, F. Scala, A. Scala*. *Dep. of Agricultural Biotechnology, Sect. Plant Pathology, University of Florence, P.le delle Cascine 28, I-50144, Firenze, Italy. Fax: +39.055.45733232; e-mail: aniello.scala@unifi.it

Cerato-platanin (CP) is a phytotoxic protein of 12.4 kDa produced by the Ascomycete *Ceratocystis fimbriata* f.sp. *platani*, the causal agent of the canker stain disease which affects *Platanus* spp. trees in Europe and north America. CP is highly homologous to three other fungal proteins: the snodprot1 protein from *Phaeosphaeria nodorum* (the causal agent of wheat glume blotch), the rAsp f13 allergen from *Aspergillus fumigatus* and the antigen AG19 from *Coccidioides immitis*. CP elicits the synthesis of phytoalexins by plane leaves and soybean cotyledons, and induces tobacco leaf cell necrosis. Immunofluorescence assays show that CP is an abundant, stable structural component of the surface of hyphae, conidia and ascospores. The presence of CP on the fungal cell surface and its early release in the medium indicates an important role of the protein in the first contact between the pathogen and host tissues. By using the 5'/3' RACE technology we completed the sequence of the *cp* gene and expressed it in heterologous systems to obtain large amounts of protein needed for its further structure and functional characterization.

ANTAGONISTIC ACTIVITY OF *PSEUDOMONAS SYRINGAE* SUBSP. *SAVASTANOI*: LOCALIZATION OF A GENETIC DETERMINANT. A. Sisto*, M.G. Cipriani, M. Morea, S.L. Lonigro, P. Lavermicocca. *Istituto di Scienze delle Produzioni Alimentari, C.N.R., V.le Einaudi 51, I-70125 Bari, Italy. Fax: +39.080.5486063; e-mail: a.sisto@area.ba.cnr.it

Strain ITM317 of *Pseudomonas syringae* subsp. *savastanoi* (*P. sav.*) shows antagonistic activity against other strains of this bacterium because of the production of a bacteriocin-like substance (BLIS). The BLIS was sensitive to heat and proteolytic enzymes, active between pH 5.5 to 7.5 and its molecular mass was estimated to be in the range of 100-300 kDa. In order to identify the genetic determinants involved in the antagonistic activity, a collection of Tn5-induced mutants from strain ITM317 was screened and mutant ITM317-636, which had lost the ability to inhibit the growth of other *P. sav.* strains, was identified. Southern blot analysis demonstrated that the genome of mutant ITM317-636 contained a single copy of the Tn5 element and that Tn5 insertion occurred on a plasmid. The responsibility of Tn5 insertion for the above-mentioned mutated phenotype was demonstrated by marker-exchange mutagenesis. The DNA regions flanking Tn5 insertion in mutant ITM317-636 were cloned and in part sequenced. Sequence similarity searches revealed that Tn5 insertion occurred within a DNA region encoding a predicted product 37% identical and 52% similar to a putative RHS-related protein of *Xanthomonas campestris* pv. *campestris*.

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SEQUENCE ANALYSIS OF THE *hrpC* OPERON AND THE *hrpE* GENE OF *PSEUDOMONAS SYRINGAE* SUBSP. *SAVASTANOI*. A. Sisto*, M.G. Cipriani, M. Morea. *Istituto di Scienze delle Produzioni Alimentari, C.N.R., Viale Einaudi 51, I-70125 Bari, Italy. Fax: +39.080.5486063; e-mail: a.sisto@area.ba.cnr.it

We have recently reported on a Tn5-induced mutant of *Pseudomonas syringae* subsp. *savastanoi* (*P. sav.*) which did not cause any disease symptom on olive plants; this mutant also failed to elicit a hypersensitive response in a nonhost plant. Molecular characterization revealed that DNA flanking Tn5 insertion in the mutant encoded a protein highly similar to the HrcC protein of *P. s. pv. syringae*. In order to define the genetic organization of the region and to identify other *hrp* genes in *P. sav.*, further sequence analysis of DNA regions flanking Tn5 insertion in the mutant was carried out. The results revealed that the gene encoding the HrcC protein in *P. sav.* was part of an operon including five genes with the same arrangement as in other phytopathogenic bacteria. On the basis of their homologies to previously characterized genes of other *P. s.* pathovars, these genes were named *hrpF*, *hrpG*, *brcC*, *hrpT* and *hrpV*. Upstream of this operon an homolog of the *hrpE* gene was also found in *P. sav. brcC* mutant of *P. sav.*, suggest that at least some *hrp/brc* genes play a key role in the pathogenicity of this plant pathogen which induces hyperplastic symptoms on its host plants.

