

PCR ANALYSIS APPLIED TO CITRUS MAL SECCO DIAGNOSIS. G. Albanese, V. Grimaldi, R. La Rosa, I. Di Silvestro and Antonino Catara*. *Dipartimento di Scienze e Tecnologie Fitosanitarie, Università di Catania, Via Valdisavoia 5, I-95123 Catania, Italy. Fax: +39.095.361487; e-mail: patvegct@mbbox.fagr.unict.it

Phoma tracheiphila, the causal agent of citrus mal secco disease, is a serious threat to lemon and may destroy any other citrus species when it infects the roots of susceptible rootstocks. The fungus may remain latent in the nursery. The pathogen is included among the quarantine organisms but specific, easy to use and fast diagnostic methods for early detection of infections in plant material are not available. Previous researches have shown the high sensitivity of polymerase chain reaction (PCR) analysis even though it presents some complexity for fungus DNA extraction from woody host tissues. By using devised oligonucleotides and modifying mixture and amplification parameters previously reported, we applied PCR directly to the mycelium. Small woody pieces (5 mm²) of symptomatic, asymptomatic and healthy shoots of lemon, were put into 1.5 ml Eppendorf tubes containing carrot broth (500 µl). The broth was centrifuged each day of incubation at 21°C, up to 8 days, and the pellet resuspended in water. PCR positive results were obtained after an incubation of 2-3 days with symptomatic shoots, after 6-8 days with the symptomless ones. Healthy samples, uninoculated broth or mycelium of different fungus species always gave negative results in PCR analysis, confirming the method specificity. The applied DNA amplification test was found to be fast, useful for handling a large number of samples, easy to non-mycologist technicians, and suitable, with the support of statistic sampling criteria, for health certification and quarantine schemes.

EFFECTIVENESS OF NATURAL PRODUCTS FOR *IN VITRO* AND *IN VIVO* CONTROL OF EPIPHYTIC POPULATIONS OF *PSEUDOMONAS SYRINGAE* PV. *TOMATO* ON TOMATO PLANTS. Giorgio Mariano Balestra*, M. Antonelli and L. Varvaro. *Dipartimento di Protezione delle Piante, Università della Tuscia di Viterbo, Via S. Camillo De Lellis, I-01100 Viterbo, Italy. Fax: +39.0761.357473; e-mail: balestra@unitus.it

Pseudomonas syringae pv. *tomato* (Okabe) Young, Dye, Wilkie is the causal agent of bacterial speck on tomato plants. Its biological cycle is characterised by an epiphytic phase on the host and an invasive phase that leads to the appearance of typical symptoms. The phyllosphere of tomato plants is an habitat largely colonised by *P. s.* pv. *tomato*. By controlling these epiphytic populations, it is possible to decrease the potential bacterial inoculum and reduce disease risk. Copper compounds show good results in preventing this disease; among alternative products for use in biological agriculture, propoli and sodium silicate are recommended. Very little is known about the efficacy of these natural products on phytopathogenic bacteria. Our tests show that *in vivo* and *in vitro*: (1) sodium silicate gives good inhibition *in vitro* (undiluted and diluted to 10%) and *in vivo* (commercial products containing sodium silicate 35%, at 1% concentration), reducing the epiphytic *P. s.* pv. *tomato* populations, even if symptoms began to appear 8 days after treatment; (2) no effects were obtained with propoli treatment *in vitro* (commercial product at different dilutions) or *in vivo* (commercial product with galangina 20 mg ml⁻¹ used, as suggested, at 0.1% concentration). Considering the new European restrictions on the use of copper compounds in biological agriculture, new research to evaluate the usefulness silicate-copper and propoli-copper combinations is needed.

FUNGITOXIC ACTIVITY OF ESSENTIAL OILS AGAINST POSTHARVEST CITRUS PATHOGENS. Giovanni Arras* and S. Arru. *CNR, Istituto per la Fisiologia della Maturazione e della Conservazione del Frutto delle Specie Arboree Mediterranee, Via Dei Mille 48, I-07100 Sassari, Italy. Fax: +39.079.232047; e-mail: G.Arras@imfpp.ss.cnr.it

We report the fungitoxic activity of 12 essential oils (EO) from medicinal plants, used at 3 different concentrations (250, 500 and 1000 ppm), against *Penicillium digitatum*, *P. italicum*, *Botrytis cinerea* and *Alternaria citri*. *In vitro* trials were performed by mixing the EO with the culture medium (PDA, Potato Dextrose Agar) and seeding the pathogen suspension. Results show strong fungitoxic activity of *T. capitatus* EO, which inhibited germ tube elongation of the 4 fungi at the 250 ppm concentration. The other 11 essences reduced the development of the cfu of the fungi tested from 95% to 9% at 250 ppm. The fungitoxic activity of *T. capitatus* EO (250, 500 and 1000 ppm) on orange fruits, inoculated with *P. digitatum*, was weak at atmospheric pressure (3-10% inhibition at the 3 concentrations), while under vacuum (0.5 bar) conidial mortality on the exocarp was high (90-97% at the 3 concentrations). These data proved to be not statistically different from treatments with TBZ (2000 ppm). In the hypobaric environment EO inhibitory activity was about 10 times higher than at atmospheric pressure. *T. capitatus* EO vapours altered the morphology of *P. digitatum* hyphae and conidia (SEM observations). Gas-chromatographic analyses of the composition of thyme EO showed a high percentage of carvacrol (74.5%), p-cymene (8.5%), γ -terpinene (5.9%), and the presence of β -myrcene (2.1%), linalool (1.7%), borneol (0.9), α -pinene (0.7%), in the oil *in toto*. Carvacrol proved to be the most important fungitoxic compound among the thyme EO constituents, but, unlike thyme EO, it caused alterations to the fruit.

EVALUATION OF RESISTANCE TO *FUSARIUM CULMORUM* OF OAT LINES AND CULTIVARS. V. Balmas, A. Santori, M. Maccaroni, A. Magnotta and Luciana Corazza*. *Istituto Sperimentale per la Patologia Vegetale, Via C.G. Bertero 22, I-00156 Roma, Italy. Fax: +39.06.86802296; e-mail: mc_ispave@www.inea.it

Foot rot is a complex disease frequently occurring on winter cereals. *Fusarium culmorum* is one of the most common and damaging causal agents in Italy. Oat is generally less susceptible to this pathogen as compared with wheat, barley and triticale, all severely affected. *F. culmorum* was also frequently isolated from symptomless oat plants, which thus contribute to increase the inoculum potential of the soil. In the greenhouse, under controlled conditions, 41 lines and 14 cultivars of oat were tested for their resistance to a strain of *F. culmorum* isolated from infected oat. Inoculum was obtained by growing *F. culmorum* in corn-meal sand at 25°C for two weeks and was added to the soil covering the seeds. The parameters observed were emergence and severity of infection on seedlings after 20 days. Five grades of infection severity (0 to 4) were defined on the basis of symptom severity on seedlings. All the varieties tested, except a new one, 'Croara', were susceptible. About 50% of the lines tested, were moderately resistant, and particular resistance was shown by lines 152-13-3, 128-9-4-3, 171-10-3 and 195-1-2. Breeding for resistance to this damaging soil-borne pathogen is thus possible. The isolate of *F. culmorum* used for the inoculation was identified by PCR using two specific primers. *F. culmorum* was also detected by PCR in artificially infected seeds.

FUSARIUM WILT OF WINTER MELON. A. Belisario, L. Luongo, V. Balmas, L. Pezza and Luciana Corazza*. *Istituto Sperimentale per la Patologia Vegetale, Via C.G. Bertero 22, I-00156 Roma, Italy. Fax: +39.06.86802296; e-mail: mc_ispave@www.inea.it

Fusarium wilt of melon is mainly caused by *Fusarium oxysporum* f. sp. *melonis*. Four races of *F. o. f. sp. melonis*, namely 0, 1, 2, and 1,2, are present in Italy. Identification of races present in winter melon-producing areas was carried out, with special regard to Sicily, where race 1 was the most frequent. Races 1 and 1,2 were the most widespread in Italian melon fields, the latter being more frequent in intensively cultivated areas. Vegetative compatibility group (VCG) identification tests were carried out with *F. o. f. sp. melonis* tester isolates. So far VCG 0134, which is also present in France, was the most frequent. No differences were found, comparing total mycelium protein profiles (SDS-PAGE), between different species and formae speciales of *F. oxysporum*, *F. solani* and *F. culmorum* were also isolated from winter melon collar and fruit respectively. Pathogenicity assays performed, under controlled conditions, on *F. culmorum* infected soil with 'Purceddu', a Sicilian local population, gave 90% of mortality in the germination stage, and over 90% of mortality of inoculated transplanted seedlings. Among *F. culmorum* isolates tested those from beet and carrot proved to be virulent on melon while those obtained from cereals (maize, oat, wheat) were found to be less virulent. On this account, the presence of *F. culmorum* could be influenced by cultural succession. This is the first report of *F. culmorum* as a pathogen of winter melon, though the fungus had already been isolated in 1937 from the fruit in USA.

