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ABSTRACTS

Summaries of oral presentations, posters and invited speeches presented at the Twelfth Hellenic Phytopathological Congress, Kastoria, Greece, October 12–14, 2004

The 12th National Phytopathological Congress, organized every two years by the Hellenic Phytopathological Society (HPS), was held in Kastoria, October 12–14, 2004 and was attended by more than 300 scientists. Fifty-five oral presentations and 57 posters were presented at the meeting, dealing with plant diseases caused by fungi, bacteria, viruses, with non-parasitic disorders, and with disease control. Three invited speakers gave papers on the following subjects: "Non parasitic disorders of cultivated plants: current approaches to determine their importance and the molecular basis of their pathogenesis, detection and control", "Current problems and developments in phytodiagnosis", "Approaches for the control of crop diseases in organic farming". Abstracts of the papers and posters are presented in this issue.

Mycology

***Avena sterilis-Drechslera avenae* f. sp. *sterilis* relationship: the involvement of phytotoxin pyrenophorin in pathogenesis.** K.A. Aliferis[1], M. Chrysayi[1] and C. Fasseas[2]. [1]*Agricultural University of Athens, Pesticide Science Laboratory, 75 Iera Odos, 118 55 Athens, Greece.* [2]*Agricultural University of Athens, Electron Microscopy Laboratory, 75 Iera Odos, 118 55 Athens, Greece.*

Elucidation of the mechanisms involved in a host-pathogen relationship is of scientific interest and also has various practical applications, mainly in crop protection. In fungal diseases secondary metabolites play an important role in the interactions between the pathogen and the host plant. Several bioactive metabolites isolated from pathogenic fungi have been studied and characterized as phytotoxins. Pyrenophorin is a non-selective phytotoxin isolated from *Drechslera avenae* f. sp. *sterilis,* a fungus pathogenic on wild oats (*Avena sterilis).* This compound occurs in cultures of the fungus within 10 days of inoculation. External application of aqueous solutions of pyrenophorin at 7×10^{-5} M on *A. sterilis* leaves results in tissue bleaching within 36 h, under illumination. Examination of the treated leaf tissues with the transmission electron microscope (TEM) showed loss of chloroplast integrity. Epidermal cells and other sub-cellular parts of mesophyll cells, such as cytoplasmic membranes, mitochondria and nuclei, remained unaffected. It has been reported that chloroplasts are sub-cellular targets during the invasion of *Drechslera* species into their gramineous hosts. The selective action of pyrenophorin towards chloroplasts, combined with the fact that it is produced *in vitro* relatively early, is indicative of an interference of this phytotoxin with the *Avena sterilis-Drechslera avenae* f. sp. *sterilis* interrelation and the process of pathogenesis.

Study of pathogenesis and eggplant root colonization by the plant pathogenic fungus *Verticillium dahliae* using transgenic technology. D.F. Antonopoulos[1], E.J. Paplomatas[1], S. Kang[2] and E.C. Tjamos[1]. [1]*Agricultural University of Athens, Laboratory of Plant Pathology, 75 Iera Odos, 118 55 Athens, Greece.* [2]*Pennsylvania State University, Department of Plant Pathology, University Park Philadelphia, PA 16802, USA.*

In order to study the pathogenesis process on eggplant root and the ramification of the fungus into plant tissue, the pathogen *Verticillium dahliae* (Kleb.) was transformed by the *EGFP* gene expressing the Enhanced Green Fluorescent Protein (EGFP). Eggplant cv. Black Beauty seedlings grown in a sand:perlite (4:1 v:v) mix were infected by drenching with a conidial suspension of a transformed strain of the pathogen at a concentration of 10^7 spores ml^{-1}. Microscopic observations started 24 hours after inoculation using fluorescent microscopy at 450–490 nm wavelength and continued at 48-hour intervals basis, for 11 days. Three days after infection, conidia were dispersed on the root surface without having germinated. Conidia started to germinate 5 days post inoculation with the developing hyphae growing on the root epidermal cells in various directions. Some of the germinating hyphae were adjacent to the cell wall junctions. A large number of conidia were concentrated around the lateral roots and the zone of elongation. At that time, some of the root hairs were infected with the germination tubes that were seen to fluoresce inside the infected tissue. Seven days after inoculation, the hyphae had grown even more and the first infections on the main roots were observed as hyphae entered the epidermal root cells. Moreover, some lignitubes that is lignin cavities around the penetration peg formed by the pathogen were also seen. By day 9, hyphae had reached the interior root cell layers, while almost all of the conidia had disappeared from the root surface. Finally, eleven days after inoculation, the pathogen had invaded the xylem vessels, which showed an intense fluorescence. Fluorescence was also very strong at the points where the lateral roots emerged. During all these days of microscope inspections the plants remained symptomless.

New records of wood-rotting macrofungi from selected forest ecosystems in central Greece. D.M. Dimou[1], G.I. Zervakis[2] and I. Sakketos[1]. [1]*Agricultural University of Athens, Laboratory of Agricultural Microbiology, 75 Iera Odos, 118 55 Athens, Greece.* [2]*National Agricultural Research Foundation, Institute of Environmental Biotechnology, 87 Lakonikis Str., 241 00 Kalamata, Greece.*

Wood-rotting macrofungi have hardly been investigated in Greece despite their high ecological and economical (phytopathological, biotechnological) significance. During a study lasting several years, some forest ecosystems of central Greece (Attiki, Evoia, Aitoloakarnania, Fthiotida, etc.) with plant vegetation dominated by *Abies cephalonica*, *Quercus* spp. and *Castanea sativa*, were inventoried. About 100 species of wood-rotting basidiomycetes were recorded, several of which are first reports for Greece, e.g. *Antrodiella romellii*, *Athelia bombacina*, *Exidiopsis candida*, *Hapalopilus salmonicolor*, *Hyphodontia alutaria*, *Peniophora rufomarginata*, *Phlebiella tulasnelloidea*, *Pycnoporellus fulgens*, *Schizopora flavipora*, *Steccherinum litschaueri* and *Xenasma pulverulentum*. Also noteworthy was the occurrence of the genera *Crustoderma* and *Protodontia* here reported for the first time in Greece. In addition, many other taxa were recorded on new hosts, e.g. *Amylocorticium cebennense* on *Pinus halepensis*, *Crepidotus mollis* var. *mollis*, *Hyphodontia arguta*, *Phlebiella sulphurea* and *Subulicystidium longisporum* on *Laurus* sp., *Ganoderma resinaceum* on *Schinus molle*, *Hyphodontia sambuci* on *Sambucus nigra*, *Irpex lacteus* on *Robinia pseudoacacia*, *Trametes gibbosa* and *Datronia mollis* on *Populus alba*, *Rigidoporus ulmarius* and *Cylindrobasidium evolvens* on *Platanus orientalis*, *Trechispora farinacea* on *Olea europea*, *Polyporus meridionalis* on *Quercus coccifera* and *Populus alba*. Furthermore, the known biodistribution of some edible wood-rotting mushrooms, including *Pleurotus dryinus*, *Fistulina hepatica* and *Laetiporus sulfureus*, was found on new substrates and in new localities.

Esca disease on citrus. K. Elena[1], D. Dimou[2] and D.M. Dimou[3]. [1]*Benaki Phytopathological Institute, 8, S. Delta Str., 145 61 Kifissia, Athens, Greece.* [2]*Prefecture of Argolis, Direction of Agricultural Development, 211 00 Nafplion, Greece.* [3]*Agricultural University of Athens, Agricultural Microbiology Laboratory, 75 Iera Odos, 118 55 Votanikos, Athens, Greece.*

A severe disease in citrus orchards (Washington navel orange and common mandarin grafted on sour orange rootstocks) was observed in the Argolis area (southern Greece) some years ago. Diseased trees decline, the leaves became yellow and fell early and finally the shoots and twigs died while damage extended towards the trunk. Cross-sections in infected branches and trunks revealed a light-colored soft rot in the center surrounded by hard brown necrotic wood, resembling the symptoms of esca on grapevine. The necrosis started from pruning areas, extended to the trunk and continued to the wood of the rootstock (sour orange). The growers, who have observed these symptoms for many years, usually remove the affected branches but the disease nevertheless proceeds to the remaining parts. In all cases, a fungus *Fomitiporia* sp. was isolated from the white rotted areas. Fungal fruiting bodies formed abundantly on the trunks of diseased citrus trees. *Fomitiporia punctata* Murril and more recently *F. mediterranea* M. Fish-

er are the main pathogens reported to cause esca of grapevine. In the last few years, there has been a dramatic increase in esca not only in old vineyards but also in younger ones and the disease has become more destructive. Recently, a similar wood disease, caused by the genus *Fomitiporia,* was observed in olive and kiwi plantations in Greece, causing severe losses. The same symptoms were also observed on other cultivated tree species. Although pathogenicity tests on citrus are in progress, the similarity of the disease on citrus, as far as symptoms and pathogens are concerned, with that on the other hosts mentioned is obvious. To our knowledge, this is the first record of the fungus *Fomitiporia* sp. causing citrus wood decay, although a similar disease has been reported on citrus in Italy.

Identification of AFLP markers linked to *Fusarium oxysporum* f. sp. *cucumerinum* resistance in cucumber (*Cucumis sativus* L.). E. Jaber[1,3], A. Srour[1,3], D.J. Vakalounakis[2] and A.G. Doulis[1]. [1]*Institute of Viticulture, Floriculture and Vegetable Crops, National Agricultural Research Foundation, PO Box 2229, 710 03 Heraklion, Greece.* [2]*Institute of Plant Protection; National Agricultural Research Foundation, PO Box 2228, 710 03 Heraklion, Greece.* [3]*Mediterranean Agronomic Institute of Chania, PO Box 85, 731 00, Chania, Greece.*

The present work was part of a research project to incorporate genetic resistance into diseases of cucumber (*Cucumis sativus* L.). Bulked segregant analysis (BSA) was employed to identify AFLP markers linked to the resistance gene (*Foc*) against Fusarium wilt caused by *Fusarium oxysporum* f. sp. *cucumerinum* (FOC). For BSA, DNA from parents as well as from selected F_2 progeny was used. The F_2 generation had been produced by crossing a plant from an isogenic line resistant to FOC (SMR18) with a plant from a susceptible isogenic line (Straight 8). DNA was screened using 21 *Eco*RI/*Mse*I primer pair combinations. At the initial stage of BSA, four AFLP markers putatively linked to *Foc* were identified. Screening the whole F_2 progeny confirmed the identification of one stably linked marker (E-AC/M-CAT_{134}), which maps at a distance of 8 cM from the resistance locus. We are currently at the stage of cloning the AFLP marker using non-radioactive technology. Once sequenced the AFLP marker could be converted to a single band marker known as a sequence-characterized amplified region (SCAR). The SCAR marker could be employed in marker-assisted patho-breeding programs as well as for comparative genomics.

Factors controlling the severity and timing of *Leveillula taurica* epidemics on tomato in Greece. A.M. Kasselaki[1], E. Markellou[2], S. Konstantinidou-Doltsinis[3], N.E. Malathrakis[1] and M.W. Shaw[4]. [1]*Technological Education Institute (TEI) of Crete, STEG, Laboratory of Biological Control of Plant Diseases, Stavromenos, 710 04 Heraklion, Crete, Greece.* [2]*Benaki Phytopathological Institute, 8 S. Delta Str., 145 61 Kifissia, Athens, Greece.* [3]*National Agricultural Research Foundation, Institute of Plant Protection, Amerikis and National Road, 260 04 Patras, Greece.* [4] *The University of Reading, School of Plant Sciences, Whiteknights, Reading RG6 6AS, UK.*

Epidemics of *Leveillula taurica* were monitored in seven tomato crops at two sites in Greece. Disease progress on each leaf cohort was approximately logistic and its rate was positively related to average temperature in a curvilinear way. Trap plants demonstrated that spores were abundant in June–November and that lesion expansion was very slow in August, the hottest month. It appeared that in established crops, disease development continued throughout the winter, but more slowly. Yield losses were small, despite severe disease. In spring crops, infection rates were low and inoculum levels did not increase progressively from one leaf to the next. It is suggested that on crops and wild vegetation natural inoculum sources decline to a very low level in winter and increase again at the end of summer. This increase, when coupled with favourable environmental conditions at the early crop stages, leads to high inoculum levels on autumn-sown crops. These observations suggest that crops planted in September should initially be treated with fungicides, which can be discontinued once the average temperature falls below about 20°C. By contrast, spring crops could be left untreated until the disease appears, after which fungicides should be used to limit the otherwise accelerating and damaging epidemic.

Expression of PR genes in tomato plants grown on suppressive composts. N. Kavroulakis and K.K. Papadopoulou. *NAGREF, Institute of Environmental Biotechnology, 87 Lakonikis Str., 241 00 Kalamata, Greece.*

Induction of pathogenesis-related (PR) proteins in various plant tissues is a major biochemical and molecular event when plants are subjected to infections with pathogens such as viroids, viruses, bacteria and fungi. Furthermore, the ability of some rhizobacteria to protect plants against pathogens has been found to be correlated with alterations in the expression pattern of the PR genes. Tomato plants grown on agricultural residues-derived compost were protected from root and foliar fungal pathogens. The degree to which the compost controlled the disease was not associated with a direct interaction with the pathogen but mainly with the induction of host systemic resistance and PR gene expression. Differential transcription patterns of genes PR1, PR3, PR5 and PR69 were detected in both the roots and leaves of tomato plants grown in compost. Transcripts of the above genes were isolated and their similarity to

already known genes was examined. In parallel, expression studies showed that the distribution of certain PR gene transcripts differed among cell types. The putative mechanism of pathogen suppression by the compost as well as the physiological role of some PR genes is discussed.

A new pathotype of the fungus *Alternaria alternata*? I.A. Laidou and C.C. Thanassoulopoulos. *Aristotelian University of Thessaloniki, Faculty of Agriculture, Plant Pathology Laboratory, P.O. Box 269, 540 06 Thessaloniki, Greece.*

Numerous isolations from necrotic spots on the leaves of celery, parsley, lettuce, cotton, spinach, root beet, sugar beet and persimmon consistently showed the fungus *Alternaria alternata* in the affected tissues. The pathogenicity of the isolates was checked on their original hosts and the eight most virulent isolates were selected. Cross-inoculation tests showed that the isolates from persimmon, sugar beet and to a less extent cotton were specific to their original hosts. These three isolates were further tested on 34 plant species, 20 cultivated and 14 non-cultivated (weeds), belonging to 14 families in order to determine their host range. At least 20 plants were used for each treatment and the experiment was replicated twice. The disease intensity was estimated by measuring the total infected leaf area in mm^2 and the number of spots per plant. Results showed that the isolate from persimmon had the highest host specificity, with serious foliage infection, whereas its specificity on the other hosts was low. Therefore, the isolate from persimmon is either a new pathotype or a new species of the fungus. Research on the identification of this pathogen is in progress.

Fungi associated with summer decline of turf grasses in Greece. D. Lascaris and A. Chantzaras. *Benaki Phytopathological Institute, Plant Pathology Department, 8 S. Delta Str., 145 61 Kifissia, Athens, Greece.*

Over the last few years, a turf grass decline, with increasing frequency and severity, has occurred during or after the hottest periods. The first symptoms appeared on older leaves as blade and sheath chlorosis and necrosis gradually advancing to younger leaves and down to crown and roots, causing weakening and death of the plants. The fungi most frequently isolated from affected plants were *Bipolaris* spp., *Drechslera* spp. and *Curvularia* spp. Lately, *Pyricularia grisea* was also recorded for the first time in Greece on turf grasses showing similar symptoms. Pathogenicity tests with *B. sorokiniana, D. dictyoides* and an unidentified species of *Curvularia* were conducted in a growth chamber on *Festuca arundinacea* seedlings (vars Duster and Renegate). Both varieties were very susceptible to *B. sorokiniana* and to a lesser degree to a *Curvularia* sp. and *D. dictyoides*. There was no significant difference in susceptibility to *B. sorokiniana* and *Curvularia* sp. between plants differing in age, variety, or plants receiving different levels of N-nutrition. All fungi produced abundant spores on plant debris, whereas relative humidity and N-nutrition were the factors that most affected spore production on plant debris. *B. sorokiniana* and *Curvularia* sp. produced spores at RH>96% and *D. dictyoides* at RH>97%. Twice as many spores were produced on dead leaves of plants receiving high N-nutrition as on leaves of N-deficient plants.

Virulence of *Verticillium dahliae* isolates to common weed species. E.K. Ligoxigakis[1], C.X. Gatjilakis[2] and D.A. Goutos[2]. *[1] National Agricultural Research Foundation, Plant Protection Institute P.O. Box 2228, 710 03 Heraklion, Crete, Greece. [2]Technological Educational Foundation of Crete, School of Agricultural Technology, P.O. Box 140, 715 10 Heraklion, Crete, Greece.*

Virulence of some *Verticillium dahliae* isolates obtained from certain hosts grown in Crete, was checked by inoculating (root-dipping technique) some widespread weed species of Crete. The aim was to assess the susceptibility of common weed species to the fungus. For this purpose, 22 *V. dahliae* isolates obtained from 11 weed species belonging to seven families and from eight cultivated plant species belonging to four families, were used. Two weeks after inoculation the first symptoms (chlorosis and slight wilting) appeared on older leaves of some plants of each susceptible species. Subsequently, symptoms became more intense (permanent wilting and desiccation). Five weeks after inoculation, a final evaluation examined leaf symptoms, restriction of plant growth and discoloration of the vascular stem tissues. Of the 18 weed species tested, 13 were infected with one or more *V. dahliae* isolates showing typical wilt symptoms and the fungus was re-isolated from the vascular stem tissues. Isolates obtained from very susceptible hosts were pathogenic to more species than were isolates from less susceptible hosts. The pathogenicity of *V. dahliae* isolates on the weed species used ranged from low to very high and was independent of the host in which they originated. Isolates from weed species were usually highly pathogenic to the weed species in which they originated.

Study of the epidemiology of esca disease on olive. E.J. Paplomatas[1], A. Paraskevopoulos[2], P. Tsopelas[3], K. Elena[4] and I. Malandraki[1]. *[1]Agricultural University of Athens, Laboratory of Plant Pathology, 75 Iera Odos, 118 55 Athens, Greece. [2]Prefecture of Messinia, Direction of Agriculture and Husbandry of Trifylia, Department of Plant Protection, 245 00 Kyparissia, Greece. [3]National Agricultural Research Foundation, Institute*

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Esca of the olive tree, caused by the fungus *Fomitiporia punctata* Murrill [*Phellinus punctatus* (P. Karst.) Pilát], causes extensive damage to olive trees in regions of Messinia Prefecture. Recently, the pathogen was also found to infect olive trees in regions of Kinouria in Arkadia Prefecture, while during the last years the pathogen has also been reported to infect kiwi and citrus trees as well as grapevine. Due to the importance of this disease, the Ministry of Rural Development and Food has funded a research project, in the framework of which a study in the region of Trifylia in Messinia Prefecture is being carried out evaluating the damage caused by the disease, the epidemiology of the pathogen and methods for disease management. Samples from diseased olive trees and isolates of the pathogen are systematically collected from different regions. The pathogenicity of a series of *F. punctata* isolates from olive, kiwi and citrus trees is being tested on 3-year-old olive trees in the grove of the Agricultural University of Athens. The trees are inoculated by inserting sawdust colonized with the fungus in a 5-cm-diameter hole at two opposite sites of the trunk. Basidiospores of *F. punctata* infect young olive trees through pruning or other wounds and are produced on perennial carposomes on the trunk or branches of diseased trees. The possibility of pathogen transmission from diseased to healthy trees through sawdust attached to pruning tools is being investigated. In order to simulate transmission of the pathogen with a chain saw, branch sections were incoculated with sawdust from infected olive trees. The pathogenicity of *F. punctata* on olive trees has already been demonstrated experimentally. Nevertheless, the pathogenicity tests of a series of isolates from different hosts on olive trees and the study on the transmission of the fungus are still in progress, due to the slow progression of esca.

Molecular characterization of isolates of the fungus *Fomitiporia punctata*, the causal agent of esca disease. E.J. Paplomatas[1], I. Malandraki[1], A. Tzima[1], K. Elena[2] and P. Tsopelas[3]. [1]*Agricultural University of Athens, Laboratory of Plant Pathology,75 Iera Odos, 118 55 Athens, Greece.* [2]*Benaki Phytopathological Institute, 8 S. Delta Str., 145 61 Kifissia, Athens, Greece.* [3]*NAGREF-Institute of Mediterranean Forest Ecosystems, Terma Alkmanos, 115 28 Athens, Greece.*

The basidiomycete *Fomitiporia punctata* Murrill [ex *Phellinus punctatus* (P. Karst.) Pilát] is the most important pathogen associated with esca disease of grapevine. During the last years, this fungus has also been found to infect other hardwood hosts, like kiwi, olive and, more recently, citrus trees. In order to assess the risk of dissemination of the disease, genetic diversity was studied in a collection of isolates of the pathogen, using molecular markers. Based on patterns obtained by RAPD-PCR (using five different primers) from three Greek and one Italian isolate from kiwi, seven from olive and five from citrus trees, a phylogenetic tree was constructed, in which a further two isolates of *Phellinus rimosus* (Berk.) Pilát (from pistachio) and *P. pini* (Fr.) A. Ames (from pine) were included for comparison. It was found that four isolates from kiwi together with five out of seven isolates from olive formed one group that was differentiated about 44% from the second group, which included the other two olive isolates plus the two Greek grapevine isolates. It is worth noticing that the grapevine isolates of this fungus have recently been classified as belonging to the new species *F. mediterranea* (M. Fischer), in which isolates of other hosts will probably also be included. Between these two subgroups there was a cluster composed exclusively of citrus isolates, which differed 41% from the first (larger) subgroup. Overall, the cluster of *F. punctata* isolates was clearly differentiated (57%) from the one that contained the isolates of the two other hosts. In this dichotomous branch, isolates of *P. pini* from pine and *P. rimosus* from pistachio were clustered in different groups. The data so far suggest a possible parallel phylogenetic evolution of *F. punctata* in three of the hosts (kiwi, olive, grapevine), whereas in citrus the fungus seems to have evolved monophyletically. This assumption is being further investigated by studying additional isolates from other geographic regions, comparing them with isolates from forest trees (which are the initial hosts of the pathogen) and performing cross inoculations among the three agricultural hosts.

Molecular characterization of *Pyricularia* isolates from ctenanthe and rice plants and their biological response to respiration inhibitors. E.J. Paplomatas[1], A.C. Pappas[2] and E. Syranidou[2]. [1]*Agricultural University of Athens, Laboratory of Plant Pathology, 75 Iera Odos, 118 55 Athens, Greece.* [2]*University of Thessaly, School of Agriculture, Crop Production and Agricultural Environment, Laboratory of Plant Pathology, 384 46 N. Ionia, Volos, Greece.*

The molecular profile of *Pyricularia oryzae* isolates from ctenanthe was characterized and compared with those from rice plants and their biological response to two strobilurins (azoxystrobin, kresoxim-methyl) and to the phenylpyridinamine fungicide fluazinam was studied. Five different isozymes (α-esterase, lactate, malate, isocitrate and sorbitol dehydrogenases) and five random decamer primers for RAPD-PCR were used as molecular markers. Using UPGMA, ctenanthe isolates were found to form a separate group that differed distinctly from that of the rice isolates with both markers. Amplified polymorphic sequences of cytochrome b that were

digested with *Fnu*4HI or *Sty*I revealed no differences between *Pyricularia* isolates at amino acid positions 143 or 129. In the presence of SHAM, an alternative respiration inhibitor, sensitivity to azoxystrobin, kresoxim-methyl and fluazinam did not differ greatly between the isolates from rice and ctenanthe. The ED_{50} values for mycelial growth, spore germination and appressorium formation were: for azoxystrobin <0.005, <0.001 to 0.002, <0.001; for kresoxim-methyl 0.015 to 0.503, <0.001, <0.001 and for fluazinam 0.1 to 0.216, 0.006 to 0.015, <0.001 mg litre^{-1}, respectively. In the absence of SHAM, the effectiveness of the three fungicides was always lower and more variable. Azoxystrobin or kresoxim-methyl concentrations sub-inhibitory for mycelial growth had no adverse effect on sporulation. The results show that appressorium formation is the main target of respiration inhibitors, and azoxystrobin the most effective fungicide in *Pyricularia*. Alternative respiration gave sufficient protection of mycelial growth and spore germination from strobilurin fungicides and fluazinam. This is the first report comparing the molecular profile and the sensitivity to respiration inhibitor fungicides of *P. oryzae* isolates from a non gramineous host with *P. oryzae* isolates from rice.

Holes in strawberry cuticle under appressoria of *Colletotrichum acutatum*. A.G. Pattas. *Directorate of Agriculture of Messinia, Prefecture of Messinia, 241 00 Kalamata, Greece*.