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BIO126: AN EFFECTIVE *METSCHNIKOWIA PULCHERRIMA* STRAIN AGAINST GREY AND BLUE MOULD ON APPLE. D. Spadaro*, F. Alloati, A. Garibaldi, M.L. Gullino. *DiVaPRA - Sez. Patologia Vegetale, Università di Torino, Via Leonardo da Vinci 44, I-10095 Grugliasco (TO), Italy. Fax: +39.011.6708541; e-mail: davide.spadaro@unito.it

The strain BIO126 of the yeast *Metschnikowia pulcherrima* proved its biocontrol potential in semi-commercial trials reducing *Botrytis cinerea* and *Penicillium expansum* incidence, two severe postharvest pathogens on apple. When apples 'Golden delicious' were dipped in a cell suspension of the antagonist and stored at 1°C, BIO126 showed rot control capability, after 8 months, similar to thiabendazole. *In vivo* and *in vitro* experiments showed that living cells of the yeast are necessary for antagonistic potential, while culture filtrates and autoclaved cells are ineffective. The main mode of action involved in the biocontrol is competition for nutrients, but a direct interaction could not be excluded. The possibilities to integrate the biological treatment were studied in controlled conditions: acibenzolar-S-methyl, ethanol and sodium bicarbonate, with or without a hot water treatment, were used before the antagonist. The yeast proved to be the key element for the control of grey and blue mould and resulted more efficient in storing at 4°C than at 23°C. In absence of the antagonist, ethanol provided a higher reduction of grey mould while sodium bicarbonate offered more consistent results against blue mould. Heat treatment, by immersion in water at 50°C for 3 or 10 minutes, was ineffective. The compatibility of the antagonist with different concentrations of ethanol and sodium bicarbonate was assessed. A correct formulation will permit a more complete control of the fruit rots.

SPREAD OF *PSEUDOMONAS SAVASTANOI* LEVAN-POSITIVE STRAINS IN TUSCANY. G. Surico*, G. Marchi, L. Gragnani. *Dipartimento di Biotecnologie Agrarie, Sez. Patologia Vegetale, Università di Firenze, P.le delle Cascine 28, I-50144 Firenze, Italy. Fax: +39.055.3288273; e-mail: giuseppe.surico@unifi.it

Pseudomonas savastanoi is a bacterium which normally forms levan-negative colonies on media containing sucrose. However, levan-positive (L+) strains of this bacterium have been isolated from ash and olive. In particular, L+ strains were isolated in 1993 in an olive grove at Villa Bigallo, in the area of Bagno a Ripoli near Florence. To ascertain whether this find was a chance occurrence a search was made for L+ strains in the grove at Villa Bigallo as well as in some other olive-groves in Tuscany. Of all the isolates obtained 47 were L+, all identified as *P. savastanoi* on the basis of their ARDRA profile, physiological characteristics, pathogenicity and the plasmid and fatty acid profiles. However, isolates varied widely in the number and size of plasmids harboured, in the type and amount of cellular fatty acids, in virulence, etc. With regard to Tuscany as a whole, L+ strains were isolated from widely dispersed locations, near and remote from the olive grove at Villa Bigallo, and even near the border of the contiguous Region of Liguria. Since one of the most effective ways for the bacterium to spread over short and long distances is by the distribution of nursery-grown plantlets, and since Tuscan olive nurseries are among the main producers of such plantlets in Italy, it is to be feared that if the L+ strains find their way into these nurseries, they will quickly become much more widespread.

RT-PCR AND ISEM DETECTION OF *GRAPEVINE RUPESTRIS STEM PITTING-ASSOCIATED VIRUS*. F. Terlizzi, R. Credi*. *DiSTA, Dipartimento di Scienze e Tecnologie Agroambientali, Università di Bologna, Via Filippo Re 8, I-40126 Bologna, Italy. Fax: +39.051.2091413; e-mail: rcredi@agrsci.unibo.it