MOLECULAR MONITORING OF HORSE CHESTNUT SOUND LEAVES AND AFFECTED BY PATHOLOGIES AND PHYSIOPATHIES. A. Bertoluzza, G. Bottura, P. Lucchi, L. Marchetti and Aldo Zechini D'Aulerio*. *Dipartimento di Protezione e Valorizzazione Agroalimentare, Università di Bologna, Via F. Re 8, I-40126 Bologna, Italy. Fax: +39.051.351414.

Results are given of a preliminary research aimed at the molecular investigation of the leaf blotch of horse chestnut incited by *Guignardia aesculi* and environmental pollution and the characterisation of the pathological modification with respect to the physiological ones. For this purpose samples of leaves affected with pathologies or physiopathies were compared with sound leaves using vibrational Fourier Transform Infrared Spectroscopy (FTIR). The comparative analysis of the FTIR spectra of horse chestnut leaves shows: gradual weakening of the band of the aromatic domain (1650-1500 cm^{-1}) passing from the sound leaves to those suffering from fungus pathology, whilst the bands of the cellulose domain (1200-950 cm^{-1} region) remains almost unchanged in all samples; inversion of the intensities of the two main bands of the aromatic domain (1610, 1650 cm^{-1} respectively) passing from the sound leaves to the one affected by serious physiopathies, indicate changes in the composition of the constituent groups of this domain. Furthermore, a weak band at 1300 cm^{-1} , present in the spectra of the sound leaves and in those affected with pathology, disappears in the spectra of the leaves showing serious physiopathy: that band, for now not easily attributable with any certainty, could be the first marker of the presence of some physiopathy. The results obtained show that vibrational FTIR spectroscopy is a promising technique that could help in understanding the biochemical processes involving the biological macromolecules of plant tissues exposed to different environmental situations.

DIAGNOSIS OF PHYTOPHTHORA CINNAMOMI FROM PERSIAN (ENGLISH) WALNUT. A. Belisario, E. Forti and Laura Luongo*. *Istituto Sperimentale per la Patologia Vegetale, Via C.G. Bertero 22, I-00156 Roma, Italy. Fax: +39.06.86802296; e-mail: mc_ispave@www.inea.it

A severe decline of Persian (English) walnut trees was observed in fruit orchards in the Veneto Region at the beginning of summer 1997. Diseased trees showed collar and root rot with widely extended stem browning, resembling the 'ink disease'. Isolations produced white mycelial *Phytophthora* colonies with abundant hyphal swellings on potato dextrose and V-8 juice agar. Non papillate sporangia (25x40 μm) were obtained on clarified V-8 juice agar. Oospores were formed when paired with A1 mating type, and antheridia were amphigynous. Mycelial growth was maximum at 27°C in the dark. No growth was observed at 5° and 40°C. *Phytophthora cinnamomi* was identified on the basis of morphological characteristics. The identification was confirmed by total mycelial protein profile comparison (SDS-PAGE) and by RFLPs of the Internal Transcribed Spacer (ITS) of ribosomal DNA with *RsaI* and *HinfI* restriction enzymes. *Phytophthora* isolates used in profile comparisons were *P. cinnamomi* from red oak, *P. cryptogea* from lentisk, *P. nicotianae* from passion fruit and kentia, *P. cactorum* from walnut and *P. palmivora* from ivy and banana trees. Koch's postulates were fulfilled by artificial inoculations on 2 year old Persian (English) walnut seedlings. This is the first characterisation of *P. cinnamomi* from walnut in Italy.

BIOLOGICAL CONTROL OF CHESTNUT INK DISEASE WITH ECTOMYCORRHIZAL FUNGI. M. Barbara Branzanti*, E. Rocca and A.M. Pisi. *Dipartimento di Biotecnologie Agrarie ed Ambientali, Università di Ancona, Via Breccie Bianche, I-60131 Ancona, Italy. E-mail: branzanti@popcsi.unian.it

Previous studies have shown that some ectomycorrhizal (ECM) fungi, capable of forming ectomycorrhizae with chestnut in greenhouse experiments, exhibit antagonistic capacity against *Phytophthora cambivora* and *P. cinnamomi* in paired cultures on agar plates. We investigated if an early infection by *Laccaria laccata*, *Hebeloma crustuliniforme*, *H. sinapizans* and *Paxillus involutus* might protect *Castanea sativa* seedlings against attacks of ink disease. At the end of the first vegetative season mycorrhizal and non-mycorrhizal seedlings were challenged with a zoospore suspension of *P. cinnamomi*. The pathogen decreased development and biomass of non-mycorrhizal chestnut seedlings with root tips poorly developed and dry weight of root system reduced by 48% in comparison to control plants. By contrast, the growth of mycorrhizal plants was not affected by *P. cinnamomi*. The values of the biomass of mycorrhizal chestnuts five months after inoculation with the pathogen showed no statistically significant difference as compared to control plants. The dry weight of roots of seedlings preinoculated with either *L. laccata* or *H. sinapizans* and infected with *P. cinnamomi* were always greater than those of control plants but without statistically significant differences. Infection by *P. cinnamomi* was observed in non-mycorrhizal plants only. In roots colonised by ECM fungi rare not germinated zoospores and some hyphae were detected in non-mycorrhizal segments only.

TOMATO SPOTTED WILT VIRUS ON MELON AND AFRICAN EGGPLANT IN THE METAPONTE AREA OF ITALY. Ippolito Camele* and G.L. Rana. *Dipartimento di Biologia, Difesa e Biotecnologie Agro-Forestali, Università della Basilicata, Via N. Sauro 85, I-85100 Potenza, Italy. Fax: +39.0971.55748; e-mail: camele@unibas.it

Plants of melon (*Cucumis melo*) and African eggplant (*Solanum aethiopicum*) showing leaf epinasty and chlorosis followed by bronzing and top wilt, were observed in experimental fields in the Metapontum horticultural area (southern Italy), in summer 1996 and 1997. Infection rates were consistently higher in melon than in *S. aethiopicum*. The symptoms recalled those of tomato spotted wilt virus (TSWV, Tospovirus, Bunyaviridae), and the following trials were carried out: (i) mechanical inoculation of *Catharanthus roseus*, *Chenopodium quinoa*, *Nicotiana glutinosa* and *Petunia hybrida* with sap from symptomatic plant tissues; (ii) immunosorbent electron microscopy (ISEM) followed by decoration, using an antiserum to the lettuce strain of the virus (TSWV-L) and extracts from young leaves of *N. glutinosa* experimentally infected with virus isolates from naturally infected melon and *S. aethiopicum*; (iii) experimental inoculation of healthy seedlings of *C. melo* and *S. aethiopicum* with the respective virus isolates. Symptoms on the indicators and the results of ISEM and decoration tests showed that the isolates from naturally infected *C. melo* and *S. aethiopicum* were very closely related if not identical to TSWV-L. The melon isolate locally infected almost all melon seedlings but systemically infected only one, which reacted with bronzing and wilting of the apical leaves. The *S. aethiopicum* isolate was latent in eggplant (*Solanum melongena*) but reproduced the field syndrome in *S. aethiopicum*. The susceptibility of *S. aethiopicum* to TSWV makes this species unsuitable as a source of resistance genes for genetic improvement of eggplant and tomato.

MECHANISMS OF ACTION INVOLVED IN THE ANTAGONISM OF AUREOBASIDIUM PULLULANS AGAINST POSTHARVEST PATHOGENS. Raffaello Castoria*, F. De Curtis, G. Lima, S. Pacifico and V. De Cicco. *Dipartimento di Scienze Animali, Vegetali e dell'Ambiente, Università del Molise, Via F. De Sanctis, I-86100 Campobasso, Italy. Fax: +39.0874.404678; e-mail: castoria@hpsrv.unimol.it

To understand the mechanisms involved in the activity of antagonists it is crucial, for the optimisation of their performances, to establish screening criteria in the search for new isolates and for the possible combined utilisation of biological control agents acting through complementary modes of action. An isolate of the yeast like fungus *Aureobasidium pullulans* (LS-30) showed a significant antagonistic activity against some of the most important postharvest pathogens on apples and table grapes, both in small scale experiments and under semi-commercial conditions. Studies on the mechanisms involved in the activity of this antagonist were performed. We examined: (i) the possible occurrence of a direct physical interaction between the antagonist cells and hyphae of *Botrytis cinerea* and *Penicillium expansum*, a phenomenon often associated with mycoparasitism; (ii) the role of competition for nutrients; (iii) the *in vitro* (i.e. in the culture filtrates) and *in vivo* (i.e. in apple wounds, one of the conditions in which the antagonist was assayed) production of exocellular chitinase and β 1,3-glucanase activities, fungal cell wall depolymerases involved in the antagonism of mycoparasites such as *Trichoderma* spp. Further, we performed plate assays to test the possible presence of antibiosis in the activity of LS-30 against *B. cinerea* and *P. expansum*, and TLC assays to assess the possible antibiotic activity against Gram positive and Gram negative bacteria of ethyl acetate extracts obtained from the antagonist culture filtrate.

