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ABSTRACTS

Summaries of oral presentations and posters presented at the 10th Hellenic Phytopathological Congress Kalamata, Greece, October 3-5, 2000

The 10th National Phytopathological Congress, held in Kalamata, October 3-5, 2000, was attended by more than 400 scientists. The meeting was organised by the Hellenic Phytopathological Society (HPS), which holds a national congress every two years. Papers presented dealt with plant diseases caused by fungi, bacteria, and viruses, and with non-parasitic disorders and their control. A round-table discussion was held on the role of phytopathology in the integrated management of plant production. A special session was also organised on alternatives to the use of methyl bromide.

Fungal diseases

Quantification of crown and root rot of tomato using a genetically modified *Fusarium oxysporum* f. sp. *radicis-lycopersici* strain expressing the β-glucuronidase gene. I. AGGELOU, M. TOURNA and K. PAPADOPOULOU. *National Agricultural Research Foundation, Institute of Kalamata, 85 Lakonikis St., 241 00 Kalamata, Greece.*

Fusarium crown and root rot, caused by *Fusarium oxysporum* f. sp. *radicis-lycopersici* Jarvis & Shoemaker, is a serious disease of greenhouse tomatoes. A wild-type isolate was cotransformed with the *E. coli* β-D-glucuronidase (*gus*A-reporter) gene and the hygromycin phosphotransferase gene (*hph*) as a selectable marker, by introducing the plasmid vectors pNOM102 and pAN7-1, respectively, into the protoplasts. Eleven transformants were isolated. PCR and Southern blot analyses confirmed incorporation of the *gus*A gene into the genome in varying copy-numbers at different integration sites. The mitotic division of nine transformants was stabilised by isolation of single conidia that were similar in growth patterns to the wild-type strain. Specific GUS activity was quantified for the transformants and differences in individual transformants were recorded. In two of the transformants, which exhibited the same pathogenicity towards tomato plants with the wild-type strain, a positive correlation was found between GUS activity levels and fresh weight of mycelium. Detection of fungal growth *in planta* was achieved by monitoring the expression of *gus*A both macroscopically and microscopically and the disease was quantified by measuring β-glucuronidase activity in extracts from infected plants.

Evaluation of resistance to *Verticillium dahliae* in rootstocks of established olive groves. P.P. ANTONIOU, E.C. TJAMOS and S.E.TJAMOS. *Agricultural University of Athens, Department of Plant Pathology, 75 Iera Odos, 118 55 Votanikos, Athens, Greece.*

A rapid resistance-evaluation method has been developed to differentiate resistance to *Verticillium dahliae* among olive cultivars. The method was based on differences in pathogen resistance between two-year-old trees of the most susceptible variety, Amfissis, and the tolerant variety Kalamon, under glasshouse conditions. The rate of symptom development, the severity and extent of the disease were significantly less in the tolerant than in the susceptible cultivar. Branches were inoculated by injecting the conidial suspension (100 µl of 10^8 conidia/ml) of a very virulent olive isolate of *V. dahl-*

iae. The conidial suspension infiltrated into holes (3 mm diameter and 5 mm length) immediately after the opening. The wounds were sealed with vaseline. Final evaluation was after three months. To apply the same technique in the orchard an irrigated olive grove of 800 trees of the susceptible Amfissis variety was selected in Scarfia, Fthiotis. The incidence of diseased trees in this area often reaches 20%. In Greece old olive orchards consist of trees grafted on wild olive rootstocks. Long standing observations by the orchard-owner over the last 30 years indicated that the orchard included trees of the susceptible variety but without visual Verticillium wilt symptoms. We therefore inoculated sprouts growing from the base of rootstocks of healthy looking trees. Eventually, 65 symptomless olive trees with an average of four sprouts per tree were selected. The sprouts of these trees were inoculated in April 2000 following the procedure described (100 µl of 2.10^7 conidia/ml). It was found that two months after inoculation 7 rootstocks remained symptomless. Further evaluation of the results, involving classical and molecular methods, are under way to obtain olive rootstocks really resistant to Verticillium wilt.

Cross-inoculation studies of *Pyricularia oryzae* isolates on rice and ctenanthe plants. R. Bontaroudi[1], M. Tsiliopoulou[1], E.J. Paplomatas[2] and A.C. Pappas[1]. [1]*University of Thessaly, Faculty of Agriculture Crop and Animal Protection, Plant Pathology Laboratory, Pedion Areos, 383 34 Volos, Greece.* [2]*Benaki Phytopathological Institute, 8 S. Delta St., 145 61 Kifissia, Athens, Greece.*

In May 1995, a new leaf-spot disease caused by *Pyricularia oryzae* Cavara was reported on *Ctenanthe oppenheimiana* ornamental plants grown in a glasshouse in Magnissia County. Preliminary pathogenicity tests on detached leaves showed that *P. oryzae* isolates from ctenanthe infected a wide range of plant species in the family Marantaceae. By contrast, isolates from rice failed to produce disease symptoms under the same experimental conditions. Isozyme and RAPDs analysis showed clear differences between fungal isolates from rice and ctenanthe. The objective of this work was to provide further information on the intra-family host specificity of the isolates from ctenanthe. Cross-inoculations of ctenanthe and rice grown under controlled greenhouse conditions were done. A spore suspension (10^4 spores/ml) of isolates from various sources was used as inoculum. Plants were inoculated either by spraying the foliage or by placing droplets of the inoculum suspension on the expanded leaves. On rice, the inoculum droplets were placed at the attachment point of the leaf blade to the stem, and on ctenanthe plants, on the lower leaf surface. The inoculated plants were incubated under moist polyethylene bags in a growth chamber at 20-22°C, 80% rh and a 12-h day until symptoms appeared. In each test two isolates from ctenanthe and one from rice were used. Plants inoculated with sterile water were used as a control. With both inoculation methods cross-infection of the plants was unsuccessful. After 4 days of incubation on ctenanthe and 5 on rice, characteristic disease symptoms appeared only on plants inoculated with the fungal isolates from the same host plant. From the artificially infected areas, the initial fungal isolate used as inoculum was re-isolated on nutrient medium supplemented with antibiotics. The results showed that *P. oryzae* isolates from ctenanthe do not infect rice and that there are physiological races which cause disease specifically on plants in the family Marantaceae.

***Pleurotus dryinus* (Pers.: Fr.) P. Kumm. in Greece: morphological and cultural characteristics.** P. Delivorias and Z. Gonou-Zagou. *National and Capodistrian University of Athens, Biology Department, Section of Ecology & Systematics, Panepistimiopolis, 157 84, Athens, Greece.*

Pleurotus (Fr.) P. Kumm. is a genus in the basidiomycetes which has been thoroughly studied on account of its wide distribution and the economic importance of many of its species. However, its taxonomic position is still unclear and the distinction of species is also often quite difficult. *Pleurotus dryinus* (Pers.: Fr.) P. Kumm. [=*P. corticatus* (Fr.) P. Kumm., *Lentodiopsis dryina* (Pers.: Fr.) Kreisel] is a well-documented species in the subgenus *Lentodiopsis* (Bubak) O. Hilber. It is usually regarded as a saprotroph on deciduous and, less often, coniferous trees, but is also considered a weak parasite. This report offers a detailed macroscopic and microscopic description of basidiocarps (teleomorph) of *P. dryinus* collected from a living trunk of *Platanus orientalis*, as well as giving cultural characteristics, with particular emphasis on the asexually produced spores (anamorph). *Pleurotus dryinus* has recently been recorded on *Platanus orientalis* worldwide. An isolation of the species in pure culture is kept at the Athens University Culture Collection of Fungi (ATHUM 4348) and a dried specimen is deposited at the Athens University Mycological Herbarium (ATHU-M 4348). This isolate and LGAM P114 from a dead trunk of *Fagus sylvatica* (Dimou, personal communication) are the first records of *P. dryinus* from Greece.

Chestnut blight. The current situation in Greece. S. Diamandis. *National Agricultural Research Foundation, Forest Research Institute, 570 06 Vassilika, Thessaloniki, Greece.*

Chestnut blight and ink disease are considered the two most destructive diseases of chestnut. In Greece, chestnut blight has spread all over the country, being first diagnosed by Biris in 1963 at Mount Pelion, central Greece. The blight is caused by *Cryphonectria parasitica* which in nature shows great variability expressed by VC groups. A virus of the genus *Hypovirus* carrying ds-RNA was first found in Italy where it infected the virulent fungus *C. parasitica,* changing it into hypovirulent. This virus was also found naturally occurring in Mount Pelion, where the disease has now started declining. In Mount Athos, northern Greece, where the disease was first diagnosed in 1988 (it was probably introduced in 1975) the Pelion virus was brought in by inoculating active cankers with hypovirulent inocula. The results were spectacular as the introduced hypovirulence is now spreading naturally. A similar treatment of chestnut orchards started in Mount Parnon, southern Greece in 1999. A crucial point for the success of this biological control method is the introduction of a hypovirulent strain belonging to the same VC group as the local virulent strain of the fungus. The Forest Research Institute has identified and mapped the VC groups

of the fungus in the whole of the country. Over the next few years it is planned to extend the treatment to other areas where the disease is causing severe damage and hypovirulence has not occurred yet. Similar efforts are being carried out in other Mediterranean countries such as Italy, France, Spain and also in Switzerland, Hungary and Austria.

***Endoptychum agaricoides* Czerniaiev: first report of a secotioid fungus in Greece.** D. Dimou[1] and G. Zervakis[2]. [1]*Agricultural University of Athens, General and Agricultural Microbiology Laboratory, 75 Iera Odos, 118 55 Votanikos, Athens, Greece.* [2]*National Agricultural Research Foundation, Institute of Kalamata, 85 Lakonikis St., 241 00 Kalamata, Greece.*

Secotioid basidiomycetes (Secotiaceae) produce hypogeous or epigeous basidiomata that share characteristics of the lamellate fungi (Agaricales) and the gasteromycetes (Lycoperdales). Usually they produce a distinct pileus and stipe, but the pileus never expands and the margin remains attached to the stipe. The hymenium is located on the surface of the lamellae which are convoluted and anastomosed, forming a daedaloid "gleba" that never becomes powdery at maturity, as happens in the order Lycoperdales. In some cases the stipe does not protrude out of the pileus but remains enclosed within the lamellar "gleba" like a columella. It has been suggested that secotioid fungi are the ancestors of the Agaricales, but the contrary opinion has also been voiced. The genus *Endoptychum* includes four species that form basidiomata resembling the early developmental stages of *Agaricus* or *Macrolepiota*. *Endoptychum (=Secotium) agaricoides* was first found in the Ukraine in 1845 and since then has been recorded in North America, Europe, Asia, North Africa (Algeria), New Zealand, and Australia. Although secotioid fungi are xerophilic, they have not been reported in Greece before. *E. agaricoides* was recorded in the autumn of 1999 on Mount Oxya, near the edge of a beech forest, on soil rich in sheep dung (around sheep-yards), at two locations (altitudes 1400 and 1650 m), the first of which has been forayed during the last 15 years. Pure cultures were established from the collected basidiomata to study the cultural characters and mating behavior. Future work will include an assessment of the phylogenetic affinity of *E. agaricoides* to genera of the family Agaricaceae using molecular techniques.

New records of macromycetes from Greece. D. Dimou[1], E. Lahouvaris[2] and G. Zervakis[3]. [1]*Agricultural University of Athens, General and Agricultural Microbiology Laboratory, 75 Iera Odos, 118 55 Votanikos, Athens, Greece.* [2]*Hellenic Mushroom Farm, Katheni, Evia, Greece.* [3]*National Agricultural Research Foundation, Institute of Kalamata, 85 Lakonikis St., 241 00 Kalamata, Greece.*

In the last few years, a well planned initiative has been in progress to set up a macromycetes inventory, avoiding the common practice of the past, which was to record macrofungi in an occasional and unsystematic way. The Laboratory of General & Agricultural Microbiology (AUA) and the Institute of Kalamata (National Agricultural Research Foundation) have directed their primary research effort towards the intensive and long-term investigation of selected ecosystems in Greece (e.g. Mount Oxya, Mount Taygetos, Mount Dirfys, several Aegean islands, etc.). In this way, fungi with different seasonality, habit and annual periodicity are recorded and results evaluated for mapping and the future compilation of red-data lists. At the same time, additional locations of particular ecological interest throughout the country are studied and new species and genera(*) are added in the national macromycete catalogue, such as: *Agaricus bitorquis* var. *valida*, *Amanita spissa*, *Ceriporia(*) purpurea*, *Coprinus tomentosus*, *Cylindrobasidium(*) evolvens*, *Exidiopsis(*) calcea*, *E. grisea*, *Fibulomyces(*) mutabilis*, *Gyroporus(*) castaneus*, *Hypoxylon mediterraneum*, *Leccinum corsicum*, *Leucocoprinus(*) birnbaumii*, *Phallus hadriani*, *Phellinus rimosus*, *Phlebia lilascens*, *Pluteus ephebeus*, *Podofomes pyrenaicus*, *Polyporus meridionalis* etc.

Macromycetes of Mount Oxya (Fthiotida, Greece). D. Dimou[1], G. Zervakis[2] and E. Polemis[2]. [1]*Agricultural University of Athens, General and Agricultural Microbiology Laboratory, 75 Iera Odos, 118 55 Votanikos, Athens, Greece.* [2]*National Agricultural Research Foundation, Institute of Kalamata, 85 Lakonikis St., 241 00 Kalamata, Greece.*

Mount Oxya (prefecture of Fthiotida, central Greece) is of special biogeographical importance as it is situated at the southernmost limit of beech distribution. Especially during the last four years a thorough and intensive study of the mycoflora associated with the Mount Oxya beech forest as well as with neighbouring fir forests and the alpine zone has revealed a high diversity of macromycetes. So far over 350 species have been recorded, assigned to 138 genera and 53 families. Approximately 90 species have been recorded for the first time in Greece, while several other species have been reported for the first time from a beech ecosystem. It is noteworthy that 10 species representing 10 genera and two families are new for Greece: *Antrodiella semisupina* on Quercus, *Byssoporia terrestris* (Atheliaceae) on *Fagus*, *Dentipellis* sp. on *Fagus*, *Diplomitoporus lindbladii* on *Abies*, *Endoptychum agaricoides* (Secotiaceae), on manured soil, *Gyrodon lividus* under *Alnus*, *Oxyporus corticola* on *Fagus*, *Physiporinus vitreus* on soil and *Fagus* plant debris, *Spongipellis delectans* on *Fagus* and *Tyromyces chioneus* on *Fagus*. Among the species reported for the first time the following wood-rotting fungi are of particular interest: *Antrodia sinuosa*, *Exidia truncata*, *Grandinia arguta*, *G. granulosa*, *Hymenochaete cinnamomea*, *Inonotus nodulosus*, *Oligoporus fragilis*, *O. subcaesius*, *O. tephroleucus*, *Phanerochaete tuberculata*, *Pholiota henningsii*, *Pleurotus dryinus*, *Pluteus petasatus*, *Polyporus badius*, *P. melanopus*, *Stereum subtomentosum* etc. Finally, the ascomycete *Gyromitra gigas* (growing under *Abies*) is also a new record for Greece.

Infection of Poinsettia *(Euphorbia pulcherrima)* by the fungus *Alternaria euphorbiicola*. A new disease in Greece. Fr. Eleftheriadou and V.I. Tahmatsidou. *Technological Educational Institute of Thessaloniki, S.TE.A, Laboratory of Phytopathology, 541 01 Sindos, Thessaloniki.*

Severe leaf spotting was noted on poinsettia (*Euphorbia pulcherrima* Willd. ex. Klotzsch) leaves in a red-bract glass-

house culture in Thessaloniki in August 1999. Lesions on the leaves were brown, irregular and up to 30 mm across. They were mostly located on the edge and tip of the leaves. A dark-brown discolouration edged the lesions while a chlorotic halo occurred between affected and healthy tissue. Dead tissues were torn and infected leaves later fell. A fungus of the genus *Alternaria* was isolated from infected tissue and identified as *Alternaria euphorbiicola* (Simmons & Engelhard). Intense sporulation occurred on the lesions. Conidia were septate, olive-brown and ellipsoid. They were solitary or in chains of two to five spores. Conidia of poinsettia isolates were 35–60 x 10–20 µm with light-brown beaks 5–10 µm in length. Conidia had 3 to 8 transverse and 1 to 4 longitudinal septa. Inoculation of Agelica and Capri, two red-bract cultivars, was carried out by spraying with conidial suspensions of a pure culture. Symptoms of the disease occured after a four-day incubation period at room temperature. As far as we know this is the first record of the disease in Greece.