Rugose wood of grapevine is a complex of viral diseases. Among these, Rupestris stem pitting (RSP) is one of the most common and is known to occur worldwide. The etiology of RSP has not been fully determined, but a putative RNA viral genome (GRSPaV, *Grapevine rupestris stem pitting-associated virus*) has recently been reported and found to be consistently associated with the disease. To detect GRSPaV in grapevines, a specific RT-PCR method, using dsRNA as template, was also developed. In our study, trying to improve diagnosis of GRSPaV by PCR technology, we compared two published primer pairs (13-14 and 2-21) using total RNA from plant samples. The RT-PCR procedure was successful in detecting the virus. Among 55 grapevine accessions from the Emilia-Romagna region, northern Italy, 40 tested positive with primer pairs 13-14 compared to only 29 using primer pair 2-21. No PCR product was observed when healthy control grapevines were assayed. This study showed that oligonucleotide pair 13-14 has broad spectrum detection for the virus and a higher sensitivity compared to primer pair 2-21. GRSPaV was successfully observed using electron microscopy. Flexous rod particles, about 800 nm in length, were detected in leaf extracts from an infected grapevine. In ISEM assays, they were positively decorated with a specific antiserum provided by B. Meng (Dept. of Plant Pathology, Cornell University, Geneva, NY). This finding represents the first recorded visualization of GRSPaV particles in Italy.

PCR-BASED METHOD FOR THE DIAGNOSIS OF *CURTOBACTERIUM FLACCUMFACIENS* PV. *FLACCUMFACIENS* IN BEAN SEEDS. S. Tegli*, A. Sereni, G. Surico. *Dipartimento di Biotecnologie Agrarie, Sez. Patologia Vegetale, P.le delle Cascine 28, I-50144 Firenze, Italy. Fax: +39.055.4573232; e-mail: stefania.tegli@unifi.it

Curtobacterium flaccumfaciens pv. *flaccumfaciens* (Cff) is a Gram-positive bacterium which causes Bacterial wilt of *Phaseolus* spp.. Natural infections also occur on *Vigna* spp., *Glycine max* (L.) Merr. and *Pisum sativum* L. The bacterium is also able to colonise *via* vascular tissue the seeds where it can survive for two years or longer. Infected seeds may be discoloured, but more often no symptoms are visible. The seeds represent the vehicle for spread of the disease over short and long distances. Up to now the majority of the available diagnostic methods have low sensitivity and specificity, are time-consuming, and difficult to apply on a large scale. Cff is a seed-borne bacterium on the EPPO A2 quarantine list and the procedure here described may be useful for routine diagnosis of Cff, overcoming the problems of conventional techniques. The proposed method for the detection of Cff in bean seeds is based on the use of a pair of PCR primers (CffFOR2-CffREV4) specific for Cff and designed upon the sequence data of a cloned fragment, previously amplified in repetitive-sequence-based PCR (Rep-PCR) experiments with *C. flaccumfaciens* pv. *flaccumfaciens* strains using enterobacterial repetitive intragenic consensus (ERIC) primers. The primer pair CffFOR2-CffREV4 succeeded in amplifying a specific fragment of 306 bp size when Cff DNA as low as 5 pg was used as template. The PCR protocol was also shown to successfully detect Cff in naturally infected bean seeds within no more than 36 hours.

CYTOLOGICAL AND MOLECULAR CHARACTERIZATION OF NECROSIS INDUCING cDNAs FROM *PHYTOPHTHORA INFESTANS* GERMINATING CYSTS. A. Testa*, S. Kamoun. *Dep. of Plant Pathology, Ohio State University-OARDC, USA-44691 Wooster, OH, USA. Fax: +1.330.263.3841; e-mail: antesta@unina.it

Potato virus X (PVX)-based vectors have emerged as robust and reliable systems to express virulence and avirulence genes from microbial and viral pathogens and to express heterologous genes from plants. We currently use this technology to carry out functional screens of *Phytophthora infestans* cDNAs in plants. Polyadenylated mRNA isolated from *P. infestans* cysts germinated for 16 hours in water was used to synthesize cDNAs. The cDNAs were cloned unidirectionally in pSPORT1 vector, and then subcloned into the PVX binary vector pGR106 using a PCR-based approach. The ligation mixture was transformed directly into *Agrobacterium tumefaciens*. We performed functional screens of 2500 clones by toothpick inoculation onto the lower leaves of *Nicotiana benthamiana* plantlets. Starting from 7 to 15 days post inoculation, most of the inoculated plants showed systemic mosaic symptoms typical of PVX infection. However, 5 clones showed localized and systemic necrosis, and an additional 5% showed stunting and/or no-symptoms. Sequencing of the five inserts revealed four different cDNAs, three of which encode predicted proteins with no significant homology in public databases. These necrogenic inserts were also cloned into the binary vector pCB301 and transformed into *A. tumefaciens* to confirm the response without the PVX vector. Diverse host and non-host plants were tested. The phenotypes induced by those clones were characterized for induction of PR proteins and accumulation of phenolic compounds and callose.