CONTROL OF FUSARIUM OXYSPORUM F.SP. GLADIOLI BASED ON THE PRODUCTION OF PATHOGEN FREE SAFFRON CORMS. Curgonio Cappelli* and G. Di Minco. *Istituto di Patologia Vegetale, Università di Perugia, Borgo XX Giugno 74, I-06121 Perugia, Italy. Fax: +39.075.5856482.

Severe attacks of *Fusarium oxysporum* Schlecht. f.sp. *gladioli* (FOG) have been recorded on saffron (*Crocus sativus* L.) cultivated near L'Aquila (central Italy). The pathogen, recorded in 65 of the 80 fields examined, was the major limiting factor of stigma yield. The disease occurred only when corms harvested from infected crops were planted. Field experiments were carried out from 1995 to 1997 in two localities of L'Aquila province to produce pathogen free corms. Corms, harvested from fields where the disease was present or absent, treated or not-treated with Prochloraz (a.i. 3.5 g l⁻¹), were planted in plots according to a randomised block design. The corms harvested from healthy fields and the infected/contaminated corms treated with Prochloraz before planting produced healthy plants and corms. The use of pathogen-free corms is the best strategy to prevent the disease in Integrated Pest Management (IPM). For this purpose, we suggest careful inspection of saffron fields during flowering (October), when it is easy to observe symptoms and to isolate FOG. Inspections carried out later are not suitable because diagnosis is more difficult. Infected plants often disappear because of development of severe rot, the presence of weeds can mask the disease and snow, frequently present during the winter, complicates the inspection. The crop could be apparently healthy, but FOG present in the soil can contaminate bulbils of healthy plants and/or mature corms before harvesting. If corms for planting are then harvested from such crop, their treatment with Prochloraz can be useful to produce healthy plants.

IDENTIFICATION OF INDIVIDUAL RACES OF PSEUDOMONAS SYRINGAE PV. PISI BY MULTIPLEX PCR. V. Catara, D.L. Arnold, G. Cirvilleri and Alan Vivian*. *Department of Biological and Biomedical Sciences, University of the West of England, Frenchay Campus, Coldharbour Lane, Bristol BS16 1QY, U.K. E-mail: a-vivian@uwe.ac.uk

A multiplex PCR protocol with four pairs of primers was developed to identify the seven races of *Pseudomonas syringae* pv. *pisi*. Two pairs of primers allowed identification of the bacterium and, on the basis of the presence of one of two possible DNA fragments, two phylogenetic groups were distinguished: I (races 1, 5 and 7 and some strains of races 3 and 4, designated 3B and 4B) and II (races 2 and 6 and the other strains of races 3 and 4, designated 3A and 4A). Two new pairs of primer were designed (on the basis of the published sequences of the avirulence genes, *avrPpiA*, present in races 2, 5, and 7 and the gene *avrPpiB*, present in the races 1, 3 and 7) to give amplification products of 348 bp and 493 bp, respectively. The four pairs of primers used in a single PCR reaction on 47 strains of *P. syringae* pv. *pisi* generated 1 to 3 DNA fragments per strain when separated by AGE. No bands were detected in a range of closely related *P. syringae* pathovars (*aptata*, *glycinea*, *phaseolicola* and *syringae*) following PCR amplification. Profiles obtained did not differentiate between races 1 and 3B, nor between races 4A and 6, for which *in planta* tests will be necessary. This method makes possible the identification *P. syringae* pv. *pisi* and most of the races with a single PCR amplification. Further work will attempt to overcome the remaining ambiguities in race differentiation.

PURIFICATION AND CHARACTERISATION OF AN ANTIFUNGAL PR4 PROTEIN FROM WHEAT SEEDS.

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We have previously purified and characterised two PR4 proteins named wheatwin1 and wheatwin 2 from the water-soluble fraction of wheat seeds. Both proteins showed strong antifungal activity toward *Fusarium culmorum*, *F. graminearum* and *Botrytis cinerea*. Immunoblot analysis of protein extracts from infected and uninfected seedlings showed the differential induction of this group of proteins in response to fungal infection. The present work describes the isolation and structural-functional characterisation of a new protein from wheat seeds named wheatwin 3, closely related to the previously described wheatwins. This protein has a single peptide chain of about 13 kDa and an isoelectric point of 7.0. Immunoblot analysis showed cross-reactivity with anti-wheatwin 1 antibody; in addition, this protein was found to be blocked at its N-terminus, as are the PR4s previously characterised. Antifungal activity was assayed to investigate the biological role of this protein. Wheatwin 3 was effective (IC₅₀ about 15 µg ml⁻¹) in inhibiting hyphal growth of *F. culmorum*, *F. graminearum*, *Aspergillus flavus*, *B. cinerea*, *Phytophthora infestans*, *Septoria tritici*, *Thielaviopsis basicola* and *Verticillium dahliae*. Changes in shape were often observed on the germinating spores after exposure to wheatwin 3 with a concomitant accumulation of exudates around the spore germ tubes. These observations, and analogy with the previously characterised PR4 wheat proteins, indicate a possible role for wheatwin 3 in the mechanism of plant defence.

USE OF APHANOCLADIUM ALBUM IN BIOLOGICAL CONTROL AGAINST SPHAEROTHECA FULIGINEA IN SQUASH. Franco Ciccarese*, A. Ambrico and D. Schiavone.

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Aphanocladium album (Preuss.) W. Games is an epiphytic microfungus able to produce hydrolytic enzymes (protease, β-glucanase and especially chitinase). In tests of biological control against the powdery mildew of tomato caused by *Oidium lycopersici* with applications of *A. album*, it was ascertained that the antagonistic fungus survives on phylloplane and exercises an appreciable control of the disease when applied before the inoculation with the pathogen. Researches on biological control using *A. album* were extended to the powdery mildew of squash caused by *Sphaerotheca fuliginea*. In greenhouse trials carried out over two years on the hybrid 'Corsair' of squash, the antagonistic activity of *A. album* towards the agents of the powdery mildew was confirmed. The plants treated weekly with conidial suspensions of *A. album* showed a percentage of infected leaf surface of 41.5% and 31%, in the first and second year of the test, respectively. The percentage of infected surface on non-treated plants was 73.5% in the first year of the test and of 74.5% in the second. The possibility of integrating the applications of *A. album* with fungicides was investigated. In preliminary tests carried out *in vitro*, Pyrifenoxy, at reduced dose, was inactive towards *A. album*. The combination, with alternated treatments, of *A. album* and Pyrifenoxy at reduced doses, improved the control of the powdery mildew in comparison with the single application of the antagonist.

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DEVELOPMENT OF SPECIFIC PRIMERS FOR PCR DETECTION OF FUSARIUM OXYSPORUM F.SP. BASILICI. A. Chiochetti, S. Ghignone, A. Garibaldi and Quirico Migheli*.

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Fifty-two isolates of *Fusarium oxysporum*, obtained from infected basil plants, seeds, flower residues and soil from different growing areas in Italy and Israel, were analysed by random amplification of polymorphic DNA (RAPD-PCR). In a pathogenicity assay, 35 isolates determined 32-92% of diseased seedlings on the highly susceptible basil cultivar 'Fine verde' 21 days after sowing in artificially infested substrate, while 17 isolates were non pathogenic on basil. All *F. oxysporum* f. sp. *basilici* (*F.o.b.*) isolates gave identical amplification patterns by using 31 different random primers, which allowed the clear differentiation of *F.o.b.* from representatives of other *formae speciales* or from non-pathogenic strains of *F. oxysporum*. Several amplification products were eluted from gel, digoxigenin-labelled and tested for *F.o.b.*-specificity in a dot blot assay. A 1 kbp primer hybridised only to DNA from all *F.o.b.* isolates but not to DNA from other *F. oxysporum* isolates or from representatives of *F. redolens*, *F. tabacinum*, *Rhizoctonia solani*, *Sclerotinia sclerotiorum*, *S. minor* and *Pythium ultimum*. This DNA fragment was cloned in pGEM, subcloned in pBS-SK and sequenced. Two pairs of *F.o.b.*-specific primers were designed based on these sequences, giving rise to amplification products of 381 and 331 bp, respectively. Our research is now targeted at the development of a PCR-based diagnostic kit for the fast and reliable screening of pathogenic isolates from plant tissues.

REACTIONS OF GERMLASM OF OLIVE TO FUNGAL DISEASE. Franco Ciccarese*, A. Ambrico and D. Schiavone.