The fungus *Colletotrichum acutatum,* the pathogen of anthracnose disease of strawberry, may cause black spots on strawberry leaves and stolons but it usually remains latent on the plant until the fruits begin to ripen. Scanning electron microscopy on inoculation sites of strawberry leaves and stolons revealed that the appressoria of the pathogen formed a pore at their ventral wall and perforated the plant cuticle underneath this pore without the formation of an infection peg. Such pre-penetration holes in the cuticle, that bring the appressorium into close contact with the host's intercellular space, may be an integral stage in the infection process of the pathogens that restrain their pathogenicity to produce latent infections.

Phytophthora *species involved in chestnut ink disease in Greece*. C. Perlerou and S. Diamandis. *National Agricultural Research Foundation, Forest Research Institute, 570 06 Vassilika, Thessaloniki, Greece*.

Two *Phytophthora* species are the main causal agents of ink disease of chestnut in Europe: *P. cambivora,* which has been reported from France, Italy and Greece, and *P. cinnamomi,* which is more aggressive and seems at present to be restricted to western Europe and in particular to France, Spain and Portugal. In recent years, besides *P. cambivora*, two more species, *P. citricola* and *P. cactorum,* have been isolated from the rhizosphere of diseased chestnut trees in Italy. The pathogenicity of the latter two species has been determined experimentally on chestnut seedlings. Since the 1990s, chestnut cultivation in Greece appears to be developing again. In many chestnut areas, however, ink disease causes considerable losses. The objective of the current work was to investigate whether other *Phytophthora* species are associated with symptomatic trees in Greece. Soil samples and bark tissues were removed from 22 diseased trees in five different areas in northern Greece. PARBhy (MEA, pimaricin, ampicillin, rifampicin, benomyl, hymexazol) was used as isolation medium. Species determination was based on growth temperature, colony appearance and the morphology of sporangia, oogonia, antheridia, chlamydospores and hyphal swellings. The three *Phytophthora* species were isolated from 17 out of the 22 trees (73%): *P. cambivora* (50%), *P. citricola* (37%) and *P. cryptogea* (25%). *P. cambivora* was found in soil and tissue in 4 out of the 5 areas screened. *P. citricola* and *P. cryptogea* were isolated from soil in only 2 areas. In some soil samples, *P. cambivora* co-existed with *P. citricola* and *P. cryptogea* with *P. citricola*. *P. cryptogea* has been reported as killing chestnut trees in Australia. The pathogenicity of the three *Phytophthora* species on chestnut seedlings is currently being determined.

Studies on fungi of the genus *Botryosphaeria* affecting different hosts in Greece. I.C. Rumbos[1] and A.J.L. Phillips[2]. [1]*National Agricultural Research Foundation, Plant Protection Institute of Volos, 380 01 Volos, Greece.* [2]*CREM, Universidade Nova de Lisboa, 2829-516 Caparica, Portugal*.

Species of *Botryosphaeria* Ces & De Not. have been reported as pathogens on several woody hosts in many temperate and tropical regions. Amongst the hosts on which the fungus has been reported in Greece are apple, peach, pistachio, kiwifruit, olive, walnut and grapevine. The species *Botryosphaeria dothidea* (Moug.:Fr.) Ces. & De Not., the causal agent of black dead arm of grapevine, produces hyaline, aseptate, fusiform conidia, 24–29×4–5 μm in size, belonging to *Fusicoccum aesculi* Corda (anamorph). The same species also infects pistachio trees, causing panicle and shoot blight. However, the isolates from California seem to be morphologically a little different fitting between *B. dothidea* and *B. ribis,* and therefore they may be a distinct species. The same species infects apple and peach. The species *B. parva* Pennycook & Samuels infects walnut and kiwifruit causing dieback of the twigs and branches and produces hyaline, thin-walled and aseptate conidia that become olivaceous with 1–2 septa with age. The anamorph stage belongs to *Fusicoccum parvum* Pennycook & Samuels with conidia 15–22×4.5–7.5 μm in size. *Fusicoccum* isolates from olive, which infect the fruits, still

cause a lot of uncertainty in their identification. Morphologically they are distinct from *B. dothidea* and may represent a new species. *Macrophoma dalmatica* should be transferred to the genus *Fusicoccum,* but the species name is not yet settled. The species *B. parva* was recently found to cause twig dieback on *Ilex aquifolium* as well. The sexual stage was found only on kiwifruit. Pathogenicity studies with isolates from kiwifruit, walnut, olive, *I. aquifolium,* grapevine and pistachio on seven different hosts showed considerable variation in aggressiveness, although the isolates from any given host were able to infect all other hosts. No evidence for host specificity was found. The growth of the different *Botryosphaeria* isolates on PDA in relation to temperature was also studied.

Dieback symptoms of kiwifruit trees (*Actinidia chinensis*) caused by *Botryosphaeria parva*. I.C. Rumbos. *National Agricultural Research Foundation, Plant Protection Institute of Volos, 380 01 Volos, Greece.*

In 2000–03, our attention was drawn to three 10-to-15-year-old commercial kiwifruit orchards cv. Hayward in the areas of Larissa and Volos, Thessaly, central Greece, showing severe dieback symptoms. In all cases, dead twigs and branches were associated with pruning wounds and cankers. In cross and longitudinal sections extensive wood discoloration was observed. In several cases the discoloration extended along the trunk, causing the death of the whole tree. It was obvious that the pathogen invades through pruning wounds and progressively destroys the vascular tissue, resulting in the death of affected twigs, branches or trees. Isolations on potato-dextrose agar (PDA) from infected phloem and wood near the margins of the invasion front consistently detected the fungus *Botryosphaeria parva* Pennycook & Samuels. The fungus was identified by Prof. Alan J.L. Phillips. The asexual form of the fungus belongs to *Fusicoccum parvum* Pennycook & Samuels and forms hyaline conidia, 15–22×4.5–7.5 μm in size, becoming darker with 1–2 septa with age. The sexual form was also found. The pathogenicity of the isolates was confirmed by a series of inoculations on kiwifruit, grape, walnut, almond, pear, cherry and quince trees. All tested isolates were virulent. This is the first record of infection of kiwifruit by *B. parva* worldwide. In New Zealand this fungus is reported to cause post-harvest fruit rot.

Molecular identification of *Leveillula taurica* isolates from different plant hosts. A. Tampakaki, A. Kasselaki, M. Fanouraki and N. Malathrakis. *School of Agricultural Technology, Technological Educational Institute of Crete, 715 00 Heraklion, Crete, Greece.*

The genus *Leveillula* of Erysiphaceae includes endoparasitic fungi that cause powdery mildew in more than 1000 cultivated and wild plants. *Leveillula taurica,* the best known species of the genus, mainly infects species in the Solanaceae family and results in severe yield loss. *L. taurica* is a complex species that urgently needs to be studied to elucidate its morphological characteristics, its specificity, its host range and other characters useful in the epidemiology of the diseases it causes. The aim of this study was to examine whether isolates of *L. taurica* from different hosts are genetically uniform and to determine the phylogenetic relationships among isolates. The study was based on rDNA analysis, the non transcribed regions (ITS) of which show variations and are useful for phylogenetic studies of closely related genera, species and races. Similar studies have been carried out on the phylogenetic analysis of several powdery mildew fungi. In the present study, we determined the sequence of about 600 nucleotides of the ITS regions including the 5.8S rDNA and identified the nucleotide sequence diversity of the nuclear rDNA for *L. taurica* isolates from: *Solanum melongena, Capsicum annuum*, *Cynara scolymus* and *Oxalis pes-caprae*. Preliminary studies indicated that isolates from hosts belonging to the same plant family showed less genetic variation than isolates from hosts belonging to different plant families. Further studies are needed to determine whether these nucleotide sequence differences are useful for the taxonomy of the fungus.

Study on the systemic resistance of *Arabidopsis thaliana* to *Verticillium dahliae* when induced by the biocontrol agent K-165. S.E. Tjamos[1], E. Flemetakis[2], E.I. Paplomatas[1] and P. Katinakis[2]. [1]*Agricultural University of Athens, Laboratory of Plant Pathology, 75 Iera Odos, 118 55 Athens, Greece.* [2]*Agricultural University of Athens, Laboratory of Molecular Biology, 75 Iera Odos, 118 55 Athens, Greece.*

Verticillium dahliae Kleb. is a destructive vascular wilt soil fungus widely distributed all over the world and causing great damage to many crops. Several rhizosphere or endophytic bacteria have been reported as biocontrol agents against *V. dahliae*. The mechanisms of action of biocontrol agents against *V. dahliae* mainly involve antibiosis, parasitism, competition, the secretion of enzymes such as glucose oxidase, chitinase and glucanase, and the induction of disease resistance in the host (Tjamos, 2000). Tjamos *et al*. (2004) reported the isolation of a plant-growth promoting rhizobacterium (PGPR) strain, identified as *Paenibacillus alvei* and designated K165, with biocontrol activity against *V. dahliae* in glasshouse and field experiments. The main objectives of the present study were (i) to determine whether induced resistance is the mode of action of the PGPR strain K165 against *V. dahliae,* using the model plant *Arabidopsis thaliana* and a novel bioassay, (ii) to elucidate the signaling pathway controlling the K165-mediated induced resistance and (iii) to monitor the gene

expression level of the genes encoding the pathogenesis-related proteins PR-1, -2, -3 and -5 and the transcription factors TGA2 and WRKY15. The study showed that the biocontrol bacterium *P. alvei*, strain K165, protected *A. thaliana* against *Verticillium dahliae*. The direct antagonistic action of K165 against *V. dahliae* could be ruled out, suggesting that K165-mediated protection results from induced systemic resistance (ISR) in the host. K165-mediated protection was tested in various Arabidopsis mutants and transgenic plants variously impaired with different defense signaling pathways. These plants included: NahG (transgenic line degrading salicylic acid, SA), *etr1-1* (insensitive to ethylene), *jar1-1* (insensitive to jasmonate), *npr1-1* (non expressing NPR1 protein), *pad3-1* (phytoalexin-deficient), *pad4-1* (phytoalexin-deficient), *eds5*/*sid1* (enhanced disease susceptibility) and *sid2* (SA-induction deficient). ISR was blocked in the Arabidopsis mutants *npr1-1, eds5*/*sid1* and *sid2* indicating that components of the pathway from isochorismate and a functional NPR1 play a crucial role in K165-mediated ISR. Furthermore, the concomitant activation and increased expression of the PR-1, -2, and -5 genes was observed in the treatment where both the inducing bacterial strain and the challenging pathogen were present in the rhizosphere of the *A. thaliana* plants.

Occurrence of canker stain disease of planes in Messinia Prefecture, Greece. P. Tsopelas, A. Angelopoulos and N. Soulioti. *National Agricultural Research Foundation, Institute of Mediterranean Forest Ecosystems, Terma Alkmanos, 115 28 Athens, Greece.*

In the fall of 2003, the fungus *Ceratocystis fimbriata* (Ellis & Halstead) Davidson f. sp. *platani* Walter that causes canker stain of plane trees, was reported for the first time in Greece. This is the most destructive disease of plane trees worldwide. It has so far been found in various localities of the Messinia Prefecture, killing plane (*Platanus orientalis* L.) trees of different age and size, in residential areas as well as along rivers and streams. The pathogen grows in the vessels of the sapwood causing tracheomycosis, but at the same time, it causes necrosis of the cambium and the bark, with the formation of cankers. When bark is peeled off, stained streaks can be distinguished, bluish-black to reddish-brown, extending longitudinally in the sapwood. The disease is spread mainly by humans when transporting planting material and infected wood. In Messinia Prefecture, the pathogen spreads along rivers and streams; dead infected logs are carried by the water, creating new infection loci. The fungus is also spreading to neighboring trees by root anastomoses. Phytosanitary measures should be applied to avoid further spread of the disease to new areas. Planting material should be obtained from regions free of the disease and the transport and use of plane wood from infected areas should be forbidden. Infected trees must be felled and if possible uprooted and the wood as well as all the debris and sawdust should be destroyed. Active surveillance is needed to detect newly established foci. Control is more effective when the disease is detected at the initial stages and the number of affected trees is still limited.

Molecular detection and characterization of *Fusarium oxysporum* f. sp. *vasinfectum* in cotton by restriction fragment length polymorphism analysis of the intergenic spacer (IGS) regions of ribosomal DNA. A.G. Zambounis[1], E.J. Paplomatas[2], K. Pasentsis[3] and A.S. Tsaftaris[1,3]. *[1]Institute of Agrobiotechnology, CERTH, 6th km Charilaou-Thermis Road, P.O. Box 361, 570 01 Thermi, Greece. [2]Agricultural University of Athens, Laboratory of Plant Pathology, 75 Iera Odos, 118 55 Athens, Greece. [3]Department of Genetics and Plant Breeding, Aristotelian University of Thessaloniki, 540 06 Thessaloniki, Greece*.

A polymerase chain reaction (PCR)-based method was developed to detect DNA of *Fusarium oxysporum* f. sp. *vasinfectum*, the cause of vascular wilt of cotton. Variation within the intergenic spacer (IGS) of the ribosomal DNA gene for twenty-two strains of *F. oxysporum* and its *formae speciales*, as well as other cotton fungal pathogens, was examined by PCR, coupled with RFLP analysis. The length of the amplified IGS regions ranged from about 1.9 kb to 3 kb in all strains and was amplified with the primers CNL12 and CNS1. Restriction digestions of IGS regions amplified by *Alu*I, *Rsa*I, *Hae*III, *Taq*I, as well as double digests with *Taq*I and *Hae*III, gave rise to different IGS haplotypes among several strains of *F. oxysporum* f. sp. *vasinfectum*.

Bacteriology

***Pseudomonas syringae* pv. *apii*: causal agent of bacterial leaf spot of celery in Greece.** D.E. Goumas and P. Lalla. *Technological Education Institute of Crete, School of Agricultural Technology, Laboratory of Phytopathology - Bacteriology, P.O. Box 1939, 710 04 Heraklion, Crete, Greece.*

In March 2003, a bacterial infection of celery crops was recorded in several regions of Crete. Symptoms were confined on the older leaves. Initial symptoms were small water soaked spots visible on both sides of the leaf. The developed lesions had a circular shape (or angular, if limited by the leaf veins), and were usually surrounded by a chlorotic halo. Later, the spots lost their water-soaked appearance and became yellow, brown to tan, necrotic and papery. Under high moisture, lesions often coalesced and caused severe blighting of the foliage. Fluorescent bacteria were consistently isolated from lesions of the leaves. In the LOPAT tests, the isolated

celery bacterial strains presented the phenotype [+ - - - +], corresponding to the Ia group of the fluorescent pseudomonads. On the basis of the analytical phenotype of morphological, physiological and biochemical characters the isolated celery strains were identified as members of *Pseudomonas syringae* pv. *apii*. This was confirmed by pathogenicity tests on celery and bean plants, on immature detached fruits of lemon and pear, as well as on bean pods. This is the first record of *Pseudomonas syringae* pv. *apii* in Greece. The fact that the pathogen was isolated in two out of fifteen tested samples of celery seed, suggested that the bacterium could be dispersed in Crete through infected seed.

First record of *Xanthomonas campestris* pv. *vesicatoria* on pepper seedlings in Greece. D.E. Goumas and A. Ziogas. *Technological Education Institute of Crete, School of Agricultural Technology, Laboratory of Phytopathology - Bacteriology, P.O. Box 1939, 710 04 Heraklion, Crete, Greece.*

In May 2003, *Xanthomonas campestris* pv. *vesicatoria,* the causal agent of bacterial leaf spot in pepper (*Capsicum annuum*), was isolated from pepper seedlings in Crete. Infections appeared primarily as small irregular, black or water-soaked spots on leaves. Later spots were often surrounded by a yellow halo and usually coalesced to create larger leaf blotches. In some cases, severe defoliation was observed. The isolated bacteria grew on the semi-selective medium Tween and formed the characteristic yellow colonies of *Xanthomonas* spp. on NAG and YDC media. All the bacterial strains tested were non-fluorescent, Gram-negative, rod-shaped, catalase-positive, oxidase-negative and tolerant to 0.02% but not to 0.1% TTC. They also metabolized glucose oxidatively, grew at 37°C, did not reduce nitrates, hydrolyzed gelatin and esculin. They produced acids from D-arabinose, mannose and D-glucose, and were amylolytic but non-pectolytic. All pepper strains induced a hypersensitive reaction on tobacco leaves. Disease symptoms were reproduced on pepper (cv. Early California Wonder) and tomato (cv. Ace) plants. Thirteen pepper cultivars or hybrids with or without the resistance genes *Bs1*, *Bs2*, or/and *Bs3* and four tomato hybrids were artificially infected with the isolated strains. Based on the phenotypic characters and the pathogenicity tests, all the strains of the bacterium belonged to group B of *Xanthomonas campestris* pv. *vesicatoria* (Stall *et al*., 1994). This is the first record of this bacterium on pepper plants in Greece.

Demonstration of a role in disease development for *Erwinia carotovora* subsp. *atroseptica* putative type III secreted effector (DspE/A) and helper (HrpN) proteins using a pooled transposon mutation grid. M.C. Holeva, K.S. Bell, L.J. Hyman, A.O. Avrova, S.C. Whisson, P.R.J. Birch and I.K. Toth. *Plant-Pathogen Interactions Programme, Scottish Crop Research Institute, Dundee, DD2 5DA, UK.*

Type III secretion system (TTSS), encoded by the *hrp* gene cluster, has been identified in soft rot *Erwiniae* and implicated in disease development. In this work, the nucleotide sequence of the *hrp* gene cluster and the adjacent *dsp* genes in *Erwinia carotovora* subsp. *atroseptica* (Eca, EcaSCRI1039) was completed and showed that the cluster was similar in content and structural organisation to that of *Erwinia amylovora* (Eam). However, putative genes of a currently unknown function located within the Eca cluster and with no homologues in the Eam cluster were also identified. Hrp box promoter elements, to which the HrpL transcriptional regulator binds, were observed upstream of dspEF, hrpW, hrpN, hrpC, hrpA and hrpJ operons. An arrayed set (mutation grid) of Eca Tn*5* insertional mutants was constructed for the first time in a plant pathogenic bacterium, and a PCR-based pooling strategy allowed the rapid isolation of *hrp*/*dsp* mutants. A potato-stem inoculation bioassay showed that Eca mutants affected in a structural (*hrcV, hrcC*) gene, a helper gene (*hrpN*) or an effector gene (*dspE*/*A*) were significantly less virulent than the wild-type strain. These results suggested that the Eca TTSS and the secreted proteins play an essential role in potato infection.

Parallels in *Pantoea stewartii* subsp. *stewartii* biofilm development and maize vascular infection. M. Koutsoudis[1], D.S. Tsaltas[2] and S.B. von Bodman[1,2]. *[1]Department of Molecular and Cell Biology, [2]Department of Plant Science, University of Connecticut, Storrs, CN, USA.*

The phytopathogenic bacterium *Pantoea stewartii* subsp. *stewartii* causes Stewart's wilt disease in maize. A small beetle vector *Chaetocnema pulicaria* transmits the disease during periods of feeding. The wilting condition is the result of xylem blockage due to the bacterium secreting a capsular/extracellular polysaccharide (EPS) virulence factor. The timing of EPS production may be important for the bacterium to establish the degree of pathogenic fitness. The control of EPS production is regulated by the quorum sensing mechanism a cell density sensing system. Delayed EPS synthesis may be necessary for the bacterium to initiate and progress through the early events of biofilm development, while EPS onset at a later stage may be important for subsequent steps including motility, microcolony formation and biofilm maturation. Our recent data support such a hypothesis.

Epiphytic unicellular algae and cyanobacteria pathogenic on ornamental house plants in greenhouses. P.E. Kyriakopoulou[1], A.E. Voloudakis[2], S.P.

CHRISTEAS[1] and H. SLUIMAN[3]. *[1]Laboratory of Phytopathology, [2]Laboratory of Plant Physiology and Plant Morphology, Agricultural University of Athens, 75 Iera Odos, 118 55 Athens, Greece. [3]Royal Botanic Garden of Edinborough, 20A Inverleith Row, Edinborough EH3 5LR, UK.*

A serious phytopathological problem was observed on the leaves of house and other ornamental plants in the commercial greenhouse of the Agricultural University of Athens, as well as in Almyros-Magnisia and Thebes-Boeotia, for at least the last five years. Plants of various species, including *Dizygotheca elegantissima*, *Ficus europa*, *F. lyrata*, *F. benjamina*, *Schefflera actinophylla*, *Philodentrum burgundii*, *Gardenia jasmisnoides*, *Yucca gloriosa*, *Camellia japonica* and *Adiantum raddianum*, showed on their leaf surfaces (mainly the upper) areas of various sizes covered with a green epiphytic growth in the form of a coating. This symptom was more prevalent under conditions of indirect light and high relative humidity, especially under mist propagation. Initial microscopic inspection of such coatings indicated the occurrence of unicellular algae. From those coatings, isolations were performed in Bold's Basal (BB) medium supplemented with vitamins. The petri dishes were placed under RT conditions (20–25°C) and under indirect natural illumination to avoid phototoxicity. Those isolations gave: a) dark green colonies, diameter 0.25–1 mm (most common result), b) thick sparse white mycelium (cotton thread-like) related in some of its areas with a dark gray-green growth (one isolation), and c) green and white netlike flat growth of thin (diameter 0.05–0.3 mm) threads with the occurrence of small whitish spherical sporophores with a cracked surface, diameter 0.5–0.8 mm on thin, coloured stalks of 2–3 mm, full of dark brown spores (two isolations). So far, two organisms have been identified from these pure cultures: 1) *Choricystis minor* (Chlorophyta) a unicellular alga, 2) a cyanobacterium (Cyanobacteria) of the order Chroococcales. For the control of the supposed fungal growth in case c) above, the fungicide flusilazole (10 ppm) was successfully used in BB medium. In the absence of the fungus the green phenotype of the culture was strongly affected, and only small colonies of pink, cream or green colour were produced .

Comparison of three laboratory methods to evaluate the pathogenicity and aggressiveness of six *Pseudomonas syringae* pv. *syringae* isolates on apple, pear, cherry and peach trees. T. THOMIDIS, C. TSIPOURIDIS, E. EXADAKTYLOU and P. DROGOUDI. *National Agriculture Reseacrh Foundation (NAGREF), Pomology Institute, 38 S. S. Naoussas, 592 00 Naoussa, Greece.*

The pathogenicity and aggressiveness of six Greek *Pseudomonas syringae* pv. *syringae* isolates originating in a number of hosts (citrus, pear, peach and cherry) were evaluated using three different laboratory methods. The results generated by these methods were in good agreement. All the isolates tested were pathogenic on apple, pear, cherry and peach trees showing no host specificity. The aggressiveness of individual isolates, however, differed markedly. The extent of tissue colonized varied considerably among the different isolates. Generally, isolates BPI 176, BPI 203, BPI 14 and BPI 2 were the most aggressive on excised shoots and twigs, while isolates BPI 4 and BPI 20 were the least aggressive. These methods are proposed as rapid and reproducible screening systems for testing the susceptibility of apple, pear, cherry and peach cultivars to this pathogenic bacterium.

Analysis of the mechanism of pathogenicity in the model interaction between *Pantoea stewartii* subsp. *stewartii* and *Zea mays*. D.S. TSALTAS[1] and S.B. VON BODMAN[1,2]. *[1]Department of Plant Science, [2]Department of Molecular and Cell Biology, University of Connecticut, Storrs, CN, USA.*

The gram-negative phytopathogenic bacterium *Pantoea stewartii* subsp. *stewartii* (*Pn.s.s.*) causes wilt disease in corn (Stewart's wilt). The insect vector *Chaetocnema pulicaria* transmits the disease while feeding on corn leaves. Symptoms of wilt are caused by bacterial occlusions in the xylem vessels from extracellular polysaccharides (EPS) secreted by bacteria. *Pn.s.s.* has a quorum sensing (QS) mechanism which regulates the EPS biosynthesis. Previously cloned and characterized genes *esaI* and *esaR* regulate QS (Bodman and Farrand, 1995). Our aim is to investigate the mechanism that the bacterium uses to cause Stewart's wilt. Modern techniques such as genomics and proteomics will be briefly described in order to present a thorough image of the research done in our lab with this model interaction.