OBTAINMENT OF MUTANTS AFFECTED IN THEIR ANTAGONISTIC POTENTIAL FROM THE *FUSARIUM OXYSPORUM* BIOCONTROL STRAIN FO47 BY *FOT1* TRANSPOSON-MEDIATED INSERTIONAL MUTAGENESIS. S. Trouvelot, C. Olivain, G. Recorbet, C. Alabouvette, Q. Migheli*. *Dipartimento di Protezione delle Piante, Università di Sassari, Via E. De Nicola 9, I-07100 Sassari, Italy. Fax: +39.079.229316; e-mail: migheli@uniss.it

Nonpathogenic strains of *Fusarium oxysporum* play an important role in soil microbial ecology and in the natural phenomenon of soil suppressiveness. To investigate the biocontrol mechanisms by which the antagonistic *F. oxysporum* strain Fo47 is active against Fusarium wilts, a *Fot1* transposon-mediated insertional mutagenesis approach was adopted to generate mutants affected in their antagonistic activity. Ninety strains in which an active *Fot1* copy had transposed were identified by using a phenotypic assay for excision and tested for their biocontrol activity against *F. oxysporum* f.sp. *lini* on flax in greenhouse experiments. Sixteen strains were affected in their capacity to protect flax plants, either positively or negatively. The molecular characterisation of these strains confirmed the excision of *Fot1* and its reinsertion in most of the cases. The phenotypic characterization of these mutants showed that they were affected neither in their *in vitro* growth habit or in their competitiveness in soil as compared to the wild type strain Fo47. These results suggest that the altered biocontrol phenotype should likely be expressed during the interaction with the host plant or the pathogen.

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GENETIC VARIABILITY OF GLRAV-3 ISOLATES. C. Turturo, P. Saldarelli*, Y. Dong, M. Digiaro, V. Savino, G.P. Martelli. *Dipartimento di Protezione delle Piante e Microbiologia Applicata and CEGBA, Università degli Studi and Istituto di Virologia Vegetale, CNR, Sez. di Bari, Via G. Amendola 165/A, I-70126 Bari, Italy. Fax: +39.080.5442911; e-mail: csvps04@area.ba.cnr.it

The genetic variability of *Grapevine leafroll associated virus 3* (GLRaV-3) isolates, originating from different countries, was analyzed by single-strand conformation polymorphism (SSCP) and nucleotide sequence analysis of the RNA-dependent RNA polymerase (RdRp), heat shock protein 70 (HSP 70) and coat protein (CP) genes. SSCP analysis revealed the existence of a predominant pattern in RdRp and CP genes, whereas two profiles, almost equally represented, were observed in the HSP 70 gene. A predominant viral variant in the RdRp and CP genes suggests the existence of constraints, likely determined by a positive selection pressure. No correlation between SSCP profiles and geographic origin of the isolates was observed. Intra-isolate genetic variability showed a population structure that agrees with the 'quasispecies' concept, since a predominant variant was observed in the majority of the clones of selected isolates. Deviations from this structure were observed in Chinese and Austrian isolates, which revealed the presence of different viral variants, possibly due to mixed infections of two GLRaV-3 strains. The agreement between sequence data and SSCP patterns confirms that SSCP analysis can be an effective and rapid procedure to study the genetic variability of viral populations.

DEHYDRINS AS NEW STRESS MARKERS IN *QUERCUS* PLANTS. E. Turco*, R.D. Fenton, T.J. Close, A. Ragazzi. *Dipartimento di Biotechnologie Agrarie- Sez. Patologia Vegetale, Università di Firenze, P.le delle Cascine 28, I-50144 Firenze, Italy. Fax: +39.055.3288273; e-mail: elena.turco@unifi.it

In the last ten years, much attention has been given to understand the role of dehydrin proteins (DHN or LEA D-11) during plant adaptation to environmental stresses that induce a dehydration status in vegetative tissues. The dehydrins are characterized by a consensus 15 amino acid sequence domain EKKGIMDKIKEKLPG always present and they tend to be glycine-rich. In the last few years, the genes that code these proteins have been characterized and mapped in some of the most important cereals species, and some information has come from studies on woody plants birch, *Populus* spp. and peach under several stresses. But until now, nothing is known about dehydrins in oak plants. Twenty-months-old holm oak (*Quercus ilex*) and turkey oak (*Q. cerris*) seedlings were grown in an environmental chamber under two periods of water stress and infected with *Phytophthora cinnamomi* to evaluate dehydrin synthesis. These proteins, found in holm oak leaves under unstressed conditions, increased in concentration when the plants showed a midday stem water potential of about -2.7 MPa observed in 'water stress' treatments. Otherwise, dehydrins are synthesised in turkey oak leaves under drought stress with -3.8 MPa as midday water potential. The apparent molecular weights are 50 and 43 kDa, for holm oak and turkey oak respectively. In relation to the results obtained, the authors hypothesize the application of the dehydrins as biochemical markers for an early identification of stress status in oak plants.