Further investigation into the defoliating pathotype of *Verticillium dahliae* on cotton after its first report in Greece. K. Elena and E.J. Paplomatas. *Benaki Phytopathological Institute, 8 S. Delta St., 145 61 Kifissia Athens Greece.*

The defoliating pathotype of *Verticillium dahliae* Kleb. on cotton was first reported in Greece in 1998, during a study of the pathogenicity, vegetative compatibility and RAPDs, of 71 isolates of the fungus. Infected strains were isolated from diseased cotton plants obtained from the main cotton cultivation areas of central Greece in 1995–1996. New isolates found in the summer of 1999 were collected from the Trikala area, where the defoliating strain was found, and a representative sample of five isolates was tested for virulence on cotton cv. Acala, and compared with previously characterized defoliating and nondefoliating Greek strains. Also, nitrate nonutilizing (*nit*) mutants from these isolates were paired against tester strains of the previously described vegetative compatibility groups (VCGs). The five strains were found to be virulent to cotton when cotton plants were inoculated at the base of the stem with a conidial suspension containing 10^7 spores/ml. None of the tested strains was of the defoliating pathotype. Pairings were performed between chlorate-resistant *nit* mutants derived from each strain. The five nondefoliating *Verticillium* strains were classified as belonging to VCG2. Moreover, the electrophoretic profiles of the PCR products with five specific primers were different from those of the defoliating strain. It seems that the defoliating race has not yet spread in Greece.

Pathogenic and genetic variability of *Fusarium oxysporum* f. sp. *phaseoli* isolates from Greek bean cultivars. K. Elena[1], A.C. Pappas[2], F. Karra[1], A. Mistriotis[1] and S. Konstantopoulou[1]. [1]*Benaki Phytopathological Institute, 8 S. Delta St., 145 61 Kifissia, Athens, Greece.* [2]*University of Thessalia, Faculty of Agriculture, Plant Pathology Laboratory, 383 34 Pedion Areos, Volos, Greece.*

A representative sample of 27 isolates of *Fusarium oxysporum* Schlechtend. from diseased *Phaseolus* plants was tested for virulence and vegetative compatibility. Virulence was tested on 7-day-old plants of six local Greek cultivars from Chryssoupoli (Gigantes, Psila, and Zarganes) and Kastoria (Chondra, Gigantes and Elephantes). Plants were inoculated by dipping their roots in a conidial suspension (10^6 conidia/ml) of each isolate. Twenty-three isolates caused mild or severe disease symptoms in one or more of the bean cultivars and were classified as *Fusarium oxysporum* Schlechtend. f.sp. *phaseoli* Kendrick and Snyder (*Fop*). Four isolates although not found pathogenic, caused a slight vascular discoloration in the inoculated bean plants. All Greek cultivars tested were found susceptible to *Fop* isolates. However, a great variation was found in the response of cultivars to various inocula. Zarganes from Chryssoupoli, Gigantes and Elephantes from Kastoria were the most susceptible cultivars and they showed severe defoliation symptoms. Complementations were carried out among the chlorate-resistant, nitrate-nonutilizing (*nit*) mutants of 27 strains of *F. oxysporum* in all possible combinations to determine their vegetative compatibility. Representative mutants were assayed in Italy against tester strains of five previously defined vegetative compatibility groups (VCGs) for comparison. Fifteen of 23 *Fop* isolates were found to belong to a new group, 0166. Isolates F9 and F10 were assigned by the Italians to VCG 0165, while six pathogenic isolates did not anastomose with the testers. None of the four isolates avirulent to *Phf, olus* isolates belonged to any of the VCGs defined.

Analysis of isozyme electrophoretic patterns in *Fusarium oxysporum* isolates from cucumber. G.A. Fragkiadakis and D.J. Vakalounakis. *National Agricultural Research Foundation, Plant Protection Institute, 711 03 Heraklion, Crete, Greece.*

Isozyme variation among protein extracts from 14 isolates of *Fusarium oxysporum* f. sp. *radicis-cucumerinum*, 14 isolates of *F. oxysporum* f. sp. *cucumerinum* and four non-pathogenic isolates of *F. oxysporum* from cucumber was investigated with the aim of developing a reliable method to distinguish between the two pathogens. Aliquots of protein extracts were electrophoresed on non-denaturing polyacrylamide gels. The enzyme bands were localised by activity staining. In the the six enzymes examined, esterase (EST), peroxidase (PRX), superoxide dismutase (SOD), acid phosphatase (ACP), alkaline phosphatase (ALP) and malate dehydrogenase (MDH), reproducible and significant differences were found mainly among the esterase isozymes. When using a mixture of α- and β-napthyl acetate as a substrate in "staining" esterase zymograms, two major bands were observed in the isolates pathogenic to cucumber. One of the bands was black (indicating specificity for α-napthols) and the electrophoretic mobility of the isolates of both *F. oxysporum* f. sp. *radicis-cucumerinum* and *F. oxysporum* f. sp. *cucumerinum* was the same. The second band was reddish (indicating specificity for α-napthols) and had a lower electrophoretic mobility in *F. oxysporum* f. sp. *radicis-cucumerinu*m than the *F. oxysporum* f. sp. *cucumerinum* isolates. The esterase zymograms of the non-pathogenic isolates were more complex.

Isolation and viability evaluation of *Rhizoctonia solani* protoplasts. A. Gialeli and M. Chrysayi-Tokousbalides. *Agricultural University of Athens, Pesticide Science Laboratory, 75 Iera Odos, 118 55 Votanikos, Athens, Greece.*

Fungal protoplasts are a biological tool to evaluate and study fungitoxic substances but the validity of the results is absolutely dependent on the viability of the obtained protoplasts. The objectives of this study were to isolate *Rhizoctonia solani* protoplasts, to determine the factors which favour a high yield of viable protoplasts and to ascertain the criteria for viability evaluation. The results showed that the length of exposure of mycelium to the enzymatic solution and the composition of the digestive preparation (enzyme-osmotic factor-buffer system) were critical factors to obtain and maintain viable protoplasts and that mycelium age, temperature and pH were less important. It was also concluded that estimating the reversion frequency of the protoplasts was a more practical and reliable method for the determination of their viability than the measurement of oxygen uptake by the protoplast suspension. Selective staining of dead or live protoplasts with safranin-O, phenosafranin, methylene blue or fluorescein diacetate did not give satisfactory results. The data for all three strains of *R. solani* tested were similar.

Preliminary results of a study of Basidiomycota in oak forests in Arkadia (Peloponnese, Greece). S. Ioannidou[1,2], E. Polemis[1], D. Tzanoudakis[2], D. Dimou[3] and G. Zervakis[1]. [1]*National Agricultural Research Foundation, Institute of Kalamata, 85 Lakonikis St., 241 00 Kalamata, Greece.* [2]*University of Patras, Department of Biology, Panepistimioupoli, 265 00 Rion, Greece.* [3]*Agricultural University of Athens, Agricultural Microbiology Laboratory, 75 Iera Odos, 118 55 Votanikos, Athens, Greece.*

About one third of Greek forest vegetation is composed of broad-leaved deciduous forests and 22% of oak forests. These particular ecosystems have not been thoroughly studied in relation to their biodiversity. Concerning the macromycetes in particular, despite their environmental importance, there are no previous studies on their ecology, distribution and the biological interactions with plant species. Over the last two years, this topic has been a subject of study in the forest region of Megalopolis and in adjacent areas where oak is the principal vegetation component. This larger area is classified as part of the para-Mediterranean *Quercetalia pubescentis* phytosociety: *Quercus conferta* is the dominant species often forming mixed groups with *Quercus pubescens*, while many other species are also present: *Fraxinus ornus, Carpinus betulus, Carpinus orientalis, Arbutus unedo, Quercus coccifera, Cistus incanus* etc. Preliminary research has recorded 69 mushroom species: *Boletus splendidus, Boletus fechtnerii, Cercorticium molare,* and *Macrolepiota heynii* are first reported for Greece, whereas *Clitopilus prunulus, Helvella acetabulum* and *Lactarius insulsus* are recorded for the first time in *Quercus conferta* and *Q. pubescens* habitats.

Fusarium root and crown rot, a new disease of tomato in Cyprus. N. Ioannou[1], T. Katan[2] and A. Hadjinicoli[1]. [1]*Agricultural Research Institute, 1516 Nicosia, 220 16 Cyprus.* [2]*The Volcani Center, Bet Dagan, Israel.*

Fusarium wilt caused by *F. oxysporum* f. sp. *lycopersici* (*Fol*), was a limiting factor to tomato production in Cyprus until the early 1990s, when commercial cultivars with resistance to the two major local races of *Fol* became widely available. Recently, however, a new Fusarium disease has been found in several tomato greenhouses in the Limassol and Larnaca districts, killing plants of the *Fol*-resistant cv. FA 179, Graziella and Bellinda. The first symptoms were observed at harvest time and consisted of wilting of the top leaves, descending gradually to the lower leaves. There was also vascular discoloration of the basal stem, which did not extend up more than 25 cm from the soil surface. Wilting was followed by the root and crown-rot syndrome from which the disease derives its name. On the surface of the basal stem there was also profuse sporulation, permitting air-borne spread of the pathogen. The pathogen was readily isolated in the laboratory from infected tissues. Although morphologically indistinguishable from *Fol*, it was identified as *F. oxysporum* f. sp. *radicis-lycopersici* (*Forl*) on the basis of field symptoms, pathogenicity tests and vegetative compatibility group (VCG) tests. Pathogenicity tests were carried out under two temperature regimes, using 10 isolates of the pathogen and 8 differential tomato cultivars. Isolates varied in pathogenicity but all indiscriminately attacked *Fol*-susceptible and *Fol*-resistant cultivars. The only exception was two cultivars reportedly resistant to *Forl*, which exhibited no or only mild infection. Disease severity was much higher under the low (18–20ºC) than the high (25–29ºC) temperature regime. Four isolates were further studied by the VCG method and all were assigned to VCG 0090 II, common among *Forl* isolates in the Mediterranean region. This is additional genetic evidence confirming the presence of *Forl* in Cyprus.

First report of Verticillium wilt of sugar beet caused by *Verticillium dahliae* in Greece. D.A. Karadimos, G.S. Karaoglanidis, and K. Tzavella-Klonari. *Aristotelian University of Thessaloniki, Faculty of Agriculture, Plant Pathology Laboratory, 540 06 Thessaloniki, Greece.*

Wilted sugar beet plants (*Beta vulgaris* L.) were noted in fields of the Larissa area in the summers of 1997 and 1998. Diseased plants initially showed general yellowing and epinasty and were sporadically distributed in the fields. As symptoms progressed, the outer leaves wilted and became desiccated. Inner leaves showed marginal and interveinal yellowing. These areas later turned brown and became necrotic. Longitudinal sections of the roots of diseased plants displayed browning of the vascular tissue. Fungal isolates obtained from the discoloured vascular tissue on potato dextrose agar medium were identified as *Verticillium dahliae* by their morphological features. Mycelium was hyaline, white to off white, becoming black with the formation of microsclerotia. Conidiophores were hyaline, verticillated, branched with 3–4 phialides at each node. Conidia borne on phialides, were ellipsoidal to short cylindrical and mainly one-celled, 2.5–8x1.4–3.2 µm. Microsclerotia were dark-brown to black

and variable in shape and size (25x50–100 µm diameter). Pathogenicity tests were carried out using the root-dip technique. Inoculated plants exhibited wilted leaves with interveinal yellowing about 30 days after inoculation, while symptoms were not observed on control plants. *V. dahliae* was reisolated from artificially inoculated plants. This is the first report of Verticillium wilt of sugar beet in Greece.

First report of Phytophthora root rot of sugar beet, caused by *Phytophthora cryptogea*, in Greece. G.S. Karaoglanidis, D.A. Karadimos and K. Tzavella-Klonari. *Aristotelian University of Thessaloniki, Faculty of Agriculture, Plant Pathology Laboratory, 540 06 Thessaloniki, Greece.*

A severe root rot of sugar beet was observed in the Amyndeon area of northern Greece in the summer of 1998. Infected plants initially showed temporary wilting which became permanent later; plants eventually died. Slightly diseased roots showed necrotic spots towards the base whereas more heavily diseased roots showed a more extensive wet rot that extended upward. Rotted tissue was brown with a distinct black margin. In most isolations, carried out on potato dextrose agar (PDA), the pathogen obtained was identified as *Phytophthora cryptogea* Pethybr. & Laferty. Mycelium consisted of fairly uniform, fine hyphae with a slightly floral growth pattern. In autoclaved soil-extract medium chains or clusters of hyphal swellings (average 12 µm diameter) were formed. Sporangia were not produced on solid media, but were abundant in soil-extract medium. Sporangia were oval to obpyriform in shape, non-papillate with rounded base and variable in size (39–80 by 24–40 µm). Oospores were plerotic, thick-walled and averaged 25 µm in diameter. When cultured on PDA the pathogen did not grow at 36°C. The closely related species *Phytophthora drechleri* Tucker has been reported to cause similar root rot symptoms on sugar beet. However, *P. dreschleri* grows well at 36°C while *P. cryptogea* does not and this is the major distinguishing feature separating the two species. To test the pathogenicity of this organism, surface-sterilised sugar beet roots cv. Rizor were inoculated with 5-mm-diameter PDA plugs of an active growing culture. *P. cryptogea* was reisolated from the rotted tissues. This pathogen has been previously recognised as a root-rot pathogen of sugar beet in Japan and Wyoming, USA. This is the first report of Phytophthora root rot of sugar beet in Greece.

Pathogenic fungi of *Sonchus oleraceus* L. E.K. Koulakiotou, K. Tzavella-Klonari and C.C. Thanassoulopoulos. *Aristotelian University of Thessaloniki, Faculty of Agriculture, Plant Pathology Laboratory, 540 06 Thessaloniki, Greece.*

Sampling to investigate the pathogens of the sow-thistle weed, *Sonchus oleraceus*, was started in Macedonia in 1999–2000. Samples of diseased plants revealed four infections caused by the fungi *Bremia lactucae* Regel (Mastigomycotina, Oomycetes), *Alternaria sonchi* J.J. Davis (Deuteromycotina, Hyphomycetes), *Sclerotinia sclerotiorum* (Lib.) de Bary (Ascomycotina, Discomycetes) and *Oidium* sp. (Ascomycotina, Erysiphales). *B. lactucae* caused long interveinal chlorotic spots on the leaves, which became brown and finally necrotic. Fruiting bodies were observed as a white mildew on the lower surface of the leaves. *A. sonchi* gave rise to numerous circular or elliptical, necrotic spots (1–6 cm in diameter) on the leaves, with a light-brown centre and a red-brown margin. Infection with *S. sclerotiorum* resulted in a soft rot at the base of the stem and lower leaves as well as postharvest rot. Infection with the imperfect stage of *Oidium* sp. was also observed. The first symptoms and indication of the disease consisted of small white spots on the leaves with the characteristic white mycelium and conidiophores. The infection spread to the whole surface of the stem and leaves. Species identification is not complete since the perfect stage has not yet appeared. This is the first report of *B. lactucae* and *S. sclerotiorum* as pathogens of *S. oleraceus* in Greece and also the first report of *A. sonchi* in Europe.

The main diseases of commercially grown white mushroom *Agaricus bisporus*, in Greece. E. Lahouvaris[1], G. Zervakis[2], A. Phillippoussis[3] and D. Dimou[4]. *[1]Hellenic Mushroom Farm, Kathenoi, Evoia, Greece. [2]National Agricultural Research Foundation, Institute of Kalamata, 85 Lakonikis St., 241 00 Kalamata, Greece. [3]National Agricultural Research Foundation, Institute of Agricultural Engineering and Constructions, 61 Democratias St., 135 61 Ag. Anargyri, Athens, Greece. [4]Agricultural University of Athens, Agricultural Microbiology Laboratory, 75 Iera Odos, 118 55 Votanikos, Athens, Greece.*

The cultivation of edible mushrooms is a controlled biotechnological process with a high level of specialisation producing a food with valuable organoleptic properties. Mushroom yield and quality depend on several interacting factors, both biotic (pests and diseases) and non-biotic (environmental parameters, substrate conditions, nutrition). The problems which arise in the cultivation of the white mushroom *Agaricus bisporus*, are due to incomplete adjustment to enviromental conditions or failure in controlling pathogens, and have deleterious and usually irreversible consequences on the cultivation process. For that reason fast and accurate diagnosis of the causes and appropriate intervention measures are important prerequisites for successful mushroom cultivation. This work presents the main mycological (pathogens: *Mycogone perniciosa, Dactylium dendroides, Verticillium fungicola, Trichoderma* spp., *Chaetomium globosum*, etc.), bacteriological (*Pseudomonas tolaasii*), and non-parasitic (stipe inflation, pileus yellowing, clusters, etc.) diseases encountered in *A. bisporus* cultivation at the Hellenic Mushroom Farm S.A., which is the largest mushroom-production establishment in Greece. The causes of the diseases and their symptoms are examined in relation to the cultivation stage at which the assessment was made.