Gene expression of NADPH-oxidase of cotton (*Gossypium hirsutum* L.) in response to *Xanthomonas campestris* pv. *malvacearum*. A.E. VOLOUDAKIS[1], P. MARMEY[2], E. DELANNOY[2] and M. NICOLE[2]. *[1]Laboratory of Plant Physiology and Morphology, Department of Agricultural Biotechnology, Agricultural University of Athens, 75 Iera Odos, 118 55 Athens, Greece. [2]IRD - UMR DGPC (Diversité et Génome des Plantes Cultivées; AGRO M, CIRAD, INRA, IRD), BP 5045, 34032 Montpellier, France.*

Free radicals (reactive oxygen species, ROS) are produced as a result of several cellular processes in plants, including processes in response to various environmental stresses. To isolate cotton (*Gossypium hirsutum* L.) NADPH-oxidase, degenerate primers were designed based on partial sequence data of an NADPH-oxidase homologue of cotton from Genbank. The designed primers were used in PCR reactions to isolate the desired gene fragment, utilizing as a template a cDNA library

constructed from RNA of cotton variety Reba 50 in response to isolate Xcm18 of the phytopathogenic bacterium *Xanthomonas campestris* pv. *malvacearum* (Xcm). To isolate the 3' and 5' ends of the NADPH-oxidase specific and designed degenerate primers (based on plant NADPH-oxidases) were used. DNA sequencing of the obtained fragments and its analysis verified the isolation of a NADPH-oxidase from cotton. Gene expression of cotton NADPH-oxidase was studied via semiquantitative RT-PCR utilizing RNA samples isolated at various times during the interaction of cotton cotyledons with the incompatible bacterium Xcm18, as well as with the compatible bacterium Xcm20. NADPH-oxidase was induced 4–6 hrs after interaction of cotton with Xcm18. The increased transcriptional activity of NADPH-oxidase of cotton, although late in comparison to the initial oxidative burst, indicated that the gene was likely to contribute to the overall oxidative burst during the hypersensitive reaction and its physiological development. In addition, digitonin (80 μM), a known elicitor of O_2^{l-} production, induced cotton NADPH-oxidase gene expression within 30 min of interaction with cotton cotyledon cells. It was also shown that NADPH-oxidase of cotton is expressed at the same levels in tissues of all plant parts such as the root, stem, leaf and flower.

Virology

Transgenic tobacco plants incorporating part of the RNA polymerase of *Tobacco rattle virus* are resistant to virus mechanical inoculation and to transmission by its nematode vector. F. Bem[1], N. Vassilakos[1], H. Barker[2], B. Reavy[2] and D.J. Robinson[2]. [1]*Benaki Phytopathological Institute, 8 S. Delta Str., 145 61 Kifissia, Athens, Greece.* [2]*Scottish Crop Research Institute, Invergowrie, Dundee DD2 5DA, Scotland, UK.*

Nicotiana tabacum plants were genetically modified using A*grobacterium tumefaciens* to incorporate the 59K read-through region of the RNA polymerase gene of *Tobacco rattle virus* (TRV). Several F5 lines were produced by sequential self-pollination. Following total RNA extraction, the mRNA of the transgene was detected by RT-PCR in plants from these lines, but not by Northern blotting analysis, indicating low level of transcription or, possibly, transgene degradation through RNA silencing. F5 plants were resistant to mechanical inoculation with the homologous TRV-SYM isolate and the nematode transmissible TRV-PpK20, which was very similar, as regards RNA1 (>99% identity at the nucleotide level). The resistance of the 59K transgenic plants to TRV was not compromised when *Cucumber mosaic virus* (CMV), *Potato virus Y* (PVY) or *Tomato spotted wilt virus* (TSWV) were inoculated before TRV. Moreover, the plants were tolerant to the severe Greek isolate TRV-GR (90% nucleotide sequence identity at the 59K part of the polymerase gene). Finally, the 59K polymerase transgenic plants remained resistant after challenge with viruliferous vector nematodes carrying TRV-PpK20, in contrast to CP-mediated resistant transgenic plants, the resistance (to mechanical inoculation) of which was overcome by natural transmission of TRV by its vector nematodes (Ploeg *et al*., 1998).

Improvement of RT-PCR for the detection of viroids ASSVd, PBCVd and PLMVd. I.N. Bouboura-kas[1], C. Arambatzis[1], C.I. Dovas[2] and P.E. Kyriakopou-lou[1]. [1]*Agricultural University of Athens, Faculty of Crop Science, Department of Plant Pathology, 118 55 Athens, Greece.* [2]*National Agricultural Research Foundation (NAGREF), Plant Protection Institute of Thessaloniki, P.O. Box 324, 570 01 Thermi, Thessaloniki, Greece.*

The research in Greece in pome and stone fruit viroids is so far very limited, despite the extent and severity of the problem. Moreover, our certification system of fruit-tree propagation material is imperfect. A basic prerequisite for the development of such a certification system is to have a sensitive, reliable and fast method suitable for large-scale routine diagnosis, which could restrict viroid spread and ensure the production of healthy and high-quality propagation material. Our research focused on *Apple scar skin viroid* (ASSVd), *Pear blister canker viroid* (PBCVd) and *Peach latent mosaic viroid* (PLMVd), which were found in Greece in cultivated and wild pear several years ago. An RT-PCR method was developed for the detection of these three viroids. Primer pairs were designed for each viroid from conserved regions of their molecules that exhibited the lowest possible secondary structure. As regards sample preparation, in order to avoid the need to handle hazardous reagents (phenol) and to save time, we applied, with minor modifications, the protocols described by Rowhani *et al*. (1995), La Notte *et al*. (1997) and Rott & Jelkmann (2001). The latter method gave the best results. In the reverse transcription (RT) step, in order to reduce the negative effects of the viroid high secondary structure in primer annealing and the activity of the reverse transcriptase, we used several additives (DMSO, Betaine, Tertamethylene sulfoxide). Results showed that the use of Tertamethylene sulfoxide, both in RT and in PCR, was necessary for the detection of ASSVd isolates. Problems with primer dimer production were overcome by applying hot-start PCR. The examination of more samples is in progress, and the performance of the method will determine its usefulness in the certification system of propagation material.

The presence of ASSVd, PBCVd and PLMVd viroids in cultivated and wild pome and stone fruits in Greece. I.N. Bouboura-kas[1], A. Hadidi[2] and P.E. Kyriakopoulou[1]. [1]*Agricultural University of Athens, Facul-*

ty of Crop Science, Department of Plant Pathology, 118 55 Athens, Greece. [2]*Agricultural Research Service, United States Department of Agriculture, Beltsville, MD, 20705, USA.*

In 1997, the presence of *Apple scar skin viroid* (ASSVd), *Pear blister canker viroid* (PBCVd) and *Peach latent mosaic viroid* (PLMVd) were recorded in cultivated and wild pear for the first time in Greece. A study has lately been started to detect these viroids in fruit trees and bushes of cultivated and wild Rosaceae species and to determine their geographical distribution in Greece. For the last two years, a large number of samples have been collected from pome and stone fruit trees and bushes from Peloponnesous (Arkadia, Argolida, Korinthia, Ilia), Etoloakarnania (Mountainous Naupaktia) and Magnisia (Pilio, Zagora). Viroid detection was first based on an ordinary Reverse Transcription-Polymerase Chain Reaction (RT-PCR) but later, a more suitable method was developed for the rapid, sensitive and simultaneous detection of all three viroids (triplex RT-PCR). The results showed for the first time in Greece the occurrence of PLMVd in apricot (Argolida and mountainous Naupaktia), PBCVd in quince (Argolida) and ASSVd in apple (Argolida, Korinthia and Pilio, Zagora), in addition to the already known occurrence of the viroids in cultivated and wild pear. We also report PBCVd (Mountainous Naupaktia) in a wild *Crataegus* sp. for the first time.

Management of *Tomato spotted wilt virus* epidemic spread in tobacco crops of the Kilkis prefecture in Greece. E.K. Chatzivassiliou. *Democritus University of Thrace, Department of Agricultural Development, Plant Pathology Laboratory, 193 Pantazidou Str., 68 200 N. Orestiada, Greece.*

Tomato spotted wilt virus (TSWV) is a serious pathogen found in several important tobacco producing areas of Greece. Following a destructive epidemic in the prefecture of Kilkis, two local Tobacco Producing Farmers' Unions initiated, in 2004, a coordinated disease management plan in an area covering more than 1000 ha of tobacco crops. Detailed studies preceded the application of the plan. In laboratory experiments, the most effective insecticides were selected for the control of the thrips-vector *Thrips tabaci* Lindeman. Samplings showed that this thrips species overwinters mainly in the soil of the wild flora close to infested fields, while the appearance of the first flying individuals was recorded using blue sticky traps (Horiver-TR) during the 13th week of the year. *T. tabaci* populations in seedbeds were kept under control by a preventive application of carbofuran, when seedlings reached the stage of four leaves, followed by two methomyl and one malathion foliage application. Viruliferous individuals were found on weeds close to the tobacco fields during week 16, while a significant increase in the number of flying individuals was recorded from the 19th week of the year. Tobacco fields were protected by applications of the following: carbofuran during transplantation, one spraying with cypermethrin or methomyl in early plantings, and subsequently two rounds of spraying with cypermethrin, methomyl and malathion. In order to achieve an effective decrease of the primary inoculum, the first round of sprayings was applied by the two Farmers' Unions in the beginning of week 23. At that time, 2500 *T. tabaci* individuals were found in the traps and up to 52% of thrips found in the neighboring flora were infected with TSWV. Afterwards, *T. tabaci* populations did not present any significant increase in TSWV infection until the end of the cropping period (up to 500 individuals per trap), while field infection with TSWV fluctuated from 5% to 14%. In tobacco fields where the recommended sprayings were not applied, up to 5700 individuals of *T. tabaci* were found per trap and TSWV infection ranged from 60% to 85%. The outcome of this effort showed that a well-coordinated collective action plan can represent an effective way of combating TSWV epidemics.

Efforts to eradicate *Citrus tristeza virus* in Greece. D. Dimou[1], E. Moschos[2], M. Tsoukaki[2], K. Nikiforakis[3], K. Spanou[1] and P. Dermatas[1]. [1]*Directorate of Agricultural Develoment, Prefecture of Argolis, 211 00 Nafplion, Greece.* [2]*Control Station for Vegetative Propagative Material, 193 00 Aspropyrgos, Athens, Greece.* [3]*Directorate of Agriculture of Chania, 731 00 Chania, Greece.*

The eradication of *Citrus tristeza virus* disease, first reported in 2000 in Argolis, Greece, is based on sanitation measures involving continuous virus testing and the uprooting of diseased trees or entire orchards. In Argolis, surveys are undertaken every year in all virus foci (10) where the disease has been identified, as well as in neighboring orange and mandarin orchards. In 2002, 16 trees were found virus-positive out of 9,129 trees tested, in 2003, 10 out of 10,229, and in spring 2004, two out of 2,446 trees tested. In three out of the 10 foci the situation is now thought to be under control, since no new infections were found in three successive surveys. In a fourth focus however, virus dissemination by aphid transmission occurred and the grove was finally eradicated by uprooting 150 orange trees in March 2004. New infections were also detected in an orchard of mandarin trees var. Clemenpons established with certified propagation material of Spanish origin that was initially found to be infected in 2001. In Crete, which was the second area, together with Argolis, that initially accepted the infected Spanish material, 93 trees were positive out of 3,715 tested in 2002, eight out of 1,441 in 2003, and four out of 180 in spring 2004. Up to now, about 4,000 trees have been uprooted in Crete. Problems concerning the unremitting effort needed to eradicate tristeza in the country will be discussed.

Generic detection and differentiation of tobamoviruses by a spot nested RT-PCR-RFLP using dI-containing primers along with homologous dG-containing primers. C.I. Dovas[1,2], K. Efthymiou[2] and N.I. Katis[2]. [1]*National Agricultural Research Foundation (NAGREF), Plant Protection Institute of Thessaloniki, P.O. Box 324, 570 01 Thermi, Thessaloniki.* [2]*Aristotle University of Thessaloniki, Faculty of Agriculture, Plant Pathology Laboratory, P.O. Box 269, 541 24 Thessaloniki, Greece.*

A spot nested RT-PCR-RFLP method to detect and identify members of the *Tobamovirus* genus was developed. It involved a one-step RT-PCR, in which a combination of degenerate deoxyinosine (dI)-substituted primers amplified part of the polymerase region of tobamoviruses, followed by a nested PCR amplification that increased specificity and sensitivity of detection. Virus species were differentiated by subsequent restriction enzyme analysis. The sensitivity of the method was further increased when along with one primer containing many dIs, another homologous primer in which dIs were replaced by dGs was used. The homologous primer was shorter than the dI-containing primer and with lower degeneracy, resulting in higher overall amplification efficiency due to the increased stability of the primer-target duplex. With this strategy, highly degenerate primers containing many dIs could be used effectively to improve detection sensitivity, alleviating problems of primer-target duplex destabilisation that occur due to many dI substitutions. Different primers were evaluated and amplification conditions were optimized. This method permitted the successful detection and differentiation of *Cucumber green mottle mosaic virus* (CGMMV), *Odontoglossum ringspot virus* (ORSV), *Paprika mild mottle virus* (PaMMV), *Pepper mild mottle virus* (PMMV), *Ribgrass mosaic virus* (RMV), *Sunn-hemp mosaic virus* (SHMV), *Tobacco mild green mosaic virus* (TMGMV), *Tomato mosaic virus* (ToMV), *Turnip vein clearing virus* (TVCV) and *Tobacco mosaic virus* (TMV). This method can be useful in the diagnosis, epidemiological studies, and characterisation of known and unidentified *Tobamovirus* species.

Spherical virus related to grapevine chlorotic vein banding. S.-M. Girgis[1], P.E. Kyriakopoulou[2], A. Avgelis[3] and A. De Stradis[4]. [1]*National Agricultural Research Foundation (NAGREF), Athens Grapevine Institute, 1 S. Venizelou Str., 141 23 Lycovryssi, Attica, Greece.* [2]*Agricultural University of Athens, Department of Plant Pathology, 75 Iera Odos, 118 55 Athens, Greece.* [3]*National Agricultural Research Foundation (NAGREF), Laboratory of Virology, P.O. Box 2228, Katsabas, 710 03 Heraklion, Crete.* [4]*Dipartimento di Protezione delle Piante e Microbiologia Applicata, Università degli Studi e Istituto di Virologia Vegetale del CNR, Sezione di Bari, Via Amendola 165/A, Bari, Italy.*

In 2002 (11th Hellenic Phytopathological Conference), a new virosis was described to which the name grapevine chlorotic vein banding was given. The disease was observed in the ampelographic collection of the Institute in Amaroussion of Attica, in vines of the variety Lehonitis, originating from Magnesia prefecture. The characteristic reaction of the grapevine indicator 5BB (*Vitis berlandieri*×*V. riparia*) was also described; late occurring (end of August) extended bright yellow discoloration in the form of sectors on both sides of the primary veins, as well as systemic mottle of mechanically inoculated *Chenopodium quinoa*. In June 2004, the woody indicator grapevine hybrid SO4, graft-inoculated in March 2003 with field material, showed symptoms similar to those of the original field symptoms of Lehonitis. A spherical virus 30 nm in diameter was purified from systemically infected *C. quinoa*, using the purification method of Ching-Ang Ong and Mink (1989) as modified by Girgis (2002). An antiserum was produced from this preparation.

Grapevine green banding, a disease related to an ilarvirus. S.-M. Girgis[1], P.E. Kyriakopoulou[2] and I.N. Bourbourakas[2]. [1]*National Agricultural Research Foundation (NAGREF), Athens Grapevine Institute, 1 S. Venizelou Str., 141 23 Lycovryssi, Attica, Greece.* [2]*Agricultural University of Athens, Department of Plant Pathology, 75 Iera Odos, 118 55 Athens, Greece.*

In 2003, a previously non-described grapevine symptomatology was observed in the Ampelographical Collection of Grapevine Institute in Syggrou farm in Amaroussion of Attica, in vines of the variety Red Soultanina, originating in Corinthia prefecture. The plants showed fine green banding of primary, secondary and tertiary veins, so as to give the impression of green banded netting. According to observations of 2003 and 2004, these symptoms occurred in June and remained until the end of growing season. The leaves developing in late June showed the opposite symptomatology, *i.e.* banded chlorotic (instead of green) netting. Using buds from diseased vines, the grapevine indicators San George, 5BB, SO4 and 420 were grafted around the end of March 2004. All these indicators reacted with various chlorotic symptoms, the most characteristic being the ones of 5BB and SO4. The symptoms of 5BB were banded chlorotic netting and mild leaf rolling downwards. SO4 showed chlorosis at sites of the primary, secondary and tertiary veins towards the perimeter of the leaf, and leaf crinkling. In addition to this graft inoculation, mechanical transmission was also done in *Chenopodium quinoa*, using homogenate of young infected tissue from vegetative progeny of the original field plants grown in the greenhouse. The homogenate was prepared using 0.1 M

phosphate buffer pH 7.2 and 2% nicotine. *C. quinoa* reacted with systemic mottle, and with such tissue as inoculum other herbaceous indicators were infected: *C. amaranticolor* (systemic leaf mottle and crinkle), *C. murale* (microphylly and dwarfing), *Nicotiana tabacum* cv. Turkish (systemic leaf crinkle) and *Spinacia oleracea* (systemic mottle and malformation). RT-PCR trials using degenerate (genus specific) primers amplifying part of the polymerase gene of ilarviruses disclosed that the virus under study was an ilarvirus. Its identification using DNA sequencing of part of the polymerase gene is under way.

Identification of an interaction between domains of the nonstructural, transmission-associated 2b protein with the viral coat protein (CP) of *Tobacco rattle virus* (TRV) based on the yeast two-hybrid assay. R.C. Holeva[1], J.F. Uhrig[2] and S.A. MacFarlane[1]. [1]*Gene Expression Programme, Scottish Crop Research Institute, Dundee, DD2 5DA, UK.* [2]*Max-Planck-Institut für Züchtungsforschung, Carl-von-Linné-Weg, D-50829 Köln, Germany.*

Viral protein-protein interactions have been implicated in nematode/insect virus transmission. In this study, the interaction of the RNA2-encoded, non-structural 2b protein with the CP of the two TRV isolates PpK20 and PaY4 which are transmitted by the trichodorid nematodes *Paratrichodorus pachydermus* (both isolates) and *P. anemones* (isolate PaY4 only) was mapped using a GAL4 transcription activator-based yeast two-hybrid assay (Y2H). The analysis provided evidence that in TRV-PpK20, 2b acts as a monomer and its central domain interacts with the N-terminal and C-terminal flexible (flexi) domains of CP. In TRV-PaY4, 2b interacts through its N-terminal domain with the CP flexi domain and with other 2b molecules (homodimerisation) through a central or C-terminal domain. Moreover, TRV-PaY4 CP cannot be functionally exchanged with that of TRV-PpK20, as suggested by the Y2H (*i.e.* no interaction with TRV-PaY4 2b) or *in planta* expression patterns (*i.e.* no accumulation of TRV-PaY4 2b in *Nicotiana benthamiana* leaves). Furthermore, even when the TRV-PaY4 CP flexi domain was fused with TRV-PpK20 CP, it could not rescue the interaction with TRV-PaY4 2b in Y2H and immunogold labelling assays. The data suggest that the differences in the CP-2b interaction account for the specificity in vector nematode transmissibility of the two isolates, elucidating some of the molecular mechanisms underlying the tobravirus-vector nematode complex.

Incidence and characterization of viruses infecting cultivated species of the family Apiaceae in Greece. M. Houliara[1], V. Maliogka[1], C.I. Dovas[1,2], K. Efthimiou[1], K. Papathanasiou[1], A. Sereti[1], A. Harou[1] and N.I. Katis[1]. [1]*Aristotle University of Thessaloniki, Faculty of Agriculture, Plant Pathology Laboratory, P.O. Box 269, 541 24, Thessaloniki, Greece.* [2]*National Agricultural Research Foundation (NAGREF), Crop Protection Institute of Thessaloniki, P.O. Box 324, 570 01 Thermi, Thessaloniki, Greece.*

In 2002–2004, a survey was carried out to determine virus incidence in celery and parsley crops showing virus-like symptoms, such as mosaic, yellowing, stunting and leaf distortion. A total of 2094 celery (*Apium graveolens*) and 221 parsley (*Petroselinum crispum*) samples from nine and three different prefectures of Greece respectively were collected. Virus diagnosis was carried out by ELISA, using polyclonal antisera against the following viruses: *Celery mosaic virus* (CeMV), *Apium virus Y* (ApVY), *Carrot thin leaf virus* (CTLV), *Parsnip mosaic virus* (ParMV), *Cucumber mosaic virus* (CMV) and *Tomato spotted wilt virus* (TSWV). The survey found infection of celery samples by CeMV (60.6%), ApVY (8.1%) and CMV (8%). Parsley samples were infected by ApVY (64.3%), followed by CMV (6.8%) and CeMV (3.6%). Isolates of CeMV, ApVY and CMV were further differentiated by amplification of the capsid protein gene by RT-PCR and subsequent restriction enzyme analysis. There were very small variations between the isolates of CeMV and ApVY in celery, while isolates of ApVY, originating in parsley, were divided into two groups. As far as CMV is concerned, both serotypes (CMV-I and CMV-II) were present but CMV-I was prevalent. Testing for satellite RNA of CMV by RT-PCR gave positive results in the majority of samples. The physical and chemical properties of ApVY were also studied: L.I.V. (1 day at 22°C), T.I.P. (45°C for 10 min), D.E.P (10^{-1}–10^{-2} at *P. cripum* and *Chenopodium amaranticolor* extracts).

Virp1, a tomato protein specifically binding potato spindle tuber viroid (PSTVd), is essential for viroid replication. K. Kalantidis[1], E. Marinou[2], M. Denti[1], M. Tabler[1] and M. Tsagris[1,2]. [1]*Institute of Molecular Biology and Biotechnology, Foundation for Research and Technology, P.O. Box 1527, 711 10 Heraklion, Greece.* [2]*Department of Biology, University of Crete, 711 10 Heraklion, Crete, Greece.*

Viroids are circular RNAs that replicate autonomously in their plant hosts. The induced disease symptoms vary in severity and depend on the viroid strain, the host and environmental conditions. Single nucleotide changes within the rod-like viroid molecule can influence symptom expression, or abolish replicability, or systemic spread, indicating an evolutionary well-established subtle interaction between the RNA and host factors, which promote replication of the pathogenic RNA, as well as cell-to-cell and systemic spread. We earlier described the tomato viroid-binding protein 1 (Virp1) which was isolated because of its specific binding to *Potato spindle tuber viroid* (PSTVd) RNA. GFP::Virp1 fusion constructs

under the control of 35S promotor have shown that Virp1 protein is located in the nucleus. We used *Nicotiana benthamiana* plants to clarify the role of Virp1. Wild type *N. benthamiana* is a symptomless host of PSTVd. First, we generated transgenic plants expressing the tomato Virp1 gene. Those plants could be infected with PSTVd in the same way as wild-type plants. In a second experiment, we transformed *N. benthamiana* with a construct expressing a dsRNA hairpin from a fragment of the tomato Virp1 cDNA. Several transgenic lines were obtained and some of them produced Virp1-specific siRNAs and had reduced or no mRNA levels of Virp1. Virp1-suppressed plants were challenged with PSTVd, but no infection could be established. In addition, protoplasts from Virp1-suppressed plants were transfected with PSTVd RNA transcripts. PSTVd did not replicate in Virp1 knock-out protoplasts, but it did in protoplasts from wild-type plants. These data suggest that down-regulation of Virp1 is active at the protoplast level and that Virp1 is necessary for replication of the viroid at the cellular level.

Rapid *in vitro* indexing of citrus viroids with bud micrografting. T. KAPARI-ISAIA, A. KYRIAKOU and L. PAPAYIANNIS. *Agricultural Research Institute, P.O. Box 22016, 1516 Nicosia, Cyprus.*

Citrus exocortis disease is caused by a viroid (CEVd) or complex of viroids and affects *Poncirus trifoliata* and its hybrids, Rangpur lime, Palestine sweet lime, some citrons and lemons. The detection of exocortis and related viroids rely on biological indexing on Etrog citron (*Citrus medica*) Arizona 861. This method is lengthy and first symptoms on inoculated citron are not observed until 3 to 12 months after inoculation. An alternative to biological indexing was developed for faster detection of citrus viroids. This new method, which was termed "*in vitro* indexing of citrus viroids", is a combination of the use of indicator plants and the shoot-tip grafting technique. Seedlings of Etrog citron Arizona 861 grown *in vitro* were micro-inoculated with viroid-infected citrus bark 15 days after planting. Viroid diagnostic symptoms, consisting of leaf epinasty, petiole browning, leaf reduction and plant stunting, appeared on the Etrog seedlings 7 to 12 days post-inoculation at a temperature of 25–28°C and a 16-h day. Viroid presence in the symptomatic seedlings was confirmed by RT-PCR.