SURVIVAL OF *TILLETIA INDICA* TELIOSPORES IN SOIL IN ITALY. M. Valvassori*, L. Riccioni, A. Inman, K. Hughes, G. Conca, G. Di Giambattista, A. Porta-Puglia. * Istituto Sperimentale per la Patologia Vegetale, Via G.C. Bertero 22, I-00156 Roma, Italy. Fax: +39.06.86802296; e-mail: epid&resist@ispave.it

Tilletia indica, which causes Karnal bunt of wheat, is a quarantine listed organism for the EU. As part of the QLRT-PL1999-1554 EC Project, the pest risk analysis (PRA) for this disease is being revised by targeted scientific study, including the survival of the teliospores under European soil conditions. Their survival is being assessed over three years in Italy (ISPave), the UK (CSL, York) and Norway (NCRI, Ås) under quarantine containment. In these studies, freshly-produced teliospores from the USA were mixed with soil, enclosed in polyester bags (20 µm mesh) and placed at three depths in soil within PVC cylinders. The cylinders were sealed with polyester mesh screens allowing water to circulate while preventing teliospore dispersal. To improve containment, each cylinder was placed into a plastic bin with drainage at the bottom. In Italy, the site was at Monterotondo, Rome where, after one year, a set of three cylinders was removed from the ground and transported to a quarantine laboratory. Teliospores were recovered from the soil by sucrose centrifugation and tested for germination on tap-water agar at two and five months from recovery. Germination of teliospore from soil was 13%, 15%, 11% when tested at two months from recovery, and 31%, 33% and 31% five months after recovery from burial at 5 cm, 10 cm, and 20 cm, respectively. These data agree with results obtained from UK and Norway. It is concluded that teliospores of *T. indica* can survive in European soils for at least one year contributing in part to favouring establishment of the fungus in Europe.

SOURCES OF RESISTANCE TO *PHYTOPHTHOTA CAMBIVORA* IN NATURALISED AND CULTIVATED PROVENIENCES OF SWEET CHESTNUT IN ITALY AND SPAIN.

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An extensive survey on genetic resources and adaptability of sweet chestnut populations in Europe was carried out within the framework of the EU project CASCADE. Among the adaptive traits, the resistance to *Phytophthora cambivora* among and within 4 chestnut populations was studied (Sicily and Piedmont in Italy; Gaucin and La Coruña in Spain). Domestication levels (coppice, orchard, naturalised) were identified within each population. Eight trees were chosen per each level and 15 to 20 seeds per tree were harvested. A total of 80 half-sib progenies were compared in a completely randomised design. Inoculation of seedlings was carried out through soil infestation with a selected isolate of *P. cambivora*. Mortality at progeny level and length of stem and root necrosis (expressed as indices) were used as evaluators. Statistical analysis of data showed a high variability in susceptibility among and within provenience. The provenience of Sicily resulted the less susceptible according to the evaluators used. In particular the orchard displayed the highest level of resistance and a relative low variability among progenies. At least 3 progenies named GO4 (Gaucin), TO2 (Piedmont) and SN 5 (Sicily) expressed a level of resistance statistically not different from that of the French hybrid CA 125 used as resistant control. The present study offers new perspectives in the utilisation of sweet chestnut germplasm in breeding programs for resistance.

PRESENCE OF PLASMIDS IN *DIAPORTHE HELIANTHI* ISOLATES FROM DIFFERENT GEOGRAPHIC ORIGIN.