Surface microflora and antagonism of four fungi on pears cv. Tsakoniki. I.A. Laidou and C.C. Thanassoulopoulos. *Aristotelian University of Thessaloniki, Faculty of Agriculture, Plant Pathology Laboratory, 540 06 Thessaloniki, Greece.*

The surface microflora of pears cv. Tsakoniki was investi-

gated and numerous fungi, bacteria and yeasts were isolated. Antagonism of *Penicillium expansum*, *Aspergillus flavus*, *Alternaria alternata* and *Stemphylium vesicarium*, *in vitro* and *in vivo*, and the penetration of these fungi into the pulp of pears were also investigated. Dual cultures showed a strong antagonistic effect of *A. flavus* against *A. alternata*, the colony of which was overgrown. A strong antibiosis effect was observed with all the other pairs. Pears were surface-disinfected and dipped in a solution of 0.1×10^6 conidia/ml of the above fungi and in a solution containing all four fungi together at same concentration. Each fruit was divided into four sections, 5 mm thick, from the surface (first section) to the area around the carpel (fourth section). Three pieces from each section were placed on dishes with potato dextrose agar. Cultures were incubated for 7 days at 25°C in the dark. *P. expansum* and *A. flavus* were isolated even from the fourth section, from diseased as well as healthy tissues, both in treatments with each fungus separately and in those with the four fungi together. *A. alternata* and *S. vesicarium* were isolated from the first section only.

Infection of blackberry (*Rubus lacinatus*) with the fungus *Phytophthora cactorum*. I.A. Laidou and C.C. Thanassoulopoulos. *Aristotelian University of Thessaloniki, Faculty of Agriculture, Plant Pathology Laboratory, 540 06 Thessaloniki, Greece.*

Root rot of blackberry caused by fungi of the genus *Phytophthora* is a serious disease. Infections extend along the rows and causes death of plants as well as yield reduction. Symptoms are not observed only on the roots, where scraping off the epidermis reveals the characteristic reddish brown discoloration, but also in the crown region and the higher parts of the canes. Infected canes have declining lateral shoots with leaves turning yellow, wilting and dying. In the St. Basilios area of Thessaloniki a severe infection of blackberry plants cv. Thornless evergreen was observed in 1998, causing yield losses of up to 60%. The disease spread in course of time to more plants. Fungi of the genus *Phytophthora* were isolated from infected plants and the fungus *P. cactorum* was identified by morphological characteristics: papillate sporangia 31.2x23.4 µm, oospores 18–34 µm with wall thickness 2 µm, and numerous chlamydospores with high viability that are produced by this strain of the fungus. This is the first report of *P. cactorum* infecting blackberry in Greece.

Stem Canker of Cotton caused by the fungus *Alternaria alternata* (Nees:Fries) Keissler. I.A. Laidou, E.K. Koulakiotu and C.C. Thanassoulopoulos. *Aristotelian University of Thessaloniki, Faculty of Agriculture, Plant Pathology Laboratory, 540 06 Thessaloniki, Greece.*

A stem blight of cotton (*Gossypium hirsutum* L.) was observed on plants of cultivar 132 in the district of Ammoudia, Serres. The symptoms of the disease were cankers on the stem, leaf spots and boll rot. Affected plants showed defoliation, early maturing and total or partial necrosis. Symptoms on the stem were dark-brown, circular spots, which enlarged rapidly and became sunken in the centre to form the canker. The spots gradually became elliptical and the tissues split longitudinally along the stem. The infected stem dried resulting in the total or partial death of the plant. The fungus isolated from infected stem tissues was identified as *Alternaria alternata*. Inoculated plants showed disease symptoms with a frequency greater than 95%, while the frequency of re-isolation of the same fungus was more than 70%. This fungus is commonly known to cause leaf spot, boll rot and seedling blight diseases of cotton, but this is the first report of *A. alternata* causing stem blight of cotton worldwide.

New hosts of *Verticillium dahliae* races 1 and 2 in Greece and worldwide. E.K. Ligoxigakis[1], D.J. Vakalounakis[1] and C.C. Thanassoulopoulos[2]. [1]*National Agricultural Research Foundation, Plant Protection Institute, 711 10 Heraklion, Crete, Greece.* [2]*Aristotelian University of Thessaloniki, Faculty of Agriculture, Plant Pathology Laboratory, 540 06 Thessaloniki, Greece.*

An extensive survey of cultivated plants and weed species with wilt symptoms was done in Crete in 1992-1997. In addition, several vegetable and forage species were grown in a field naturally infested with *Verticillium dahliae*. The result of these studies was 33 new hosts of *V. dahliae* to be recorded. Of these, 15 were new hosts worldwide, and 18 new hosts in Greece. Among 28 species grown in the naturally infested field, 14 new hosts of *V. dahliae* were found. Of these, six: *Anethum graveolens*, *Beta vulgaris* var. *sicla*, *Cicer arietinum*, *Lathyrus ochrus*, *Vicia sativa* and *Lens culinaris* were new worldwide, while eight: *Brassica oleracea* var. *botrytis*, *B. oleracea* var. *capitata*, *B. oleracea* var. *italica*, *Raphanus sativus*, *Cichorium endivia*, *C. intybus*, *Foeniculum vulgare* ssp. *vulgare* and *Pisum sativum* were new to Greece. The first five worldwide new hosts and the first six new hosts for Greece were also found to be infected under natural field conditions. In a sampling of 98 plant species grown under natural field conditions, 50 hosts were found. These included a) 14 worldwide new hosts, including six cultivated species: *A. graveolens*, *B. vulgaris* var. *sicla*, *C. arietinum*, *L. ochrus*, *Tagetes erecta* and *Vicia sativa*, and eight weed species: *Anthemis melanolepis*, *Cardaria draba*, *Convolvulus arvensis*, *Erodium* sp., *Euphorbia helioscopia*, *Helminthotheca echioides*, *Lactuca serriola*, *Sinapis alba*; b) 16 hosts new for Greece, including 11 cultivated species: *B. oleracea* var. *botrytis*, *B. oleracea* var. *capitata*, *B. oleracea* var. *italica*, *Cichorium endivia*, *C. intybus*, *Cucurbita pepo*, *Cucurbita* sp., *Impatiens balsamina*, *Lactuca sativa* var. *longifolia*, *Raphanus sativus*, *Spinacia oleracea*, and five weed species: *Raphanus raphanistrum*, *Sinapis arvensis*, *Sonchus oleraceus*, *Euphorbia* sp. and *Trifolium* sp.; and c) 20 known hosts. Among these 50 hosts, seven were new for Crete, including two cultivated species: *Pistacia vera* and *Vitis vinifera* ssp. *vinifera*, and five were weed species: *Amaranthus* sp., *Capsella bursa-pastoris*, *Chenopodium album*, *Senecio vulgaris* and *Malva sylvestris*. Also, five worldwide new hosts of *V. dahliae* race 2 were found under conditions of natural infection including four cultivated species: *Lactuca sativa* var. *longifolia*, *C. pepo*, *C. arietinum* and *O. europaea*, and one common weed species: *S. nigrum*. To the best of our knowledge, one tree and one weed species are here reported for the first time as hosts of *V. dahliae* race 2.

Studies on the biology of the fungus *Leveillula taurica* Lev. N.E. Malathrakis and M.N. Fanouraki. *Technological Educational Institute of Crete, School of Agricultural Technology, 715 00 Heraklion, Crete, Greece.*

The fungus *Leveillula taurica* infects about 1000 plant species including some valuable agricultural crops. It is a major pathogen of tomatoes, eggplants and peppers grown in greenhouses. Yet its biology has not been properly studied and its control is difficult. To obtain biological data for more effective control, the effect on conidia germination of: a) different agar substrates b) water c) temperature and d) age of conidia, was studied. The conidia germination rate was about 60% on all 7 agar substrates tested. Germination on the most suitable substrate, agar dextrose manitol was the same when conidia were suspended in water or applied dry. The greatest conidia germination was obtained at 21–26°C. It started at about 4 h from the start of the incubation period and gradually increased up to 24 h. There is some evidence that conidia germinability was highest just after their development and gradually decreased in the following ten days. Among the several hosts of *L. taurica* examined, cleistothecia were detected only in artichoke, from many different parts of Crete. Many cleistothecia produced mature asci and ascospores.

Evaluation of resistance of cotton cultivars to *Verticillium dahliae*. E.I. Paplomatas and I. Orfanos. *Benaki Phytopathological Institute, 8 S. Delta St., 145 61 Kifissia, Athens, Greece.*

Nineteen cotton cultivars were tested under greenhouse conditions for their resistance to *Verticillium dahliae* Kleb. Three isolates of the pathogen were used, two representing races 1 and 2 of the fungus on tomato and one from an infected cotton plant. Inoculations were performed by dipping cotton roots in a water suspension of 10^7 conidia/ml. Disease assessment was based on the height, the fresh weight of the above-ground part and the foliar symptoms. All three isolates of the pathogen had a statistically significant effect on the height of the plants. Differences among cultivars in the weight of the above-ground part of plants were evident only with race 2 of the fungus, not with race 1 or the cotton isolate. Cultivars infected with race 1 or 2 of the pathogen were statistically separated by their foliar symptoms. However, in pathogenicity tests with the cotton isolate which in previous tests had been found to be highly virulent, all cultivars were equally susceptible.

Identification of fungicide-resistant phenotypes of *Botrytis cinerea* using biochemical and molecular techniques. E.J. Paplomatas[1], D.G. Antoniadis[2] and A.C. Pappas[2]. [1]*Benaki Phytopathological Institute, 8 S. Delta St., 145 61 Kifissia, Athens, Greece.* [2]*University of Thessaly, Faculty of Agriculture Crop and Animal Production, Plant Pathology Laboratory, Pedion Areos, 383 34 Volos, Greece.*

Over 200 selected *Botrytis cinerea* Pers. single-spore isolates from various greenhouse crops were examined for resistance to the fungicides dicarboximide, benzimidazole and phenylcarbamate. On the basis of the mode of spore germination and mycelial growth on fungicide-amended media, isolates were classified into 6 groups of resistant phenotypes: PcmHR, phenylcarbamate highly resistant (spore germination and mycelial growth at 100 mg/l diethofencarb); DicMR PcmHR, dicarboximide moderately and phenylcarbamate highly resistant (spore germination and mycelial growth at 3 mg/l iprodione and 100 mg/l diethofencarb); BenHR, benzimidazole highly resistant (spore germination and mycelial growth at 100 mg/l carbendazim); BenHR RcmHR, benzimidazole and phenylcarbamate highly resistant (spore germination and mycelial growth at 100 mg/l carbendazim and 100 mg/l diethofencarb); DicMR BenMR PcmHR, dicarboximide and benzimidazole moderately resistant and phenylcarbamate highly resistant (spore germination and mycelial growth at 3 mg/l iprodione, 1 mg/l carbendazim and 100 mg/l diethofencarb); DicMR BenHR, dicarboximide moderately and benzimidazole highly resistant (spore germination and mycelial growth at 3 mg/l iprodione and 100 mg/l carbendazim). RAPD analyses of 3 representative isolates of each group and construction of dendrograms revealed the clear division of the various resistant phenotypes into the 6 groups. The first group included strains with high resistance to diethofencarb and carbendazim and formed a dichotomous branch with the phenotypes DicMR BenMR PcmHR. All the above strains were distinct from the group with high resistance to the benzimidazoles. Finally, the wild strain was placed on a separate branch at a large genetic distance from the two groups with resistant strains. The data showed a positive correlation between conventional and molecular techniques in identifying fungicide-resistant phenotypes and supported earlier findings on the genetic basis of fungicide resistance.

Infection of rose plants by the fungus *Cryptosporella umbrina*: first report for Greece. E.J. Paplomatas, K. Elena and A. Tzima. *Benaki Phytopathological Institute, 8 S. Delta St., 145 61 Kifissia, Athens, Greece.*

In February 2000, a sample of diseased rose plants from a greenhouse culture at Krya Vryssi, Yannitsa, Pella county, was sent to the Benaki Phytopathological Institute. The main canes of some plants were dead, but in others the damage was more extensive and had caused the death of the whole plant. Small black dots on the infected canes were identified as pycnidia under the microscope. Their spores were brown, ellipsoid, single-celled with an average size of 18x12 µm. Fungus isolated from the tissues produced two types of pycnidia in culture. One type formed spores similar to those found on the diseased canes, while the other formed much smaller spores, with an average size of 8x3 µm. From both the morphology of the fungus and the symptoms on the plant, it was deduced that the disease was Brown canker of rose caused by the fungus *Cryptosporella umbrina* (Jenkins) Jenkins and Wehm. To fulfil Koch's postulates, rose canes were artificially inoculated with the fungus. Part of the bark was removed and was replaced by a plug of fungal culture bearing both fungal mycelium and pycnidia. To prevent dehydration, the infection sites were covered with plastic tape and vaseline. Subsequently, the canes were placed in the greenhouse at 22°C with a 12-h day in glass containers with water. In the control canes, uninoculated culture medium was substituted for the fungal culture in the incisions. About

ten days later, inoculated canes showed the first symptoms, which progressively developed like those found on the diseased sample. The same fungus was reisolated from those canes. This is the first report of the fungus *Cryptosporella umbrina* on rose in Greece.

Contribution to the investigation into macrofungal biodiversity in selected islands of the Cyclades region (Aegean Sea, Greece). E. Polemis[1], D. Dimou[2] and G. Zervakis[1]. [1]*National Agricultural Research Foundation, Institute of Kalamata, 85 Lakonikis St., 241 00 Kalamata, Greece.* [2]*Agricultural University of Athens, Agricultural Microbiology Laboratory, 75 Iera Odos, 118 55 Votanikos, Athens, Greece.*

The biodiversity of macrofungi in the island-cluster of the Cyclades forms a new area of research of significant scientific interest because of the particular constitution of the local plant communities and the high percentage of plant endemism. Indicative of its importance is the fact that until now, no references to the subject are to be found in the international literature. Only in the last few years have Pavel Lizon and the authors of the present work begun to gather data on the geographic distribution of fungal taxa in the Cyclades, and to inventory macromycetes previously unknown in this and other parts of Greece. On the islands of Andros and Naxos, 102 species of Basidiomycetes (assigned to 57 genera) and 13 species of larger Ascomycetes (10 genera) have been identified. First records for mycoflora in Greece are: *Agaricus iodosmus, Agaricus cupreonrunneus, Colus hirudinosus, Crinipellis stipitaria, Hebeloma cistophilum, Lactarius cistophilus, Lactarius tesquorum, Perenniporia rosmarini, Stropharia thrausta* and *Pholiota highlandensis*. The following taxa are reported on new host-plants: *Ganoderma adspersum* on *Alnus glutinosa*, *Auricularia auricula-judae* on *Mellia* sp., *Pisolithus arhizus* on *Quercus macrolepis* and *Quercus coccifera, Phellinus punctatus* on *Q. coccifera, Paxillus involutus* on *Platanus orientalis* and *Alnus glutinosa, Omphalotus olearius* on *Quercus ilex* and *Trametes versicolor* on *Alnus* sp. Of particular interest are the interactions observed between macromycetes and typical host-plants of the local flora.

Young grapevine decline caused by *Phaeoacremonium* spp. and *Cylindrocarpon* spp. I.C. Rumbos. *National Agricultural Research Foundation, Plant Protection Institute, 380 01 Volos, Greece.*

Numerous samples of declining young vines from different grapevine-growing areas of the Peloponnese, Macedonia and Thessalia were examined at the Plant Protection Institute of Volos between 1997 and 2000. Declining grapevines showed significantly lower vigour, reduced foliage, shortened internodes, smaller leaf size and interveinal chlorosis. In cross section declining rootstocks exhibited dark-brown to black dots and in longitudinal section streaks in the vascular elements, particularly at the base where the roots start, and at the union point of scion and rootstock. In this last case the graft union was dark and dark streaks sometimes extended from the union downwards. The vines exhibited symptoms of graft failure and the graft was sometimes easily broken. In the first year of planting losses were between 2-25%. In subsequent years significant numbers of vines exhibited more definite decline symptoms and sometimes died. In some cases growers were forced to replant the whole vineyard after the first year. From infected plants we consistently isolated fungi in the genera *Phaeoacremonium* and *Cylindrocarpon* from symptomatic young vines of various cultivars and on a variety of rootstocks (Sirah/SO_4, Roditis/1103 Paulsen, Moschoudi/1103 Paulsen, Korinthiaki/3309, Agiorgitico/1103 Paulsen, Muscat d'Hamburg/110R, $SO_4$1103 Paulsen). In order to fulfil Koch's postulates, cuttings of different cultivars and rootstocks as well as young vines from tissue culture were inoculated with spore suspensions of the isolated fungi. Inoculated plants are still under observation for symptom development. The distribution of diseased propagated material from nurseries is also being studied.