First report of *Maize rough dwarf virus* (MRDV) on maize crops in Greece. N.I. KATIS[1], C.I. DOVAS[1,2] and K. EFTHYMIOU[1]. *[1]Aristotle University of Thessaloniki, Faculty of Agriculture, Plant Pathology Laboratory, P.O. Box 269, 541 24 Thessaloniki, Greece. [2]National Agricultural Research Foundation, Plant Protection Institute, P.O. Box 324, 570 01 Thermi, Thessaloniki, Greece.*

In summer 2002, maize crops grown in northern Greece, began to show severe dwarfing and reduced corncob size. These symptoms were different from those caused by *Maize dwarf mosaic virus,* which is endemic to maize crops in Macedonia. The dwarfing disease has been epidemic in some regions (Imathia and Serres), where crop losses are estimated to be over 70%. Disease symptoms were similar to those caused by two closely related members of the genus *Fijivirus, Maize rough dwarf virus* (MRDV) and *Rice black-streaked dwarf virus* (RBSDV). The assumed virus could not be transmitted mechanically to indicator plants. A RT-PCR was developed and optimized for the detection of both MRDV and RBSDV. Two primers were designed from highly conserved regions within both viral genomes (segment eight from MRDV and nine from RBSDV). RT-PCR using total RNA from 15 plants showing typical dwarfing symptoms gave the expected 568 bp product, which was subsequently cloned and sequenced. Sequence comparisons revealed 96% identity with genome segment eight of an Italian isolate of MRDV (L76561), whereas identity with genome segment nine of two RBSDV isolates from China (AF459812, AY050486) was 85%. MRDV occurrence was also confirmed serologically (DAS-ELISA) by specific polyclonal antibodies. More than 50 samples showing MRDV symptoms gave a positive reaction with ELISA. This is the first report of MRDV in Greece.

Seed-borne viruses of the genus *Tobamovirus* and sphaerical viruses in spinach crop in Marathon, Attica, Greece. P.E. KYRIAKOPOULOU, M.E. GRATSIA, I.N. BOUBOURAKAS and A. GRIGORIOU. *Agricultural University of Athens, Faculty of Crop Science, Department of Plant Pathology, 118 55 Athens, Greece.*

In January 2004, in a spinach crop in Marathon, characteristic yellow ringspot symptoms were observed, in the form of rings, ringspots, concentric line patterns, oakleaf patterns and yellow veins, in high proportion of plants (more than 50%). The plants were derived from spinach seeds of the variety Spinaker, from the Dutch company Bejo Zaden b.v. Samples of symptomatic plants from the affected crop were tested by mechanical inoculations on indicator plants and by electron microscopy. Spherical particles about 25 nm in diameter, as well as rod-like particles reminiscent of tobamovirus were observed. Indexing showed that *Chenopodium quinoa* reacted to the spherical virus with numerous local chlorotic lesions of a diameter ~1 mm eight days post inoculation, and with systemic mottling and malformation about 5 days later. The reactions of *C. murale*, *C. amaranticolor* and *C. foetidum* were similar, but that of the last two was milder. Of the solanaceous indicators examined, *Nicotiana tabacum* cv.. Turkish, Xanthi, Samsun, Σ 53, *N. tabacum* × *N. glutinosa* cv. Glurk, *N. benthamiana, N. glutinosa* and *Petunia hybrida*, only the last became infected and reacted with local diffuse

chlorotic lesions. Spinach indicators of the same variety Spinaker reacted with local chlorotic lesions of a diameter ~1 mm 4–8 days post inoculation, and with systemic chlorotic vein netting about five days later. On the other hand, the rod-shaped virus reacted on all *Chenopodium* and *Nicotiana* species, as well as on Spinaker, with numerous necrotic local lesions ~1 mm in diameter 1.5–2 days post inoculation. In the *Nicotiana* species, these small local necrotic lesions were surrounded 2–3 days later by a characteristic fine whitish necrotic ring 0.5–1 cm in diameter. RT-PCR tests, using degenerate primers (genus specific) containing deoxyinosines (dI) at the loci of full degeneration amplifying part of the polymerase gene of tobamoviruses, proved that the rod-shaped virus belonged to the genus *Tobamovirus*. Seeds of Spinaker from the same shop in Marathon, which had supplied the grower of the affected spinach field, produced infected plants that reacted to the indicators in the same way and had similar rod-shaped virus particles. The identification of this spinach tobamovirus by sequencing part of the polymerase gene and the comparison of the two isolates, from the field and from the seed respectively, is under way, as is the identification of the spherical viruses.

Detection and differentiation of picorna-like plant viruses using a semi-nested RT-PCR and a phylogenetic analysis based on the polymerase protein gene. V. Maliogka[1], C.I. Dovas[1,2], K. Efthimiou[1] and N.I. Katis[1]. *[1]Aristotle University of Thessaloniki, Faculty of Agriculture, Plant Pathology Laboratory, P.O. Box 269, 541 24 Thessaloniki, Greece. [2]National Agricultural Research Foundation (NAGREF), Plant Protection Institute of Thessaloniki, P.O. Box 324, 570 01 Thermi, Thessaloniki, Greece.*

A semi-nested RT-PCR method was developed in which a combination of degenerate deoxyinosine (dI)-substituted primers amplified part of the RNA-dependent RNA polymerase (RdRp) domain of *Comoviridae* species and other bipartite picorna-like plant viruses. The RdRp fragment, which was amplified, identified and used in phylogenetic analysis, allowed the detection, molecular characterization and classification of these viruses. The method was successfully applied to seven known picorna-like viruses and it identified known and characterized as yet unknown viruses. Neighbour–joining trees, calculated from the homologous RdRp amino acid part of 25 published sequences, were used to establish the taxonomic relationships between members of picorna-like viruses, revealing lineages that classified all species within their respective genera and clearly distinguished each genus from other genera, proving the usefulness of the specific gene domain for the classification of this virus group. Previously non-characterised RdRp domains of *Potato black ringspot virus* (PBRSV), *Cherry leafroll virus* (CLRV) and Arracacha virus B (AVB) were amplified and sequenced. Phylogenetic analysis confirmed the classification of PBRSV and CLRV within the genus *Nepovirus*, whereas AVB was placed into a cluster different from the *Nepovirus*, *Comovirus* and *Fabavirus* lineages, and was proposed to form a new virus genus (ALSV-like viruses).

Partial characterization of *Artichoke yellow ringspot virus* infecting onion crops in southern Greece. V. Maliogka[1], C.I. Dovas[1,2], D.E. Lesemann[3], S. Winter[3] and N.I. Katis[1]. *[1]Aristotle University of Thessaloniki, Faculty of Agriculture, Plant Pathology Laboratory, P.O. Box 269, 541 24 Thessaloniki, Greece. [2]National Agricultural Research Foundation, Plant Protection Institute, P.O. Box 324, 570 01 Thermi, Thessaloniki, Greece. [3]Biologische Bundesanstalt für Land- und Forstwirtschaft, Institut für Biochemie und Pflanzenvirologie, Braunschweig, Germany.*

In 1999, a virus was isolated from onion crops growing in the area of Korinthia (southern Greece, Peloponnessus) and that were showing mainly yellow stripes on the leaves. The virus was mechanically transmitted from symptomatic onion plants to a large number of test plants belonging to six plant families, including Solanaceae, Leguminosae and Chenopodiaceae, where it caused ringspots, malformations and top necrosis. The virus was readily seed-transmitted in onion (20%). Electron microscopical observations of plant extracts and ultra-thin leaf sections revealed isometric particles 24–25 nm in diameter and also intracellular alterations similar to those caused by virus species of the genus *Nepovirus*. By applying a RT-PCR assay using degenerate primers specific to the *Comoviridae* family, part of the viral RdRp gene was amplified and sequenced. The corresponding RdRp amino acid sequence showed 60% homology to the equivalent region of *Black currant reversion associated virus* (BRAV) and 100% identity with two AYRSV isolates originating in artichoke and *Vicia faba*, whose homologous gene domain was identified using the same procedure. Subsequent phylogenetic analysis confirmed the classification of AYRSV in the genus *Nepovirus*. For the detection of the different AYRSV isolates, specific primers were designed and a RT-PCR method was developed. This is the first report of a nepovirus infecting onion in the field and also the first of a seed-borne virus of the *Allium* genus.

A unique virus associated with blueberry fruit drop disease. R.R. Martin[1] and I.E. Tzanetakis[2]. *[1]Horticultural Crops Research Laboratory, USDA-ARS, Corvallis, OR, 97330 USA. [2]Dept. of Botany and Plant Pathology & Center for Gene Research and Biotechnology, Oregon State University, Corvallis, OR, 97331 USA.*

In the last three years, several blueberry plantations in

British Columbia (Canada), Oregon and Washington (USA) have exhibited unusual symptoms. Fruit develops up to about 5 mm in diameter and is aborted. Physiological disorders may cause such symptoms, but the fact that the number of symptomatic plants is increasing in areas without a history of nutritional disorders and that plants exhibit symptoms year after year indicated that a pathogen may be associated with the disease. DsRNA was extracted from symptomatic plants and cloned. The sequence obtained belongs to a virus without homology with any plant virus found in the database. Several primer sets from sequences of the putative causal agent were developed and all amplified the expected virus region from symptomatic plants, in contrast with healthy plants that failed to produce any amplicons. The lack of homology with any other plant virus is a concern, since many pathogenic fungi may carry viruses. Although unlikely, we are currently investigating the possibility that the virus is infecting an endophyte that is the causal agent of the disease.

Molecular characterisation of *Citrus tristeza virus* (CTV) isolates from Cyprus. L. Papayiannis[1], C. Santos[2], C.I. Dovas[3], A. Kyriakou[1], T. Kapari-Isaia[1] and G. Nolasco[2]. *[1]Agricultural Research Institute, 1516, Nicosia, Cyprus. [2]Universidade do Algarve, 8000 Faro, Portugal. [3]National Agricultural Research Foundation (NAGREF), Plant Protection Institute of Thessaloniki, P.O. Box 324, 570 01 Thermi, Thessaloniki, Greece.*

Citrus tristeza virus (CTV) was first reported in Cyprus in 1968. So far, virus detection has been mainly based on Mexican lime (*Citrus aurantifolia*) indexing and ELISA tests. Recently, a nested Reverse Transcription (RT) Polymerase Chain Reaction (PCR) was developed to allow rapid and sensitive amplification of the viral Coat Protein Gene (CPG). For template preparation, a simple and rapid protocol involving the spotting of plant sap extracts on a nylon membrane, was used. Twelve isolates originating in different parts of the island and causing a wide diversity of symptoms were tested. Symptoms on field trees ranged from inconspicuous to twig dieback, and the decline and death of sweet orange or grapefruit on sour orange rootstock. Similarly, on Mexican lime, symptoms ranged from barely noticeable leaf vein clearing to vein corking, stem pitting and plant stunting. CPG amplicons were digested by *Hinf* I restriction enzyme and characterised with single strand conformational polymorphism (SSCP). Strain-group specific biotinylated probes were hybridized with the PCR products in an asymmetric RT-PCR/ELISA. Isolates were classified into strain groups according to their reaction with the probes. In addition, amplicons of RT-PCR were cloned in the pTZ57R plasmid vector and sequenced. Both sequencing analysis and the probe hybridisation reaction gave similar results concerning the classification of Cyprus isolates into the various strain groups. Four isolates reacted positively with Group 5 probes and showed high affinity with strain B249, which causes quick decline on trees without stem pitting symptoms. Three isolates gave a positive hybridisation with Group 3b probes and presented a similar sequence homology with strain 28C originating in Portugal. One isolate causing quick decline (QD) symptoms on Mexican lime reacted with Group 1 probes, a group of which strain T36 from Florida is a type member. Another QD-inducing isolate was classified in Group 4 whose type member is strain T3. Finally, mixed infections were found in three isolates, which seemed to belong to Group 4 and 5, Group 2 and M (mild), and Group 1,3 and M respectively. These results provide molecular information about the presence of mild, stem pitting and QD-inducing isolates of CTV in Cyprus. The presence of the QD isolates is of special concern because of the wide use of the susceptible sour orange rootstock in the island.

Production of healthy grapevine propagative material of the local varieties in Rapsani, Greece. I.C. Rumbos[1], A. Gliatis[2], A. Chatzaki[1], G. Salpigidis[2], I. Adamopoulos[1] and A. Anthomelidis[3]. *[1]NAGREF, Plant Protection Institute of Volos, 380 01 Volos, Greece. [2]Directorate of Agricultural Develoment, Prefecture of Larissa, 411 00 Larissa, Greece. [3] Winery E. Tsantali, 680 80 Agios Pavlos, Chalkidiki, Greece.*

A project was undertaken in the Prefecture of Larissa to establish an integrated production system at the grape producing area of Rapsani. In the framework of this project, a study was initiated to produce healthy grapevine propagation material of the local varieties Stavroto, Krasato and Xinomavro, and the French variety Sirah. In summer 2002, vine stocks were selected in 15–20–year–old vineyards on the basis of macroscopic criteria. In winter 2002–03, canes of the selected stocks were collected and tested for viruses, fungi and bacteria distributed by propagated material. Virus detection was realized by ELISA and regarded the following six viruses: *Grapevine fan leaf virus* (GFLV), *Grapevine leafroll associated virus 1 and 3* (GLRaV-1 and GLRaV-3), *Arabis mosaic virus* (ArMV), *Grapevine virus A* (GVA) and *Grapevine fleck virus* (GFkV). The results showed that out of 267 vines tested, 123 stocks (46.06%) were infected with one or more viruses. GFkV was the most widespread virus (31.08%), followed by GFLV (9.36%), GLRaV-3 (9.36%), GLRaV-1 (5.24%) and GVA (1.87%). ArMV was not detected in any sample. The Sirah variety had the highest virus infection rate (56%), followed by Stavroto (50%), Xinomavro (43.83%) and Krasato (42.5%). The most worrying situation concerned the variety Sirah, 48% of which was infected with GFLV. Healthy genotypes of the varieties examined were propagated in aseptic media and preserved in tissue culture

in the Genetic Bank of Greek Local Grape Varieties, which exists at the Plant Protection Institute of Volos.

Preliminary studies on the etiology of small apple fruit disorder occurring in commercial orchards in mount Pelion. I.C. Rumbos[1], G. Nanos[2] and I. Boutla[3]. *[1]NAGREF, Plant Protection Institute of Volos, 380 01 Volos, Greece. [2]University of Thessaly, Fytoko, 384 46 N. Ionia, Volos, Greece. [3]Agricultural Cooperative of Zagora, 370 01 Zagora, Greece.*

Since 1999, it has been noted that in apple producing areas of mount Pelion, an increasing number of trees were bearing unusually small fruits without any commercial value. Initially, the phenomenon appeared in high-altitude areas of Zagora region but then it spread to the lower areas, as well as to the regions of Makryrachi, Anilio, Drakia, Vyzitsa and Chania. Small fruits appeared in either a few or all the trees of the orchards. Trees produced small fruits in only one twig/branch or throughout the crown. The cultivar Starking Delicious, widely cultivated in mount Pelion, appeared to be the most sensitive. A smaller proportion of small fruits appeared in the cv. Red Chief and Starkrimson. Trees did not bear small fruits every year but might resume production of normal fruits one growing season, only for small fruits to appear again the following year. Most of the symptomatic trees were grafted on seedling rootstocks and only a few trees on rootstock MM 106. They usually had a good appearance with vigorous shoots and a normal coloration, as is also the case with "apple chat fruit", a disease of unknown etiology. Preliminary studies on the etiology of the small fruits showed a high concentration of Mn in the small fruits and a high concentration of ozone (0_3) during fruit set and growth. Injections with oxytetracycline-HCL in a small number of trees, carried out in September–October of 2002 and 2003, led in the following year to a remission of symptoms in 50–60% of cases. Research continued into 2004 using fluorescence microscopy (DAPI), ELISA and PCR in order to detect phytoplasmas, responsible for apple proliferation disease. Various cultural techniques and chemical agents to alleviate the symptoms were also applied.

Complete nucleotide sequence of *Fragaria chiloensis latent virus*, a member of the genus *Ilarvirus*. I.E. Tzanetakis[1] and R.R. Martin[2]. *[1]Department of Botany and Plant Pathology and Center for Gene Research and Biotechnology, Oregon State University, Corvallis, OR, 97331 USA. [2]Horticultural Crops Research Laboratory, USDA-ARS, Corvallis, OR, 97330 USA.*

Ilarviruses (type member: *Tobacco streak virus* (TSV)) belong to the family *Bromoviridae* and are transmitted vertically through seed and horizontally by pollen and thrips. Two ilarviruses infect strawberry, *Strawberry necrotic shock virus*, previously thought to be a strain of TSV (Tzanetakis *et. al*, 2004) and *Fragaria chiloensis latent virus* (FClLV). In the process of developing molecular tests for these strawberry viruses, we cloned and sequenced the genome of FClLV. RNA 1 encodes the virus replicase with methyltransferase and helicase motifs, while RNA 2 is monocistronic and encodes the virus polymerase. RNA 3 encodes the movement and coat proteins of the virus and a putative ORF of 9 KDa, without any homology with the ilarvirus genes found in the databases. Phylogenetic analysis of the enzymatic motifs of RNAs 1 and 2 as well as that of the movement and coat protein genes revealed that FClLV is related most closely to *Prune dwarf*, *Prunus necrotic ringspot* and *Apple mosaic* viruses. Phylogenetic analysis also revealed a close relationship between FClLV and *Alfalfa mosaic virus* (AlMV), further evidence that AlMV is an aphid-borne ilarvirus.

A new method to clone viral RNA. I.E. Tzanetakis[1], K.E. Keller[2] and R.R. Martin[2]. *[1]Department of Botany and Plant Pathology and Center for Gene Research and Biotechnology, Oregon State University, Corvallis,OR, 97331 USA. [2]Horticultural Crops Research Laboratory, USDA-ARS, Corvallis, OR, 97330 USA.*

Single-stranded (ss) or double-stranded (ds) RNA can be used for RNA virus cloning and sequencing. RNA viruses produce dsRNA as a replication intermediate which is extracted easily from infected tissue. DsRNA is the preferable template for cloning and sequencing RNA viruses with low titer and a variety of methods have been developed for this purpose. Although dsRNA extraction is straight-forward, inhibitory compounds are often co-extracted with the nucleic acids. The traditional methods used for cloning from dsRNA include reverse transcription followed by second strand synthesis utilizing *Escherichia coli* DNA polymerase I. Previous work has shown that DNA polymerases are inhibited in the presence of various plant extracts while reverse transcriptase (RT) can perform enzymatic activities in the presence of these compounds. Reverse transcriptases are RNA and DNA-dependent DNA polymerases. Both polymerization activities of RT in combination with RNAse H and restriction endonucleases were utilized to clone nine different viruses from either ss- or dsRNA, extracted from six plant species, including a virus from pea that failed to give any sequence information when four of the previously described protocols were applied.

Studies on two new closteroviruses and a potexvirus from mint. I.E. Tzanetakis[1], J.D. Postman[2] and R.R. Martin[3]. *[1]Department of Botany and Plant Pathology and Center for Gene Research and Biotechnology, Oregon State University, Corvallis, OR, 97331 USA. [2]National Clonal Germplasm Repository, USDA-ARS,*

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Mentha × gentilis L. cv 'Variegata' has been used as an ornamental for over 200 years. Some symptoms to which this plant is subject, including vein banding and leaf yellowing, have been attributed to virus infection since they are graft transmissible and can be eliminated by heat therapy. The putative causal agents of the symptoms are being studied. Two newly described viruses as well as *Strawberry latent ringspot virus,* family *Sequiviridae,* have been associated with the symptoms. A Potexvirus, family *Flexiviridae,* related to *Potato virus X* and *White clover mosaic virus,* has been sequenced completely. Sequence analysis of more than 8 Kb of a second virus belonging to the family *Closteroviridae* revealed a virus that shared homology with all three genera of the family, making it a candidate for the origin of diversity for the family, an ancestral member, or perhaps a product of recombination between members of all three genera. A mint plant marketed as 'Variegata' that exhibited yellowing symptoms similar but not identical to the clone obtained from the USDA-ARS National Clonal Germplasm Repository, and used in this study was also examined for the virus. A second closterovirus of mint, with similarity to *Citrus tristeza virus* and *Beet yellows virus*, the type member of the genus, was identified in this plant. Transmission studies are under way to identify an aphid vector for the latter virus.

Viroids, "small but mighty". M. Tabler[1] and M. Tsagris[1,2]. *[1]Institute of Molecular Biology and Biotechnology, Foundation of Research and Technology, P.O. Box 1527, 711 10 Heraklion, Greece. [2]Department of Biology, University of Crete, 711 00 Heraklion, Greece*

Over 30 years have passed, since viroids were first described as a disease caused by a simple nucleic acid. From the beginning of their career, viroids have not stopped surprising us. They were the first pathogens consisting of a naked nucleic acid, they were the first natural circular RNA molecules described, one of the first RNAs to be sequenced, and they have been extensively subjected to mutagenesis in order to study the effect of the mutations on pathogenicity and replicability. They were one of the first non-coding genomes, a term which has lately flooded scientific literature. In more recent years, the mechanisms of replication and pathogenesis have been elucidated and proteins have been described, which bind to viroid RNA and play specific roles in their life cycle. Recent developments from our and other laboratories will be discussed and a model on viroid evolution will be presented.

Study on the etiology of potato tuber necrotic ringspot disease (PTNRD). C. Varveri and N. Klavdianos. *Benaki Phytopathological Institute, Laboratory of Virology, 8 S. Delta Str., 145 61 Kifissia, Greece.*

Potato virus Y (PVY), the most serious virus in potato, forms three strain groups, PVY°, PVY^{C} and PVY^{N}. During the last two decades new diseases and isolates have emerged, often possessing intermediate properties between PVY° and PVY^{N}. The most serious new disease is potato tuber necrotic ringspot disease (PTNRD) which is caused by PVY^{NTN} isolates, which are a subgroup of PVY^{-}. Despite numerous attempts, several characteristics of the disease as well as the discrimination between necrotic and non-necrotic isolates have remained unclear. The various PVY^{NTN} detection protocols have also proved unreliable. In the framework of an international work-group created to elucidate the etiology of PTNRD, the reaction of three potato cultivars (cv. Nadine, Nicola, Hermes) to infection with eight single-lesion type isolates belonging to all virus strain groups and subgroups (PVY^{-}, PVY^{C}, PVY^{N}, PVY^{-TN}, PVY^{N}-W) was studied. A severe Greek NTN-like isolate, PVY-GR-24, possessing a highly recombinant genome, was also included. The experiment was carried out under screenhouse conditions and at the end, a disease index (DI) varying from 0 to 1 was calculated, based on the proportion of tubers with necrosis and the surface area affected. All PVY° and PVY^{NTN} isolates caused PTNRD symptoms in the three cultivars with various DI values (0.004–0.754). Isolate PVY^{N}-W caused a low DI value (0.054) only in cv. Nadine, as did PVY^{C} in cv. Nicola (0.021). PVY° was the only isolate that did not cause any symptoms at all. In conclusion, it seems that all PVY^{N} isolates can cause PTNRD symptoms to some degree and under certain circumstances, and that it may be necessary to set a DI threshold to separate PVY^{NTN} causing significant tuber necrosis.

Properties of and interactions between viral citrus pathogens for disease expression. G. Vidalakis, S.M. Garnsey, J.S. Semancik and D.J. Gumpf. *Department of Plant Pathology, University of California Riverside, CA 92521, USA.*

Biological indexing of graft-transmissible pathogens of citrus is a vital component of every citrus-budwood certification program. The probability of symptom expression, the efficacy of the bioindexing tests and the number of citrus indicators required for pathogen detection was statistically evaluated. Multiple infections did not preclude symptom expression or reduce the diagnostic efficacy of the primary indexing hosts for *Citrus tristeza virus* (CTV), *Citrus psorosis virus* (CPsV) and citrus tatter leaf virus (*Apple stem grooving virus*). Symptoms of citrus vein enation virus and the diagnostic efficacy of Mexican lime (*Citrus aurantifolia*) were suppressed by the T30 group of CTV isolates but not by the other CTV isolates tested. CPsV suppressed symptom expression

and the diagnostic efficiency of sweet orange (*C. sinensis*) and sweet tangor (*C. reticulata* × *C. sinensis*) for concave gum. The hypothesis of the viroid quasi-species RNA population structure was also tested as a probable source for intra-population interactions affecting disease expression. The population structure of the two disease causing viroids, *Citrus exocortis viroid,* the causal agent of exocortis disease, and citrus viroid II (*Hop stunt viroid*), the causal agent of the cachexia disease, was investigated.