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Diaporthe helianthi is the causal agent of sunflower stem canker and it is threatening in Europe, although sporadically recorded in Italy. In a program aimed to investigate molecular variability among *D. helianthi* isolates from several countries, our approach was focused on the identification of extrachromosomal genetic determinants. Twenty-six isolates from different geographic origin, 14 deriving from France, 3 from ex-Yugoslavia, 3 from Argentina, 1 from Romania, 5 from Italy, have been studied. Extrachromosomal bands have been detected in most isolates. Rnase A and DNA exo-nucleases (exo-III and exo-I) digestions suggested they were DNA plasmid-like elements apparently with a circular conformation. More detailed analysis was then performed on a selected plasmid derived from a French isolate presumably present, as inferred by molecular weight, in all French and Yugoslavian isolates (countries where the disease has a heavy incidence). Its intracellular localisation resulted to be mitochondrial. In order to check its specificity, it was used in Southern blot experiments as a probe against DNAs derived from all twenty-six *D. helianthi* isolates. Further investigations are needed to characterise that plasmid (cloning and sequence analysis) and to make a comparison (e.g. RFLP) with plasmids from the other *D. helianthi* isolates.

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PHYTOTOXICITY EVALUATION OF NEW AMINOACID AND PEPTIDE COPPER CHELATES AND ULTRASTRUCTURAL STUDY OF THE INDUCED LEAF ALTERATIONS.

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Aminoacid and peptide copper chelates could be used in reduction of copper input in plant protection, particularly in organic and IPM viticulture, because they have the same good fungicide activity with a copper dosage reduction. In field trials phytotoxicity has sometimes been observed on grapevine. The aim of this research was to determine, in controlled conditions, the factors that induce copper hydroxide chelate phytotoxicity on grapevine and to study, at the ultrastructural level, the leaf alterations caused by it. The effect of the number of treatments, temperature and leaf wetness on the incidence of leaf lesions has been evaluated. The increase in the number of treatments, low and high temperatures associated with leaf wetness are positively correlated with the severity of leaf damages. Ultrastructural cytological observations initially show chloroplast alteration and occasional presence of precipitated; heavy plasmolysis is present on the most altered tissues.

SEPEDONIUM CHRYSOSPERMUM AS BIOCONTROL AGENT.

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Sepedonium chrysospermum strains isolated from wild basidiomycetes, but having no pathogenic activity on cultivated mushroom, have been studied in the present work for their antimicrobial activity. In vivo bioassays showed that one strain (704) has a strong biocontrol effect against *Rhizoctonia solani* and other pathogens. In order to study the mechanism of antagonism, we found evidence indicating that a concurrent production of antibiotics and hydrolytic enzymes, which has been shown to be important in mycoparasitism, is involved in this biocontrol process. We have purified and partially characterised a few chitinases and glucanases acting synergistically with a metabolite able to strongly inhibit spore germination and hyphal growth of *Botrytis cinerea*. The metabolite was extracted from culture filtrates of *S. chrysospermum*, purified and chemically characterised. It was found to be 2,4-dihydroxy-3,6-dimethylbenzaldehyde and to have a potent inhibitory effect on test fungi (active concentration started from 0.01 ppm), with germ tube elongation being more affected than spore germination. A low level of phytotoxicity, tested by infiltration in bean leaves of the purified antibiotic, was observed at a concentration higher than 50 ppm. We are working on the chemical synthesis of this antifungal metabolite and the cloning of the enzyme-encoding genes.

EVALUATION OF RESISTANCE OF NEW POTATO GENOTYPES TO DRY ROT IN POST-HARVEST. S. Vitale*, L. Corazza, B. Parisi, A. Pentangelo. *Istituto Sperimentale per la Patologia Vegetale, Via C.G. Bertero 22, I-00156 Roma, Italy. Fax: +39.06.86802296; e-mail: mal.crit@ispave.it

Many species of fungi can cause severe damage to potato both in the field and during storage. *Fusarium* tuber rot of potato is one of the most economically important diseases of stored potatoes. The most common species of *Fusarium* involved in this disease are *F. sambucinum*, *F. solani* var. *coeruleum* and *F. avenaceum*. In this work, 18 breeding clones from the National Project 'Potato Breeding' were screened for resistance to *F. sambucinum* 1228MC ISPaVe, resulted highly virulent in previous artificial inoculation tests. Potato tubers utilised in this tests were grown in three different Italian environments: Brunico in northern Italy, Angri and Battipaglia in southern Italy. Genotype 'CS 90-157-24' resulted resistant to *F. sambucinum* in all tests, while genotype 'MN 285' resulted resistant when grown in Brunico and had an acceptable degree of resistance when grown in Angri and Battipaglia. 'ISCI 67' resulted resistant when grown in two environments of southern Italy. This genotype had a good agronomical performance and will be included in the Varietal List of Agronomical Species.