New species of genus *Cercospora*, potential agents for biological control of the weeds *Chenopodium album* and *Heliotropium europaeum*. V.K. Saltzis[1], K. Tzavella-Klonari[2] and P. Lolas[3]. [1]*Technological Educational Institute of Epirus, Faculty of Agricultural Technology, Department of Plant Production, 471 00 Arta, Greece.* [2]*Aristotelian University of Thessaloniki, Faculty of Agriculture, Plant Pathology Laboratory, 540 06 Thessaloniki, Greece.* [3]*University of Thessaly, Department of Agriculture, Plant and Animal Production, Weed Science Laboratory, Pedion Areos, 383 34 Volos, Greece.*

In autumn 1999, as part of a survey to record the main fungi that attack weeds in tobacco and cotton fields in the area around New Ephesos, Katerini, a severe infection, of the genus *Cercospora*, with spotting on the weeds *Chenopodium album* and *Heliotropium europaeum*, was noted. Spots on the leaves were small circular and greyish with clear red-brown margins. Expansion of the spots caused the drying of a large part of the leaf area, and eventually foliage loss. On the spots of *Chenopodium album* leaves formed olivaceous-brown fasciculated conidiophores measuring 30–60x3–6 µm and hyaline conidia with 1–4 septa, 35–55 µm long x5–7 µm wide and were indentified as belonging to the species *Cercospora chenopodii* Fresen. The fungus was also isolated and indentified on cultures from infected tissues. Conidia of *Cercospora heliotropii* Ell. & Ev. were observed in spots on leaves of *Heliotropium europaeum*. These two diseases are already known to occurr in Canada, USA, India, Pakistan and some countries in Europe, but as far as we know this is their first report in Greece. The study of disease severity and losses is in progress.

Resistance of wheat to Fusarium head blight. E. Siranidou and H. Buchenauer. *Institute of Phytomedicine, University of Hohenheim, 70593 Stuttgart, Germany.*

Infection of wheat spikes with fungi of the genus *Fusarium* causes not only yield losses but also contaminate the grains with mycotoxins, which can damage human and animal health. Until now no wheat cultivars have been found immune to Fusarium infection, most are susceptible and a few

are resistant. In an experiment with seven winter-wheat cultivars (*Triticum aestivum* L.) and one spring-wheat cultivar the role of phenolic compounds in inhibiting infection of the spikes with *F. culmorum* and the spread of the pathogen through spike tissue was investigated. The spring wheat cv. Frontana was, compared with the other cultivars examined, highly resistant to primary infection of the spikelets and to spread of the pathogen through the spike tissue. The cv. Frontana contained significantly greater amounts of free phenolic compounds at all samplings times than the other cultivars examined. Furthermore, two days after inoculation there was a significantly greater amount of p-coumaric acid in the cv. Frontana than in the uninoculated control. In all the other cultivars no increase in phenolic compounds was detected two days after inoculation. The higher content of phenolic compounds (e.g. ferulic acid and p-coumaric acid) in spike tissue before inoculation, and the increase of p-coumaric acid in infected spike tissue after inoculation may be significant factors in the resistance of cv. Frontana to Fusarium head blight.

Fusarium rot of asparagus and crop-loss assessment. C.C. Thanassoulopoulos and A. Tsoumaki. *Aristotelian University of Thessaloniki, Faculty of Agriculture, Plant Pathology Laboratory, 540 06 Thessaloniki, Greece.*

Fusarium rot has tended to become a limiting factor in the asparagus industry of northern Greece. A study to identify the pathogenic agent and assess losses from this rot was undertaken in 1998-1999 in Chryssoupoli, Kavala, in 10 fields with asparagus cv. Larac. Disease incidence was estimated as percentage of ferns with symptoms. A disease index (D.I.) of 1–5 was applied, in three samplings, June, July, August, with 100 plants/sample, and a total of 2000 plants per sampling. Total losses in each field were expressed as percentage/ acre referred to the field with 0 disease. *Fusarium moniliforme* Sheld was identified as the rot agent. The three samplings of disease severity showed that in the course of the survey healthy plants decreased by 46.6% and dead plants increased by 39.4%. This difference is expressed by the changing of the sum of the areas of the trapezoids and triangles which are formed with perpendicular ribs of the percentage of each scale of D.I. The integral of these areas, according to the Trapezoid rule of integration, expresses the final D.I. The D.I. of the third sampling against crop loss percentage of the next year had the best coefficient of regression r=0.776, (r^2=0.602), and was expressed by the equation: $Y=51.28(\pm12.78)+1.15(\pm0.54)X- 0.011(\pm0.005)X^2$ which could be used for a disease prognosis of the next year.

The susceptibility to powdery mildew of different cucumber cultivars grown in Greece. E. Topalidou[1], A. Markellou[2,3] and S. Konstantinidou-Doltsinis[1]. *[1]National, Agricultural Research Foundation, Plant Protection Institute, 260 04 Patras, Greece. [2]Benaki Phytopathological Institute, 8 S. Delta St., 145 61 Kifissia, Athens, Greece. [3]National, Agricultural Research Foundation, Athens, Greece.*

A study was conducted to investigate the susceptibility to powdery mildew *(Sphaerotheca fuliginea)* of commonly grown cucumber cultivars in Greek greenhouses. Twenty-two cultivars were examined in a total of 7 experiments. Cucumber plants in the greenhouse were either naturally infected or artificially inoculated (1st leaf only). Infection was defined as percent leaf area covered with powdery mildew on the leaves by position, with leaf 1 as the first full leaf. Disease severity was measured once on the 6th fully-grown leaf of the naturally infected plants. In artificially inoculated plants disease severity was measured four times. The data were used to construct an Area Under Disease Progress Curve (AUDPC) for percent infected leaf area (total duration of the disease monitoring period was 28 d). Data were subjected to analysis of variance followed by Duncan's test. Results of the trials showed significant differences in susceptibility to *S. fuliginea* among cultivars. All cultivars were listed according to their average disease severity on the leaves (natural infection) and were grouped in three different classes: low (0–10%), medium (10–25%) and high (25–50%) susceptibility to *S. fuliginea*. The AUDPC (artificial inoculation) was used to verify the results. In the low-susceptible cultivars disease progress was slower than in the more susceptible ones (moderate/high) as indicated by the significantly lower values of the AUDPC (%-days).

***Heterobasidion annosum* and *Heterobasidion abietinum* in forests of black pine and fir of Mount Taygetos and Mount Parnon.** P. Tsopelas. *National Agricultural Research Foundation, Institute of Mediterranean Forest Ecosystems and Forest Technology Products, Terma Alkmanos, 115 28 Athens, Greece.*

The occurrence of *Heterobasidion* species in fir (*Abies cephalonica* Loud.) and black pine [*Pinus nigra* Arn. ssp. *pallasiana* (Lamb.) Holmboe] forests of Mount Taygetos and Mount Parnon was examined. Species were identified by pairing homokaryotic and heterokaryotic isolates with homokaryotic tester strains. Thirty-nine out of 42 isolates from fir belonged to *Heterobasidion abietinum* Niemelä & Korhonen, and 3 to *H. annosum* (Fr.) Bref. On the other hand, 21 out of 26 isolates from black pine belonged to *H. annosum* and 5 to *H. abietinum*. In the fir forests of both mountains, extensive tree mortality due to *H. abietinum* was observed. Disease centres of various sizes were observed where most of the trees were dead. These mortality centres originated in many different infection points, as was seen by examining the fungal genotypes (clones) in somatic compatibility tests. *H. annosum* was very common in black pine stands and in mixed pine and fir stands. However, no significant damage by this pathogen was observed in black pine forests. *H. annosum* was mostly found as a saprophyte in stumps, seldom killing young pine trees.

***Alternaria solani* Sorauer isolates from tomato and potato: variability in cultural characteristics, *in vitro* sporulation, virulence and reaction to fungicides**. I. Vloutoglou and E. Stamelou. *Benaki Phytopathological Institute, 8 S. Delta St., 145 61 Kifissia, Athens, Greece.*

In vitro and *in vivo* experiments on *Alternaria solani* isolates from potato and tomato showed that isolates belong to

two groups that vary in cultural characteristics, sporulation *in vitro*, virulence and the EC_{50} values of various fungicides used against them. Although linear growth of mycelium from these isolates on V-8 agar did not differ significantly ($P<0.05$), isolates from potato were characterised by light grey colonies, aerial mycelium and the absence of conidia, whereas isolates from tomato by black colonies, no aerial mycelium and the production *in vitro* of great amounts of conidia. On S-medium, isolates from tomato produced significantly ($P<0.05$) more conidia ($8.1–9.6x10^3$ conidia/cm^2) than isolates from potato ($0.7–2.6x10^4$ conidia/cm^2). Cross-inoculation isolates from tomato cv. Ace 55VF with isolates from potato cv. Spunta plants showed that *A. solani* isolates from tomato were highly virulent on both tomato and potato (disease severity 70–90% and 78–90%, respectively) while isolates from potato were more virulent on potato (disease severity 95–100%) than on tomato (disease severity 33–60%). Isolates from tomato had significantly ($P<0.05$) higher EC_{50} values for mancozeb, chlorothalonil, azoxystrobin and prochloraz than those from potato.

The importance of macrofungi diversity in Greece: how many species exist? G. Zervakis[1] and D. Dimou[2]. [1]*National Agricultural Research Foundation, Institute of Kalamata, 85 Lakonikis St., 241 00 Kalamata, Greece.* [2]*Agricultural University of Athens, Agricultural Microbiology Laboratory, 75 Iera Odos, 118 55 Votanikos, Athens, Greece.*

Available information on the fungal biodiversity in Greece is scarce and fragmentary. Most of it refers to species of phytopathological interest or to macromycetes. The total number of fungal species recorded in Greece until now is about 2800, which includes 1200 mushroom species. However, these figures represent only a very small part (much less than 10%) of the true size of the mycoflora in Greece; for geomorphological, biogeographical and climatological data suggest on a conservative estimate a total of some 30.000 species (including 6.000–7.000 macrofungi). Of special importance is the richness of the Greek flora, as well as the high proportion of species endemism or rarity, outside Greece a consequence of the fact that most fungi develop highly specialised interactions with higher plants. A limited knowledge of this field of mycology can be attributed to a lack of pertinent research, the pausity of scientists working on these subjects, and the very low priority of this type of research has at both educational and funded-project level. Therefore, current objectives should include to inventory the mycoflora in selected ecosystems, to investigate unknown fungal groups, to apply integrated taxonomic approaches for identification purposes, to establish and/or expand herbaria and culture collection facilities, to promote collaboration schemes for mapping purposes, and subsequently to develop of red-data lists that will promote the conservation of biodiversity in Greece.

Inventory of wood-rotting fungi in Mount Taygetos (Peloponnese, Greece). G. Zervakis [1], E. Polemis[1] and D. Dimou[2]. [1]*National Agricultural Research Foundation, Institute of Kalamata, 85 Lakonikis st., 24100 Kalamata, Greece.* [2]*Agricultural University of Athens, Agricultural Microbiology Laboratory, 75 Iera Odos 118 55 Votanikos, Athens, Greece.*

Although Mount Taygetos is one of the most biodiverse natural ecosystems in Greece, its mycoflora has never been investigated. Since 1996, the study of macromycetes in this area has been a significant research priority, and preliminary data are of particular ecological and economic interest. Especially as regards wood-rotting fungi, 60 species of the orders Ganodermatales, Hericiales, Hymenochaetales, Poriales and Stereales have so far been recorded. Twenty of them are first reports for Greece, including *Amylocorticium cebennense, Athelia decipiens, Columnocystis abietina, Tubilicrinis borealis*, etc. Some others (e.g. *Heterobasidion annosum, Phaeollus schweinitzii*) are considered to be among the factors responsible for lethal infections in *Abies cephalonica* and *Pinus nigra* trees, while *Armillaria mellea* and *A. tabescens* have been observed growing on *Quercus conferta* and *Q. pubescens* in the northern part of Mount Taygetos. In addition, several other species, which attack dead wood and cause white or brown rots (e.g. *Agrocybe paludosa, Gloeophyllum sepiarium, Ischnoderma benzoinum, Lentinellus castoreus, Oligoporus caesius, Peniophora piceae, Pleurotus ostreatus* and *Trametes versicolor*) are evaluated as regards their ability to produce extracellular enzymes for the biodegradation of lignocellulose agro-industrial wastes and residues.

Ectomycorrhizal fungi associated with coniferous forests in Messinia (Peloponnese, Greece). G. Zervakis[1], E. Polemis[1], K. Papadopoulou[1] and D. Dimou[2]. [1]*National Agricultural Research Foundation, Institute of Kalamata, 85 Lakonikis St., 241 00 Kalamata, Greece.* [2]*Agricultural University of Athens, Agricultural Microbiology Laboratory, 75 Iera Odos, 118 55 Votanikos, Athens, Greece.*

Coniferous forests cover limited parts of Messinia, mainly in the southeast (Mount Taygetos, with its dominant species *Abies cephalonica* and *Pinus nigra*, and some minor groups of *P. halepensis*) and northwest (coastal areas with *P. halepensis*). Those trees are of particular environmental and economic importance, but they are also very susceptible to human intervention and especially forest fires. Their growth on relatively poor soils and under xerothermic conditions is achieved through highly specialised symbiotic relationships that the trees develop with ectomycorrhizal fungi (ECMF). ECMF also contribute significantly to the recycling of organic matter and the biocontrol of plant diseases. Recently, detailed studies on the mycoflora associated with conifers in Messinia has led to more than 150 ECMF being recorded, most of them in the following genera: *Amanita, Boletus, Cortinarius, Entoloma, Hydnellum, Inocybe, Laccaria, Lactarius, Lyophyllum, Pisolithus, Ramaria, Rhizopogon, Russula, Suillus* and *Tricholoma*. More than 30 species are reported for the first time in Greece, including *Camarophyllus pratensis, C. virgineus, Cortinarius odorifer, Lyophyllum fumosum, Myxomphalia maura, Ramaria largentii, Russula acrifolia, Sacrodon leucopus, and Tricholoma stans*. Of great significance seems to be the role of ECMF in speciation and coevolution processes, as well as in the rehabilitation of forest ecosystems after the serious environmental disturbances that have recently been recorded in Mount Taygetos.

Facultative biotrophic basidiomycetes produce high-quality edible mushrooms. G. Zervakis[1], P. Katsaris[1], S. Ioannidou[1], E. Lahouvaris[2] and A. Phillippoussis[3]. [1]*National Agricultural Research Foundation, Institute of Kalamata, 85 Lakonikis St., 241 00 Kalamata, Greece.* [2]*Hellenic Mushroom Farm, Kathenoi, Evoia, Greece.* [3]*National Agricultural Research Foundation, Institute of Agricultural Engineering and Constructions, 61 Democratias St., 135 61 Ag. Anargyri, Athens, Greece.*

Pleurotus eryngii (Basidiomycotina, Poriales) and its allied taxa are typical species of the Mediterranean mycoflora. This fungal group is unique within the genus since its members can grow as facultative biotrophs on Umbelliferae and Compositae plants. The mycelium colonises the lower portions of the plant (root-system and underground portion of the stem), is mainly nourished by the oil-depository substances of the host, and produces basidiomata after three to twelve weeks. *In vitro* infection trials on Umbelliferae plants demonstrated that effective fungus attack was highly specific to particular host species, and this was also the case *in situ*, with the result that a sympatric speciation process was observed. Fungi of the *P. eryngii* species-complex also grew very satisfactorily as saprotrophs on plant residues. Therefore, since the basidiomata posses valuable organoleptic properties, a research project is currently under way to exploit agricultural residues and by-products by growing *P. eryngii* mushrooms on them. Laboratory-scale experiments provided evidence that this particular fungus could make good use of a large array of lignocellulose wastes, while larger-scale cultivation trials with bulk substrate pasteurisation have also had promising.

Virus diseases

Detection and identification of *Cucumber green mottle mosaic virus* with immunological and molecular techniques. F. Bem, C. Varveri and N. Vassilakos. *Benaki Phytopathological Institute, 8 S. Delta St., 145 61 Kifissia, Athens, Greece.*

Cucumber green mottle mosaic virus (CGMMV) has recently caused serious losses in watermelon production. To develop sensitive laboratory detection techniques of this disease the virus was isolated, propagated in melon and purified with the method of Tung and Knight. Rabbit antiserum was prepared by 5 weekly injections of purified virus (1 mg/ml). The antiserum had a titre of 1:1024 in the microprecipitin test and was used to prepare reagents for applying an $F(ab')_2$ based ELISA. The sensitivity of this test was 100 pg/ml of purified virus preparation, i.e. a 100–fold unprovement over commercial DAS-ELISA reagents. Virus isolates of different origins were all efficiently detected. To develop even more sensitive molecular virus detection methods, such as the polymerase chain reaction (PCR), specific primers were designed based on published virus sequences. The application of immunocapture-PCR permitted the amplification of the expected fragment of the virus coat protein gene from the different isolates, which were further characterised by restriction fragment length polymorphism (RFLP) analysis of their PCR products.