Studies on the function of the 2b protein of *Pea early-browning virus*. E.K. Vellios[1], D.J.F. Brown and S.A. MacFarlane[2]. [1]*Laboratory of Plant Pathology, Department of Agriculture Crop Production and Rural Environment, University of Thessaly, 38 446 Nea Ionia, Magnessia, Greece.* [2]*Scottish Crop Research Institute, DD2 5DA Invergowrie, Dundee, UK.*

Pea early-browning virus (PEBV) is naturally transmitted by nematodes belonging to the Trichodoridae family. The viral coat protein and the non-structural proteins 2b and 2c are responsible for virus transmission. The genes encoding these proteins are located at the viral RNA2. The present study aims to elucidate the function of the 2b protein. The 2b protein of the TpA56 isolate (nematode-transmissible) differs in two amino acids (aa) from the 2b protein of the isolate SP5 (very poorly transmissible by nematodes). These aa substitutions result from single base changes at positions 1736 and 1997 in RNA2. The lower transmission rate of the SP5 isolate was shown to be caused solely by the base change at position 1997. It also appears that the 2b protein is related to the aggregation pattern of the virus particles in the plant cells. In leaves and roots of *Nicotiana benthamiana* plants inoculated with TpA56 isolate, clumps of virus particles were found in the cell cytoplasm, joined together end to end and side by side. However, in plants inoculated with mutants lacking the 2b gene (which are not transmitted by nematodes), sheets of virus particles were found in the cytoplasm, joined together side by side only. Furthermore, it was found that the 2b protein was present in the cytoplasm in soluble form and was observed in high concentrations at the clumps of virus particles. Conclusively, it is possible that the 2b protein acts as a 'bridge' between the virus particles and the nematode mouthparts, and that the presence of virus clumps is a result of this function. Alternatively, the transmission of the virus isolates by their nematode vectors may be determined by the aggregation pattern of the virus particles. Finally, based on the number of virus particles counted at the root-tip cells of *N. benthamiana* plants, it is likely that protein 2b and/or protein 2c are involved in the long distance movement of the virus particles.

Nematology

An innovative rapid diagnostic test for the detection of the *Tobacco rattle virus* (TRV) vector nematodes, *Paratrichodorus allius* and *P. teres*. E. Karanastasi[1], E. Riga[2], C.M.G. Oliveira[3,4] and R. Neilson[3]. [1]*Washington State University, IAREC, Prosser, WA, USA.* [2]*Benaki Phytopathological Institute, 8, S. Delta Str., 145 61 Kifissia, Athens, Greece.* [3]*Scottish Crop Research Institute, Dundee, Scotland.* [4]*Instituto Biológico, Campinas, SP, Brazil.*

One of the main problems affecting the potato industry during the last few years, especially in the Pacific Northwest of USA and in several areas of Great Britain has been infestation by plant parasitic nematodes of the family Trichodoridae that transmit *Tobacco rattle virus* (TRV). Two of the trichodorid nematode species that are predominant TRV vectors are *Paratrichodorus allius* and *P. teres*. At present, the non-qualitative method that is being used for the detection of trichodorid nematodes takes up to several days, and to detect viruliferous specimens requires a bioassay that lasts about six weeks. The present study reports on the development of a rapid and reliable method that can identify these trichodorid species immediately after soil extraction and within a few hours. Species-specific diagnostic primers were designed to detect the two target nematode species, *P. allius* and *P. teres*, using a one-step PCR amplification. The selectivity of the two diagnostic primer sets was verified by applying them to non-target nematode species and the efficacy of the method was further enhanced when it was combined with a multiplex test.

Molecular identification of the plant-parasitic nematode species *Trichodorus* and *Paratrichodorus*. E. Karanastasi[1], I. Duarte[2], M.T.M. Almeida[3], C.M.G. Oliveira[4], E. Riga[5] and R. Neilson[6]. [1]*Benaki Phytopathological Institute, 8 S. Delta Str., 145 61 Kifissia, Athens, Greece.* [2]*Escola Superior Agrária de Coimbra, Coimbra, Portugal.* [3]*Universidade do Miñho, Braga, Portugal.* [4]*Instituto Biológico, Campinas, SP, Brazil.* [5]*Washington State University, IAREC, Prosser, WA, USA.* [6]*Scottish Crop Research Institute, Dundee, Scotland.*

Some trichodorid species are natural vectors of specific strains of *Tobacco rattle virus* (TRV), and the combined effect of these two pathogens has a deleterious effect upon several agronomically important crops, such as for example potato and tobacco. Considering the complicated relationship between the various TRV strains and trichodorid nematodes, it is very important to have a rapid and accurate method to identify the nematodes to species level and that can be incorporated into a crop husbandry regime for a more effective use of control measures. Morphological identification sometimes depends on subjective judgement and usually requires

well-trained specialists. Moreover, during the last few years there has been a steady decline in the number of invertebrate-group taxonomists, including nematode experts, in Europe. The present study describes a PCR-RFLP method that determines the variability of the 18S rDNA gene in order to distinguish different trichodorid species. The resulting PCR product is 614 bp in length, and can be located at the 3' end of the 18S rDNA gene. Seven restriction enzymes were used to digest the PCR product and the generated pattern was consistent among populations of the same species and successfully discriminated trichodorids down to species level. The proposed method was tested with seventeen trichodorid species and proved to be effective.

Phylogenetic relationships among species of the plant parasitic nematodes *Trichodorus* and *Paratrichodorus*. E. Karanastasi[1], I. Duarte[2], M.T.M. Almeida[3], C.M.G. Oliveira[4], E. Riga[5] and R. Neilson[6]. *[1]Benaki Phytopathological Institute, 8 S. Delta Str., 145 61 Kifissia, Athens, Greece. [2]Escola Superior Agrária de Coimbra, Coimbra, Portugal. [3]Universidade do Miñho, Braga, Portugal. [4]Instituto Biológico, Campinas, SP, Brazil. [5]Washington State University, IAREC, Prosser, WA, USA. [6]Scottish Crop Research Institute, Dundee, Scotland.*

The plant parasitic nematodes that belong to the genera *Trichodorus* and *Paratrichodorus* are known for their ability to vector *Tobacco rattle virus* (TRV) to many crops of high economic importance, such as potato and tobacco. Despite the fact that the *Trichodorus* and *Paratrichodorus* genera have been described in detail, very little is known regarding their molecular phylogenetic relationships. The aim of the present study was to determine these phylogenetic relationships based on 18S rDNA gene sequences. A comprehensive trichodorid survey was carried out in Portugal and several other areas, Greece being also included. Four *Trichodorus* and nine *Paratrichodorus* species, including three undescribed (and potentially new) species, were examined by classical taxonomy. Representative specimens from each species were selected for molecular study and DNA was extracted from at least two individual nematodes from each species and/or population. 18S rDNA was isolated using appropriate newly designed primer sets, sequenced, and subsequently a multiple sequence alignment was produced and used as a basis of a phylogenetic analysis. With only one exception, the phylogenetic tree resulting from the above study, clearly separated the two genera and the species belonging to them into groups that were in agreement with the currently accepted taxonomy of the Trichodoridae family. However, populations of *P. minor* were more closely associated with *Trichodorus* species than were other, more relative *Paratrichodorus* species, concurring with a previous study of trichodorid populations.

Non parasitic diseases

Effects that different Fe levels and N forms in the nutrient solution have on growth, leaf nutrient concentration, and the Fe^{3+} reduction capacity of root in spinach. A. Assimakopoulou. *Benaki Phytopathological Institute, 8 S. Delta Str., 145 61 Kifissia, Athens, Greece. Present address: District Laboratory of Agricultural Extension and Fertilizer Analysis, 204 00 Xylokastro, Greece.*

Spinach plants (var. Viroflay) were grown in synthetic nutrient solutions with three Fe levels (0 μM Fe; 20 μM Fe; 3 μM Fe + 10 mM $NaHCO_3$), and two N forms at two ratios: 100% NO_3-N; and 80% NO_3-N: 20% NH_4-N. Three harvests were examined in total. Leaf chlorosis in plants grown with 3 μM Fe +10 mM $NaHCO_3$ was more severe than it was in plants receiving no Fe in the nutrient solution (0 μM Fe). A significant interaction was found between Fe concentration and the nitrogen form. Total dry weight was lower in plants grown with 0 μM Fe and 100% NO_3-N than in plants grown with 0 μM Fe and 80% NO_3-N plus 20% NH_4-N. The concentration of Fe in the leaves was significantly higher in plants grown with 20 μM Fe and 80% NO_3-N: 20% NH_4-N than it was in plants grown with 20 μM Fe and 100% NO_3-N. Plants deprived of Fe (0 μM Fe) had a significantly lower concentration of Fe and higher concentrations of Ca, Mg, Mn and Zn, as compared to plants grown with sufficient Fe (20 μM Fe). The relatively high concentration of bicarbonates in the nutrient solution (10 mM NaHCO3 + 3 μM Fe) decreased leaf concentrations of P, Mn and Zn and increased concentrations of K, Ca and Mg. Also, biocarbonates significantly increased the Fe^{3+} reduction capacity of the root. In regard to the N form, plants grown with 80% NO_3-N: 20% NH_4-N had significantly higher concentrations of P, Fe, Mn, Zn and Cu, and a lower concentration of Ca, than plants grown with 100% NO_3-N.

Susceptibility of pistachio (*Pistacia vera* L.) to *Camarosporium pistaciae* in relation to the tree nutrient status. A. Assimakopoulou[1,3], K. Elena[1], D. Vlachoyannis[2] and V. Georgopoulos[3]. *[1]Benaki Phytopathological Institute, 8 S. Delta Str., 145 61 Kifissia, Athens, Greece. [2]16 F. Matsagoura Str., 322 00 Thiva, Greece. [3]District Laboratory of Agricultural Extension and Fertilizers Analysis, 204 00 Xylokastro, Greece.*

In 2002, in the district of Thiva (Boeotia), in two pistachio orchards severely affected with *Camarosporium pistaciae*, a greater infection was found in high-yield than in low-yield trees. Diagnostic leaf analysis for N, P, K, Ca, Mg, Fe, Mn, Zn, Cu and B showed that trees with a greater incidence of infection had a significantly lower K concentration than trees with a lower incidence. In the following year 10 trees from these orchards were

selected and treated with increased K fertilization and 10 trees with no K application in order to investigate the relationship between K nutrition and disease severity. The chemical analysis revaled an increasing K concentration in the leaves of the plants fertilized with K. However, it was not possible to evaluate the effect of the improved K status of trees on their susceptibility to the fungus, as environmental conditions were unfavorable for the development of the disease in 2003.

Effect of different rates of nitrogen fertilization on the concentration of micronutrients in field-grown lettuce. A. Assimakopoulou[1,2], G. Troyanos[1] and C.H. Tsougrianis[2]. *[1]Benaki Phytopathological Institute, 8 S. Delta Str., 145 61 Kifissia, Athens, Greece. [2]District Laboratory of Agricultural Extension and Fertilizer Analysis, 204 00 Xylokastro, Greece.*

In a field experiment, lettuce plants (Romaine, var. Toledo) were grown at six different nitrogen fertilization rates: 0 (N_1) - 45 (N_2) - 90 (N_3) - 135 (N_4) - 180 (N_5) - 225 (N_6) kg N ha^{-1}. Nitrogen was applied as ammonium nitrate, using a Latin square (6 × 6) experimental design. Five harvests were conducted at weekly intervals, and the concentrations of Fe, Mn, Zn and Cu in the leaves were determined at the 5th harvest. Leaf concentration of Zn was significantly higher at the N_5 fertilization level than at N_1, N_2, N_3, N_4 and N_6. At the highest nitrogen level (N_6) a reduction of plant growth occurred, probably due to a negative effect of excess fertilizer applied to the soil. Leaf Cu increased significantly at the N_4 and N_5 levels. A statistically significant positive correlation was found between fresh plant weight and concentrations of Zn and Cu in the leaves.

Critical concentrations of nitrogen and nitrate in leaves of Romaine (cv. Toledo) and Butterhead (cv. Divina) lettuce. Y.E. Troyanos, E. Moustaka and A. Assimakopoulou. *Laboratory of Non-parasitic diseases, Benaki Phytopathological Institute, 8 S. Delta Str., 145 61 Kifissia, Athens, Greece.*

In sand culture experiments, critical concentrations of total and nitrate nitrogen in the leaves of lettuce plants were estimated, on the basis of the existing relationship between yield and the concentration of nitrogen in the leaves. All cultivars had similar critical concentrations for total N, approximately 4% on a dry weight basis, as well as for nitrates, approximately 350 ppm. The concentration of nitrates referred to the sap extracted from the base of petioles of young leaves, according to the Merckquant method. A linear relationship was found between yield and the leaf nitrogen concentration of plants.

First measurements and biomonitoring of phytotoxic ozone levels within Messinia district, Greece. D. Velissariou, I. Salmas, K. Katsampoulas, E. Panageas and G. Kelaidi. *Technological Educational Institute of Kalamata, 241 00 Antikalamos, Kalamata, Greece.*

In the spring and summer of 2003, phytotoxic levels of tropospheric ozone were monitored for the first time at the Messinia district, southern Greece, using instruments and biomonitors. An ozone monitor ("Dashibi" chemiluminescent ozone analyzer) was used for this purpose and ozone means in ppbs were recorded hourly on a data logger from May to September 2003. The monitoring site was at the grounds of the Technological Education Institute in the area of Antikalamos, 6 km W-NW from the city of Kalamata. At the same time, the toxic effects of ozone on plants were 'biomonitored' within the framework of an international project (ICP Vegetation, UN-ECE, Convention on Long-range Transboundary Air Pollution), aimed to assess ozone effects on plants in 25 European countries. Evaluation of these effects was carried out in each country by means of the dry biomass losses of a sensitive clover (*Trifolium repens*) biotype against a resistant clone of the same biotype, as well as by scoring ozone toxicity symptoms on clover leaves. In 2004, in addition to this project, another biomonitoring programme was conducted at 13 sites within Messinia district using 'Bel-W3' tobacco plants, an internationally used specific ozone biomonitor. Results showed extremely high phytotoxic ozone concentrations. For the period 22/05/2003–31/07/2003, the monitor recorded cumulative ozone concentrations as high as 16,108 ppb/hours, which were more than five times the European critical level of 3,000 ppb/hours. In the 2003 and 2004 experiments, statistically significant differences in the dry biomasses were obtained between the sensitive and resistant biotypes of clover by means of a specific biomass ratio index calculated each month. This index was positively correlated to the ozone levels recorded. In all cases, ozone toxicity symptoms were observed on the sensitive biotype. Finally, the first assessment of spatial ozone dispersal within the Messinia district using the Bel-W3 biomonitoring tobacco plant confirmed the presence of phytotoxic ozone levels from an altitude of 1,300 m in the fir forest ecosystem of mount Taygetos to the agricultural area of the Messinian coast. This probably explains the severe ozone-like symptoms observed on crops such as potato, bean, and watermelon in the agricultural area as well as on Greek fir (*Abies cephalonica*) at mount Taygetos.

Biological and non-chemical control

Evaluation of Greek bean cultivars for resistance to *Colletotrichum lindemuthianum*, the causal agent of anthracnose disease. G.A. Bardas and K. Tzavella-Klonari. *Aristotelian University of Thessaloniki, Faculty of Agriculture, Plant Pathology Laboratory, 541 24 Thessaloniki, Greece.*

Colletotrichum lindemuthianum Sacc. & Magnus, a fungus causing anthracnose on bean, was isolated from field-grown bean plants, cv. Zargana Chrissoupolis, in Kavala Prefecture. Forty bean cultivars, collected from several regions of Greece, were evaluated for their resistance to the specific isolate. The experiments showed that five of the cultivars (Ithomi, Larissa, Myrssini, Short Barbouni and Pastalia) did not show any disease symptoms. Several biological characteristics of the specific isolate, such as growth and sporulation on different substrates and conidial germinability at various temperatures, were studied. Fungal growth was better on potato dextrose agar (PDA) than on bean pod agar (BPA), Mathure's medium or glucose peptone agar (GPA). Sporulation of the pathogen was also more abundant on PDA (10.3×10^6 conidia ml^{-1}) than on BPA (5.13×10^6 conidia ml^{-1}), Mathure's medium (4.6×10^6 conidia ml^{-1}) or GPA (2.4×10^6 conidia ml^{-1}). The percentage of *C. lindemuthianum* conidia that germinated was higher at 22°C (88.8%) than at 20°C (81%), 24°C (80.4%), 18°C (60.4%) or 12.5°C (19.2%).

Evaluation of watermelon rootstocks for resistance to the phytopathogenic fungi *Verticillium dahliae* and *Fusarium oxysporum* f. sp. *niveum*. G.A. Bardas[1], G. Parousi[2] and K. Tzavella-Klonari[1]. [1]*Aristotelian University of Thessaloniki, Faculty of Agriculture, Plant Pathology Laboratory, 541 24 Thessaloniki, Greece.* [2]*National Agricultural Research Fondation, 570 01 Thermi, Thessaloniki, Greece.*

The phytopathogenic fungi *Verticillium dahliae* Kleb. and *Fusarium oxysporum* f. sp. *niveum* (E.F. Smith) Snyder & Hansen were isolated from watermelon plants in Thessaloniki Prefecture. Five watermelon rootstocks, Calago, Dako, Max–2, Astra and Mammouth, were inoculated with these isolates during transplanting, using the dipping method and were grafted with the susceptible watermelon cv. Crimson sweet. The results showed that cv. Crimson sweet was highly susceptible to *V. dahliae* and *F. oxysporum* f. sp. *niveum*. On the contrary, all the rootstocks showed resistance to *F. oxysporum* f. sp. *niveum*, while the rootstocks Calago, Astra and Mammouth were also resistant to *V. dahliae*.

Use of ozone and a biological product to control soil-borne diseases in greenhouse tomato. V.A. Bourbos and E.A. Barbopoulou. *National Agricultural Research Foundation, Institute of Olive Tree and Subtropical Plants of Chania, Laboratory of Plant Pathology and Ecotoxicology of Plant Protection Products, Agrokipio, 731 00 Chania, Crete, Greece.*

Control of soil-borne diseases is mainly based on methyl bromide, which however has well-known side-effects on the agro-ecosystem and on humans. The aim of this experiment was to study alternatives to methyl bromide. The experiment was conducted in tomatoes grown in an unheated plastic greenhouse. One or two applications of ozone, alone or in combination with Trianum-G, a commercial biological product based on *Trichoderma harzianum* strain T22, at a dose of 150 g m^{-2} was used. In all treatments, yield per plant increased considerably compared to the control and ranged between 39.3 and 40.6%. Daily plant growth was higher (12.3%) compared to the control in plots where firstly ozone and secondly the biological product were applied. Examination of the plant root system showed that the percentage of infection (3.3%) by the pathogens *Fusarium oxysporum* f. sp. *radicis-lycopersici, F. oxysporum* f. sp. *lycopersici* and *Pyrenochaeta lycopersici* was lower in plots treated with two applications of ozone or with a combination of ozone and the biological product.

Use of a specific biostimulator to control *Sphaerotheca fuliginea* in greenhouse cucumber. V.A. Bourbos and E.A. Barbopoulou. *NAGREF, Institute of Olive Tree and Subtropical Plants of Chania, Laboratory of Plant Pathology and Ecotoxicology of Plant Protection Products, Agrokipio, 731 00 Chania, Crete, Greece.*

Cucumber powdery mildew, caused by the fungus *Sphaerotheca fuliginea* (Schlechtend. Fr) Pollacci, under certain conditions causes serious losses to greenhouse crops. The control of the fungus presents serious problems in practice due to the appearance of fungal strains with a lowered sensitivity to plant protection products. The possibility of controlling the fungus with harpin Ea, a specific biostimulator of the plant defence system, at a dose of 0.51 g l^{-1} of the commercial product Messenger®, was studied. The fungicide pyrifenox at a dose of 20 ml hl^{-1} of the commercial product Dorado 20 EC was used as a reference product. Efficacy was determined on the basis of the number of lesions that appeared on the leaves. The protein harpin Ea reduced disease incidence by 99.54 and 99.81% in the first and second cultivation period respectively, as compared with the control. .

Ultraviolet-C radiation as an alternative method to suppress *Botrytis cinerea* on cut freesia flowers (*Freesia hybrida*, L.). A.I. Darras[1], N.E. Pompodakis[1], L.A. Terry[1], D.C. Joyce[2] and I. Vloutoglou[3]. [1]*Cranfield University, Institute of Bioscience and Technology, Plant Science Laboratory, Silsoe, Bedfordshire, MK45 4DT, UK.* [2]*The University of Queensland, School of Agronomy and Horticulture, The Centre for Native Floriculture, Gatton,. 4343, Australia.* [3]*Benaki Phytopathological Institute, Plant Pathology Department, 8 S. Delta Str., 145 61 Kifissia, Athens, Greece.*

The effect of post-harvest application of ultraviolet type C radiation (UV-C, λ=254 nm) on infection of cut freesia

flowers by *Botrytis cinerea* Pers.: Fr. was studied. Freesia (var. Cote d'Azur) flowers were exposed at the bud stage to 0.5, 1, 2.5 or 5 kJ m^{-2} UV-C radiation either 24 h prior to or immediately after inoculation with a *B. cinerea* conidial suspension (1×10^4 conidia ml^{-1}). Disease severity as well as the number and diameter of lesions were recorded 24 and 48 h after inoculation. The effect of radiation on flower vase life, wilting and fresh weight was also studied. Flower exposure to UV-C irradiation prior to inoculation did not significantly decrease disease severity and number of lesions. However, flower exposure to 0.5, 1, 2.5 or 5 kJ m^{-2} immediately after inoculation reduced disease severity by up to 44, 70, 74 and 59% and lesion numbers by up to 37, 62, 68 and 60%, respectively compared to the controls. Radiation with doses ≤2.5 kJ m^{-2} immediately after inoculation had no effect on either vase life or fresh weight. Exposure of flowers to UV-C radiation at 2.5 or 5 kJ m^{-2} caused necrosis of epidermal cells and increased disease severity compared to the controls.

Resistance test of Greek field elm (*Ulmus minor*) against Dutch elm disease. S. Diamandis and C. Perlerou. *National Agricultural Research Foundation, Forest Research Institute, 570 06 Vassilika, Thessaloniki, Greece*.

Three indigenous species of elm occur in Greece: *Ulmus minor* Miller, the most commonly found in plains and lowland all over the country, *U. glabra* Huds. which prefers mountainous, moist habitats, and *U. laevis* Pall. which is found only in riparian ecosystems. *U. canescens,* which also occurs in Greece, is considered a variety of *U. minor*. Exotic species, such as *U. pumila* and *Ulmus×hollandica* (syn. *U. vegeta*), have also been introduced to Greece. Two major epidemics of Dutch elm disease devastated European elm populations in the 20th century. The first, caused by *Ophiostoma ulmi* in 1920–1940, was rather milder than the second and still ongoing pandemic, caused by *O. novo-ulmi*. As part of a RESGEN European research project, Greek genotypes of *U. minor* were tested for resistance to *O. novo-ulmi*. At this first attempt, eight surviving ortets were selected from areas where the disease had caused severe losses. Saporo and Lobel were used as resistant controls, and the French clone CEM085 as a susceptible control. Ramets were produced by root cuttings. Two replications of four 4-years-old plants each were inoculated with a 10^6 spore suspension on May 13, 2002. Foliage wilting was assessed 4 and 10 weeks after inoculation on an 8-step scale: 1=0%, 2=1–10%, 3=11–25%, 4=26–33%, 5=34–50%, 6=51–75%, 7=76–99%, 8=100%. In the first assessment, wilting ranged between 1 and 50% (only 2 out of 64 individuals were assigned to step 6), while in the second assessment no significant deterioration was observed. Saporo and Lobel were highly resistant (0% wilting), whereas clone CEM085 was susceptible (34–75% wilting). Among the Greek ortets, FRI902 (1–25%), FRI905 (1–25%, with one individual assigned to step 4) and FRI 910 (0–25%, with one individual in step 6) stood out. The results showed that there was significant variability in the severity of wilting among the Greek genotypes, which encourages further research. Already, 18 new elm ortets are being tested.

Effectiveness of the biocontrol agents *Trichoderma koningii* and *Pseudomonas chlororaphis* PCL 1391 against the disease caused by *Phytophthora capsici* on pepper plants. I. Halkias, A.L. Lagopodi and K. Tzavella-Klonari. *Aristotle University of Thessaloniki, Faculty of Agriculture, Plant Pathology Laboratory, 541 24 Thessaloniki, Greece*.