GENETIC IMPROVEMENT OF ANTAGONISTIC FUNGI AND THEIR ABILITY TO INDUCE SYSTEMIC DISEASE RESISTANCE IN THE PLANT. S. L. Woo, M. Ruocco, S. Lanzuise, V. Scala, F. Vinale, F. Scala, M. Lorito*. *Department Ar.Bo.Pa.Ve., Section of Plant Pathology, Biocontrol Lab, University of Naples Federico II; and CNR Institute for Plant Protection, Via Università 100, I-80055 Portici (NA), Italy. Fax: +39.081.7755320; e-mail: lorito@unina.it

The interaction between *Trichoderma* spp., other soil or leaf microbes (including phytopathogens) and the plant is much more complex than it was thought. These beneficial fungi seem to have the remarkable ability to function at the same time both as microbial antagonists and plant symbionts, by using a variety of molecular factors and specialized structures. For example, seed treatments with various *Trichoderma* strains provide increased resistance to infection on the leaves by *Botrytis cinerea*. Recent understanding of the mechanisms of fungal antagonism at the molecular level is providing new tools to genetically improve the performance of biocontrol agents. A number of antifungal compounds have been identified and their specific role assessed by gene disruption. Further, a few promoters induced during mycoparasitism and biocontrol have been cloned and characterized, and they can be used to express in an inducible manner foreign genes that may augment biocontrol ability. For instance, we have expressed in *T. atroviride* a gene of *Aspergillus nidulans* encoding for a glucose oxidase under a biocontrol-related promoter, and have obtained mutants more powerful than the wild type both in pathogen biocontrol and induction of systemic disease resistance in the plant.

THE CONTROL OF A DIEBACK OF HAZELNUT USING DIFFERENT COPPER COMPOUNDS. G. Vuono, G.M. Balestra*, L. Varvaro. *Dipartimento di Protezione delle Piante, Università della Tuscia, Via S. Camillo de Lellis, I-01100, Viterbo, Italy. Fax: +39.0761.357473; e-mail: balestra@unitus.it

Within the problems related to the cultivation of hazelnut, a dieback disease, of bacterial aetiology, is one of the most important. The control of this disease is carried out by different preventive actions, as agronomical practices and treatments using copper compounds. The aim of the research was to compare the effectiveness of different copper compounds, in controlling the dieback of hazelnut. The chemical compounds used were: copper oxychloride, tribasic copper sulphate and pentahydrate copper sulphate. The effectiveness of different copper compounds was verified by *in vitro* and *in vivo* tests. In *in vitro* tests, the growth of bacterial isolates was evaluated in broth and on agar media supplemented with selected copper compounds. *In vivo* tests were carried out for three consecutive years in two hazelnut orchards in northern Latium. In each one five experimental plots were delimited, and the following treatments were applied: a) copper oxychloride; b) tribasic copper sulphate; c) tribasic copper sulphate supplemented with an aminoacidic compound; d) pentahydrate copper sulphate; e) untreated. The results obtained, in addition to confirming the effectiveness of copper oxychloride, revealed the effectiveness of the pentahydrate copper sulphate. Even characterized with a copper concentration five time lower with respect to the copper oxychloride used, and sprayed in reduced field-doses, it gave similar results in controlling the dieback of the hazelnut.

GENETIC VARIABILITY OF *XANTHOMONAS CAMPESTRIS* PV. *CAMPESTRIS* POPULATIONS. FIRST RESULTS. M. Zaccardelli*, A. Del Galdo, C. Caglioti, R. Buonauro. *Istituto Sperimentale per le Colture Industriali, Strada statale 18, n. 156, I-84091, Battipaglia (SA), Italy. Fax: +39 0828.340169; e-mail: tscibattipaglia@tiscalinet.it

Xanthomonas campestris pv. *campestris* (Pammel) Dowson (Xcc), is the causal agent of Black rot of crucifers, disease also known as Vascular blackening. The symptoms consist of marginal chlorosis of the leaves and blackening of the vascular tissue. The genome of the bacterium is completely sequenced. It consist of an unique circular chromosome of 5.076.187 bp, it haven't plasmids but is rich of transposable elements (109 insertion sequences). To evaluate genetic variability of Xcc, in this work were preliminarily characterized, for DNA polymorphism, 29 out 150 isolates obtained from different crucifers plants collected from different localities of central and southern Italy. The characterization, performed by PCR using the primers ERIC and BOX, distinguished the 29 isolates in 8 and 11 aplotypes respectively. The characterization performed by M13 primer, showed a number of aplotypes almost equal to the number of isolates. These results indicate a large genetic variability of the population of this phytopathogenic bacterium.