Investigation of the causal agent of leaf chlorosis and internal fruit deterioration of watermelon plants. H.N. Boubourakas[1], E. Hatziloukas[1], C.I. Dovas[1], A. Gliatis[2] and N.I. Katis[1]. [1]*Aristotelian University of Thessaloniki, Faculty of Agriculture, Plant Pathology Laboratory, 540 06 Thessaloniki.* [2]*Directorate of Agricultural Development of Larissa, Plant Protection Department, Larissa, Greece.*

In summer 1999, a disease with a composite symptomatology, affecting grafted watermelon plants in the field, appeared in various regions of northern and central Greece. The most common symptoms were petiole necrosis or characteristic chlorotic spots on the leaves, later coalescing into irregular chlorotic patterns, or both. Attempts to detect *Watermelon chlorotic stunt virus* (WCSV) by PCR using *Geminivirus* genus-specific primers in plants showing leaf chlorosis were unsuccessful. However, all plants bearing any of the above symptoms reacted positively in DAS-ELISA tests using polyclonal antiserum raised against *Cucumber green mottle mosaic virus* (CGMMV). Using this antiserum, the rod-shaped viral particles measuring 300x18 nm were decorated. ELISA with this antiserum produced a strong reaction with Greek isolates of watermelon CGMMV, and a very weak one with Greek isolate of CGMMV, from cucumber. Plant-sap preparations originating in plants with all three types of symptoms were used to infect *Chenopodium amaranticolor*, functioning as an intermediate indicator, to purify the virus through the formation of local lesions. Plant sap from these local lesions was used to infect watermelon plants and reproduced all three types of symptoms. The virus was transmitted mechanically to cucumber, melon, watermelon, *Luffa acutangola*, *C. amaranticolor* and *Gomphrena globosa* plants. Serological examination of 297 wild plants collected from the interior part of infected watermelon fields revealed the virus in 17 samples belonging to five species. Melon and bottlegourd (*Lagenaria* sp.) plants, collected from the periphery of the same fields, were infected with CGMMV in 63.5% and 75% of cases respectively. Lastly, using RT-PCR, the virus was detected in pollen of watermelon, melon and cucumber plants.

Transmission parameters of *Tomato spotted wilt virus* (TSWV)by *Thrips tabaci* Lindeman. E.K. Chatzivassiliou[1], D. Peters[2] and N.I. Katis[1]. [1]*Aristotelian University of Thessaloniki, Faculty of Agriculture, Plant Pathology Laboratory, 540 06 Thessaloniki, Greece.* [2]*Department of Virology, Wageningen Agricultural University, Binnenhaven 11, 6709 PD Wageningen, The Netherlands.*

The developmental stages at which thrips larvae of an arrhenotokous tobacco population of *Thrips tabaci* Lindeman efficiently acquire *Tomato spotted wilt virus* (TSWV), the median acquisition access period (AAP_{50}) and the latent period (LP_{50}) were determined for both males and females using the petunia leaf disc method. The fact that larvae of different ages acquired the virus showed that it was acquired during both the first and the second larval stages, although the percentage of transmitters decreased with increasing larval age. The highest transmission rate occured when thrips adults acquired the virus as newborn (0-24-h-old) lar-

vae (50.6% for females and 61.1% for males). However, the virus could also be transmitted at low percentages after acquisition by 120–144-h-old female larvae (1%) or 96–120-h-old male larvae (17.8%). The AAP_{50} at 25ºC was 41 min for the entire population, 65 min for males and 35 min for females. The majority of thrips started to transmit after becoming adults. Transmission rates depended on the temperature. When populations were kept at 20, 24 and 27ºC, males transmitted the virus at 45.0, 54.6 and 64.6% respectively, females at 53.6, 65.5 and 67.2%, and the entire population at 53.6, 63.5 and 66.8%. The latent period was also temperature-dependent. The LP_{50} values for thrips transmitting the virus only as adults were 351, 280, and 223 h for males, 405, 310, and 241 h for females, and 378, 300 and 235 h for the total population at the three temperatures.

Temporal and spatial spread of *Tomato spotted wilt virus* (TSWV) in relation to thrip populations in tobacco crops. E.K. Chatzivassiliou[1], I. Zintzaras[2], J.A. Tsitsipis[2] and N.I. Katis[1]. [1]*Aristotelian University of Thessaloniki, Faculty of Agriculture, Plant Pathology Laboratory, 540 06 Thessaloniki, Greece.* [2]*University of Thessaly, Faculty of Agriculture Crop and Animal Protection, Entomology and Agricultural Zoology Laboratory, Pedion Areos, 383 34 Volos, Greece.*

The temporal and spatial spread of *Tomato spotted wilt virus* (TSWV) was studied in fields of tobacco cv. Virginia in Kilkis, one of the most important tobacco-producing areas of northern Greece, for three years (1995–1997). Thrips and infected plants were sampled bi-weekly, both in the seedbeds and in the field. The thrips population was recorded using blue sticky traps (Horiver-TR). In the seedbeds, TSWV-incidence was estimated by testing 100 randomly selected leaves with ELISA using polyclonal antibodies against the N protein of TSWV (BR-01). In the field, plants showing typical symptoms of TSWV infection were counted, their position in the plot was marked and ten plants per plot were randomly collected and tested by ELISA to confirm infection. The maximum thrips population in both plots was recorded in the 22nd and 23rd week of 1995 at approximately 5000 adults per trap. At the end of this period, only 7.4% of plants were infected. In 1996 the thrips population peaked in the 18th and 19th week, in 1997 it peaked two weeks later, in the 20th and 21st week, at 1400 and 1800 thrips per trap respectively. In both years at the end of the season all plants were infected with TSWV. The statistical analysis of the spatial spread of the virus with the Monte Carlo test revealed that the pattern of infected plants was not random ($P<0.01$). Thrips identification showed that *Thrips tabaci* Lindeman was the predominant thrips species and the only vector of TSWV collected on the traps.

Differential *Tomato spotted wilt virus* infection and vector-species infestation of weeds in greenhouses and tobacco fields. E.K. Chatzivassiliou[1], I. Boubourakas[1], E. Drossos[2], I. Eleftherohorinos[3], G. Jenser[4], D. Peters[5] and N.I. Katis[1]. [1]*Aristotelian University of Thessaloniki, Faculty of Agriculture, Plant Pathology Laboratory, 540 06 Thessaloniki, Greece.* [2]*Aristotelian University of Thessaloniki, School of Biology, Systematic Botany and Phytogeography Laboratory, 540 06 Thessaloniki, Greece.* [3]*Aristotelian University of Thessaloniki, Faculty of Agriculture, Department of Agronomy, 540 06 Thessaloniki, Greece.* [4]*Plant Protection Institute, Hungarian Academy of Sciences, Budapest, Hungary.* [5]*Wageningen Agricultural University, Department of Virology, Binnenhaven 11, 6709 PD Wageningen, The Netherlands.*

A survey to determine the reservoir of weed plants hosting *Tomato spotted wilt virus* (TSWV), was conducted in Macedonia (Greece) on three tobacco fields and in a greenhouse complex where vegetables (lettuce, peppers) and ornamentals (chrysanthemum, gerbera, aster, anemone) were grown. A total of 6172 samples, 3909 from tobacco fields and 2263 from the greenhouses, were collected and identified as 208 plant species from 137 genera in 42 families. Samples were assayed for TSWV infection using the double-antibody enzyme-linked immunosorbent assay and by inoculation of indicator plants. Plants belonging to 86 species from 63 genera in 27 families became infected, leading to the identification of 40 additional species as new hosts of TSWV. A relative potential infection index was calculated for each weed species in order to evaluate its potential as a virus-source with each of the two cultivation systems. Seventeen species in the tobacco fields and nine in the greenhouses had a relative potential infection index higher than one. Most infected plants belonged to the Compositae and were found predominantly in tobacco crops. Some weed species occurred in both the tobacco fields and the greenhouses, but they were infected in only one of these two sites. Thrips populations sampled from weeds differed greatly in their virus transmission rate between fields and greenhouses: *Frankliniella occidentalis* Pergande was the most common thrips species on weeds and crops in the greenhouses, but *Thrips tabaci* Lindeman was the only thrips vector found on the tobacco plants and weeds in the fields.

Incidence of viruses in tobacco crops in Greece. E.K. Chatzivassiliou[1], K. Efthimiou[1], Th. Matsi[1], N. Zissopoulos[1], M. Papadimitriou[1], I. Roumbos[1], M. Tsombana[1], M. Chouliara[1], A. Papadopoulou[3], G. Poimenidis[3] and N.I. Katis[1]. [1]*Aristotelian University of Thessaloniki, Faculty of Agriculture, Plant Pathology Laboratory, 540 06 Thessaloniki, Greece.* [2]*Aristotelian University of Thessaloniki, Faculty of Agriculture, Applied Soil Science Laboratory, 540 06 Thessaloniki, Greece.* [3]*Tobacco Institute of Greece, 661 00, Drama, Greece.*

The incidence of viruses in tobacco seedbeds and fields was studied in several tobacco-producing areas of Greece. More than 9000 samples were collected randomly from seedbeds in 1997 and 1998, and an equal number from field plants with virus-like symptoms (stunting, mosaic, leaf distortion and necrotic patterns) from 1997 to 1999. Samples were tested either by ELISA, electron microscopy (EM) and/or artificial inoculation onto indicator plants. Among the viruses identified, *Cucumber mosaic virus* (CMV) and *Potato virus Y* (PVY) were the most common, occuring in all sampling areas and cultivars, and in both fields and seedbeds. CMV was a problem in Karditsa, Pella, Kozani, Larissa and Trikala, and PVY in Agrinio, Karditsa, Pella and Katerini. *Alfalfa mosaic virus* (AMV) appeared also to be a problem in

some regions, mainly in central Greece, such as Larissa, where alfalfa is cultivated in the vicinity of tobacco crops. *Tomato spotted wilt virus* (TSWV) is a major problem mainly in northern Greece, especially Kilkis, Kavala, Thessaloniki, Xanthi, Serres, and Drama, where infection rates reached 100% of symptomatic plants in individual fields. TSWV was not detected in Agrinio, Argolida, Karditsa, Kozani, Larissa, Pieria, Trikala or Imathia. In 2000, a TSWV epidemic occurred in Kozani and Kastoria. *Tobacco mosaic virus* (TMV) was detected in all areas and especially in oriental tobacco cultivars in Argolida, Trikala and Drama. *Eggplant mottled dwarf virus* was detected at a very low (<0.01%) incidence in Lamia, Kilkis, Drama, Karditsa and Komotini. All these viruses infected both oriental and flue-cured tobacco cultivars. In Pieria, *Tobacco rattle virus* (TRV) was detected in a small number of oriental tobacco crops whereas a new, semi-persistently transmitted aphid-borne closterovirus was isolated from the oriental tobacco cv. Basmas in Macedonia (Drama).

Problems concerning clonal selection of grapevine associated with viral infection. C.I. Dovas[1], N. Leventakis[2], H. Spinthiropoulou[2], Ã.Õ. Stavrakakis[3], and N.I. Katis[1]. [1]*Aristotelian University, Faculty of Agriculture, Plant Pathology Laboratory, 540 06 Thessaloniki, Greece.* [2]*VITRO HELLAS, 593 00 Nisseli Imathias, Greece.* [3]*Agricultural University of Athens, Viticulture Laboratory, 75 Iera Odos, 118 55 Votanikos, Athens, Greece.*

During a project on the preservation and clonal selection of *V. vinifera* wine-grape varieties of Macedonia, Thrace and Hepirus, 91 biotypes were collected. Plant collection was based on the Ampelographic descriptions and only plants without virus-like symptoms were collected. Grapevine plants were marked before harvest. Ripe canes were collected in winter and cortical scrapings were used for virus indexing. Virus diagnosis was based on serology (ELISA) using polyclonal antisera and monoclonal antibodies to detect the following viruses: *Grapevine fanleaf virus* (GFLV), *Tomato black ring virus* (TBRV), *Arabis mosaic virus* (ArMV), *Grapevine leafroll-associated virus -1,-2,-3,-5,-6,-7,* (GLRaV-1,-2,-3,-5,-6,-7), *Grapevine fleck virus* (GFkV) and *Grapevine virus A, B,* (GVA, GVB). In addition, 40 plants were tested by PCR (using two primer pairs), for *Grapevine rupestris stem pitting associated virus-1,* (RSPaV-1). Results indicated a high incidence of GLRaV-1 (12%), GLRaV-2 (27.7%), GLRaV-3 (22.2%), GVA (23.3%), GFkV (52%) and GFLV (11.1%), a lower incidence of GVB (2.2%), GLRaV-5 (2.2%), GLRaV-6 (1.1%) and GLRaV-7 (5.5%). No infection was found with ArMV or ToBRV. Lastly PCR revealed high infection levels with RSPaV-1 (80%). Results clearly indicate the difficulties inherent in the clonal selection of grapevine when selection is based only on visual inspection.

Virus interactions in garlic plants with multiple viral infections. C.I. Dovas[1], A.P. Mamolos[2], Ch. Papachristos[1], G. Patsiavouras[1] and N.I. Katis[1]. [1]*Aristotelian University of Thessaloniki, Faculty of Agriculture, Plant Pathology Laboratory, 540 06 Thessaloniki, Greece.* [2]*Aristotelian University of Thessaloniki, Faculty of Agriculture, Ecology & Environmental Protection Laboratory, 540 06 Thessaloniki, Greece.*

Onion yellow dwarf virus (OYDV), *Leek yellow stripe virus* (LYSV), *Garlic virus-C* (GarV-C), and *Garlic virus-D* (GarV-D), infect garlic crops with a high incidence. The aim of this study was to investigate the interaction between viruses in garlic plants with multiple virus infections. Viral concentrations were determined during the growing period, in different plant parts using quantitative ELISA. Results indicated an uneven distribution of all viruses in the plant. The presence of the potyviruses (OYDV, LYSV) in garlic did not affect allexivirus (GarV-C, GarV-D) concentrations in multiply infected plants, but the allexiviruses lowered the potyvirus concentrations (OYDV concentration was lower in the presence of allexiviruses, while LYSV was lower until mid-March, after which it increased slightly). The presence of GarV-C in garlic plants dramatically decreased concentrations of GarV-D, but the presence of LYSV did not have any influence on concentrations of OYDV.

Identification of *Cymbidium mosaic virus* (CyMV) and *Odontoglossum ringspot virus* (ORV) in orchids in Greece, and the development of multiplex spot-PCR for their detection. C.I. Dovas[1], I.N. Smirnioudis[1], E.K. Chatzivassiliou[1], S. Konstantinidou-Doltsinis[2] and N.I. Katis[1]. [1]*Aristotelian University of Thessaloniki, Faculty of Agriculture, Plant Pathology Laboratory, 540 06 Thessaloniki, Greece.* [2]*National Agricultural Research Foundation, Plant Protection Institute, 260 04 Patra, Greece.*

In 1998, orchid plants belonging to the Genera *Cymbidium* and *Phalenopsis* and showing chlorotic and necrotic stripes and lesions were found in a greenhouse near Patra (Achaia). Virus diagnosis was carried out based on serology (ELISA), electron microscopy, artificial inoculation of indicator plants, and the polymerase chain reaction (PCR). None of the samples reacted serologically (ELISA) with antisera prepared against *Tomato spotted wilt virus, Impatiens necrotic spot virus, Iris yellow spot virus, Potato virus Y, Alfalfa mosaic virus* and *Cucumber mosaic* virus. Electron microscopy revealed rod-shaped (300x18 nm) and filamentous (450x13 nm) virus particles, which were decorated with antisera prepared against *Cymbidium mosaic virus* (CyMV) and *Odontoglossum ringspot virus* (ORSV). Artificial inoculation with ORSV-infected *Cymbidium* plant tissue caused: (a) chlorotic local lesions in *Chenopodium amaranticolor, C. quinoa, Nicotiana glutinosa* and *Gomphrena globosa*; (b) Necrotic local lesions followed by systemic infection in *N. rustica*; (c) chlorotic rings and mosaic in *N. tabacum* cv. Samsun and (d) general chlorosis in *Vigna sinensis*. Furthermore, (e) latent infection was detected in *Phaseolus vulgaris* cv. Burlotto and *Cucurbita pepo* cv. Jedida F1. No infection was detected in *Datura stramonium* or *Vicia faba*. None of the abovementioned indicator plants were infected after artificial inoculation with CyMV-infected *Odontoglossum* plant tissue. Two primer pairs designed for the detection of CyMV and ORSV by PCR produced amplicons of the expected size.

Lastly, a multiplex spot-RT-PCR assay was developed using filter paper for the simultaneous detection of both viruses with a single assay.