The effectiveness of the biocontrol agents *Trichoderma koningii* and *Pseudomonas chlororaphis* PCL 1391 against the disease caused by the oomycete *Phytophthora capsici* on pepper plants was tested *in planta*. Pathogenicity tests of the biocontrol agents on pepper plants showed no negative effect on plant growth. Test of the effectiveness of the biocontrol agents against *P. capsici* on pepper plants showed that both agents reduced the disease under the experimental conditions. Specifically, plants grown in the presence of *P. chlororaphis* and the pathogen showed no statistical differences in the disease progress, when compared with plants grown without *P. capsici* during the first three weeks. However, by the end of the fourth week, *P. chlororaphis* effectiveness was significantly reduced. Plants grown with *T. koningii* and the pathogen also showed disease reduction, though smaller than that caused by *P. chlororaphis* and the pathogen for the first four weeks. However, later on the effectiveness of *T. koningii* actually became greater than that of *P. chlororaphis*. The application of the two biocontrol agents together reduced the effectiveness of both, so that with this combination after the end of the second week the percentage of dead plants with combined treatment did not differ from that of the positive control.

Nematosymbiotic bacteria for the control of cotton damping-off caused by *Pythium* spp. A.V. Kapsalis[1], F.T. Gravanis[2] and S.R. Gowen[1]. *[1]The University of Reading, Earley Gate, P.O. Box 236, Reading, RG6 6AT, UK. [2]Technological Education Institute (TEI) of Larissa, Department of Plant Production, 411 10 Larissa, Greece*.

The bacteria *Pseudomonas* (=*Flavimonas*) *oryzihabitans* and *Xenorhabdus nematophilus,* which are symbiotes of entomopathogenic nematodes, were evaluated *in vitro* and *in vivo* for their effectiveness in controlling damping-off of cotton seedlings caused by *Pythium* spp. The reduction in *Pythium* spp. mycelial growth was positively

related to the concentration of bacterial cells applied. Both suspensions and extracts of bacterial cells proved to be effective in controlling cotton seedlings damping-off caused by *Pythium* spp. under controlled environmental conditions.

Induction of tomato resistance against powdery mildew (*Leveillula taurica*) by *Acremonium alternatum*, a hyperparasite of *Sphaerotheca fuliginea*. A.M. Kasselaki[1, 2], M. Shaw[2], N.E. Malathrakis[1] and J. Haralambous[3]. [1]*Technological Education Institute (TEI) of Crete, STEG, Laboratory of Biological Control of Plant Diseases, Stavromenos, 710 04 Heraklion, Crete, Greece.* [2]*The University of Reading, School of Plant Sciences, Whiteknights, Reading RG6 6AS, UK.* [3]*Greek Centre of Maritime Research, Institute of Maritime Resources, Athens, Greece.*

The fungus *Acremonium alternatum*, a hyperparasite of *Sphaerotheca fuliginea*, was tested against powdery mildew of greenhouse tomato caused by *Leveillula taurica*. A moderate reduction in the disease was achieved, which was slightly higher when the applied fungal spores were killed by heat and it remained unchanged when the spores were killed by UV irradiation. Presumably, this effect was due to the induction of host resistance by substances released by the fungal spores, especially when the spores were killed by heat. Experiments in the growth chamber showed that the effect was systemic, as the untreated leaves were also protected. The effectiveness depended on the leaf position and the level of plant infection by *L. taurica,* and was greater at 24–27°C and at low disease pressure.

Antagonistic microflora of composted agricultural residues to fungal pathogens of tomato. N. Kavroulakis[1], N. Tripolitsioti[1], A. Damaskinou[1], S. Ntougias[1], C. Ehaliotis[2], G. Zervakis[1] and K.K. Papadopoulou[1]. [1]*NAGREF, Institute of Environmental Biotechnology, 87 Lakonikis Str., 241 00 Kalamata, Greece.* [2]*Agricultural University of Athens, Laboratory of Soils and Agricultural Chemistry, 75 Iera Odos, 118 55 Athens, Greece.*

The effectiveness of composted organic amendments against various plant diseases, especially those caused by soil-borne pathogens, have been demonstrated in numerous studies. Nevertheless, the isolation of the biocontrol micro-organisms from compost is rather limited. In the present report, antagonistic bacterial and fungal isolates from agricultural-residues derived compost mixtures that suppressed soil and foliar fungal pathogens of tomato plants, were tested *in vitro* for their inhibition of these pathogens. Selective media for *Pseudomonas, Trichoderma, Fusarium* sp. etc. were used for the isolation. Fermentation conditions and methods of application to potted plants were investigated and optimised to enhance biocontrol activity against *Fusarium oxysporum* f. sp. *radicis-lycopersici*. Disease incidence was recorded in all experiments. Disease reduction, in the presence of the antagonistic isolates, was in some cases >90% as compared to the controls. Putative modes of action of the most promising antagonists, in *in vitro* tests or *in planta*, were also examined.

Antibiotic action of volatiles from 'Isabella' grapes on *Botrytis cinerea*. E.K. Kulakiotu[1], C.C. Thanassoulopoulos[1] and E.M. Sfakiotakis[2]. [1]*Aristotle University of Thessaloniki, Faculty of Agriculture, Laboratory of Plant Pathology, 540 06 Thessaloniki, Greece.* [2]*Aristotle University of Thessaloniki, Faculty of Agriculture, Laboratory of Pomology, 540 06 Thessaloniki, Greece.*

The fungus *Botrytis cinerea*, one of the most harmful pathogens of grapes, does not cause disease on 'Isabella' grapes, a variety of the species *Vitis labrusca*. Instead, it is the pathogen itself that becomes diseased in the presence of 'Isabella' grapes. The volatile substances of 'Isabella' act as biocontrol agents of *B. cinerea* by decreasing both the inoculum and the pathogenicity of the fungus. Antibiosis was identified as the mechanism by which 'Isabella' volatiles acted as biocontrol agents of the fungus. 'Isabella' volatiles caused endolysis of the mycelial hyphae and deformation of the cell walls, thus causing *B. cinerea* to become "diseased", even though the formation of chlamydospores, which was also observed, allowed the fungus to survive.

Potential use of *Arthrobotrys* spp. for biocontrol of root-knot nematodes. D. Lascaris[1], E. Karanastasi[2] and I. Koutsoumaris[1]. *Benaki Phytopathological Institute,* [1]*Department of Phytopathology,* [2]*Department of Entomology and Plant Zoology, 8 S. Delta Str., 145 61 Kifissia, Greece.*

Fungi of the genus *Arthrobotrys*, isolated from the soil or from commercial hydroponic cultivations were evaluated *in vitro* for their predatory ability against saprophytic and plant parasitic nematodes. One isolate of *A. oligospora* and one of *A. dactyloides* were assessed for their effectiveness in suppressing root-knot nematode populations in the soil and symptom development on tomato plants in the growth chamber. Pots filled with soil naturally infested with *Meloidogyne* spp. and amended with two application rates of each of the two fungal species, were planted with tomato plants and kept at 25°C, 12 h day and a soil moisture of 60% field capacity. Six weeks later, there was a reduction in the number of plant parasitic as well as of saprophytic nematodes in all treatments. *A. dactyloides* reduced the number of the 2nd stage juveniles of *Meloidogyne* spp. by 69 to 72% and 2nd stage juveniles of *A. oligospora* by 30 to 52%. Similar reductions were seen in the number of saprophytic nematodes. The number and size of root galls on plants grown in soil amended with the two fungal spe-

cies was reduced whereas total root length was greater compared with that of the control plants. Neither fungus had any adverse effect on plant growth.

The biological activity of *Pseudomonas oryzihabitans* on the root-knot nematode *Meloidogyne javanica* of tomato. S.V. Leontopoulos[1], I.K. Vagelas[2], F.T. Gravanis[2] and S.R. Gowen[1]. [1]*The University of Reading, Earley Gate, P.O. Box 236, Reading, RG6 6AT, UK.* [2]*Department of Plant Production, Technological Education Institute (TEI) of Larissa, 411 10 Larissa, Greece.*

The bacterium *Pseudomonas oryzihabitans* is a particularly effective biological agent against root-knot nematodes (*Meloidogyne* spp.) *in vitro*. Studies *in vivo* demonstrated the efficacy of *P. oryzihabitans* in preventing second stage juveniles (J2s) of *M. javanica* from invading tomato roots treated with bacterial cells. A concentration of 10^5 ml^{-1} bacterial cells provided sufficient nematode control. The number of J2s invading tomato roots was reduced to such an extent that the number of egg masses was reduced, although the average number of eggs per egg mass remained the same.

Evaluation of olive rootstocks for resistance to Verticillium wilt. E.A. Markakis, S.E. Tjamos, P.P. Antoniou, E.I. Paplomatas and E.C. Tjamos. *Agricultural University of Athens, Phytopathology Laboratory, 75 Iera Odos, 118 55 Athens, Greece.*

Verticillium wilt is a destructive fungal disease that poses an increasing threat to olive orchards and tends to become epidemic in many regions across the Mediterranean. As with any other big problem that occasionally appears, prevention is the best management. To prevent Verticilium wilt, it is important to grow varieties and rootstocks that eliminate or as much as possible restrict the infection and its symptoms, and to minimise the spread of inoculum. A rapid evaluation of olive trees for wilt resistance was carried out following injection with a conidial suspension (100 μl, 10^8 conidia ml^{-1}) in 5-mm-deep (3-mm diameter) hole on the trunks of young olive trees growing in Amvrakia (M/10), Aggeloupoli (A/29) and Nikopoli (F/12) areas. Some rootstocks did not show any symptoms and the pathogen was not isolated, although the trees had been inoculated twice in 2000–2002. Rooted grafts, derived from these maternal trees, were inoculated via the soil with *Verticillium dahliae* microsclerotia (20 microsclerotia g^{-1} soil). After one year isolations and PCR amplifications were performed on the young olive trees although did not show any symptoms. *V. dahliae* was present in one type of rootstocks, whereas in the other two types the pathogen was not detected. A large number of grafts, derived from these two types of rootstocks, have been rooted and will be inoculated with microsclerotia via the soil, in order to confirm their resistance to Verticillium wilt. For this reason olive trees of the varieties Amfissis, Kalamon, Koroneiki and Gaidourelia were inoculated with *V. dahliae* microsclerotia at 3, 10 and 20 microsclerotia g^{-1} soil in order to evaluate their resistance to Verticillium wilt. The symptoms as well as the isolations made from these trees showed that the varieties Kalamon, Koroneiki and Gaidourelia were more resistant than the known susceptible variety Amfissis, while Koroneiki was a little more resistant than Gaidourelia.

Biocontrol of aflatoxin in pistachios and figs in California. T.J. Michailides[1], M.A. Doster[1], P.J. Cotty[2] and L. Boeckler[1]. [1]*University of California Davis, Department of Plant Pathology, Kearney Agricultural Center, 9240 South Riverbend Ave., Parlier CA 93648, USA.* [2]*USDA, ARS, 1140 E. South Campus Drive, Tucson, AZ 85721, USA.*

A multi-year research project was undertaken at the Kearney Agricultural Center of the University of California, funded by the United States Department of Agriculture. Aflatoxins are a group of closely related toxins produced by *Aspergillus flavus* and *A. parasiticus,* fungi that grow in various crops. Aflatoxins are widely regulated by governments, who have set very low tolerances for aflatoxins in food and animal feed. The aflatoxin-producing fungi, *A. flavus* and *A. parasiticus,* are widespread and probably occur in every pistachio (*Pistacia vera*) and fig (*Ficus carica*) orchard in California. Not all strains of *A. flavus* produce aflatoxins. In one study of isolates from California orchards, only 43% of *A. flavus* isolates produced aflatoxins. Naturally occurring non-aflatoxin producing ("atoxigenic") strains of *A. flavus* have to a large extent been used to displace toxigenic isolates of *A. flavus* from cottonseed and corn and *A. parasiticus* from peanuts. The most successful use of the atoxigenic strains to displace toxigenic *A. flavus* is that of atoxigenic strain AF36, which has been widely used in commercial cotton fields in Arizona and Texas. In 2001 the atoxigenic strain AF36 was found to be naturally occurring throughout the pistachio and fig growing regions of California and to make up approximately 6% of *A. flavus* isolates in orchards. In 2002 and 2003, we carried out preliminary experiments with AF36 in pistachio and fig orchards. It was concluded that AF36 could displace *A. flavus* from the soil of orchards and reduce the contamination of pistachio nuts and figs with aflatoxin in California.

Study of the combined efficacy of the entomopathogenic fungus *Metarhizium anisopliae* (Metschinkoff) Sorokin (Deuteromycotina: Hyphomycetes) and diatomaceous earth against larvae of the stored-product beetle *Tribolium confusum* Du Val (Coleoptera: Tenebrionidae). M.P. Michalaki[1], N.G. Kavallieratos[2], C.G. Athanassiou[1], G.N. Balotis[3], H.A.

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The insecticidal efficacy of the entomopathogenic fungus *Metarhizium anisopliae* (Metschinkoff) Sorokin (Deuteromycotina: Hyphomycetes), against larvae of the stored-product beetle *Tribolium confusum* Du Val (Coleoptera: Tenebrionidae) was examined in laboratory bioassays. A solid fungal formulation was prepared and applied to wheat, in three doses, alone or in combination with a diatomaceous earth formulation in two doses. The efficacy of the application of the diatomaceous earth and the entomopathogenic fungus alone was assessed as well. The efficacy was based on larval mortality counts, after 24 h and 48 h and 7 days and 14 days of exposure of the treated wheat. Mortality was positively correlated to the length of the exposure interval of the treated substrate. Furthermore, mortality was significantly affected by temperature and relative humidity during the bioassays. Finally, the addition of diatomaceous earth in the fungal formulation had a synergistic effect in some cases.

The use of host resistance and solarization in the management of root-knot nematodes (*Meloidogyne* spp.) and other soilborne pathogens of greenhouse tomato. G. NEOPHYTOU[1], N. IOANNOU[1] and D.J. WRIGHT[2]. *[1]Agricultural Research Institute, 1516 Nicosia, 22016 Cyprus. [2]Imperial College, Silwood Park, Ascot, Berks, SL5 7PY, UK.*

The use of tomato cultivars resistant to plant-parasitic nematodes and to other major soilborne pathogens has been largely overlooked, mainly due to the effectiveness of soil fumigation with methyl bromide (MB). However, with the imminent phasing out of MB, the use of resistance needs to be re-examined. To this end, three trials were conducted at Zygi Experimental Station to evaluate the effectiveness of tomato cultivars resistant to root-knot nematodes (*Meloidogyne* spp. [RKN]). In the first trial, the effectiveness of four resistant tomato cultivars (3161, 1402, Nefeli and 1410) and a susceptible cultivar (Graziella) in combination with 4-weeks solarization was evaluated. The second trial evaluated the nematode-susceptible, cherry-type cultivar Bar 138-8, grafted on the resistant rootstock 'Super'. The third trial tested the nematode-susceptible tomato cultivars Graziella and FA 179, also grafted on the resistant rootstock 'Super'. The 4-weeks solarization treatment significantly increased plant growth and cumulative fruit yield by 42%, and decreased the percentage of root galling compared to the uncovered control. All resistant cultivars kept nematode damage to very low levels and increased cumulative yield compared to the susceptible cultivar Graziella. In the solarized plots, the resistant cultivars 3161 and 1402 gave significantly greater cumulative fruit yield than in non-solarized plots, suggesting a synergistic effect between resistance and solarization. Both solarization and the resistant cultivars significantly reduced the severity of corky root rot (*Pyrenochaete lycopersici*) and vascular wilts (*Fusarium oxysporum* f. sp. *lycopersici* and *Verticillium dahliae*). In the second trial, the infections by RKN and other soilborne pathogens were significantly decreased in the susceptible cultivar Bar138-8 grafted on the resistant rootstock 'Super'. In contrast, percent root galling on non-grafted plants was 70%. The grafted plants increased shoot fresh weight and cumulative fruit yield by 60 and 16% respectively compared with non-grafted plants. In the last trial, the cultivars Graziella and FA 179 grafted on the nematode resistant rootstock 'Super' kept RKN damage to very low levels and increased shoot fresh weight by 30% compared with the non-grafted cultivar FA 179. The study showed that both solarization and the use of nematode resistant cultivars or rootstocks were powerful plant protection methods and could be utilized as alternatives to MB. The combination of both methods, through their synergistic and additive effects, could provide a significant advantage to farmers.

Effect that grafting of tomato onto commercial rootstocks has on the development of Verticillium wilt. A.M. PAPADAKI[1], A.L. LAGOPODI[1] and F.A. BLETSOS[2]. *[1]Aristotelian University of Thessaloniki, Faculty of Agriculture, Plant Pathology Laboratory, 540 06 Thessaloniki, Greece. [2]National Agricultural Research Foundation (NAGREF), Agricultural Research Center of Macedonia and Thrace, Department of Vegetables, P. O. Box 60458, 570 01 Thermi, Thessaloniki, Greece.*

Grafting of tomato onto commercial rootstocks is an environmentally friendly method for controlling Verticillium wilt. The susceptible cv. Early pack was grafted onto the rootstocks He-man, 48-S-548, Beufort, Primavera, Nova, Packmore and Vigomax on the premises of the company "Agrotikos Oikos Spyrou". Self-grafted plants of the same cultivar were used as controls. The experiment was conducted in 2004 in an unheated plastic greenhouse in the NAG.RE.F Agricultural Research Center of Macedonia and Thrace, Thessaloniki.

Twenty-seven grafted plants from each rootstock were dipped in *Verticillium dahliae* inoculum (10^6 spores ml^{-1}) for 10 min and transplanted to soil disinfected with methyl bromide. An equal number of grafted plants was transplanted without inoculation to the same soil and used as controls. One month after transplanting a disease index (DI) was estimated for each plant, on a 1–6 scale (1=healthy plant, 6=practically dead plant) and their height was measured. Assessments were made at 10-day intervals. It was found that the rootstocks Packmore and Vigomax were more susceptible to Verticillium wilt and promoted disease development (DI=3.297 and 3.257 respectively), while rootstocks Beufort and Primavera were more resistant (DI=2.890 and 2.740 respectively) and delayed disease development. All commercial rootstocks inoculated with *V. dahliae* were 2.54 to 13.78% taller than the self-grafted plants, while in the controls, the plants that were grafted on the rootstocks Vigomax, He-man, 48-S-548, Beufort and Nova were 2.17 to 6.43% taller than the self-grafted plants.

Efficacy of three essential oils on root-knot nematodes (*Meloidogyne* spp.) of tomato. D. Paraschi, V. Palamiotou, I.K. Vagelas and F.T. Gravanis. *Department of Plant Production, Technological Education Institute (TEI) of Larissa, 411 10 Larissa, Greece*.

Three essential oils extracted from oregano (*Origanum vulgare*), citronella (*Citronella* sp.) and lavender (*Levandulla officinalis*) were tested *in vitro* and *in vivo* against a mixed population of root-knot nematodes (*Meloidogyne* spp.) derived from a glasshouse in the Larissa region, Greece. All three essential oils immobilized second stage juveniles (J2s) and inhibited *in vitro* hatching. Essential oil of oregano showed the highest *in vitro* nematicidal activity. *In vivo* tests showed that a significantly lower number of J2s invaded treated tomato plants compared to the controls. Oregano oil, but not citronella or lavender essential oil, prevented J2s from invading tomato roots to a significant extent. Moreover, treatment of tomato plants with oregano oil resulted in lower numbers of nematode egg masses and fewer eggs per egg mass.

Suppression of Verticillium wilt of strawberry with commercial products of mycorrhizae and *Bacillus subtilis*. V.I. Tahmatsidou[1], J. O'Sulivan[2], A.C. Cassells[2], D. Voyiatzis[3] and G. Paroussi[4]. *[1]Prefectural Department of Agricultural Development in Serres, Department of Plant Protection and Quality Control, Terma Omonoias, 621 25 Serres, Greece. [2]National University of Ireland, Department of Plant Science, Cork, Ireland. [3]Aristotle University of Thessaloniki, Department of Horticulture, Laboratory of Biology of Horticultural Plants, 541 24 Thessaloniki, Greece. [4]NAGREF, Agricultural Research Center of Macedonia-Thrace, 570 01 Thermi, Thessaloniki, Greece*.

Experiments were conducted in a field of NAGREF at Thermi, Thessaloniki, Greece, in order to study the control of Verticillium wilt in strawberry by biological means. A commercial product containing spores of the fungus *Glomus* (VAMINOC-G®) and another containing spores of the non-pathogenic micro-organism *Bacillus subtilis* FZB24® (*Bacillus subtilis* FZB24®-WG) were used. Strawberry plants of the cultivars Elvira and Selva derived from: (a) runners of certified stock plants, (b) runners of microplants and (c) micropropagation, were planted. Inoculation with VAMINOC-G® was done with 6 g of the commercial product for (a) and (b), and 3 g per plant before acclimatization for (c). Inoculation with *Bacillus subtilis* FZB24® was by dipping plant roots in 0.1% w:v suspension of warm sterile distilled water at planting for (a) and (b) and before acclimatization for (c) and it was repeated by watering with 50 ml of a 0.02% w:v suspension per plant seven days after planting. Ten ml of *Verticillium dahliae* inoculum was used to inoculate each plant 2 weeks after planting. Marketable fruit yield per plant and plant and root fresh weight were significantly higher in plants treated with either VAMINOC- G® or *Bacillus subtilis* FZB24®-WG before inoculation with the pathogen than in those treated only with *V. dahliae*, with only a few exceptions.

Effectiveness of essential plant oils against postharvest diseases caused by *Botrytis cinerea, Penicillium italicum* and *P. digitatum*. A.G. Vitoratos, D.J. Anagnostou, G.Z. Baferos, A.N. Markoglou and B.N. Ziogas. *Agricultural University of Athens, Laboratory of Pesticide Science, 75 Iera Odos, Votanikos, 118 55 Athens, Greece*.

Essential oils are naturally occurring terpenic mixtures (mainly carvacrol, thymol, *p*-cymene, α-pipene and γ-terpinene) with plant protection activity against important plant pathogens and extreme environmental conditions. In this study the effectiveness of plant essential oils from *Origanum vulgare* L. subsp. *hirtum* and *Citrus limon* against grey mould disease caused by *Botrytis cinerea,* and thymus oil from *Thymus vulgaris* against green and blue mould caused by *Penicillium digitatum* and *P. italicum* respectively were investigated. *In vitro* experiments showed that all essential oils were more effective against mycelial growth and conidial germination when they were applied as a vapour, rather than when they were added to the fungal growth medium. It was found that: (a) thymus oil totally inhibited mycelial growth and spore germination of *P. digitatum* and *P. italicum* at concentrations of 0.134 and 0.5 $\mu l\ cm^{-3}$ respectively; (b) oregano and lemon oil inhibited the mycelial growth of *B. cinerea* at concentrations

of 0.017 and 16.7 μl cm^{-3} respectively and spore germination at concentrations of 0.017 and 21.7 μl cm^{-3} respectively. As regards the effectiveness of essential oils to control mould caused by *P. digitatum*, *P. italicum* and *B. cinerea* on fruits and vegetables it was shown that: (a) thymus oil controlled blue and green moulds on orange fruits at a relatively low concentration of 0.313 μl cm^{-3}; (b) oregano and lemon oils controlled grey mould from *B. cinerea* on tomatoes at concentrations of 0.313 and 0.125 μl cm^{-3} respectively, and (c) lemon oil inhibited rot symptoms caused by *B. cinerea* on cucumbers and strawberry at the relatively low concentrations of 0.125 and 0.047 μl cm^{-3} respectively. These results indicate that after suitable formulation and application essential oils could be used for the control of post-harvest diseases caused by *Botrytis* and *Penicillium* species.

Control of potato late blight (*Phytophthora infestans* [Mont] de Bary) in organic production using resistant varieties and bio-products. S. Zarifi[1], K. Papakyriakidou[2], I.K Vagelas[2], I. Papastylianou[1] and F.T. Gravanis[2]. [1]*Agricultural Research Institute of Cyprus, P.O. Box 22016, 1516 Nicosia, Cyprus.* [2]*Technological Education Institute (TEI) of Larissa, Department of Plant Production, 411 10 Larissa, Greece.*

In a field trial conducted in the Frenaros region of Cyprus, ten tomato cultivars were tested for their resistance to natural infection with late blight. Late blight severity differed significantly between cultivars. It was significantly higher in the cv. Nicola and Rania, followed by cv. Chantal, and severity in this cv. was significantly higher than that in cv. Cara. Late blight severity was very low in the cv. Remarka, Orla, Timate and Appel, while no disease developed in cv. Cicero and Derby. Two independent experiments were conducted to test the efficacy of various plant protection treatments permitted in organic agriculture for controlling *Phytophthora infestans*. A field trial in the Frenaros region in Cyprus tested the efficacy of copper oxychloride, garlic oil and the commercially available products BioVital, Biomax and EM against naturally infected plants. All these substances proved ineffective in controlling the disease. The other experiment was conducted in the Larissa region in Greece, where potatoes (cv. Spunta) grown in pots were artificially inoculated with *P. infestans*. Potato plants were treated (10 replicates per treatment) with copper oxychloride, a local isolate of *Trichoderma viride,* a local isolate of *Gliocladium virens* and the commercial product Seamac. Ten plants were kept as controls. All these treatments controlled late blight. The plants were not left to mature but were harvested one month after planting and the number and weight of the tubers plus the fresh weight of the above-ground plant parts and the disease percentage were determined. A high disease pressure on potato plants artificially inoculated with *P. infestans* and with no other treatment resulted in severe disease symptoms and the premature production of a high number of heavy tubers, statistically different from those with the other treatments and the control. There was no significant difference between any of the treatments and the control in the fresh weight of the above-ground plant parts. Control plants did not produce tubers.