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Within a research program for the improvement of olive-growing in Southern Italy, studies on olive resistance to *Verticillium dahliae* and *Spilocaea oleagina* were carried out. Preliminarily, isolates of *V. dahliae* from several localities in southern Italy were collected and their aggressiveness on susceptible olive trees assessed. Inoculations were carried out with the Vd-302 isolate by dipping the roots into a fungal suspension at the concentration of 4x10⁷ cfu. The severity of the external symptoms and the vascular discoloration were estimated. Screening of olive seedlings originating from seed showed characteristics of resistance in some plants. In previous observations, sources of resistance to *V. dahliae* in *Olea europaea* L. *sativa* and *Olea europaea* L. *oleaster* germplasm were found. The results of resistance tests of self-rooted cuttings of selections supplied by the Institute of Research on Olive-growing in Perugia (Italy) indicated promising reactions of resistance in DA 12 I and Yusti selections. Surveyings on reactions of olive germplasm to 'leaf spot' by *Spilocaea oleagina* have included investigations in olive-groves in several areas of southern Italy. The cultivars 'Carolea' and 'Cassanese' (or 'Rossanese') were highly susceptible, the cultivar 'Leccino' confirmed its intermediate susceptibility. The disease manifestation on local selection 'Nocellara of the Belice' consisted in brown ring-like symptoms, chlorotic areas were not present and defoliation was not observed. The local cultivar 'Roggianella' was highly resistant to 'leaf spot' of olive.

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CHARACTERIZATION OF TOMATO RESISTANCE (*OL-2* GENE) TO *OIDIUM LYCOPERSICI*. Franco Ciccicarese*, A. Ambrico and D. Schiavone. *Dipartimento di Patologia Vegetale, Università di Bari, Via G. Amendola 165/a, I-70126 Bari, Italy. Fax: +39.080.5442906; e-mail: fciccicare@agr.uniba.it

During screening of tomato germplasm for resistance to *Oidium lycopersici*, a monogenic recessive resistance to powdery mildew was found and designated as gene *ol-2*. A research program was started to characterize this resistance with the following particular aims: in particular, (1) to isolate the *ol-2* resistance gene; (2) to investigate the cyto-histological and physiological mechanism of resistance (3) to assess the validity of this source of resistance towards several populations of *O. lycopersici*; and (4) to investigate the influence of endogenous and exogenous factors on the phenotypic expression of resistant plants. For this last aspect, research was carried out to assess the influence of leaf age and nutritional state of the plants (different levels of nitrogen) on the expression of resistance conferred by the *ol-2* gene. Tests were carried out in a greenhouse with temperatures of 24±2°C and relative humidity 100%. Inoculations were made by shaking heavily-mildewed tomato leaves over each test plant. Disease development on the susceptible and resistant plants was assessed periodically, removing the fungal structures from the leaf surface with the 'celloidine film' technique. On susceptible plants progress of the powdery mildew was not affected by leaf age or nutritional status. In resistant genotypes, expression of resistance was conditioned by leaf age. Nitrogen applications had no marked effects on disease progress in resistant plants.

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IMPAIRED CELL-TO-CELL MOVEMENT OF POTATO VIRUS Y IN TRANSGENIC PLANTS EXPRESSING THE 5' AND 3' TERMINAL REGIONS OF THE VIRAL GENOME. Aniello Crescenzi*, M. Nuzzaci, S. Comes, L. D'Aquino, P. Piazzolla and J. Burgyán. *Dipartimento di Biologia, Difesa e Biotecnologie Agro-Forestali, Università della Basilicata, Via N. Sauro 85, I-85100 Potenza, Italy. Fax: +39.0971.55748; e-mail: crescenzi@unibas.it

Nicotiana tabacum plants were transformed with the linked 5' and 3' terminal sequences of a Hungarian necrotic isolate (PVYN-H) of potato virus Y via the *Agrobacterium*-mediated leaf disc transformation procedure. When challenge-inoculated with PVYN-H, transgenic plants displayed various levels of resistance, and three lines were selected based on their reaction to infection. The first was a resistant line (R) with no visible symptoms. The second intermediate line (I) showed a response delayed by 7-10 days, and unusual chlorotic-necrotic lesions (hypersensitive response) in which the challenge virus was localised and cell-to-cell transfer was limited; however, vascular movement was not affected because the lesions also developed in uninoculated, newly formed leaves. The third susceptible (S) line showed the same symptoms as untransformed plants. The same behaviour was seen with other PVY strains that caused vein necrosis in non-transgenic *N. tabacum*. By contrast, all plants from the lines R, S and I were completely susceptible to PVY-O strains, which induce mild symptoms in *N. tabacum*. Northern blot analysis of the PVYN-H-inoculated plants showed efficient virus replication in plants with symptoms, but no viral RNA was found in symptomless tissues. PVYN-H replicated efficiently in protoplasts prepared from 'R' transgenic plants, suggesting that the resistant phenotype of 'R' transgenic plants is due to an impaired cell-to-cell movement of the challenge virus, rather than to interference with virus replication.

DOWN-REGULATION AND CO-SUPPRESSION: TWO MECHANISMS OF TRANSGENIC TOLERANCE MEDIATED BY CUCUMBER MOSAIC VIRUS SATELLITE RNA. F. Cillo, M. Papanice and Donato Gallitelli*. *Dipartimento di Protezione delle Piante dalle Malattie, Università degli Studi di Bari, Via G. Amendola 165/a, I-70126 Bari, Italy. Fax: +39.080.5442911; e-mail: gallitel@agr.uniba.it

Variants of cucumber mosaic virus satellite RNA (CMV satRNA) with attenuating effects on symptom expression (benign satRNAs) were studied as potential biocontrol agents of CMV-induced diseases. Tomato plants transformed with a single copy of the full-length sequence of a benign satRNA (Tfn-satRNA) recovered within three weeks from infection by either a satRNA-free strain of CMV (CMV-FL) inducing shoestringing or a CMV strain supporting a necrogenic variant of satRNA (CMV-77 plus 77-satRNA) causing lethal necrosis. We suggest that in both cases recovery is the result of two independent mechanisms operating in the same transgenic line. With the first mechanism, the Tfn-satRNA transcript affects the replication of both CMV strains (CMV-FL and CMV-77), outcompeting genomic RNAs for viral replicase. Evidence for this is the reduction of CMV concentration in transformed plants to about 25% that of untransformed controls, and the great accumulation of single-stranded and double-stranded Tfn-satRNA in infected tissues. The second mechanism may specifically operate against the necrogenic 77-satRNA, since recovery was correlated with disappearance of this molecule from infected plants. This latter mechanism could be an example of homology-dependent gene silencing or co-suppression. This appears to be the first report of transgenic tolerance mediated by CMV-satRNA against a CMV strain supporting a necrogenic satRNA variant.

ANALYSIS OF PECTINOLYTIC ENZYMES AND RELATED GENES IN *PYRENOPHORA* SPP. C. Cristani, M.R. Vergara, F. Favaron and Giovanni Vannacci*. *Dipartimento di Coltivazione e Difesa delle Specie Legnose, Sez. Patologia Vegetale, Università di Pisa, Via del Borghetto 80, I-56124 Pisa, Italy. Fax: +39.050.543564; e-mail: gvann@agr.unipi.it

Pyrenophora graminea Ito and Kuribayashi and *P. teres* (Died.) Drechs. respectively cause leaf stripe and net blotch on barley leaves. Since both pathogens show intercellular growth in part of their life cycle, pectinolytic enzymes could be important for host colonization. A 1.5 kbp PCR product (Pg2-PCR), previously obtained from *P. graminea* DNA with primers from the *Fusarium moniliforme* endo-PG gene sequence, was used as a homologous probe in Northern blot experiments. A strong constitutive expression (about 1 kb transcript) was detected in *P. graminea* and *P. teres* grown on pectin or barley cell walls. Furthermore the probe Pg2-PCR was apparently specific for the *Pyrenophora* spp. genome since it did not recognize *F. moniliforme* sequences in Northern nor in Southern blot experiments. Analysis of secretion of *P. graminea* polygalacturonases showed that endo-PG activity was very high in cultures containing pectin, but ten times lower in those containing cell walls, and only barely detectable in control cultures. Isoelectrofocusing patterns were equivalent in both inductive systems. Two main endo-PG isoforms were identified at about 8.5 and 7.5 Ip, while minor isoforms were found between 7.0 and 8.3 Ip and between 4.5 and 5.2 Ip. The two predominant endo-PGs had masses of 42 kDa and 40 kDa respectively, and cross-reacted with an *F. moniliforme* anti-PG polyclonal antibody. However the significance of this difference in results obtained with molecular and biochemical approaches is still an open question.