Identification of viruses infecting spinach (*Spinacea oleracea*) crops in Greece. C.I. Dovas[1], V. Fotopoulos[2], A. Theocharopoulos[1], I. Loumbourdis[1], G. Diamandidis[3], S. Winter[4] and N.I. Katis[1]. [1]*Aristotelian University of Thessaloniki, Faculty of Agriculture, Plant Pathology Laboratory, 540 06 Thessaloniki, Greece.* [2]*Imperial College of Science, Technology and Medicine, University of London, Biology Department.* [3]*Aristotelian University of Thessaloniki, Faculty of Agriculture, Laboratory of Agricultural Chemistry, 540 06 Thessaloniki, Greece.* [4]*DSMZ AG Pflanzenviren c/o BBA Institut fur Biochemie und Pflanzenvirologie, D-38104 Germany.*

A survey for viruses infecting spinach crops was carried out in Greece. A total of 744 spinach plants exhibiting virus like symptoms were collected from five districts (Evia, Imathia, Thessaloniki, Pieria and Halkidiki). Virus identification was based on ELISA, electron microscopy and decoration. Serological tests were performed for the following viruses: *Beet western yellows virus* (BWYV), *Cucumber mosaic virus* (CMV), *Turnip mosaic virus* (TuMV), *Lettuce mosaic virus* (LMV), *Broad bean wilt virus 1* and *2* (BBWV), *Spinach latent virus* (SpLV), *Beet yellows virus* (BYV), *Tomato spotted wilt virus* (TSWV) *and Beet mosaic virus* (BtMV). Results indicated high incidence of BWYV (18.7%), and a lower incidence of CMV (11.8%) and TuMV (7.0%). BWYV was common in most areas. CMV and TuMV were found with a high incidence in Thessaloniki and Evia, while CMV occurred in all samples collected from Pieria.

***Beet western yellows virus* (BWYV) in Greece**. C. Dovas[1], A. Theoharopoulos[1], I. Loumbourdis[1], E. Smyrnioudis[1], A. P. Sklavounos[2], P.E. Kyriakopoulou[2] and N.I. Katis[1]. [1]*Aristotelian University of Thessaloniki, Faculty of Agriculture, Plant Pathology Laboratory, 540 06 Thessaloniki, Greece.* [2]*Agricultural University of Athens, Department of Plant Pathology, 75 Iera Odos, 118 55 Votanikos, Athens, Greece.*

Beet western yellows virus (BWYV) is a polyhedral, diameter *circa* 26 nm, aphid-borne virus, with a wide range of natural hosts including vegetables, ornamentals and arable weed plants. Known in the USA since 1958, is also widespread in Europe, causing problems especially in the crucifers. It has now also been found in Greece. In spring 2000, yellows symptoms were noted on various plant species in diverse parts of Greece: Thessaloniki, Chalkidiki, Fthiotida, Evia, Attica and the Eleia counties. ELISA detected BWYV in various cultivated and arable weeds, such as: *Ballota acetabulosa* in Lehaina of Eleia, *Cicer arientinum* in Livanates of Fthiotida, *Bignonia radicans, Canna indica, Cotoneaster integerrima, Cyperus rotundus, Flomis fruticosa, Gazania splendens, Malva sylvestris, Plantago major, Reseda alba, R. lutea, Scandix pecten-veneris, Sinapis alba, Spinacia oleracea, Spiraea media, Vicia faba,* and two other unidentified species in Votanikos, Kiphissia, and Polydrosso of Attica, *Spinacia oleracea* in Halastra and Vassilika of Thessaloniki, Plagia and Triglia of Chalkidiki and Psachna of Evia, and *Sinapis alba* in Triglia of Chalkidiki. A severe yellows problem with a high frequency infection was observed in spinach crops of the Marathon area in Attica, in the above-mentioned areas of Thessaloniki, Chalkidiki and Evia, and in one chickpea crop (*Cicer arientinum*) in Livanates of Fthiotida. The spinach crops in Marathon were particularly affected by a high BWYV-infection frequency and by mixed infection with *Cucumber mosaic virus* (CMV). In addition to the bright-yellow symptomatology of BWYV, plants showed severe stunting, leaf malformation, and a certain degree of plant death. In Marathon, too, BWYV was common on the perennial forest shrub *Flomis fruticosa* and the perennial herbs *R. alba, R. lutea* and *Malva sylvestris*. These plants are obviously infected throughout the year, providing a continuous source of BWYV inoculum which then infects crop plants such as spinach and arable weeds, annual and perennial, through the aphids. Considering the Marathon area, therefore with in extensive vegetable production, BWYV sources of inoculum are available for the infection of these plants in widely dispersed areas throughout the year. There were also many BWYV infected plants in the Votanikos area of Attica, including the grounds of the Agricultural University of Athens, with both perennial and annual species, and especially *Sinapis alba*, which is commonly infected throughout Attica. The virus was experimentally transmitted from field-infected chickpea to *Raphanus sativus* by *Myzus persicae*, in the persistent way. The data collected so far suggest that BWYV is by now widespread and common throughout Greece on an extensive host range and poses a significant phytopathological threat to the country.

Screening tobacco varieties or genetic lines for resistance to viruses non-persistently transmitted by aphids. K. Efthimiou[1], E.K. Chatzivassiliou[1], E. Efthimiou[1], E. Kontezaki[1], V. Maliogka, V. Michalak[2], K. Seitos[3] and N.I. Katis[1]. [1]*Aristotelian University of Thessaloniki, Faculty of Agriculture, Plant Pathology Laboratory, Thessaloniki 540 06, Greece.* [2]*Tobacco Research Station of Katerini, 8 Fleming St., 601 00, Katerini, Greece.* [3]*Tobacco Research Station of Karditsa, 10 Agriniou St., 431 00 Karditsa, Greece.*

Two experimental plots were established in the Katerini and Karditsa areas in 1998 and 1999. To screen tobacco cultivars or genetic lines for resistance to viruses non-persistently transmitted by aphids: *Potato virus Y* (PVY), *Alfalfa mosaic virus* (AMV) and *Cucumber mosaic virus* (CMV). No insecticide was applied in these experimental plots. Nine tobacco cultivars were included in the study; the oriental Kolindrou, Σ53, KE 26/2, KI 26/10 and Σ79, the Virginia type VE-9 and Niki 3 and the Burley type B21 and TN86. Leaf-samples were collected in September and tested by ELISA using polyclonal antisera against PVY, AMV and CMV. In both years, AMV infection was too low to draw reliable conclusions pertaining to the cultivars tested. In 1998 infection rates in Karditsa ranged from 4.4 to 25.8% and from 0.0 to

9.6% for PVY and CMV respectively while in Katerini they were from 0.7 to 25.7% and from 6.8 to 68%. However, infection rates varied considerably among replicates. In 1999, CMV infection was higher in Karditsa (max. 97.9%) and PVY infection higher in Katerini (max. 99.2%). The Virginia and Burley tobacco types were almost 100% infected with both CMV and PVY, except TN-8, which was not infected with PVY. However, this cv. had shown some infection by PVY (26.4%) in the previous year. Oriental cultivars had lower infection rates than other cultivars. Infection rates of CMV in the tobacco cv. KE 26/2 and KI 26/10 tested in Karditsa were 46.9% and 49.8%, but the cv. Kolindrou, Σ53 and Σ79 tested in Katerini were the most resistant to PVY (7.9%-11.8%).

***Ilarvirus* related to grapevine angular paraveinal mosaic disease, a new viral disease of grapevine in Greece.** S.M. Girgis[1], F. Bem[2], P.E. Kyriakopoulou[3], C.I. Dovas[4], A.P. Sklavounos[3], A. Avgelis[5], N. Katis[4], A.M. Tsagris[6] and S. Tzortzakaki[6]. [1]*National Agricultural Research Foundation, Grapevine Institute, 1 S. Venizelou St., 141 23 Lycovrisi Attikis, Greece.* [2]*Benaki Phytopathological Institute, 8 S. Delta St., 145 61 Kifissia, Athens, Greece.* [3]*Agricultural University of Athens, Department of Plant Pathology, 75 Iera Odos, 118 55 Votanikos, Athens, Greece.* [4]*Aristotelian University of Thessaloniki, Plant Pathology Laboratory, 540 06 Thessaloniki, Greece.* [5]*National Agricultural Research Foundation, Plant Protection Institute, 711 03 Heraklion, Crete, Greece.* [6]*Institute of Molecular Biology and Biotechnology and University of Crete, Greece.*

In 1994, a new viral symptomatology was reported on grapevine in the collection of the Grapevine Institute in Athens, Greece, on the hybrid Baresana x Baresana. Symptoms consisted of distinctive sharp angular mosaic connected with the veins or vein angles, small leaves, leaf malformation and reduced vine size. Affected vines produced no or very little fruit and had smaller, wrinkled and non-germinating seeds. A virus was isolated from young infected leaves by mechanical inoculation of *Gomphrena globosa*, causing both local and systemic dark-red or necrotic lesions. The virus was single-lesioned and was mechanically transferred to *C. quinoa*, where it caused necrotic local lesions and systemic mottle, and to 3 cultivars of tobacco, on which it caused sharp necrotic local lesions 1–3 mm in diameter. Pollination of *C. quinoa* with pollen from infected *C. quinoa* plants gave about 30% infected seedlings. The virus was purified from *C. quinoa* by differential centrifugation using, for virus extraction, 0.02 M phosphate buffer pH 8.0, containing 0.01 M DIECA and 0.01 M sodium thioglycolate. In a purified preparation, quasispherical virus particles of about 29 nm diameter were observed. Electrophoretic mobility of the viral coat protein showed a molecular weight of 30,000 daltons. Using purified preparations containing about 5 mg/ml virus, an antiserum was obtained with a titre of 1:1024 in the microprecipitin test, and an optimum IgG dilution end point in PTA-ELISA of 10,000-20,000 for maximum absorption at 405 nm. Using degenerate primers designed from homologous regions of RNA-2 of Ilarviruses, the expected 381-bp PCR-product was obtained. This product was cloned and sequenced. Comparisons with sequence data from the homologous regions of the RNA-2 of other known Ilarviruses showed that the sequence of the above 381-bp amplicon shared a sequence similarity of 72% with *Tobacco streak virus*, 67% with *Citrus variegation virus* and *Spinach latent virus*, 66% with *Asparagus virus 2* and *Elm mottle virus*, and 65% with *Citrus rugose leaf virus*. From the above data it is concluded that the isolated virus is an *Ilarvirus* from grapevine. To our knowledge, this is the first positive record of an *Ilarvirus* infecting *Vitis*.

Resistance of American varieties of grapevine rootstock to mealybug–transmitted leafroll–associated virus 3. N. Ioannou and A. Hadjinicolis. *Agricultural Research Intitute, 1516 Nicosia, 22016 Cyprus.*

Leafroll is the most common virus disease of grapevine in Cyprus, with an average incidence of 45% in traditional varieties and 80% in introduced ones. The cause of leafroll has been attributed to seven different closteroviruses. Although four of these viruses have been identified in Cyprus, only one grapevine leafroll-associated virus 3 (GLR·V-3) appears to be of economic importance. This virus is transmitted by two mealybug species, *Planococcus ficus* and *P. citri*. The natural spread of GLR·V-3 has been monitored in recent years in many vineyards and variety collections around Cyprus, using ELISA for virus detection in plant tissues and in the mealybug vector. The results indicate that in areas and/or years of severe mealybug infestation, the rate of GLR·V-3 spread is alarmingly high, leading to practically 100% virus infection within a few years. Such high levels of infection are found in all European grapevine varieties *(Vitis vinifera)*. By contrast, levels of infection in American rootstock varieties are always very low. Thus, virus incidence in *V. rupestris* grown at Acheleia was only 7%, compared with 73-94% in six vinifera varieties grown on the same plot. On another plot at Zygi, the average virus incidence in 17 American rootstock varieties was 3.5%, compared with 92% in 45 vinifera varieties. The incidence in rootstock varieties increased from 3.5% to 8.2% when GLR·V-3 was measured biologically, by graft-inoculation on a Cabernet Franc indicator, rather than by ELISA. The low levels of GLR·V-3 infection in American rootstock varieties may therefore to some extent be the result of the difficulty of ELISA-detection of the virus in rootstock tissue. Our data also indicate however that American rootstock varieties possess considerable resistance (lower susceptibility) to the mealybug–GLR·V-3 complex. The main reason for such resistance appears to be the poor development of mealybugs on the American rootstocks.

Tomato apical necrosis: a new virus disease of tomato caused by a strain of *Parietaria mottle virus* (PmoV). N.I. Katis[1], P. Roggero[2], C.I. Dovas[1] and E.K. Chatzivassiliou[1]. [1]*Aristotelian University of Thessaloniki, Faculty of Agriculture, Plant Pathology Laboratory, 540 06 Thessaloniki, Greece.* [2]*Istituto di Fitovirologia Applicata CNR, Strada delle Cacce 73, 101 35 Torino, Italy.*

A necrotic disease of tomato causing apical necrosis, necrotic spots on the leaves, and corky rings and brown patches on

the fruit surface was occasionally observed in an industrial tomato variety in Arethousa (Thessaloniki) and a table variety in the Saint Gregory Monastery in Mount Holly (Mount Athos). Necrotic areas on the leaves may break away from the surrounding tissue and drop out. Fruits do not show internal browning. Disease symptoms initially resemble those caused by *Tomato spotted wilt virus* (TSWV) or *Cucumber mosaic virus* with satellite RNA (CARNA 5-CMV). TSWV and CMV are widespread in tomato crops in Greece and this does not facilitate virus diagnosis. Disease symptoms are generally observed in June to July in young tomato plants. Apical necrosis is usually followed by the appearance of new leaves without any symptoms. The causal agent of the disease is a virus that belongs to the genus *Ilarvirus*, and is serologically related to *Parietaria mottle virus* (PmoV). Mechanical inoculation of *Chenopodium quinoa*, using sap from symptomatic tomato tissue, produced systemic infection similar to that caused by PmoV. In addition, mechanical inoculation onto tomato plants in the laboratory with purified preparation of the virus resulted in field-symptom reproduction.

Incidence of insect-borne cucurbit viruses in Greece. C. Papavasiliou[1], E. Hatziloukas[1], C.I. Dovas[1], K. Efthimiou[1], A.D. Avgelis[2] and N.I. Katis[1]. [1]*Aristotelian University of Thessaloniki, Faculty of Agriculture, Plant Pathology Laboratory, 540 06 Thessaloniki, Greece.* [2]*National Agricultural Research Foundation, Plant Protection Institute, 711 00 Heraklion, Crete, Greece.*

In 1999 and 2000, a survey was carried out to determine virus incidence in cucurbit crops. A total of 1442 leaf samples were collected in 1999 and 740 in 2000 from 16 prefectures in Greece, and from plants showing virus-like symptoms such as yellowing, mosaic, blistering, stunting, and leaf and fruit malformations. Virus diagnosis was based on serology (ELISA) using polyclonal antisera. In 1999, tests were carried out to detect the following viruses: *Cucumber mosaic cucumovirus* (CMV), *Zucchini yellow mosaic potyvirus* (ZYMV) and *Watermelon mosaic 2 potyvirus* (WMV-2). In 2000, the tests were extended to include detection of the following viruses: *Zucchini yellow fleck potyvirus* (ZYFV), *Squash mosaic comovirus* (SqMV) and *Cucurbit aphid-borne yellows luteovirus* (CABYV). Infection incidences were as follows: a. on watermelon samples collected in 1999 37% by ZYMV and 13% by WMV-2; on those collected in 2000 7% only by WMV-2. CMV was not detected on watermelon. On melon samples in 1999 67% predominantly by WMV-2, 33% by CMV and 9% by ZYMV; in 2000 48% by WMV-2, 45.5% by CMV, 25.5%, by SqMV, 10.5%, by CABYV, 1.5%, by ZYMV, 1.5% and none by ZYFV. c. on Zucchini in 1999 76% by WMV-2, 29.5% by ZYMV, and 10,5% by CMV; in 2000 83.5% by WMV-2, 39.5% by CMV, 14% by CABYV, 4.5% by ZYMV, and 0.5% by ZYFV.

Transgenic tobacco plants expressing CMV coat protein show resistance to mechanical inoculations with the virus. V. Plastira[1], E.I. Paplomatas[2] and F. Bem[2]. [1]*National Agricultural Research Foundation, Institute of Mediterranean Forest Ecosystems and Forest Technology Products, Terma Alkmanos, 115 28 Athens, Greece.* [2]*Benaki Phytopathological Institute, 8 S. Delta St., 145 61 Kifissia, Athens, Greece.*

Transgenic tobacco plants were produced using *Agrobacterium tumefaciens*-mediated transfer. Leaf disks of Σ53 tobacco plants were co-cultivated with a disarmed strain of *Agrobacterium tumefaciens* transformed by the binary vector plasmid pROK2 , which contained the coat protein gene of a Greek CMV strain and a kanamycin-resistance selection gene. The cultures, were then transferred to an MS medium containing cefotaxime for the control of *Agrobacterium*, kanamycin for the selection of transformed plants and plant growth regulators NAA and BA in order to induce shoot regeneration. The regenerated shoots were transferred to an MS medium containing kanamycin, cefotaxime, IAA, kinetin and folic acid, where they developed roots. The rooted plants were micropropagated in the same medium and potted up in the greenhouse. PCR and southern blot were used to verify the transfer of genes. Five clones of Σ53 tobacco plants were produced expressing the CMV coat-protein gene and the kanamycin-resistance gene, and 5 clones expressing only the kanamycin-resistance gene. The plants were mechanically inoculated with a very infectious Greek strain of CMV (Trikala strain). The transgenic plants expressing the CMV coat-protein gene did not show any symptoms, while the transgenic plants expressing only the kanamycin resistance gene showed very strong symptoms.