Integrated and Chemical Control

Efficacy evaluation of EPOK 600 EC against *Phytophthora infestans* de Bary on a potato crop. M. Antonakou, T. Arapogiannis and P. Roussos. *Hellafarm S.A, 15 Fleming Str., 151 23 Maroussi, Athens, Greece.*

In autumn 2003, the Field Trial Unit of Hellafarm S.A. conducted a field trial in the Corinthia area to evaluate the efficacy of EPOK 600 EC (fluazinam 40% + metalaxyl-M 20% w:v) against *Phytophthora infestans* on a potato crop. The trial was based on EPPO guideline No 2 (1989) and the instructions of ISK Biosciences Europe, the producer of EPOK 600 EC. Ridomil Gold MZ WP (metalaxyl-M 4% + mancozeb 64% w:w) was used as a reference compound. Three foliage sprays were applied. EPOK 600 was applied at the rate of 400 ml ha^{-1} and Ridomil Gold MZ at 2.5 kg ha^{-1}. The extent and severity of the infection, the marketable yield of visually healthy tubers at harvest and after 35 days of storage were evaluated in nine assessments. EPOK 600 EC reduced infection (2.5% compared to 96.25% in the untreated plants), increased marketable yield (22.386 t ha^{-1} compared with 15.903 t ha^{-1} when untreated) and reduced storage losses (1.6% compared with 14.62% in untreated plants). No statistically significant differences between EPOK 600 EC and Ridomil Gold MZ WP were found. No toxicity symptoms were observed. The trial was repeated in 2004.

Efficacy evaluation of the fungicide Rizolex SC (tolclofos-methyl 50% w:v) against the soil-borne fungi *Sclerotinia sclerotiorum* (Libert) de Bary and *Sclerotinia minor* (Jagger) on lettuce. M. Antonakou, P. Roussos and T. Arapogiannis. *Hellafarm S.A, 15 Fleming Str., 151 23 Maroussi, Athens, Greece.*

In 2003–2004, the Field Trial Unit of Hellafarm S.A. conducted two trials in the Attiki area to evaluate the efficacy of Rizolex SC (tolclofos methyl 50% w:v) against the soil-borne fungi *Sclerotinia* spp. on lettuce, based on EPPO guideline No. 1/80 (1984) and the instructions of Sumitomo Chemical Agro Europe S.A., producer of Rizolex SC. One trial was conducted in a glasshouse and another in a field crop, where *Sclerotinia minor* and *S. sclerotiorum* were identified respectively. Rovral FL (iprodione 25.5% w:v) was used as a reference compound. Both products were applied by soil spraying two days

after transplanting. Rizolex was applied at rates of 2 and 4 l ha^{-1} and Rovral at 0.3 l hl^{-1}. To evaluate the efficacy of the products, three assessments were carried out on the percentage of infected plants and the severity of infection. Final yield was also estimated. Rizolex significantly reduced the number of infected plants compared to the untreated control (from 35 to 16–20% in the field trial and from 55 to 16–20% in the glasshouse trial) with a corresponding increase in yield. No statistically significant differences in efficacy were found between the two fungicides and Rizolex at the applied rates. No phytotoxicity symptoms were observed on lettuce.

Fusarium wilt of alfalfa and methods to control it. D.F. Antonopoulos and K. Elena. *Benaki Phytopathological Institute, 8 S. Delta Str., 145 61 Kifissia, Athens, Greece.*

Fusarium wilt of alfalfa caused by the fungus *Fusarium oxysporum* f. sp. *medicaginis* (Weimer) Snyder & Hansen was first observed in Greece in August 2000. Disease symptoms were chlorosis, yellowing, premature defoliation, wilting, vascular discoloration (dark brown), stunting and death of plants. Inoculations of alfalfa and clover seedlings by root-dipping (10^7 microconidia ml^{-1} for 20 min) demonstrated that all alfalfa varieties were susceptible, while clover varieties remained healthy. In order to find a way to control the disease, Bion, Baba, carbendazim, 231F, a mutant strain of *F. oxysporum* resistant to benomyl and 4F3, a *nit* (nitrate-non utilizing) mutant strain of *F. oxysporum* were tested on alfalfa seedlings, cv. Yliki. To inoculate the alfalfa seedlings, 25 ml of a microconidial suspension of *F. oxysporum* f. sp. *medicaginis* at a concentration of 10^7 ml^{-1} was poured into the soil of each pot. Thirty days after inoculation, carbendazim, 231F and 4F3 were effective in controlling the wilt, while Baba and Bion were ineffective.

Potential use of a specific biostimulator to control *Phytopthora infestans* in greenhouse tomato. V.A Bourbos and E.A. Barbopoulou. *NAGREF, Institute of Olive Tree and Subtropical Plants of Chania, Laboratory of Plant Pathology and Ecotoxicology of Plant Protection Products, Agrokipio, 731 00 Chania, Crete, Greece.*

Tomato late blight, caused by the fungus *Phytopthora infestans* (Mont.) de Bary, is a serious problem for field and greenhouse crops. In this trial the possibility of controlling the pathogen with the protein harpin Ea, a specific biostimulator of the plant defence system at a dose of 0.51 g l^{-1} of the commercial product Messenger® was studied. The fungicide Aliette 80 WP (fosetyl-Al) at the dose of 200 g hl^{-1} was used as a reference product. Efficacy was judged on the basis of the number of lesions on the leaves and stems. Harpin Ea reduced disease incidence significantly by 98.8–100% compared with the untreated control.

The effect of acetic acid and ethanol fumigations on the *in vitro* growth of *Botrytis cinerea*. K. Cetiz[1], A.D. Koukounaras[2], A.L. Lagopodi[3] and E.M. Sfakiotakis[2]. *[1]Mediterranean Agronomic Institute of Chania, 731 00 Chania, Greece. [2]Laboratory of Pomology, Department of Horticulture, Aristotle University of Thessaloniki, 541 24 Thessaloniki, Greece. [3]Laboratory of Phytopathology, Department of Horticulture, Aristotle University of Thessaloniki, 541 24 Thessaloniki, Greece.*

Grey mould, caused by *Botrytis cinerea,* is the most common postharvest disease of vegetables, ornamentals and fruits. The present study was undertaken to investigate the effect of acetic acid and ethanol fumigations on *in vitro* growth of *B. cinerea*. The fungus was grown in petri dishes on potato dextrose agar. Fumigations with 10, 20, 50, 100 and 200 μl l^{-1} of ethanol for 3 and 6 min each neither enhanced nor inhibited *B. cinerea* mycelial growth. Fumigations with different concentrations of acetic acid however sometimes enhanced, and sometimes delayed or inhibited *in vitro* growth of *B. cinerea*, depending on the concentration. Fumigations with 1 and 2 μl l^{-1} of acetic acid for 3 and 6 min each and with 4 μl l^{-1} of acetic acid for 3 min caused a threefold increase in growth (%) compared with the control for the first 2–4 days. However, from the fifth day the mycelial growth rate was the same for all treatments. Finally, fumigation with 8 μl l^{-1} of acetic acid for 6 min, completely inhibited fungal growth.

Dissipation of famoxadone in the cultivated mushroom *Agaricus bisporus*. M. Chrysayi[1], S. Coward[1], M. Kastanias[2], P. Diamantopoulou[3] and A. Filippoussis[3]. *[1]Agricultural University of Athens, Pesticide Science Laboratory, 75 Iera Odos, 118 55 Athens, Greece. [2]Ministry of Rural Development and Food, General Directorate of Plant Produce, Directorate of Plant Produce Protection, Department of Pesticides, 3-5 Ippokratous Str., 101 64 Athens, Greece. [3]National Agricultural Research Foundation, Institute of Agricultural and Environmental Technology, Laboratory of Edible Fungi, 61 Democratias Str., 135 61 Athens, Greece.*

The need for fungicides to be highly selective, as well as other crop-related limitations, has led to a scarcity of fungicides suitable specifically against fungi infecting cultivated mushrooms. Fungicides used against mushrooms must not only be effective but must also be of low persistence because of the short crop cycle and the consecutive production of flushes. A study was undertaken to investigate the effectiveness of famoxadone, a new fungicide belonging to the oxazolidinediones, against dry bubble disease of *Agaricus bisporus* caused by *Verticillium fungicola*. Efficacy and residue trials were conduct-

ed in mushroom growing rooms to establish optimal formulations and dosages. Formulations used were: as single (5 days after casing) or split (5 and 12 days after casing) drench applications at rates from 0.1 to 1 g a.i. m^{-2} of culture area. For residue analysis, mushroom samples were taken from three consecutive flushes, on the day of peak production of each flush. Famoxadone residues were determined using GC-ECD. The limit of the quantification of the method used was 0.009 mg kg^{-1}. The dissipation rate of famoxadone in mushrooms is rather fast. The residues detected in all samples were below 0.1 mg kg^{-1} even in cultures that had been exposed to the highest rate. The established MRL for famoxadone in mushrooms is 0.02 mg kg^{-1} (Directive 03/60/EC) which is the limit of quantification due to the lack of residue studies for this compound. The above value has been provisionally set mainly for residue monitoring and it will be valid until July 2007, unless new data come to light. Based on residue trials, the Community established MRLs for famoxadone in other crop commodities such as tomatoes and table grapes are 0.2 and 2 mg kg^{-1} respectively.

Dissipation of trifloxystrobin in the cultivated mushroom *Agaricus bisporus*. M. Chrysayi[1], M. Kastanias[2], P. Diamantopoulou[3] and A. Filippoussis[3]. *[1]Agricultural University of Athens, Pesticide Science Laboratory, 75 Iera Odos, 118 55 Athens, Greece. [2]Ministry of Rural Development and Food,General Directorate of Plant Produce, Directorate of Plant Produce Protection, Department of Pesticides, 3-5 Ippokratous Str., 101 64 Athens, Greece. [3]National Agricultural Research Foundation, Institute of Agricultural and Environmental Technology, Laboratory of Edible Fungi, 61 Democratias Str., 135 61 Athens, Greece.*

In a project to investigate new compounds against dry bubble disease of *Agaricus bisporus* caused by *Verticillium fungicola,* the selective activity of trifloxystrobin against the pathogen was evaluated. The effectiveness of this new fungicide, which belongs to the oximinoacetates, was evaluated and residue trials were conducted in mushroom growing rooms. Determination of the residues is important due to the short crop cycle and the consecutive production of flushes, which begins about 18 days after casing. Trifloxystrobin was used in single (5 days after casing) or split (5 and 12 days after casing) drench applications at rates from 0.4 to 1.8 g a.i. m^{-2} of culture area. For residue analysis, mushroom samples were taken from three consecutive flushes, on the day of peak production of each flush. Samples were analyzed for the parent compound and for the metabolite CGA 321113 as well. Determination of residues was made using GC-ECD, and the limits of the quantification of the method used were 0.001 and 0.003 mg kg^{-1} for trifloxystrobin and CGA 321113, respectively. The dissipation rate of both compounds in mushrooms was rather fast. The residues detected in all samples were below 0.08 mg kg^{-1} for the parent compound and 0.05 mg kg^{-1} for CGA 321113, even in samples from cultures that had been exposed to the highest rate. Trifloxystrobin has been included in Annex I of Directive 91/414/EEC, but Community MRLs have not been established. In Greece the fungicide has been registered to be used on apple, pear and grapevine and the national MRLs have been set for the corresponding produce at 0.5 mg kg^{-1}.

Effect of flusilazole and fludioxonil-resistant mutations on aflatoxin production by *Aspergillus parasiticus* Speare. E.G. Doukas, A.N. Markoglou and B.N. Ziogas. *Agricultural University of Athens, Laboratory of Pesticide Science, 75 Iera Odos, Votanikos, 188 55 Athens, Greece.*

Mutants of *Aspergillus parasiticus* resistant to: (i) the triazole fungicide flusilazole, and (ii) the phenylpyrrole fludioxonil, were isolated from a wild-type strain, after UV-mutagenesis. Study of fitness parameters in wild-type and representative mutant isolates showed two phenotypes in mutant isolates for both fungicides: (a) strains with good mycelial growth and conidial production, and (b) strains with low mycelial growth and sporulation. Cross-resistance studies with other fungicides showed that: in the first phenotypic class, the mutation(s) for resistance to flusilazole affected the sensitivity of mutant strains only to the sterol biosynthesis inhibitors (SBIs) imazalil and tebuconazole and the mutation(s) for resistance to fludioxonil reduced sensitivity only to the aromatic hydrocarbons and to the dicarboximide fungicides (AHDs) iprodione and tolclofos-methyl. By contrast, in the case of mutants from the second phenotypic class, the mutation(s) for resistance to triazole and phenylpyrrole fungicides reduced the sensitivity of mutant strains to the benzimidazole benomyl, the anilinopyrimidine cyprodinil, and the phenylpyridinamine fluazinam, but not to the strobilurin azoxystrobin and the non-specific fungicides chlorothalonil and maneb. These results show the function of two mechanisms of resistance, site modification and increased efflux, for the first and second phenotype respectively. When the effect of the mutations on aflatoxin production by the wild-type and representative mutant strains of *A. parasiticus*, with chromatographic (TLC, HPLC-FLD and HPLC-MS) and immunochemical techniques (ELISA), was studied, the aflatoxins B_1, B_2, G_1 and G_2, were produced, with B_1 and G_1 at higher concentrations. Aflatoxin production by the first phenotypic class of flusilazole-resistant strains was five times higher than that by the wild-type. A correlation between conidial and aflatoxin production was not observed in the case of fenpiclonil-resistant strains, as aflatoxigenic or non-aflatoxigenic strains were found in the first

phenotypic class. The productivity of aflatoxins by some of the mutant strains was even higher when flusilazole or fludioxonil occurred in the culture medium. However, all mutant isolates of the second phenotypic class did not produce aflatoxin. The mechanism of increased efflux due to ABC or MFS-transporters probably causes the increase in aflatoxin production and the non-specific multidrug resistance. However, it seems that these two phenomena are not connected, apparently because of the function of different protein-transporters.

Compatibility of chlorothalonil and chlorpyrifos-ethyl formulations. M. Georgitsanakou and M. Chrysayi. *Agricultural University of Athens, Pesticide Science Laboratory, 75 Iera Odos, 118 55 Athens, Greece.*

The compatibility of plant protection products with each other is an important consideration in agricultural practice. Tank mixes of two or more products are often used, but sometimes such mixed applications have undesirable effects, mainly reduced pest control and damage to non-target plants. The objective of this work was to study the physical and biological compatibility of a wettable powder formulation of the fungicide chlorothalonil with an emulsifiable formulation of the insecticide chlorpyrifos-ethyl, which are often tank partners. Tests on the stability, homogeneity, pH and temperature of the mixture showed that the components were mixable in terms of physical compatibility. Possible changes in the biological efficacy of the combination were evaluated by phytotoxicity and fungitoxicity tests. Tomato, cucumber and string bean seedlings were used as plant indicators in the phytotoxicity tests. The plants were sprayed either with mixtures of both formulations or with their active ingredients. Each product was sprayed at the full recommended and at half and one fourth the full rate. Seedlings sprayed with water and preparations of each formulation at the full recommended, half and twice the full recommended rates were used as controls. All seedlings sprayed with the formulation combination showed phytotoxicity symptoms even at the lowest application rate. By contrast, plants exposed to a single product developed normally, even when the product was sprayed at twice the recommended rate. The severity of the phytotoxicity symptoms was directly proportional to the application rate of the formulations. Since such effects were not observed on seedlings exposed to mixtures of active ingredients, the phytotoxicity of the formulations cannot be attributed to the incompatibility between chlorothalonil and chlorpyrifos-ethyl, but must be due to the adjuvants, solvents or diluents present in the formulations. In order to evaluate the fungitoxicity of the mixture, the *in vitro* activity of chlorothalonil on *Botrytis cinerea* was examined in the presence of chlorpyrifos-ethyl. The fungus was grown on PDA amended with chlorothalonil and chlorpyrifos-ethyl at concentrations ranging from 1 to 50 μg ml^{-1}. Fungal cultures on medium containing each fungicide alone were also examined. The evaluation of the fungitoxicity was based on the inhibition of mycelium growth and on the morphology of hyphae and conidia. The results indicated a synergistic effect because chlorothalonil fungitoxicity was enhanced by 39.8% in the presence of chlorpyrifos-ethyl, whereas chlorpyrifos-ethyl by itself had no effect on the growth of *B. cinerea*.

Integrated management of soil-borne pathogens of greenhouse tomato with solarization and other soil treatments. N. Ioannou and G. Neophytou. *Agricultural Research Institute, P.O. Box 22016, 1516 Nicosia, Cyprus.*

There is a pressing need to find alternative methods for the effective and sustainable management of soil-borne pathogens in view of the forthcoming ban on methyl bromide for soil fumigation purposes. To this end, a series of field experiments were carried out in Cyprus, evaluating soil solarization, alone, or in combination with other soil treatments, including: 1) mild soil fumigants such as 1,3 dichloropropene (1,3 D), metham sodium and dazomet, 2) natural products such as organic matter, chitin and plant extracts, and 3) biological control agents, including the bacteria *Bacillus firmus* and *Burkholderia cepacia* and the fungi *Myrothecium verrucaria* and *Paecilomyces lilacinus*. All experiments were carried out in tomato greenhouses, naturally infected with root knot nematodes and major soil-borne fungi, such as: i) *Fusarium oxysporum* f. sp. *lycopersici* and *Verticillium dahliae* causing vascular wilt diseases, ii) *F. oxysporum* f. sp. *radicis-lycopersici* and *Phytophthora* spp. causing root and crown rots, and iii) *Pyrenochaeta lycopersici* causing corky root rot. Various types of soil solarization were tried for optimal effectiveness against soil-borne pathogens. Best results were achieved with virtually impermeable film (VIF) for soil mulching and by carrying out solarization in a fully enclosed greenhouse. Combining these two improvements reduced treatment to 3–4 weeks compared with the 6–8 week normally required. The three mild soil fumigants tested were fully compatible with soil solarization, broadening its range of activity and improving its effectiveness against soil-borne pathogens. Thus, the combined use of solarization and mild soil fumigants appears to be an effective, economically viable and environmentally acceptable alternative to methyl bromide fumigation in greenhouse–grown tomatoes. In contrast, all natural and biological products tested, alone or in combination with solarization, gave inconsistent results and their use in practice will still require more research.

Effectiveness of new fungicides against *Camarosporium pistaciae* in pistachio. A. Kalamarakis,

E. Markellou and K. Elena. *Benaki Phytopathological Institute, 8 S. Delta Str., 145 61 Kifissia, Athens, Greece.*

The fungus *Camarosporium pistaciae* has caused severe damage to pistachio orchards in Greece over the last few years. The fungus infects shoots and leaves but infections of clusters, which start in spring, are of greater importance. If secondary infections occur close to harvest time under conditions of high temperature and rain, they will cause very considerable yield losses. For this reason, the Ministry of Rural Development and Food funded a research project for the control of the disease. In the framework of this project, *C. pistaciae* strains were collected from isolates obtained from commercial orchards in 2003–2004. Bioassays were carried out to test the sensitivity of the fungus (mycelial growth) to a number of fungicides. *C. pistaciae* was moderately to highly sensitive to fungicides of the following groups: 1. QoIs (former name strobilurins), azoxystrobin, pyraclostrobin, trifloxystrobin and famoxadone (EC_{50} 0.0237, 0.224, 0.158 and 0.272 μg ml^{-1} respectively); 2. sterol biosynthesis inhibitors/subgroup triazoles, tebuconazole (EC_{50} 0.141 μg ml^{-1}); 3. anilides, boscalid (EC_{50} 0.692 μg ml^{-1}) and to the mixture pyraclostrobin+boscalid (EC_{50} 0.032 μg ml^{-1}). At the same time, preliminary field trials were conducted in the area of Fthiotida to test the effectiveness of several fungicides in commercial pistachio orchards. These trials are still in progress.

Comparison of deterministic and probabilistic models for dietary risk assessment to plant protection products. M. Kastanias[1], K. Kokkinaki[1], K. Machera[2] and D. Nikolopoulou[2]. [1]*Ministry of Rural Development and Food, General Directorate of Plant Produce, Directorate of Plant Produce Protection, Department of Pesticides, 150 Syngrou Ave., 176 71 Athens, Greece.* [2]*Benaki Phytopathological Institute, Department of Pesticides Control, Laboratory of Pesticides Toxicology, 7 Ekalis Str., 145 61 Kifissia, Athens, Greece.*

The use of plant protection products (ppps) in agricultural practice may leave residues in agricultural products. Therefore, maximum residue limits (MRLs) have been established. MRLs define the maximum legally admissible concentrations of ppps in food. Dietary exposure to ppps residues does not necessarily cause undesirable health effects and therefore it is essential to perform dietary risk assessment, a multidimensional procedure that links residue studies with mammalian toxicology. The aim of this project was the parallel application and comparison of the mathematical models (deterministic and probabilistic) used for dietary risk assessment. The deterministic models, which are currently used officially at E.U. level, are based on extreme trigger values reflecting a "worst-case scenario". The probabilistic models, which are still under development, incorporate the distribution of the uncertainties of all the factors involved and estimate the probability of a value to lie within a specific range. From the parallel application and comparison of the two models it was concluded that whilst the deterministic models are easy to comprehend and apply, they are likely to overestimate risk and produce unrealistic results. Probabilistic models on the other hand are more realistic and reliable indicators of the dietary risk of ppps residues. However, as far as residue and dietary data are concerned, probabilistic models are more demanding and complex in their use and interpretation.

Use of (E)-2-hexenal to control postharvest rot of table grapes caused by *Botrytis cinerea*. D. Lascaris and A. Lambrou. *Benaki Phytopathological Institute, Department of Phytopathology, 8 S. Delta Str., 145 61 Kifissia, Greece.*

The effectiveness of (E)-2-hexenal, a naturally occurring aldehyde, in controlling post harvest rots of table grapes (cv. Sultanina), caused by *Botrytis cinerea* was studied in comparison with the commonly used SO_2. Grapes or grape berries naturally infected or artificially inoculated with *B. cinerea* spores were placed in small plastic containers and fumigated with (E)-2-hexenal or SO_2 at 3°C. Mould development and berry quality were assessed after aeration and incubation for 3–7 days at 20°C. Fumigation with 9.1 mg l^{-1} (E)-2-hexenal for 30 and 60 min reduced mould incidence by 47 and 83%, respectively, compared with the control. (E)-2-hexenal at 4.5 and 9.1 mg l^{-1} for 24 h reduced mould incidence by 57 and 75% respectively, compared with the control. Doses of 27.3 and 91 mg l^{-1} of (E)-2-hexenal inhibited mould development by 100%. Fumigation with 4.5 mg l^{-1} for 72 h reduced mould incidence by 62%, whereas concentrations ≤ 2.2 mg l^{-1} were not effective. (E)-2-hexenal also inhibited *Penicillium* spp., *Aspergillus* spp. and *Rhizopus* spp. Higher doses of (E)-2-hexenal or SO_2 had to be applied in containers filled up with grapes to achieve equal effectiveness. Although during the initial stages of infection, SO_2 was more effective than (E)-2-hexenal in inhibiting or delaying symptom appearance, none of these substances controlled well-established infections. (E)-2-hexenal and SO_2 were phytotoxic at high doses. Direct fumigation with SO_2 was less effective than fumigation with the commercial in-package SO_2 generators. Grapes treated with (E)-2-hexenal had a slight odour, which disappeared after aeration for 24 h.