IDENTIFICATION OF *ARMILLARIA* SPECIES COLLECTED IN ITALY USING A PCR-BASED TECHNIQUE. T. de Gioia, Nicola Luisi* and P. Lerario. *Dipartimento di Patologia Vegetale, Università di Bari, Via G. Amendola 165/a, I-70126 Bari, Italy. Fax: +39.080.5442906; e-mail: luisin@agr.uniba.it

Armillaria is a genus of root infecting basidiomycetes, which includes seven European Biological Species (EBS), five of them occurring in Italy. Isolates of *Armillaria* spp. were collected from across the peninsula and were identified to species by pairing with haploid tester strains. Four to five isolates of each *Armillaria* species (*A. mellea*, *A. gallica*, *A. cepistipes*, *A. tabescens* and *A. ostoyae*) were selected and used for the PCR-based technique described by Harrington and Wingfield properly modified: a small section of Intergenic Spacer (IGS) of ribosomal RNA was amplified using the polymerase chain reaction. Direct amplification performed from scrapes of living mycelium from cultures without DNA extraction was successful with all isolates, however, some of them required more than one attempt. The size of the amplified product was 875 bp for all isolates of *A. mellea* and 920 bp for the other four species. All isolates were identified to species by digestion of the PCR products with the restriction enzyme *AluI*. The analysis of the Restriction Fragment Length Polymorphism (RFLP) showed an intraspecific uniformity of the bands deriving from digestion, but a different restriction pattern for each species, that allow us to distinguish the five *Armillaria* species.

A RELIABLE BIOLOGICAL INDICATOR FOR PEAR DECLINE DISEASE. Paola Del Serrone*, R. Barrale, A. Liberatore, E. Bianchi and M. Barba. *Istituto Sperimentale per la Patologia Vegetale, Via C.G. Bertero 22, I-00156 Roma, Italy. Fax: +39.06.86802296; e-mail: ispave@flashnet.it

To prevent the spread of pear decline (PD), an economically important phytoplasma disease, all international certification schemes require its absence from certified propagative material. The diagnostic method officially recognised internationally consists of indexing based on field assays using *Pyrus communis* cv. 'Doyonne du Comice' as indicator. In order to accelerate and optimise detection of PD by biological assay, the sensitivity of different indicators to PD was evaluated under controlled conditions. Cuttings from infected pear trees, collected in autumn in different pear-growing areas, were tongue-grafted on *Pyronia veitchii*, *Cydonia oblonga* 'C 7-1', and *Pyrus communis* 'A-20' and 'Abate fetel'. Four plants of each indicator were grafted and maintained in a greenhouse. Disease symptoms were recorded every two days and the experiment was repeated twice. The presence of PD phytoplasma in naturally infected pears or in experimentally grafted indicators was ascertained by PCR and RFLP assays. Only *C. oblonga* 'C 7-1' gave symptoms (rosetting, leaf distortion and yellowing) 40 days after grafting. The only doubtful symptom observed in the other indicators was the presence of elongated leaf petioles. On the basis of these results, the biotest using *C. oblonga* 'C 7-1' seems useful for screening candidate stocks. This test requires about one month for a reliable response instead of the two years of field testing needed when *P. communis* 'Doyonne du Comice' is used, as recommended by current certification schemes.

EUROPEAN STONE FRUIT YELLOWS PHYTOPLASMA: MONITORING BY BIOLOGICAL AND MOLECULAR METHODS IN *PRUNUS DOMESTICA* AFFECTED BY LEPTONECROSIS. Paola Del Serrone*, R. Barrale, E. Bianchi, A. Liberatore and M. Barba. *Istituto Sperimentale per la Patologia Vegetale, Via C.G. Bertero 22, I-00156 Roma, Italy. Fax: +39.06.86802296; e-mail: ispave@flashnet.it

We report on the spatial and temporal distribution of European stone fruit yellows (ESFY) phytoplasma in plum trees (*Prunus domestica*) naturally affected by leptonecrosis. Cuttings for propagation and samples for DNA analysis were collected at six different times of the year from apical, middle and basal parts of four branches from each plant and from healthy plants. DNA was extracted and analysed for the presence of ESFY. The cuttings were grafted (chip budding, tongue grafting and root grafting) to *Prunus persica* 'GF 305' indicators maintained under controlled conditions, and these were later tested for the presence of phytoplasmas. A phytoplasma was detected and identified as ESFY by PCR (f01/r01, fAT/rPRUS) and RFLP (*RsaI*). No products were amplified from healthy tissue. ESFY was detected at all seasons of the year considered. Even though all branches of the plant showed symptoms, ESFY was not detected in all by PCR, and this was confirmed by the biological assays. Following tongue grafting, symptoms appeared in 100% of the indicators 30-40 days later. The results show that both methods can detect ESFY in propagative material but because of uneven distribution of the phytoplasma in the plant, several samples must be tested to avoid false negatives.

EXPRESSION OF THE *A. NIDULANS atrB* GENE IN *S. CEREVISIAE* CONFERS RESISTANCE TO CHEMICALLY UNRELATED FUNGICIDES. Giovanni Del Sorbo*, M. Ruocco, A.C. Andrade, A. Decottignies, M. Lorito, M. De Waard and F. Scala. *Dipartimento di Arboricoltura, Botanica e Patologia Vegetale, Sez. Patologia Vegetale, Università di Napoli "Federico II" e CETELOBI, Via Università 100, I-80055 Portici (Napoli), Italy. Fax: +39.081.7755114; e-mail: delsorbo@unina.it

The gene *atrB* of *Aspergillus nidulans* encodes a membrane protein of the ABC (ATP-binding cassette) superfamily of membrane transporters, which utilise the energy derived from ATP hydrolysis to transport endogenous and exogenous substrates through biological membranes. The cDNA of *atrB* was cloned in the yeast plasmid pYEura3 under the control of the galactose-inducible promoter *GAL10* to produce plasmid pYatrB, which was used to transform a yeast strain (AD1-8) disrupted in several genes encoding ABC transporters (*i.e.* *PDR5*, *SNQ2* etc.) and, therefore, displaying hypersensitivity to a number of toxic drugs. Under conditions which promote the *GAL10*-driven expression of *atrB* (*i.e.* in presence of galactose) the yeast transformed with pYatrB exhibited an increased level of resistance to several groups of fungicides including DMI inhibitors (imazalil, fenarimol, myconazole and tebuconazole), dicarboximides (iprodione), phenylpyrroles (fenpiclonil) and polyene antibiotics (nystatin). This evidence demonstrates the involvement of energy-dependent efflux mechanisms mediated by AtrBp-like transporters in multidrug resistance to agricultural fungicides. Preliminary results indicate that the mechanism of multidrug resistance conferred by *atrB* expression relies on a decreased intracellular accumulation of toxicants.

CONTROL BY SPACE SOLARIZATION AND GENETIC VARIABILITY OF *FUSARIUM OXYSPORUM* F. SP. *RADICIS-LYCOPERSICI*. Pietro Di Primo* and G. Cartia. *Dipartimento di Agrochimica e Agrobiologia, Difesa delle Piante, Università di Reggio Calabria, Piazza S. Francesco 2, I-89061 Gallina (Reggio Calabria), Italy. Fax: +39.0965.682755; e-mail: gcartia@csi-ins.unirc.it

Fusarium crown and root rot caused by *F. oxysporum* f. sp. *radicis-lycopersici* (FORL) is the most harmful soilborne disease of tomato in protected cultivations in Italy. A promising disease management strategy is greenhouse 'space solarization', which is complementary to soil disinfestation for the elimination of inoculum surviving in the greenhouse structure. Space solarization is carried out by closing the greenhouse during summertime, and raising air temperatures to 60-65°C. 34 days trials, induced a significant reduction (98% mortality) in the population of FORL chlamydospores placed in bags above the soil. Moreover, soil solarization treatment carried out in the same greenhouse by mulching soil with black films induced a high mortality (98%) of the chlamydospores buried in the soil at 15 cm depth. In order to optimise the disease control, it is necessary to thoroughly investigate the pathogen. Studies on the genetic variability may help to obtain useful information on FORL. Twenty-five isolates of FORL were compared for vegetative compatibility grouping (VCG) using complementation among nitrate non utilising (*nit*) mutants. The *nit* mutants were generated from all isolates and used for complementation tests. Representative mutants were tested against Nit M mutants of FORL isolates from Israel, Belgium, Canada, Florida, France. The Italian isolates of the pathogen were assigned to three different vegetative compatibility groups (VCG: 0090, 0091 and 0092). It is concluded that the data demonstrate genetic diversity of the screened FORL isolates.