Strain emergence in *Cucumber mosaic virus*. A.P. Sklavounos[1], P.E. Kyriakopoulou[1] , D. Euthymiopoulos[1], M. Tzamopoulou[1] and C. Arabatzis[2] [1]*Agricultural University of Athens, Department of Plant Pathology, 75 Iera Odos, 118 55 Votanikos, Athens, Greece.* [2]*Agricultural University of Athens, Department of Biotechnology, 118 55 Votanikos, Athens, Greece.*

Cucumber mosaic virus (CMV) is very damaging to agriculture, as it is extremely polyphagous, extremely epidemic and causes severe symptoms; it is known to infect more than 1000 botanical species, is transmitted quickly (stylet-borne) by about 100 aphid species and to have inoculum sources available throughout the year. In the last 20–30 years, it has caused severe problems in tomato and other crops in southern European countries, including Greece. It is a multicomponent virus, consisting of 4 RNA molecules encapsulated in 3 particles, thus allowing for easy reassortments. CMV expresses high genetic plasticity, as seen in nature by the frequent emergence of new strains, some of which are highly pathogenic and damaging. This genetic plasticity was studied in the laboratory by analyzing the progeny of one pure CMV isolate derived from an infected tomato plant in Gastouni, Eleia county (isolate CMV-G) lacking CARNA 5; this study was based on the classical experiments of Price (1934). From a tobacco plant in which CMV-G was maintained, the virus was mechanically transferred to the local lesion host *Chenopodium quinoa*, and single lesions were mechanically inoculated on tobacco "Xanthi nc", to obtain one isolate from each lesion. Sharp differences in symptoms occured among these plants, ranging from extensive bright-yellow to mild-green mosaic; these symptoms were stable, as shown after their 4 successive passages in plants of the same cv. (tobacco "Xanthi nc"), during a 4-month experiment in an air-conditioned greenhouse. Various CMV isolates were thus obtained,

ranging from strongly pathogenic to very mild, with intermediate degrees in between. Similar results were obtained when transmission from tobacco "Xanthi nc", pepper, cucumber, and tomato was performed with the aphid *Myzus persicae* Sulzer. Here one aphid individual per tobacco indicator was used, and aphid transfer was done after its first 40 sec probing on the virus source. The tobacco indicator reactions ranged from bright-yellow to green mosaic. From the bank of these CMV isolates, maintained in the laboratory at 4°C as $CaCl_2$-dried samples, nine, G1-G9, were chosen to study their host range and symptoms, the electrophoretic mobility of their intact particles, the RFLPs of their capsid protein gene (900 nt) and of a 650-nt-long 5' end of their polymerase gene (RNA-2), and their transmissibility by *M. persicae*. The samples did not differ in their particle electrophoretic mobility or in the RFLPs of their capsid protein and polymerase genes, and belonged to the same CMV subgroup, 1a, as their mother isolate CMV-G. However, they expressed striking symptomatological differences, especially in their solanaceous hosts. The nucleotide sequences of the capsid protein gene of the 4 mild green mosaic isolates were identical, but minor differences were found in their non-coding regions. The present symptomatological and molecular analysis shows the genetic plasticity of CMV and explains the natural phenomenon of frequent emergence of new strains. In a CMV-infected plant, various isolates of the virus may emerge through random mutations, recombinations or reassortments, or combinations of these phenomena, which can be selected for by mechanical or aphid transmission. The importance of vector aphids in CMV strain-emergence in nature, by selecting, reassorting and spreading, is obvious.

The effect of temperature on variation in transmission of a BYDV PAV-like isolate by clones of *Rhopalosiphum padi* and *Sitobion avenae*. I.N. Smyrnioudis[1,2], R. Harrington[1], M. Hall[1], N.I. Katis[2], and S.J. Clark[3]. [1]*Department of Entomology and Nematology, IACR-Rothamsted, Harpenden, Herts AL5 2JQ, UK.* [2]*Aristotelian University, Faculty of Agriculture, Plant Pathology Laboratory, 540 06 Thessaloniki, Greece.* [3]*Department of Statistics, IACR-Rothamsted, Harpenden, Herts AL5 2JQ, UK.*

Variation in the transmission efficiency of one PAV-like isolate from Greece among seven clones of *R. padi*, four clones of *S. avenae* collected from different sites in northern Greece and one clone of *S. avenae* obtained from IACR-Rothamsted, Harpenden, Herts, UK, was assessed at three temperatures, and the epidemiological implications of the results were investigated. The transmission efficiency of the PAV isolate differed between *R. padi* clones and between temperatures. Examination of the vectoring efficiency of the *S. avenae* clones suggested that there was no difference between them but there was a significant difference between temperatures. *R. padi* had almost the same vectoring efficiency pattern at 5° and 10°C, but a different one at 15ºC. However, for *S. avenae* the most and least efficient clones changed between temperatures. Temperature had an effect on vectoring efficiency for both species with significantly greater transmission at the highest temperature. The mean transmission efficiencies for *R. padi* at 5º C, 10º C and 15º C were 53.0, 71.7 and 83.3% respectively. For *S. avenae* the mean transmission efficiencies at 5, 10 and 15ºC were 8.9, 21.4 and 26.4% respectively. The results of the transmission studies demonstrated that temperature plays a significant role in of vectoring efficiency among clones of *R. padi*.

Aphid population fluctuations and the spread of aphid-borne viruses in tobacco crops in Greece. J.A. Tsitsipis[1], I. Gargalianou[1], E.K. Chatzivassiliou[2], K. Efthimiou[2] and N.I. Katis[2]. [1]*University of Thessalia, Faculty of Agriculture, Entomology and Agricultural Zoology Laboratory, 383 34, Pedion Areos, Volos, Greece.* [2]*Aristotelian University of Thessaloniki, Faculty of Agriculture, Plant Pathology Laboratory, 540 06 Thessaloniki, Greece.*

The spread of two aphid-borne viruses**,** *Cucumber mosaic virus* (CMV) and *Potato virus Y* (PVY) was studied in relation to the aphid population in two tobacco-growing areas for two years (1998-1999). In the first area (Karditsa), an aromatic-type tobacco variety (Virginia Mc Nair 944) was grown, in the second (Katerini) an oriental-type variety (Samsous). In both areas**,** the alate aphid population was monitored weekly by Moericke yellow traps. Virus incidence was evaluated by randomly collecting biweekly 100 tobacco leaf samples twice a week and testing them serologically by ELISA using polyclonal antisera. The first sampling was carried out one month after transplanting. In Karditsa 132 and 117 aphid species were recorded in 1998 and 1999 respectively. In 1998 the most abundant species captured were *Brevicoryne brassicae, Aphis spiraecola, Phorodon humuli, A. gossypii, Myzus persicae* and *Lipaphis erysimi;* in 1999 *Aphis gossypii, M. persicae, A. spiraecola, A. craccivora, Chaitophorus populialabae* and *L. erysimi.* In 1998, the incidence of CMV and PVY was relatively low ranging from 7.0 to 14.0 and from 1.0 to 8.0% respectively. In 1999, the incidence of both CMV and PVY was high, from 2.0 to 65.0 and from 25.0 to 98.0 % respectively. Absolute aphid numbers were higher in 1999 than in 1998. In Katerini, 122 and 117 aphid species were recorded in 1998 and 1999 respectively. In 1998**,** the most abundant species here were *Microlophium* sp., *Tetraneura akinire, P. humuli, A. spiraecola, M. persicae* and *A. fabae*; in 1999 they were *A. gossypii, M. persicae, A. spiraecola, A. craccivora, C. populialabae* and *L. erysimi*. Infection rates of CMV ranged from 1.8 to 6.0% in 1998**,** and from 2.0 to 8.0% in 1999. PVY incidence was even lower, 1.0–2.4% in 1998 and 1.8–4.0% in 1999. In 1998 and 1999**,** the maximum aphid populations appeared in the 19th and 22nd week respectively, four to six weeks before tobacco was sampled to evaluate virus incidence. The importance of these results on the epidemiology of CMV and PVY is discussed.

The NTN strain of *Potato virus Y* (PVY^{NTN}): a new threat for potato cultivations in Greece. C. Varveri and F. Bem. *Benaki Phytopathological Institute, 8 S. Delta St., 145 61 Kifissia, Athens, Greece.*

The severe NTN strain of *Potato virus Y* (PVY^{NTN}), causing the potato tuber necrotic ringspot disease (PTNRD), appeared for the first time in Greece in 1994 on a limited scale in the Nevrokopi area. Measures taken at that time halted further

disease spread. In 1998 however, the disease reappeared in a more epidemic form, mainly in potato cv. Hermes cultivations established with seed potatoes imported from Scotland. Specific detection of PVY^{NTN} was achieved with the PCR technique, the only method capable of distinguishing the NTN strain from the rest of the N group, where it belongs. All symptomatic tubers of the cv.. Hermes and Spunta tested gave the characteristic PCR product of 835 bp. The same product was also obtained with 60% of tubers of cv. Fabola, Santana and Irvila bearing non-typical symptoms. In 1999 cultivations with basic potato seed cv. Spunta imported from Holland were established in fields contaminated in the previous period. ELISA on 110 tubers from these fields showed an overall PVY infection rate of 52%. The PCR test of the infected samples showed that PVY^{NTN} was responsible for 96% of these infections. Furthermore, certified potato seed cv. Hermes also planted in contaminated fields and of 113 tubers tested, PVY was detected in 36% of which PVY^{NTN} was responsible for 34%, while tests on 75 tubers from non-contaminated fields revealed infection rates of 5% for PVY, 50% of which from PVY^{NTN}. The above data show that this severe strain is established in the region and has strong epidemic characteristics.

In-*trans* action of the 2b protein of *Tobacco rattle virus* (TRV) in the transmission of the virus by nematodes. N. VASSILAKOS, D.J.F. BROWN and S.A. MACFARLANE. *Scottish Crop Research Institute, Invergowrie, Dundee DD2 5DA, Scotland, UK.*

Tobacco rattle virus (TRV) and *Pea early-browning virus* (PEBV) are transmitted by nematodes belonging to the genera *Trichodorus* and *Paratrichodorus* in a highly specific manner. The 2b gene and the coat-protein gene, which are both carried in the smaller RNA2 segment of the *Tobravirus* genome are essential for nematode transmission. Transgenic tobacco plants were constructed, incorporating either the 2b gene of the TpA56 isolate of PEBV or the 2b gene of the PaY4 isolate of TRV, which has recently been sequenced. The transgenically expressed 2b protein did not complement nematode transmission of a homologous virus that was engineered to abolish expression of the same (2b) protein. However, in mixed infections, the 2b protein expressed by a nematode-transmissible (2c) mutant of TRV-PaY4 complemented transmission of a 2b mutant of the same virus (which on its own is not transmitted by nematodes). In contrast, nematode transmission of the TRV PaY4 2b mutant was not complemented by co-infection with a transmission-competent PpK20 isolate of TRV. The results of the mixed-infection test suggest that the 2b protein acts in-*trans*, and indicate that it is specifically associated with the coat protein for transmission of TRV by nematodes. The unsuccessful transmission complementation of the 2b-mutant viruses by the transgenically expressed 2b protein is discussed.

Bacterial, non-parasitic diseases, various aspects

Records of bacterial diseases in Crete. D.E. GOUMAS and A.K. CHATZAKI. *National Agricultural Research Foundation, Plant Protection Institute 710 03 Heraklion, Crete, Greece.*

Small spots with a chlorotic halo initially appeared on the leaves of an ornamental *Magnolia* sp. As the spots enlarged they became papery in the center with brown-black margin. Eventually the whole leaf died. Isolated bacteria from the spots were identified as members of the species *Pseudomonas syringae* pv. *syringae*. Losses of 70% of eggplant seedlings at the 3rd to 4th true-leaf stage were observed in a nursery. Infection of the plants was noted in February and March of two successive years. Primary symptoms consisted of water-soaked lesions on leaves of the seedlings. These lesions rapidly covered the entire leaf and then progressed systemically to the stem and whole plant, which became rotted or blighted. From affected eggplants the bacterium *Pseudomonas viridiflava* was repeatedly isolated and identified as the causal agent. Galls of varying size were observed on the roots of native bushes of *Atriplex humilus* removed from the Venetian walls of Heraklion city. Biotype II of *Agrobacterium tumefaciens* was isolated and identified as the causal agent of this disease. Small round or irregular spots with or without a chlorotic halo were observed on the leaves of carnation plants, and gradually destroyed part of the foliage. The disease was observed at the area of Arvi, Heraklion Prefecture, but has not been recorded subsequently. *Pseudomonas andropogonis* was isolated and identified as the causal agent of this disease.

Ring rot of potato four years after its first record in Crete. D.E. GOUMAS[1], A.K. CHATZAKI[1], J. TROULAKIS[2]. [1]*National Agricultural Research Foundation, Plant Protection Institute, 710 03 Heraklion, Crete, Greece.* [2]*Center of Plant Protection, 710 00 Heraklion, Crete, Greece.*

The spread of potato ring rot in Crete over the last three years, since its first record on the Lassithi plateau, was studied. The manner of its spread was deduced from analysis of a large number of laboratory tests on randomly selected tuber samples. More than 2000 samples were collected from the main potato producing area in Crete in the last three or four cropping seasons and analysed according to EU directive 93/85/1993. Preliminary data on the epidemiology of *Clavibacter michiganensis* subsp. *sepedonicus* are presented and related to its survival in asymptomatic weed host plants and potato volunteer plants and to its possible dissemination by insects. It was confirmed that the bacterium can be detected as early as 60 days after planting the potato crop.

Effects of iron deficiency on the morphology of the root system of some peach rootstocks. A. ASSIMAKOPOULOU[1] and C. FASSEAS[2]. [1]*Benaki Phytopathological Institute, 8 S. Delta St., 145 61 Kifissia, Athens, Greece.* [2]*Agricultural University of Athens, Electron Microscopy Laboratory 75 Iera Odos, 118 55 Votanikos, Athens, Greece.*

Lack of iron from the nutrient solution reduced the length of young roots, increased the width of the subapical root zone (1 mm from the apex and few cm below), and increased the number of root hairs in five peach rootstocks (the peach-almond hybrids K.I. D 2, PR 204/84 and GF 677, plum M29C

and peach GF 305). These changes were apparent from the initial stages of Fe deficiency and were unrelated to the type of rootstock and the intensity of the chlorotic symptoms. Light and electron microscope examination of cross-sections of the root zone 2–10 mm from the root tip showed that the xylem vessels of Fe-deficient plants were more differentiated and were surrounded by xylem parenchyma cells containing a dense, organelle-rich cytoplasm and smaller vacuoles. In Fe-sufficient plants the parenchyma cells surrounding the xylem vessels at the same distance from the root apex had larger vacuoles and less cytoplasm. Repeated observation do not reveal any differences in the morphology of cell walls and cytoplasm of the rhizodermis and hypodermis between rootstocks grown with or without Fe.

Screening different peach rootstocks, grown in nutrient solution, for iron efficiency by using the iron-reducing capacity of the roots. A. Assimakopoulou[1] and C.D. Holevas[2]. [1]*Benaki Phytopathological Institute, 8 S. Delta St., 145 61 Kifissia, Athens, Greece.* [2]*Agricultural University of Athens, Plant Physiology and Morphology Laboratory, 75 Iera Odos, 118 55 Votanikos, Athens, Greece.*

In two experiments in a hydroponic system the iron efficiency of five peach rootstocks (peach-almond hybrids K.I. D 2, PR 204/84 and GF 677, plum M29C and peach GF 305) was studied using the iron-reducing capacity of the roots. The results showed that the absence of iron from the nutrient solution caused significant increases in the iron-reducing capacity of the roots in all rootstocks. This response was observed from the initial stages of iron deficiency. The tolerance of the rootstocks to Fe deficiency, as indicated by the intensity of the chlorotic symptoms, was found to be negatively correlated with the iron reducing capacity of the roots, expressed per unit root fresh weight. In addition, it was observed that the absence of iron from the nutrient solution increased the iron-reducing capacity of the roots of the more susceptible rootstock GF 305 7.5 times, of the moderately resistant M29C 3.0 times and of the more resistant peach-almond hybrids PR 204/84 and GF 677, 2.5 and 2.0 times respectively, compared with that of the complete nutrient solution.