Assessment of spray operator exposure to plant protection products and other requirements for their safe use. K. Machera[1], E. Kapetanakis[2] and A. Charistou[1]. [1]*Benaki Phytopathological Institute, Department of Pesticides Control, Laboratory of Pesticides Toxicology, 7 Ekalis Str., 145 61 Kifissia, Athens Greece.* [2]*Technological Education Institute of Crete, School of*

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For use of plant protection products (ppps) to be safe to the spray operator and to farm workers in general the following are required: (1) reliable assessment of the exposure of all farm workers to ppps, with emphasis on the spray operator. In the European Union, existing models are applicable only to spray-operator exposure. These models have not been externally verified, they are not suitable for all ppp applications and they are over-simplified. Recently there has been a tendency to use probabilistic models, which take into account the variability and uncertainty of the main exposure assessment parameters. For example, it was found that during penconazole spraying of vineyards, operator exposure levels varied from 6.6 to 122 ml h^{-1} and that during penconazole spraying of greenhouse tomato it varied from 47 to 137 ml h^{-1}. When comparing these experimental values to those calculated on the basis of the models, it is evident that the actual operator exposure levels are significantly higher than the values used for risk assessment. (2) Correct and complete ppp label. The information included on the label today, although in compliance with national and E.U. legislation regarding hazards, it does not include requirements regarding personal protective equipment (ppe). (3) Determination of acceptable procedures for the choice of the ppe required for the recommended uses of the ppp. (4) Availability of the recommended ppe and other means to limit exposure. The ppe recommended on the label must be suitable for the environmental conditions and farming procedures of the country, with specified levels of protection. These levels should be arrived at by appropriate evaluations under local conditions. (5) Compliance of farm workers with ppp application safety procedures and correct use of ppe. Compliance with good agricultural practices, including worker training on safe use of ppp, is necessary.

Study of the inherent resistance risk to pyraclostrobin in *Botrytis cinerea*. A.A. Malandrakis, A.N. Markoglou and B.N. Ziogas. *Agricultural University of Athens, Laboratory of Pesticide Science, 75 Iera Odos, Votanikos, 188 55 Athens, Greece*.

After chemical mutagenesis with *N*-methyl-*N*-nitrosoguanidine (MNNG) one phenotype that was moderately resistant (Rf: 10–20, based on MICs) and one that was highly (Rf: 70–200) resistant to pyraclostrobin (strobilurin-QoIs), were isolated from a wild-type strain of *Botrytis cinerea*, with a high mutation frequency of 6.2×10^{-4}. Cross-resistance studies with other fungicides showed that the mutation(s) for resistance to pyraclostrobin reduced the sensitivity of mutant isolates to azoxystrobin (strobilurin-QoIs) (Rf: 30–70), but not to famoxadone (azolone/oxazolidinedione-QoIs), to cyazofamid (cyanoimidazole-QiIs) or to antimycin A (QiIs). Furthermore, there was no reduction of sensitivity to the hydroxyanilidine fenhexamid, to the benzimidazole benomyl or to the phenylpyridinamine fluazinam, which affect other steps of the cellular pathways. The pyraclostrobin-resistant strains were more sensitive to the anilide boscalid and the anilinopyrimidine cyprodinil (Rf: 0.2 to 0.4). A study of fitness parameters of the pyraclostrobin-resistant isolates of both phenotypes showed that these mutation(s) had no effect on mycelial growth and pathogenicity, and may or may not have affected some other phytopathogenic fitness parameters, such as sporulation, conidial germination and sclerotia production. Preventive applications of a commercial product of pyraclostrobin, were effective against lesion development on cotyledons by the wild-type, but were ineffective against disease caused by the pyraclostrobin-resistant isolates, even at a concentration of 1,500 μg ml^{-1} Experiments on the stability of pyraclostrobin-resistant phenotypes showed a significant reduction of resistance when the mutants were grown on an inhibitor-free medium. There was a recovery of the resistance level after mutants were returned to the selection medium. *In vitro* studies on the competitive ability of resistant isolates against wild-type strain of *B. cinerea* by applications of a mixed population showed a significant reduction of resistant isolates with a corresponding increase in the wild-type population. The risk of resistance arising during the commercial use of pyraclostrobin is discussed in the light of these results.

Study of the effectiveness of flavone against *Botrytis cinerea, Penicillium digitatum, Aspergillus flavus* and *A. parasiticus*. A.N. Markoglou, S.E. Fragkiadakis and B.N. Ziogas. *Agricultural University of Athens, Laboratory of Pesticide Science, 75 Iera Odos, Votanikos, 118 55 Athens, Greece*.

Many plants, particularly from the hot regions, excrete from their leaf surfaces a complicated mix of organic substances, mainly terpenoids, flavonoids and simple phenolic compounds, which mainly accumulate in the epicuticula. These secondary plant products have a wide range of biological and ecological properties. Besides other roles, they are involved in the chemical communication between organisms, in the defence of plants against micro-organisms and in the determination of competitive relations in the ecosystem. The antioxidant activity of flavonoids is an interesting case. The *in vitro* toxicity of flavone to *Botrytis cinerea*, *Penicillium digitatum*, *Aspergillus flavus* and *A. parasiticus* and its effectiveness in controlling grey mould on cucumber seedlings was investigated in this study. *In vitro* experiments with isolates of *B. cinerea* sensitive and resistant to the aromatic hydrocarbon, dicarboximide and phenlpyrrole fungicide group, hydroxyanilidines and strobilurin fungi-

cides showed that flavone totally inhibits mycelial growth and spore germination of the wild-type and mutant isolates of *B. cinerea* at a concentration of 25 μg ml^{-1} . Furthermore, at a concentration of 5 μg ml^{-1} flavone considerably delayed the germination of conidia and reduced the number of germ tubes. By contrast, flavone did not control *P. digitatum*, *A. flavus* and *A. parasiticus in vitro*, even at concentrations over 200 μg ml^{-1}. *In planta* pot experiments with preventive applications of flavone showed that the formation of lesions on cucumber seedlings by the wild-type strain of *B. cinerea*, was completely inhibited at a concentration of 1,500 μg a.i. ml^{-1}. A study to determine the mechanism of action of flavone showed that it inhibited alternative respiration in *B. cinerea*. Respiration of germinated conidia was inhibited up to 80% by pyraclostrobin (0.5 μg ml^{-1}) and up to 0% by flavone (50 μg ml^{-1}). When pyraclostrobin (0.5 μg ml^{-1}) and flavone (5 μg ml^{-1}), or pyraclostrobin (0.5 μg ml^{-1}) and SHAM (1 mM) were present in the respiratory substrate, oxygen uptake by germinated cells of *B. cinerea* was completely inhibited. These results show that alternative oxidase in *B. cinerea* is active in the presence of inhibitors of the cytochrome pathway, which is inhibited effectively by flavone or SHAM. SHAM is known to be a specific inhibitor of the alternative respiratory pathway. Similar results were also observed with fludioxonil and fenhexamid-resistant mutants. These results show that flavonoids may play an important role in the effectiveness of cytochrome-III inhibitors against plant pathogens. However, oxygen consumption by pyraclostrobin resistant mutants was not reduced in the presence of flavone or SHAM, indicating that the resistance mechanism to pyraclostrobin was not due to the function of alternate oxidase.

Management of panicle and shoot blight caused by *Botroysphaeria dothidea* in California pistachios. T.J. Michailides, Z. Ma, D.P. Morgan and D. Felts. *University of California Davis, Department of Plant Pathology, Kearney Agricultural Center, 9240 South Riverbend Ave., Parlier CA 93648, USA.*

Since 1998, a severe epidemic of panicle and shoot blight caused by *Botryosphaeria dothidea* has become a major concern of the California pistachio industry. During the same time period, there were reports that the same disease also caused major losses in pistachios grown in Makri, Lamia Prefecture, Greece. A personal visit of the first author to these pistachios in March 2001 confirmed the presence of multiple cankers bearing characteristic pycnidia of the pathogen. During 1999 to 2004 with financial support of the California Pistachio Industry, research in our laboratory has developed a) techniques for monitoring latent infections by the pathogen in host tissues; b) species-specific PCR (polymerase chain reaction) primers that can detect *B. dothidea* from California pistachio and other plant hosts, but not those from Greek pistachios; c) microsatellite-PCR and vegetative compatibility grouping techniques for analyzing spatio-temporal changes in the populations of *B. dothidea* from pistachio orchards, which suggested that *B. dothidea* populations are spatially and temporally stable, and d) methods for the cultural, chemical, and integrated control management of the disease. Strobilurins are the most effective fungicides for controlling the disease. *B. dothidea* has not developed resistance after multiple applications of strobilurins for more than 5 years now.

Sensitivity of *Septoria pyricola* Desm. isolates to strobilurins, benzimidazoles and ergosterol biosynthesis inhibitors. I.S. Mylonopoulos, A.C. Pappas and E.K. Vellios. *University of Thessaly, Department of Agriculture Crop Production and Rural Environment, Laboratory of Plant Pathology, 384 46 N. Ionia, Magnissia, Greece.*

In summer 2003, a serious septoriosis infection appeared in pear orchards var. Krystalli in Magnissia prefecture. Benzimidazoles (Ben) and ergosterol biosynthesis inhibitors (EBIs) are mainly used for the control of the disease. In the present work, the sensitivity of *Septoria pyricola* was tested *in vitro* to the above groups of fungicides and to the recently introduced strobilurins (QoI), which have not been used against pear septoriosis in agricultural practice so far. Ten single-spore isolates from commercial pear orchards were randomly selected and their sensitivity to carbendazim (Ben), bitertanol (EBIs, triazoles) and kresoxim methyl (QoI) was tested. The assays were carried out in Petri dishes with PDA enriched with five different concentrations of commercial formulations of the above fungicides dissolved in dimethyl sulfoxide. In certain assays with kresoxim methyl, 150 mg l^{-1} of SHAM (an alternative respiration inhibitor), were added to the medium. A hundred and fifty μl of a spore suspension (3,000 spores ml^{-1}), prepared from 3-weeks-old cultures, was used as inoculum. After 3 and 8 days of incubation at 24°C in the dark, the number of germinated spores (in a sample of 150 spores dish^{-1}) and the number of colonies per dish, were recorded respectively. With the exception of one isolate of *S. pyricola* (1 M) that was isolated from an unsprayed orchard, the concentration of 100 mg l^{-1} carbendazim had no effect on normal spore germination and colony growth. The isolate 1 M, that was characterized as sensitive to the benzimidazoles, failed to grow even at a concentration of 0.1 mg l^{-1} of carbendazim. The other two fungicides were equally effective against all isolates of *S. pyricola* tested. Concentrations of 0.01 mg l^{-1} of bitertanol and 0.1 mg l^{-1} of kresoxim methyl or 0.001 mg l^{-1} of kresoxim methyl + 150 mg l^{-1} of SHAM inhibited normal spore germination and colony growth of all isolates tested. In this work, *S. pyricola* strains with resistance

to benzinidazoles were characterized and detected in high frequency for the first time, worldwide, and a proper method was developed for monitoring sensitivity changes to the above fungicides in the wild population of *S. pyricola*.

Preliminary study on hymexazol efficacy to control root and stem rot of cucumber caused by *Fusarium oxysporum* f. sp. *radicis-cucumerinum*. G.C. Pavlou. *National Agricultural Research Foundation, Olive and Horticultural Crops Institute, 85 Lakonikis Str., 241 00 Kalamata, Greece*.

Hymexazol (Tachigaren 36 SL) controls various *Fusarium* spp. and other soil-borne fungi. In the present study, the efficacy of hymexazol on *Fusarium oxysporum* f. sp. *radicis-cucumerinum,* which causes root and stem rot of cucumber, was studied. The following two treatments were applied in 10 l pots containing soil disinfected with methyl bromide and then artificially inoculated with conidia of *F. oxysporum* f. sp. *radicis-cucumerinum:* (a) application of hymexazol every 10 days via irrigation at a rate of 0.25 ml commercial solution/cucumber plant (pot), and (b) no application of hymexazol (control). Between the 10-day intervals a soluble fertilizer 20-20-20 with microelements at the rate of 1‰ was added to the irrigation water. The completely randomized experimental design was carried out in an unheated greenhouse at the Ierapetra Agricultural Research Station during the crop season from November 1998 to March 1999 (from transplanting to plant death on account of the disease). Each treatment consisted of six pots with one cucumber plant (Brunex F1) per pot. Results showed that plant growth (final average plant height and number of leaves/plant) at the end of the cultivation period was significantly higher with treatment (a). Disease incidence with treatments (b) and (a) was 16.7 and 0% one month after transplanting, and 83.3 and 16.7% after two months respectively. After three months the disease incidence was 83.3% for both treatments while the percentage of dead plants was 83% with treatment (b) and 33.3% with treatment (a). It was concluded that hymexazol at the dose recommended by the manufacturer significantly reduced disease incidence for a period of 2 months after transplanting. Further trials should be conducted, under commercial conditions to evaluate the efficacy of hymexazol against *F. oxysporum*. f. sp. *radicis–cucumerinum*

Integrated disease management on vegetables using the new fungicide Signum (pyraclostrobin/boscalid): field trial data on tomatoes, carrots and asparagus crops. D. Servis, S. Bitivanos, K. Bozoglou and K. Stavropoulos. *BASF Agro Hellas SA., Technical Department, 48 Egialeias Str., 151 25 Athens, Greece.*

Signum is a novel multi-spectrum fungicide formulated as wettable granules containing 6.7% pyraclostrobin and 26.7% boscalid, and suitable for foliar applications on tomatoes, carrots and asparagus. Signum protects these crops from a complex of foliar diseases. The average application rate is 0.15% (w:v), to be applied in a preventive program against grey mould (*Botrytis cinerea*) and powdery mildew (*Leveilulla taurica*) with secondary action against late blight (*Phytophthora* spp.) and early blight (*Alternaria* spp.). In carrots, Signum is applied at the same rate to control blight (*Alternaria dauci*) and powdery mildew (*Erysiphe umbelliferarum*), while on asparagus it protects the foliage against rust (*Puccinia asparagi*) and stemphylium *(Stemphylium versicarium*). Signum applications should begin in a preventive scheme, prior to infection by the target pathogens, or at the very early stages of infection when symptoms are not yet visible, or when environmental conditions are conducive for the diseases and the crops are at a susceptible stage. In Greece, Signum was tested in replicated field trials during the 2001–2004 growing seasons. Signum showed high efficacy following natural infection or artificial inoculation as compared to standard fungicides and improved the economic value of the crop due to its wide disease control spectrum. The performance features of Signum in terms of its biological efficacy, spectrum of activity, selectivity and environmental safety will contribute significantly to the protection of vegetable crops and support the integrated production of high quality agricultural products.

Integrated disease management on fruit trees using the new fungicides Bellis and Signum (pyraclostrobin/boscalid). Field trial data on apples and cherries. D. Servis, S. Bitivanos, K. Bozoglou, K. Stavropoulos and K. Tsakiri. *BASF Agro Hellas SA., Technical Department, 48 Egialeias Str., Athens, Greece*.

The novel active ingredients pyraclostrobin and boscalid, formulated as Bellis (12.8% pyraclostrobin and 25.2% boscalid) 300 SC (suspension concentrate) and Signum (6.7% pyraclostrobin and 26.7% boscalid) 334 WG (wettable granules), are new multi-spectrum fungicides, developed for horticultural crops to control scab (*Venturia inaequalis, V. pyrina*), powdery mildew (*Podosphaera leucotricha*) and Monilia fruit blight (*Monilia* spp.). In field trials during the 2001–2004 growing seasons, Bellis was tested in foliar spray programs starting before flowering, at the green bud stage and repeated at 14-day spray-intervals. Fungicide evaluation was based on season-long spray programs involving continuous use of the fungicide, as well as on practical spray programs with Signum and Bellis alternating with other fungicides having different modes of action. Bellis was highly effective in controlling apple scab at 1.0 l ha^{-1} and showed good activity against powdery mildew, as compared to the untreated plots and standard products. In

cherries, Signum was applied at the rate of 0.75 kg ha^{-1} three times at 7-day intervals, starting at the beginning of flowering. These formulations showed significantly greater efficacy than the standard triazole fungicides and were similar in efficacy to prochloraz. The two fungicide formulations were tested in field and semi-field selectivity trials on beneficial fauna. Bellis and Signum were safe on the predators *Euseius finlandicus* and *Coccinella semptepunctata* and were moderately toxic to *Orius insidiosus* and *Kampimodromus aberans*, at levels not significantly different from a wettable sulfur formulation. The improved features of the two fungicide formulations in terms of biological efficacy, selectivity and toxicology of the active ingredients will greatly contribute to the protection of these crops and will support integrated fruit production in Greece.

***Aspergillus niger* and *A. carbonarius* in Corinth raisin and wine-producing vineyards in Greece: population composition, ochratoxin A production and chemical control.** S.E. Tzamos[1], P.P. Antoniou[1], A. Kazantzidou[2], D.F. Antonopoulos[1], I. Papageorgiou[2] and E.K. Tzamos[1]. *[1]Agricultural University of Athens, Laboratory of Plant Pathology, 75 Iera Odos, 118 55 Athens, Greece. [2]Syngenta Hellas S.A., 153 49 Anthoussa, Athens, Greece.*

Vineyard surveys of the Corinth raisin cultivar carried out in the Peloponnese region of Greece during 2002 and of the wine-producing grape cultivars Cabernet Sauvignon and Grenache Rouge on the island of Rhodes, Greece, in 2003, detected various *Aspergillus* spp. in berries of bunches at harvest. *Aspergillus niger* and *A. carbonarius* were predominantly isolated from the berries sampled. Although the prevailing *Aspergillus* spp. isolates belonged mainly to the *A. niger* aggregate, isolates of *A. carbonarius* were by far the most efficient Ochratoxin A (OTA) producers as revealed by the enzyme-linked immunosorbent assay. This study provides the first evidence concerning the composition of *Aspergillus* populations in raisin and wine-producing vineyards and offers convincing data for their ability to produce various levels of OTA in Corinth raisins and wine-producing grapes in Greece. Furthermore, it demonstrates that chemical applications with the fungicide Switch, especially at low to intermediate severity of *Aspergillus* infection of vineyards, could both significantly reduce the occurrence of OTA-producing *Aspergillus* spp. and restrict sour rot severity. In contrast, vineyard applications with the fungicides Carbendazim or Chorus were ineffective in controlling these fungi in Corinth raisin cultivar.

The possibility of utilization of bioactivators against downy mildew of grapevine. G. Tzagaraki, S. Milla, A. Sclavounos and I. Samaras. *Technological Educational Institute of Ionian Islands, Branch of Argostoli, Department of Biological Agriculture, 281 00 Argostoli, Cephalonia, Greece*

The efficacy of salicylic acid (SA,1 mM, pH=6,5, BION MX 44 WG (acibenzolar-S-methyl 4%+metalaxyl-m 40), BIOCYT (extract from seeds of citrus fruits) and BEST BASE (extract from the seaweed *Ascophyllum nobosum*) against downy mildew was tested on European vine plants (*Vitis vinifera*) variety Zakynthino and compared with copper oxychloride. The above products were applied as sprays at weekly intervals, with the first spraying carried out when plant height was about 8 cm. By the fourth week of spraying, treated plants showed statistically significant differences in the number of infected leaves and leaf lesions compared with the untreated control. By the sixth week, plants treated with SA, BTH, Biocyt and Best Base had respectively only 65, 41, 62 and 40% of the number of infected leaves of the untreated control, while with copper oxychloride the decrease in infected leaves was 68%. Moreover, with SA, BTH, and Biocyt the number of leaf lesions decreased by 68, 42, and 33% respectively, compared with the untreated control, while with copper oxychloride it was 68%.

Preliminary results on fungicide evaluation for the control of Septoria leaf spot on pear (var. Krystalli). E.K. Vellios, I.S. Mylonopoulos and A.C. Pappas. *University of Thessaly, Department of Agriculture Crop Production and Rural Environment, Laboratory of Plant Pathology, 384 46 Nea Ionia, Magnissia, Greece.*

Septoria leaf spot (*Septoria piricola* Desm.) is an endemic disease in the Magnissia Prefecture (Greece), and has reached epidemic levels in the last few years. Fungicides of the benzimidazole (Ben) and ergosterol biosynthesis inhibitors (EBIs) classes are commonly used to control leaf spot. The aim of this study was to evaluate four widely used fungicides, as well as a fungicide belonging to the strobilurin (QoI) family, which has not yet been commercially used to treat Septoria leaf spot. The fungicides assessed were: (a) one bezimidazole, carbendazim (Carbendazim 50WP 90 g 100 l^{-1}), (b) two triazoles of the EBI family, bitertanol (Baycor 25 WP 75 g 100 l^{-1}) and flusilazol (Punch 40 EC 6.5 ml 100 l^{-1}), (c) chlorothalonil (Daconil 75 WP 130 g 100 l^{-1}) and kresoxim-methyl (Stroby 50 WG 20 g 100 l^{-1}) of the QoI family. Three trees were used for each fungicide treatment, while a group of three trees was used as an unsprayed control (eighteen trees in total, approximately 15 years old). At each spraying the trees were totally covered with fungicide solution (15 l of fungicide solution per tree) using a mist blower (450 psi). The treatments started from the 90% petal fall stage (03/04/2004), and were repeated every 12–17 days until the fruit reached a diameter of 2.0–2.5 cm (27/05/2004). Five sprayings were carried out in all (3/4/04, 15/4/04, 29/4/04, 11/5/04 and

27/5/04). The first necrotic lesions on the leaves of the unsprayed trees (control) appeared on 21/04/2004, seven days after the second treatment, when the fruits were 0.7–1.0 cm in diameter. The number of infected leaves and the number of necrotic lesions per leaf was counted in a random sample of 100 mature leaves per tree and the infection rate and severity were evaluated. The percentage of leaves with lesions after the last treatment was: 67% on the control trees, 15% on trees treated with bitertanol, 14% on trees treated with flusilazol, 43% on trees treated with carbendazim, 36% on trees treated with chlorothalonil and 21% on trees treated with kresoxim-methyl. The results so far indicate that the fungicides belonging to the EBI and QoI classes are the most effective in controlling Septoria leaf spot on pear, while carbendazim (Ben) and chlorothalonil are not effective.

Evaluation of the fungicide Fenomen (fenamidon) against oomycetes on cucumber, tobacco and potato crops in Greece. T. Veloukas, A. Parentis and R. Chantzigeorgiadis. *Bayer CropScience Hellas, 18-20, Sorou, 151 25 Marousi, Greece.*

Fenomen is a fungicide derived from the new chemical group of imidazolinone. In all compounds of fenomen there is an S-enantiomer of fenomidone, which is more active than the R one or the racemic mixture. The mode of action of fenamidone is specific. It inhibits the transport of electrons in the internal membrane of mitochondrion in complex III of the cytochrome C, interrupting the production of ATP. Fenomen is used for the control of diseases caused by fungi belonging to the genera *Phytophthora, Plasmopara, Peronospora, Pseudoperonospora* and *Bremia*. It also controls various other pathogens such as *Pythium* spp. and *Alternaria* spp. During the years 2000–2002, the following trials took place in Greece: five trials against *Phytophthora infestans* on potato, five trials against *Peronospora tabacina* on tobacco and seven trials against *Pseudoperonospora cubensis* on cucumber. Two types of formulations of the product were used: EXP-10810A, in which fenamidone is in mixture with mancozeb and EXP-10745D, in which fenamidone is in mixture with fosetyl-Al. Both compounds were sprayed on the leaves of the crops, EXP-10810A on potato and EXP-10745D on cucumber and tobacco. Against *P. infestans* on potato, the product was very effective at a dose of 125 g fenamidone + 625 g mancozeb per ha with a spray interval of 7–10 days, regardless of disease pressure. Against *P. tabacina* on tobacco, product efficacy was adequate at a dose of 107 g fenamidone + 1600 g fosetyl-Al per ha with a spray interval of 10–15 days. Against *P. cubensis* on cucumber, where the disease pressure was high and the spray interval was seven days, a dose of 78 g fenamidone + 1170 g fosetyl-Al per ha was very effective.

Study of the inherent resistance risk to the amidocarbamates iprovalicarb and benthiavalicarb in *Phytophthora infestans*. B.N. Ziogas, A.N. Markoglou, D. Theodosiou and A. Anagnostou. *Agricultural University of Athens, Laboratory of Pesticide Science, 75 Iera Odos, Votanikos, 118 55 Athens, Greece.*

Mutants of *Phytophthora infestans* highly resistant to the amidocarbamate fungicides iprovalicarb and benthiavalicarb were isolated from a wild-type strain, at a low mutation frequency of 4×10^{-14} and 2.2×10^{-7}, after UV-mutagenesis and selection on a medium containing iprovalicarb or benthiavalicarb. Cross-resistance studies with other fungicides showed that the mutation(s) for resistance to the amidocarbamates also affected the sensitivity of the mutant isolates to the phenylamide metalaxyl, strobilurins: pyraclostrobin and azoxystrobin, azolone famoxadone, antimycin A and cyanoimidazole cyazofamid, phenylpyridinamine fluazinam, acetamide cymoxanil, morpholine dimethomorph, and to the dithiocarbamate propineb, which affects other steps of cellular pathway. Study of fitness parameters of mutant isolates showed that the mutation(s) for resistance to amidocarbamates had no apparent effect on the rate of mycelial growth, but may or may not have affected some other phytopathogenic fitness characteristics such as sporulation, sporiangial germination and zoospore formation. Preliminary studies did not show a loss of the pathogenicity of resistant strains.