MONITORING OF *TRICHODERMA* ISOLATES FOLLOWING DELIVERY INTO THE ENVIRONMENT. PRELIMINARY RESULTS. S. Fanti, A. Barbieri and Giovanni Vannacci*. *Dipartimento di Coltivazione e Difesa delle Specie Legnose, Sez. Patologia Vegetale, Università di Pisa, Via del Borghetto 80, I-56124 Pisa, Italy. Fax: +39.050.543564; e-mail: gvann@agr.unipi.it

Previous experiments permitted the identification of two *Trichoderma* isolates with antagonistic activity against *Cytospora cincta* on peach twigs and the evaluation of their genetic variability by the PCR technique using 10mer random primers, microsattelites and minisattelites (consensus sequences of M13 phage). With minisatellite M13 the two isolates could be distinguished from other *Trichoderma*. Sixty-five *Trichoderma* isolates naturally present in the area of the trials (peach orchard) at ground level (soil, grass leaves, roots) and on the aerial apparatus (branches, twigs of peach trees) were isolated on selective medium. These isolates were molecularly characterised, after a 'miniprep' DNA extraction, using the minisatellite M13 primer. Results confirmed that electrophoretic patterns of the two isolates to be delivered into the environment were characteristic with one of them showing an isolate-specific band of about 390 bp. Field experiments were initiated with the distribution of antagonists as 'germlings' on the aerial apparatus and will proceed with selective re-isolation of *Trichoderma* from treated areas, molecular characterisation of all *Trichoderma* strains by analysis of their electrophoretic patterns and comparison of the latter with patterns shown by released antagonists. We are evaluating the ability of the method to give positive results when DNA of selected isolates is mixed in different proportions with DNA from other isolates.

This work was supported by the MURST, Project "Difesa delle colture in sistemi agricoli eco-compatibili".

SALICYLIC ACID-INDUCED SYSTEMIC SUSCEPTIBILITY OF *CHENOPODIUM AMARANTICOLOR* TO TOBACCO NECROSIS VIRUS. Gianni Faccioli*, A. Raiola and R. Armuzzi. *U.C.I. "S.T.A.A." Istituto di Patologia Vegetale, Università di Bologna, Via F. Re 8, I-40126 Bologna, Italy. Fax: +39.051.351434; e-mail: faccioli@agrsci.unibo.it

Salicylic acid (SA) is often regarded as a signal molecule involved in systemic acquired resistance (SAR) against plant virus infections. When infiltrated into *Chenopodium amaranticolor* leaves 12 h prior to inoculation with tobacco necrosis virus (TNV), it caused an increase in virus synthesis. We determined the level of systemic resistance against a TNV challenge inoculation, in plants allowed to absorb 3.5 or 5.0 mM SA into the lower leaves, in comparison with that of untreated controls and of plants that had been inoculated with TNV in the lower leaves. As expected, TNV-inoculated plants acquired systemic resistance, as shown by the 46% reduction of TNV synthesis in the challenge-inoculated upper leaves. However, SA-treated plants showed increased susceptibility in proportion to the SA concentration, i.e. 57% and 72% increase in TNV synthesis, respectively. Extracts of antiviral factors from the upper uninoculated leaves of plants whose lower leaves had been inoculated with TNV, reduced the infectivity of a TNV inoculum (0.2 µl ml⁻¹) by 48%. By contrast, comparable extracts from SA-treated plants increased infectivity of inoculum by 77% and 103% for 3.5 and 5.0 mM SA virus synthesis were probably present in these extracts. Preliminary determination of the SA content of the upper leaves of the differently treated plants showed no differences between TNV-inoculated and control plants. It seems therefore, that in our system, SA is involved in a mechanism of acquired susceptibility rather than acquired resistance to TNV.

DNA SEQUENCES OF THE APPLE PROLIFERATION PHYTOPLASMA FOUND IN PSYLLIDS COLLECTED FROM DISEASED APPLE TREES. Maria Stella Grando*, D. Forti and M.E. Vindimian. *Istituto Agrario di San Michele all'Adige, Via E. Mach 1, I-38010 San Michele all'Adige (Trento), Italy. E-mail: Stella.Grando@ismaa.it

Apple proliferation (AP) is the most important graft-transmissible and vector-borne disease of apples in Europe. Recently the Trentino apple orchards of Val di Non and Val di Sole suffered serious AP epidemics, mainly on cvs 'Golden Delicious', 'Florina' and 'Renetta Canada' grafted on different rootstocks. At the time of symptom appearance there was a notable increase in psyllid populations. During summer 1997, individuals of *Cacopsylla* sp. were collected from trees of 'Golden Delicious' and 'Florina' that over the past year had shown severe proliferation symptoms. The insects were allowed to feed on healthy apple trees, and were then subdivided into groups of 20-30 individuals at different growth stages and stored at -80°C until DNA extraction to check if they carried the AP phytoplasma. DNA was amplified by direct PCR using the universal primer pairs R16 F2/R2 and the specific primers fAT/rAS generated from the AP sequence. Nested PCR was done with primers R16 F2/R2 followed by the phytoplasma group-specific primers R16 (X) F1/R1. Products of the expected size for the AP group were obtained with most samples. RFLP analysis of the PCR product of the universal primers digested with *RsaI* indicated the presence of a phytoplasma of the 16S rDNA group X-A. DNA fragments amplified from psyllids and from infected apple leaves showed almost identical sequences.

STUDIES ON THE ETIOLOGY OF *PLEUROTUS OSTREATUS* AND *P. ERYNGII* YELLOWING. Nicola Sante Iacobellis* and P. Lo Cantore. *Dipartimento di Biologia, Difesa e Biotecnologie Agro Forestali, Università della Basilicata, Via N. Sauro 85, I-85100 Potenza, Italy. Fax: +39.0971.55748; e-mail: iacobellis@unibas.it

Bacteria forming precipitates in agar when grown next to *Pseudomonas 'reactans'* (a bacterial entity not yet classified) were consistently isolated from reddish-brown blotches on *Pleurotus ostreatus* sporocarps. The isolates caused pitted brown lesions on blocks of *Agaricus bisporus* tissue and sunken yellowish lesions on sporocarps of *P. ostreatus*. These features and their biochemical and nutritional characters were typical of *Pseudomonas tolaasii*. Other fluorescent pseudomonads, capable of causing less pronounced alterations of blocks of *A. bisporus* tissue and whole sporocarps of *P. ostreatus*, were also obtained from the same specimens. These isolates were identified as *P. 'reactans'* and *Pseudomonas* sp., respectively. The latter isolates were negative in the white line assay and differed from both *P. tolaasii* and *P. 'reactans'* in some nutritional characters. Bacteria identified as strains of *Pseudomonas* sp. were isolated from the yellowed *P. ostreatus* sporocarps. Bacteria identified as strains of *P. tolaasii* and/or *P. 'reactans'* were also obtained but only in a few cases. These results suggest that the bacteria described above could account for *P. ostreatus* yellowing. In particular, *P. tolaasii* seems to cause blotches surrounded by yellowish discoloration whereas *P. 'reactans'* and *Pseudomonas* sp. appear to be mainly responsible for yellowing of mushroom tissues. Bacteria identified as *P. 'reactans'* and *Pseudomonas* sp. were consistently obtained from sporocarps of *P. eryngii* showing typical yellowing. The pathogenicity features of *P. 'reactans'* on whole sporocarps and on growing mushrooms clearly indicate that this bacterium accounts for the yellowing in *P. eryngii*.

OBSERVATIONS ON ITALIAN POPULATIONS OF *PYRENOCHAETA LYCOPERSICI* AIMED AT BREEDING FOR RESISTANCE. Alessandro Infantino*, M. Aragona, E. Lahoz, A. Oliva and A. Porta-Puglia. *Istituto Sperimentale per la Patologia Vegetale, Via C.G. Bertero 22, I-00156 Roma, Italy. E-mail: ispave@flashnet.it

Corky root of tomato (*Lycopersicon esculentum* L.) caused by *Pyrenochaeta lycopersici* Schneider et Gerlach is a major soilborne disease in many growing areas in the world. In Italy the disease, formerly important in the greenhouses, is now of concern in the open field, where it was observed in nine districts. Twenty-four isolates were collected and characterised for pathogenicity, sporulation capability and growth temperature. Pathogenicity was confirmed on the susceptible cultivar 'Corbarino'. The optimum growth temperature was 22°C. Double strength V-8 agar was the best medium for sporulation. Variability of *P. lycopersici* was studied by isozyme and RAPDs analyses. First results showed polymorphism for esterase by which single isolates could be distinguished. For RAPDs analyses, twenty primers (Operon Technologies) were chosen from among sixty 10mer primers screened. Up to now, our *P. lycopersici* collection, two reference isolates of the same species and two of related species (*Phoma terrestris* and *Herpotrichia parasitica*), were analysed using six of these primers. All the *P. lycopersici* isolates showed very similar electrophoretic profiles, clearly distinct from those of the other species. The overall results confirmed that the fungus is widespread in Italy. The incidence of corky root, very high in some areas, seems to be more related to environmental conditions than to the variability of the pathogen. Biochemical and biomolecular analyses are promising for characterisation of *P. lycopersici* populations.