SUSCEPTIBILITY OF TOMATO GENOTYPES TO *PSEUDOMONAS SYRINGAE* PV. *TOMATO* IN THE FIELD CONDITIONS. M. Zaccardelli*, M. Parisi, I. Giordano.

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Pseudomonas syringae pv. *tomato* (Pst) causes Bacterial speck of tomato. The damages concern quality of the fruits (necrotic spots on the skin) used as fresh or transformed as peeled tomatoes. Not all the genotypes of tomato are susceptible: in fact, resistant cultivars are available. In this work, the susceptibility of 12 different tomato genotypes to natural infections of Pst, was evaluated in the field. The phytopathometric analysis, performed on leaves collected 4 weeks after transplanting (about one week before bloom) inferred slight or not attack for 4 genotypes (S. Marzano 2, Ranco F1, Hypeel 244, Piccadilly F1) whereas for other 4 genotypes (Corbarino ISCI 05, Vesuviano, Faino F1 and Principe Borghese), the attack was intense (from 10 % to over 14 % of diseased leaf surface). At harvesting, the higher incidence and intensity of attack, was observed on the fruits of the genotypes Vesuviano and Faino F1. These genotypes were the most early and the most attacked before bloom. The genotypes that did not show damages on fruits were S. Marzano 2, Sorrento, Cuore di Bue and Ibrido Insalataro, slightly or meanly attacked before bloom and with mean-late or late cycle. The resistance of the early genotype Piccadilly F1 was very high.

PRELIMINARY CHARACTERIZATION OF ANTAGONISTIC BACTERIA ISOLATED FROM TOMATO PHYLLOPLANE. M. Zaccardelli*, A. Del Galdo.

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In plant pathology, the study and use of control methods, as biological control, alternative to the use of chemicals compounds, is of great interest. A lot of studies inferred the use of antagonistic fungi and/or bacteria in the soil, to control soil-borne plant pathogen. Conversely, a minor number of studies inferred the use of antagonists directly on the aerial part of the cultures. In this work, a preliminary characterization of antagonistic bacteria, isolated from tomato phylloplane, was performed. From ten leaf samples, collected from different genotypes of tomato cultivated in two localities of the Campania region, 53 bacterial isolates were chosen. Twenty-one, mostly genetically different, as resulted by DNA polymorphism analyses, were able to inhibit the growth of *Alternaria* spp. *in vitro*. In particular, 5 of them were able to reduce considerably (from 58% to 35%) the fungal growth. The 21 isolates were all, except one, gram positive and, the greater number, able to produce endospores (*Bacillus* spp.). At least three of them were able to produce volatile substances that inhibit the growth of the fungus and, for other three isolates, the production of siderophores was observed. None antagonistic isolates was able to produce chitinolytic enzymes. The bacterial isolates most active *in vitro* and genetically different, will be evaluated in greenhouse and/or in the field.

INCIDENCE OF VIRAL DAMAGES ON 36 GENOTYPES OF SMALL TOMATO IN THE FIELD. M. Zaccardelli*, A. Pentangelo, I. Giordano.

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Viruses are the biotic factors mainly limiting the cultivation of tomato. *Cucumber Mosaic Virus* (CMV) is the most diffused virus in Italy, anywhere tomato is cultivated. In the last years, especially in southern Italy, *Tomato Spotted Wilt Virus* (TSWV) caused damages too. The control against these viruses is based on the use resistant or tolerant genotypes. In this work was evaluated, in the field conditions and during two years (2000 and 2001), the incidence of viral damages on 36 different genotypes of small tomato cultivated in the Campania region. For both years, all the genotypes were infected (ELISA test) by CMV whereas 5 genotypes in 2000 and one genotype in 2001, were infected by TSWV. In 2000, the incidence of damaged fruits was lower than 5% for 18 genotypes and between 5% and 10% for the remaining 18. In 2001, the incidence of damaged fruits was lower than 5% for 9 genotypes, between 5% and 10% for 21 genotypes, between 10.1% and 15% for 3 genotypes and between 15.1% and 20% for 2 genotypes; for one genotype, the incidence of damaged fruits was higher than 20%. All the 18 genotypes with incidence of damaged fruits lower 5% in 2000, showed incidence of damage not highest 10% in 2001, year with the highest viral infection. For 6 genotypes (5 S.C.-SA., Principe Borghese, Small Fry V.F.N., Grappolino, Lilliput V.F.N. F1, Remo), the lowest incidence (< 5%) registered in 2000, was confirmed in 2001.