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TRANSGENIC TOMATO: EVALUATION OF SOME POTENTIAL RISKS. V. Ilardi, L. Tomassoli and Marina Barba*. *Istituto Sperimentale per la Patologia Vegetale, Via C.G. Bertero, 22, I-00156 Roma, Italy. Fax: +39.06.86802296; e-mail: mc0540@mclink.it

A primary concern for large scale commercialization and growing of transgenic crops is the escape of transgenes into untransformed plants. We report analysis of the spontaneous flow of functional transgenes from homozygous transgenic tomato cv. 'UC82B' expressing genes for cucumber mosaic virus coat protein (CMV-CP) and neomycin phosphotransferase II (NPTII), to the corresponding untransformed controls growing in the field. Two field trials were analyzed. In at least 2000 progeny plants of 'wild type' 'UC82B' field controls, hybrids were examined for the transgenic proteins. Two plants were found from each field of plants expressing the transgenes. These plants, however, proved to be homozygous for the transgenes, indicating that they did not originate by cross-pollination between transgenic and 'wild type' parents but probably derived from transgenic seeds that by chance contaminated the untransformed seed stock during picking. The main perceived risk involved in commercialization of transgenic products is food safety. For this reason the level of the CMV-CP gene and its expression product (CMV-CP) was measured by PCR, DAS-ELISA and Western blotting in canned transgenic tomatoes. Neither the transgene nor the transgenic protein were detected in processed tomatoes, while fresh tomato controls contained both. The same tests are now underway for the NPTII transgene. We conclude that, in our conditions and in the context of cv. 'UC82B', no gene flow occurred, and that in canned transgenic tomatoes the CMV-CP gene and its protein product did not survive the canning process.

XANTHAN GUM AS ADJUVANT IN CONTROLLING TABLE GRAPE ROTS WITH *AUREOBASIDIUM PULLULANS*. Antonio Ippolito*, F. Nigro, G. Lima, G. Romanazzi and M. Salerno. *Dipartimento di Protezione delle Piante dalle Malattie, Università di Bari, Via G. Amendola 165/a, I-70126 Bari, Italy. Fax: +39.080.5442911; e-mail: antonio.ippolito@agr.uniba.it.

The results of a two-year investigation aimed at evaluating the activity of the yeast-like fungus *Aureobasidium pullulans*, isolate L47, in combination with Xanthan gum (0.05% w/v) (Xg) against sour and *Botrytis* rots of table grapes are reported. The antagonist was also applied in combination with CaCl₂ (1% w/v) or with Procymidone at 1/10 of full dose. The antagonist alone or in combination was sprayed on bunches in the field 40 (first year) and 21 (second year) days before harvest. Untreated bunches or those treated with Procymidone at full dose (100 g hl⁻¹) served as controls. Rots were evaluated at harvest and after 30 days cold storage (0°C, 95-98% RH). The epiphytic population of yeasts (including yeast-like fungi) and filamentous fungi on the berries was evaluated at harvest and after cold storage. Control of *Botrytis* rot and survival of isolate L47 at harvest were significantly enhanced when the antagonist was applied in combination with Xg; the improvement was less evident after cold storage. L47 was more active in combination with CaCl₂ than alone, but not significantly so. A significant improvement of L47 activity was observed at harvest when the antagonist was applied in combination with Procymidone at 1/10 of full dose. Sour rot, appreciable only in the field at harvest, was significantly reduced by all treatments, with the lowest values on bunches treated with Xg. The improved activity of isolate L47 in combination with Xg seems to be associated with its greater survival on the berries.

ADDITIVES AND NATURAL PRODUCTS AGAINST POSTHARVEST PATHOGENS AND COMPATIBILITY WITH ANTAGONISTIC YEASTS. Giuseppe Lima*, F. De Curtis, R. Castoria, S. Pacifico and V. De Cicco. *Dipartimento di Scienze Animali, Vegetali e dell'Ambiente, Università del Molise, Via F. De Sanctis, I-86100 Campobasso, Italy. Fax: +39.0874.404678; e-mail: lima@bpsrv.unimol.it

The utilization of antagonistic microorganisms and/or natural derived products seems a suitable strategy to reduce or replace synthetic fungicides in the control of postharvest diseases of fruits and vegetables. However, it is necessary to find additives to optimize the activity of the biocontrol agent for preparing commercial formulations. In this work we screened, *in vitro* and *in vivo*, additives and natural products, including different organic and inorganic salts, organic acids, gums, propolis and chitosan, for antifungal activity against *Botrytis cinerea* and *Penicillium expansum*, and for compatibility with two selected antagonistic yeasts (*Cryptococcus laurentii* LS-28 and *Aureobasidium pullulans* LS-30). Among the tested substances, NaHCO₃, Na silicate, NH₄HCO₃, K₂CO₃, KNaCO₃ (1% w/v), sorbic acid (0.5% w/v), acetic acid (1% w/v), benzoic acid (0.1% w/v), propionic acid (0.5% w/v) and chitosan (5 mg ml⁻¹) completely inhibited the mycelial growth of *B. cinerea* and *P. expansum* *in vitro*. Moreover, also other substances, such as lactic acid and calcium silicate (1% w/v), potassium sorbate (0.5% w/v) and propolis (0.5% w/v) showed a high antifungal activity, particularly against *B. cinerea*. As regards compatibility, the antagonists were able to grow in the presence of some substances, in particular lactic acid, sodium and calcium silicate. Some thickeners (gums, pectin, sodium alginate, etc.) appeared to be compatible with the antagonists and they could be used in a formulation with the two biocontrol agents. In *in vivo* experiments, performed on different fruits, several substances, among those selected *in vitro*, significantly reduced the infections caused by *B. cinerea* and/or *P. expansum*.

ECOPHYSIOLOGICAL RESPONSE OF SUSCEPTIBLE AND RESISTANT TOMATO PLANTS INOCULATED WITH *FUSARIUM OXYSPORUM* F. SP. *LYCOPERSICI*. Giacomo Lorenzini*, C. Nali, L. Guidi and G.F. Soldatini. *Dipartimento di Coltivazione e Difesa delle Specie Legnose, Sez. Patologia Vegetale, Università di Pisa, Via del Borghetto 80, I-56124 Pisa, Italy. Fax: +39.050.960622; e-mail: glorenz@agr.unipi.it

Experiments were conducted to study the effects on gas exchange and chlorophyll *a* fluorescence in near-isogenic susceptible and resistant tomato lines of cv. 'Craigella' (C) inoculated with *Fusarium oxysporum* f. sp. *lycopersici* race 1. Thirty days after the inoculation, a difference was observed between the youngest, fully expanded, uncolonized and symptomless leaf of resistant 'Craigella FR' (CFR) and that of the susceptible line as regards photosynthetic activity at saturation light. Resistant tissue was unaffected while the susceptible tissue suffered a 54% reduction compared with controls. Stomatal conductance and intercellular CO₂ were increased in both lines (+38 and +11% for the first parameter and +20 and +45% for the second, in resistant and susceptible material, respectively). Light-use efficiency was affected in a different way: an 18% increase in 'CFR' and a 50% reduction in 'C'. Water-use efficiency was decreased in 'CFR' (-18%) and even more in 'C' (-59%). In 'CFR', the parameters related to the fast kinetics of fluorescence did not change, whereas photochemical and non-photochemical quenching (q_{np}) increased (+20 and +38%, respectively). In 'C', ground, maximum and variable fluorescence were depressed (-10, -9 and -11%, respectively) and q_{pp} increased (+83%). Photosynthesis in susceptible inoculated plants was reduced by mesophyll limitations, whereas in resistant plants defence mechanisms were evident.

***FUSARIUM PROLIFERATUM*: AN EMERGING PHYTOPATHOLOGICAL AND MYCOTOXICOLOGICAL PROBLEM.** Antonio Logrieco*, A. Moretti, G. Mulè, A. Ritieni and A. Bottalico. *Istituto Tossine e Micotossine da Parassiti Vegetali, CNR, Viale L. Einaudi 51, I-70126 Bari, Italy. Fax: +39.080.5486063; e-mail: itmpal02@area.ba.cnr.it

Fusarium proliferatum (T. Matsushima) Nirenberg, a member of the section *Liseola*, is taxonomically a well-documented species. However, limited information exists on its host range and geographical distribution. This may be due in part to the fact that *F. proliferatum* is a rather recently described species and has often been misidentified as *F. moniliforme* Sheld. Both species are anamorphs belonging to the *Gibberella fujikuroi* complex teleomorph, and they are differentiated as distinct mating populations (biological species). Relevant differences between the two species, were also confirmed by RAPDs-PCR and sequence analyses. We identified several strains of *F. proliferatum* isolated world-wide from a large range of plant hosts including maize, asparagus, onion, date palm, tomato, some weeds (e.g. *Arundo donax*), most of which showed symptoms of disease. The strains were examined for their fertility (ability to form fertile perithecia *in vitro* crosses) and ability to produce mycotoxins *in vitro* on maize kernels. The investigations showed that the *F. proliferatum* populations from the different plant hosts are highly fertile (belonging to mating population D) and toxigenic, being able to produce fumonisins, moniliformin, beauvericin and fusaproliferin. The data confirm *F. proliferatum* as an important pathogen of several crops, and as a species causing increasing concern due to its toxicological potential.

IN PLANTA EXPRESSION OF A SINGLE GENE FROM A BIOCONTROL FUNGUS CONFERS HIGH RESISTANCE AGAINST VARIOUS PATHOGENIC FUNGI. Matteo Lorigio*, S.L. Woo, E. Filippone, A. Zoina and F. Scala. *Dipartimento di Arboricoltura, Botanica e Patologia Vegetale, Università di Napoli "Federico II", Via Università 100, I-80055 Portici (Napoli), Italy. Fax: +39.081.7755129; e-mail: lorigio@unina.it

For the first time, a gene from a biocontrol fungus has been used to improve disease resistance in transgenic plants. An antifungal endochitinase sequence from the antagonistic fungus *Trichoderma harzianum* was transferred to tobacco and potato. Plants were transformed with different constructs bearing either the genomic or the cDNA copy of the gene, and high levels of expression of the fungal sequence were obtained in leaves, stems, roots and flowers. A secretion signal peptide from *Trichoderma* was able to trigger extracellular secretion of the transgenic enzyme, which maintained its activity *in planta*. Some transgenic lines were much more chitinolytic than the wild type potato and tobacco plants, although substantial differences in endochitinase activity were detected in the transformants. High expression of the fungal gene was without apparent effect on plant growth and development. Transgenic lines were found which were tolerant or completely resistant to the leaf pathogens *Alternaria alternata*, *A. solani*, *Botrytis cinerea* and the soil-borne fungus *Rhizoctonia solani*. There was a correlation between the level of endochitinase activity in the transgenic lines and the level of resistance to fungi attacking the leaves. These results revealed a new and rich source of disease resistance genes, and may overcome the problems related to use of living biocontrol organisms by transferring the microbial disease control genes directly into plants.

GLUCOSINOLATE-MYROSINASE SYSTEM OF BRASSICACEAE FOR CONTROLLING SOILBORNE PATHOGENS. Luisa M. Manici*, L. Lazzeri, O. Leoni, G. Baruzzi and S. Palmieri. *Istituto Sperimentale per le Colture Industriali, Via di Corticella 133, I-40129 Bologna, Italy. Fax: +39.051.374857; e-mail: istsci4@iperbole.bologna.it

Glucosinolates (GL) are thioglucosides with a variable side chains consisting of aliphatic, aromatic or heteroaromatic residues variable, on their origin. The glucosinolate-myrosinase system is present in all *Brassicaceae* tissues. In intact plant cells, in fact, GLs and the myrosinase system are kept separate, coming into contact only as a result of mechanical wounding or pathogen attack. In this case, the degradation products (DP) are quickly elicited *in situ* thus determining the well known anti-fungal activity. *In vitro* studies on the fungitoxic activity of DPs from GLs on *Fusarium culmorum* revealed an inhibition activity that was significantly different depending on the GL side chain structure. The DPs from thiofunctionalized glucosinolates and benzylic GLs showed, *in vitro*, an inhibition activity significantly higher than that of DPs from alkenyl or hydroxy-alkenyl-GLs. In addition, a different sensitivity of several fungi as a function of their taxonomic class was observed. In particular, *Phytophthora* spp., *Pythium* spp., *Rhizoctonia* spp., and *Sclerotium rolfsii* are particularly sensitive to DP-GLs. The high content of GLs and myrosinase in *Brassicaceae* suggests the possibility of controlling soilborne pathogens. Trials on green manure in pots, on naturally infested soil, showed that plants, selected for a high content of thiofunctionalized GLs, can reduce the *Pythium* inoculum at a significantly higher level than hydroxy-alkenyl GLs containing ones. GLs containing plants reduce significantly the inoculum if compared with glucosinolate free ones commonly used as green manure.

HIGHLY EFFICIENT GENETIC TRANSFORMATION IN STRAWBERRY AND TRANSFER OF THE *rolC* GENE TO CV. 'CALYPSO'. Marco Mazzara*, D.J. James and B. Mezzetti. *Dipartimento di Biotecnologie Agrarie ed Ambientali, Università di Ancona, Via Breccie Bianche, I-60100 Ancona, Italy. Fax: +39.071.2204858; e-mail: Mazzara@popcsi.unian.it

Agrobacterium rhizogenes *Rol* genes can be used to modify plant growth processes as they interfere in hormone metabolism and sensitivity. They have been used to obtain transgenic strawberry plants with a modified *habitus* and enhanced tolerance toward the most important fungal pathogens. In particular, *rolC* gene expression in plants leads to a 'cytokinin-like' effect with reduction in apical dominance, bushy phenotype, and smaller leaves and flowers. Using *Agrobacterium*, the *rolC* gene, controlled by its own promoter, was introduced into 'Calypso', a cultivar with a high regeneration rate. In order to optimise transformation, the effect of different *Agrobacterium* strains and selection strategies on transformation efficiency were evaluated. As a result, more than 250 *rolC* transgenic clones were isolated and tested by PCR. Transformation efficiency (no. transgenic shoots/no. explants infected x 100) was between 8.9 and 72, depending on the strain and selection strategy used. The *rolC* plants showed a peculiar phenotype both *in vitro* and *in vivo*: high proliferation rate in absence of PGRs, reduced apical dominance, higher branching rate, smaller leaves and petioles. We plan to collect further data about physiology (photosynthesis, sugar content, transpiration rate), vegetative-reproductive parameters (number of shoots, leaves, flowers, pollen fertility etc.), and perform bioassays to evaluate tolerance to the main fungal pathogens. Field experiments will be carried out following the rules defined by the National Biotechnology Committee.

DsRNA HOMOLGY IN ITALIAN AND EUROPEAN HYPOVIRULENT STRAINS OF *CRYPHONECTRIA PARASITICA*. G. Maresi, R. Pastorelli and Tullio Turchetti*. *Istituto per la Patologia degli Alberi Forestali CNR, P.le Cascine 28, I-50144 Firenze, Italy. Fax: +39.055.354786, e-mail: Turchetti@ipaf.fj.cnr.it

135 white hypovirulent strains of *Cryphonectria parasitica* from several European populations were tested for their dsRNA contents. From healing cankers were obtained respectively: 90 white strains in Italy and the remaining 45 in some European countries (Austria, Albania, France, Switzerland, Greece, Spain, Hungary and Turkey). DsRNA electrophoretic profile was the same, in all strains, for the common presence of a L (large)-dsRNA band of about 12 kbp. Hybridisation tests were carried out using a probe obtained from the conservative domain ORF A domain of the dsRNA of CHV1-EP747 dsRNA (B. I. Hilfmann courtesy). This is one of the five defined hypoviruses belonging to *Hypoviridae* family and is closely related to type member of the family, CHV1-EP713. The origin of both these hypoviruses is European: CHV1-EP713 from France and CHV1-EP747 from central Italy. All the tested strains hybridised with the CHV1-EP747 probe, confirming a strong homology in dsRNA contents. Also the 4 hypovirulent strains, previously selected for biological control purposes executed by means of artificial combined inoculations, showed the same dsRNA pattern and hybridised with the tested probe. These results suggest the spreading of only one or two hypovirus types in the tested *C. parasitica* H strains collected in some European countries and emphasised the broad conversions ability showed by most of the tested strains. No evidence of dsRNA contents was found in several virulent pigmented strains belonging to the investigated populations.

MOLECULAR DETECTION OF PHYTOPLASMAS IN EUROPEAN PLUMS SHOWING LEPTONECROSIS SYMPTOMS. M. Morando, M.S. Grando and Maria Elisabetta Vindimian*. *Istituto Agrario San Michele all'Adige, Via E. Mach 1, I-38010 San Michele all'Adige (Trento), Italy. E-mail: Elisabetta.Vindimian@ismaa.it

Symptoms of leptonecrosis have long been known in Japanese plums. Recently the disease was observed in cv. 'Dro', a European variety close to cvs 'Hauszweitsche' and 'Pozegaca', grown in Germany and the Balkans. The affected plums were noted in the Sarca valley, a Trentino area where this cultivar is widely grown. Decline symptoms generally began in one or a few branches and gradually spread to the whole tree. Diseased plants showed off-season growth from buds at the ends of affected branches, and shoots had shortened internodes and green leaves. Summer leaves were smaller than normal, chlorotic and becoming reddish-bronze, and sometimes rolled upward. Flowering could be staggered and could also occur in winter. Fruit was poor and undersized, and affected branches showed phloem necrosis and decline. RFLP analysis of samples collected in winter 1995 from symptomatic trees of cv. 'Dro' detected a phytoplasma of the apple proliferation (AP) cluster, closely related to other phytoplasmas present in *Prunus* species in Europe. This finding further justifies the grouping of all European stone fruit phytoplasmas under the name European stone fruit yellows (ESFY). We also confirmed the presence of AP cluster phytoplasmas in samples collected in summer. PCR products from symptomatic samples, amplified with the ribosomal primer pairs rU5/rU3 and (X)F1/R1 were analysed with restriction endonucleases, revealing the presence of phytoplasmas of group X and subgroups A and B.