Tropospheric ozone monitoring in a pine forest close to Korinthos - Greece. C.J. Saitanis, M.G. Karandinos and D.B. Lekkas. *Agricultural University of Athens, Ecology and Environmental Sciences Laboratory, 75 Iera Odos, 118 55 Votanikos, Athens, Greece.*

Preliminary results of 16 days (June 18 to July 3, 2000) of ozone monitoring, carried out instrumentally in a pine forest near Korinthos (Bogdani hill, located between Athikia and Almiri; altitude 280 m) are presented. The phytotoxicity of the ozone levels was checked using plants of subterranean clover (*Trifolium subterraneum* L.), known to be highly sensitive to ozone, placed there during the same period. The maximum hourly-mean of O_3 concentration recorded was 100 ppb. During the monitored period (384 hours) the thresholds of 30, 40, 50, 60, 70 and 80 ppb were exceeded 366, 323, 207, 92, 25 and 10 hours respectively. The diurnal pattern consisted in a period of gradual ozone increase from about 08:30 to about 18:30, at which it peaked (mean 70 ppb), followed thereafter by a gradual decrease till the next morning. The minimum ozone concentrations occurred from 04:00 to 08:00, when hourly mean concentrations ranged from 40 to 45 ppb. The accumulated ozone concentrations above the threshold of 40 ppb (AOT40), during the daylight hours from 09:00 to 17:00 of the 16 monitored days, were 2563 ppb◊hours. This suggests that the ozone levels in the monitored region should be considered highly phytotoxic since the AOT40 for non-injury to plants has been determined to be less than 3000 ppb-hours over a period of three months. The phytotoxicity of the monitored ozone concentrations was further confirmed by *Trifolium subterraneum* (L.), which exhibited the characteristic ozone-induced symptoms. This work is still in progress and additional results are expected.

Phytotoxic effects of ambient ozone on two indicator clover clones in Attica Greece. D. Velissariou and L. Skretis. *Benaki Phytopathological Institute, 8 S. Delta St., 145 61 Kifissia, Athens, Greece.*

In the framework of the International Cooperative Programme on Effects of Air Pollution on Natural Vegetation and Crops of the UN-ECE (Convention on Long-range Transboundary Air Pollution) and with the participation of 18 European countries, a monitoring network has been established in order to monitor and assess the effects of ambient ozone on natural vegetation and crops. The main method of assessment consists in exposure to the ambient air of two special clover clones (*Trifolium repens*), one sensitive to ozone and one resistant. Twenty plants of each clover clone are raised every year following a common protocol and are exposed to the ambient air at each test site in the 18 countries, including Greece. The data of this work refer to the exposure of the plants at Kifissia, Attica (Benaki Phytopathological Institute) in June–August 1997. In that period three harvests, one every 28 days, were done, the above-ground d wt was measured and the ratio of the mean d wt of the sensitive clone (NCS) to the resistant ones (NCR) was calculated. The ratio for each month was: June = 0.82, July = 0.55 and August = 0.74. The ozone levels above 40 ppb in ppb/h (AOT40) for these months, as recorded at the nearby State monitoring Station at Maroussi, were: June = 6444 ppb/h, July = 7586 ppb/h and August = 6710 ppb/h. R^2 of the correlation was -0.9861, showing a) the sensitivity of the method in defining a polluted environment, and b) the expected effects on sensitive plant species due to the particularly high ozone levels in the Attica basin during the test period.

Language problems in Plant Pathology. P.E. Kyriakopoulou. *Agricultural University of Athens, Department of Plant Pathology, 75 Iera Odos, 118 55 Votanikos, Athens, Greece.*

There is a widespread tendency to alter the standard pronunciation of certain established scientific terms in the narrow field of Plant Pathology, as well as more generally. In particular, inroads are being made by an anglicided pronunciation of Latin scientific terms and Greek words are interspersed with words from English and other languag-

es. Latin was the official language of Roman Empire until the sixth century A.D. and still keeps its "official" character in the scientific area, especially in the biological sciences, with an unvarying, indestructible order of things; it is Latin which is used for officially naming organisms (the Latin binomial: genus-species) and their taxa, and it is in Latin that new species and taxa have been officially described and registered since the time of Linnaeus (*Systema naturae, sive regna tria naturae systematice proposita*). In the international phytopathological and other scientific literature, therefore, whatever its language, when the name of an organism (plant, fungus, bacterium, *etc.*) is given for the first time, the Latin binomial is immediately appended in parenthesis, so that whoever reads knows the exact identity of the organism under discussion, and scientists can communicate safely with each other. Now, Latin has strict rules of grammar and pronunciation. These rules must be followed precisely when using Latin especially in science, where accuracy is absolutely fundamendal. So, the currently common "English" pronunciation of Latin names or terms, the borrowing of English words by Greek, and, in general, a looseness of pronunciation, from a desire to be modern or merely to conform, when using phytopathological or other scientific terms and expressions, signify a deviation from the language rules and is therefore to be deplored. This phenomenon is partly due to the scientific predominance, for many decades now, of the English-speaking countries, particularly the USA, where Latin is not taught in the basic curriculum. So scientists from these countries naturally pronounce Latin as if it were English, and scientists from other countries, who study in the USA or other English-speaking countries, follow their anglicised pronunciation of Latin, unconciously, or in order to conform to them. Since English is the international language of today, this tendency has become a worlwide phenomenon, and if we also consider that many non-native speakers do not even pronounce English properly, the mispronunciation of Latin often reaches a point where Latin words are no longer recognisable. Whatever the reason for this tendency, however, whether from plain ignorance, from laxness, or from a desire to be conform or to be trendy, it is not consistent with scientific accuracy and it ignores the fact that scientific knowledge and research are fields and temples of truth, harmony and youth, which have an eternally modern texture in and of themselves and need no makeup. The faulty and fashionable ornamentations thus forced upon the scientific language confound the listener and are heard with repugnance in the temple of science. It is especially so here in Greece. Greek is the mother of the western languages, to a large extent mainly through Latin. Most scientific names, such as *Phytophthora, Rhizoctonia, Xylophilus* etc. are commonly understood in Greece if they are pronounced properly; if not they are not understood, or sound repellent. The need for a constant, standard and rigorously pronounced terminology when definig concrete meanings and terms was always one of the demands of scientific gnosiology, and the significance of this demand is obvious. The story of Babel in the Bible presents us with a clear and convincing example of what happens when language confusion is allowed to reign.

Alternatives to methyl bromide

Grafted seedlings in vegetable culture. A. Balokas, A. Perdikaris, M. Kastanias and G. Kontosfiris. *Spyrou Co, 5 Markoni St., 122 42 Athens, Greece.*

Spyrou Co evaluated the effect of soil fungal pathogens on Cucurbitaceous rootstocks through the production of grafted seedlings that could be grown without chemical soil disinfectants. We present the fieldwork done in order to determine the resistance of these rootstocks to *Fusarium oxysporum* f. sp. *radicis-cucumerinum*, which is a major problem in Crete and the Peloponnese. Cucumber seedlings as well as seedlings grafted on three different rootstocks were evaluated in eight different naturally infected fields in Ierapetra, Crete. In these experiments, one rootstock and two hybrids were found to be resistant to the pathogen. The resistant rootstock is a *Cucurbita maxima* x *C. moschata* hybrid. Furthermore, the agronomic characteristics (productivity, maturity and fruit quality) of commercial hybrids grafted on the resistant rootstock are currently being evaluated in at least three large-scale field-experiments in northern, central and southern Greece. Laboratory evaluation of the resistance of the Cucurbitacea to the above pathogen is under way.

Control of *Rhizoctonia solani* damping-off in tomato seedbeds by methods other than methyl bromide. I.E Marouli and K. Tzavella-Klonari. *Aristotelian University of Thessaloniki, Faculty of Agriculture, Plant Pathology Laboratory, 540 06 Thessaloniki, Greece.*

The use of methyl bromide in soil disinfection is common in tomato seedbeds and in crops grown in greenhouses of northern Greece because of its effectiveness against soilborne pathogens such as *Rhizoctonia solani* (Kühn), causing damping-off diseases. In a research program to explore alternatives to methyl bromide, two chemical and one biological agent were tested. The four treatments examined were: methyl bromide (68 g/m^2); metham sodium (200 ml/l of water); quintozene+etridiazole as Terrachlor Super-X (2 applications with 0.3 g/l of water/m^2); and the fungus *Trichoderma koningii* (incorporated in the soil at 4 g wheat brans six-day-old culture in 300 g soil). The experimental seedbeds were established at the greenhouse of the Aristotelian University of Thessaloniki from November 1999 to March 2000. They were artificially inoculated with *Rhizoctonia solani* cultures in a cornmeal, peat and water medium (8 g of culture for 300 g soil). Each seedbed was seeded with 80 seeds of tomato cv. Ace 55. The greenhouse was heated at night in order to keep the temperature at 20°C. The experiment was repeated twice. Samples were taken every 15 days. The first sampling began 36 days after sowing. Treatment evaluation was by counting the healthy plants good enough to be transplanted. There was no significant difference in percentage of healthy plants between the methyl bromide and metham sodium treatments in the first samples. Methyl bromide continued giving steady protection in subsequent samples. There was no significant difference between Terrachlor and the biological agent, but both these treatments were less effective than methyl bro-

mide. Moreover treatment with *T. koningii* gave well-developed plants.

Control of *Sclerotinia sclerotiorum* in tomato seedbeds, by methods other than methyl bromide. The influence on plant height. I.E Marouli. and K. Tzavella-Klonari. *Aristotelian University of Thessaloniki, Faculty of Agriculture, Plant Pathology Laboratory, 540 06 Thessaloniki, Greece.*

In a study to explore alternatives to methyl bromide, two chemical and one biological agent were tested. The four treatments examined were: methyl bromide (68 g/m^2); metham sodium (200 ml/l of water); quintozene+etridiazole as Terrachlor Super-X (2 applications with 0.3 g/l of water/m^2); and the fungus *Trichoderma koningii* (incorporated into the soil at 4 g wheat brans six-day-old culture in 300 g soil). The experimental seedbeds were established at the greenhouse of the Aristotelian University of Thessaloniki from November 1999 to March 2000. They were artificially inoculated with *Sclerotinia sclerotiorum* (Lib.) de Bary cultures on carrot slices (10 g/300 g of soil). Each seedbed was seeded with 80 seeds of tomato cv. Ace 55. The greenhouse was heated at night to keep the temperature at 20°C. The experiment was repeated twice. Samples were taken every 15 days, starting 36 days after sowing. Treatment evaluation was by measuring the height of tomato plants. The biological agent influenced growth of infected plants to the same extent as methyl bromide or metham sodium. Plants irrigated with Terrachlor Super-X remained as short as the control plants which had not received any chemical or biological treatment.

Methyl bromide replacement to control Verticillium wilt of eggplant. A.M. Moustafa[1], F.A. Bletsos[2] and C.C. Thanassoulopoulos[1]. [1]*Aristotelian University of Thessaloniki, Faculty of Agriculture, Plant Pathology Laboratory, 540 06 Thessaloniki, Greece.* [2]*National Agricultural Research Foundation, Agricultural Research Center of Macedonia and Thrace, 570 01 Thermi-Thessaloniki, Greece.*

The use of methyl bromide (MB) as a soil fumigant has been suspended worldwide until 2005 for environmental reasons. Grafting and mulching were studied as alternatives to MB for controlling Verticillium wilt of eggplant, which is the most serious disease of this crop. Eggplant seedlings cv. Tsakoniki were transplanted in 1999 to an unheated-plastic covered greenhouse in which eggplants seriously infected with *Verticillium dahliae* had been grown for three years previously. Four types of treatments were carried out (control; methyl bromide 68 g/m^2; fumigation; lateral grafting on *Solanum torvum* rootstock, mulching with a transparent polyethylene sheet 0.05 mm thick) in three replications with 10 plants/replication. Evaluation was by measuring the following parameters: the Leaf-Symptom Index (LSI), Vascular Discoloration Index (VDI) and Disease Index (DI = LSIxVDI), plant height, early and total yields (commercial and uncommercial), fruit number of early and total yield, plant weight, above-ground plant weight and root weight. The grafting and MB treatments differed significantly from the control in all the measured characteristics, while mulching differed significantly in early and total yield and in fruit number. It has been concluded that grafting and mulching are practical alternatives to methyl bromide for control of Verticillium wilt of eggplant.

Evaluation of tomato and cucurbit rootstock resistance to *Verticillium dahliae*. E.J. Paplomatas[1], K. Elena[1], A. Tsagarakou[2] and M. Fotopoulou[2]. [1]*Benaki Phytopathological Institute, 8 S. Delta St., 145 61 Kifissia, Athens, Greece.* [2]*Spyrou S.A., 5 Markoni St., 142 22 Athens, Greece.*

In view of the phase-out of methyl bromide, there is an urgent need to find alternatives that may protect plants from soilborne fungal pathogens, especially in covered crops. The aim of the present study was to evaluate the resistance of tomato and cucurbit plants to *Verticillium dahliae* Kleb., so that they could be used as rootstocks for solanaceous and cucurbitaceous plants. Nineteen tomato and thirty-three cucurbit rootstocks were tested for their resistance to *V. dahliae* using artificial inoculations under greenhouse conditions. The pathogenicity tests were performed by dipping the plant roots into a conidial suspension (10^7 spores/ml) of the pathogen. For the cucurbit tests, a pathogen isolate from a diseased cucumber plant was used, while for the tomato tests two different isolates were selected to represent the two races (1 and 2) of the pathogen in that host. Disease incidence was scored by foliar symptoms. In plants with ambiguous symptoms, the presence of the pathogen in plant tissues was confirmed by PCR using DNA primers specific for *V. dahliae*. Of the cucurbit rootstocks, six were very resistant, twenty-one slightly susceptible, four moderately susceptible and two very susceptible. The tomato rootstocks likewise fall into four groups when tested with the race 1 of the pathogen. Three rootstocks were very resistant, ten slightly susceptible, four moderately susceptible and two very susceptible. By contrast, only three groups were formed, when tomato plants were inoculated with race 2 of the pathogen. With this race one of the tomato rootstocks was resistant to infection, seventeen were moderately susceptible and one was very susceptible. Based on the above findings, twelve combinations (scion-rootstock) of commercial tomato varieties were evaluated for their response to race 1 infection, six for response to race 2 infection and seventeen combinations of cucurbits for response to the cucumber isolate. Among the grafted cucurbits, four were resistant, three moderately susceptible, nine slightly susceptible and one very susceptible. Three of the grafted tomato combinations were resistant to race 1, seven slightly susceptible and two very susceptible, while all six combinations were equally susceptible to race 2. The above combinations are being evaluated under field conditions.

Current implementation of soil solarization in plastic houses or in open fields in Greece. E.C. Tjamos[1], P.P. Antoniou[1], S.E. Tjamos[1] and A. Paraskevopoulos[2]. [1]*Agricultural University of Athens, Department of Plant Pathology, 75 Iera Odos, 118 55 Votanikos, Athens, Greece.* [2]*Directorate of Agricultural Development of Kyparissia, Plant Protection Department, 245 00 Kyparissia, Greece.*

A twenty-three-year study of soil solarization in Greece dem-

onstrated that 4–6 weeks summer soil tarping with transparent polyethylene sheets effectively controls vascular wilts such as *Fusarium oxysporum* f. sp. *cucumerinum*, *F. oxysporum*. f. sp. *melonis* and *Verticillium dahliae* of tomatoes and artichokes. *Pyrenochaeta lycopersici* and *Clavibacter michiganensis* ssp. *michiganensis* of tomatoes may also be controlled. The control of Verticillium wilt of tomatoes and globe artichokes with a single application of soil solarization lasted for more than 2 to 3 years. This indicated the involvement of heat-tolerant fungal or bacterial antagonists. Reduced doses of methyl bromide and short-term solarization for 15 days with impermeable plastic sheets gave effective control of *F. oxysporum* f. sp. *cucumerinum*, while a single solarization for 30 days reduced the symptoms of *C. michiganensis* ssp. *michiganensis* of tomatoes. In a recent attempt to overcome the major disadvantage of soil solarization, land occupation for several weeks, we applied short-term solarization with unpermiable plastic sheets (15–20 days) in conjuction with a bacterial antagonist (*Paenibacillus* sp.) already shown to be effective in our department against *F. oxysporum* f. sp. *radicis-cucumerinum*. This combination successfully controlled the disease in western Greece. Soil solarization in its classical form is currently used mainly against soil-borne pathogens of vegetables in several parts of Greece.

Biological and integrated control

Effect of rhizosphere bacteria on the germination of V*erticillium dahliae* microsclerotia in tomato and eggplant roots. D.F. Antonopoulos, S.E Tjamos. and E.C. Tjamos. *Agricultural University of Athens, Department of Plant Pathology, 75 Iera Odos, 118 55 Votanikos, Athens, Greece.*

Verticillium dahliae survives in the soil in the form of microsclerotia (ms). Ms germination is stimulated by root exudates. To study the effect of rhizosphere bacteria on the germination of ms, tomatoes cv. Early Pack were transplanted to plexiglass boxes at the stage of six fully developed leaves in a soil mixture of peat:field soil:sand (1:1:1) according to Mol L. and Van Riessen H.W. (1995). Since the root-observation boxes, were transparent, roots in contact with ms could easily be collected. On the removable side of the boxes, a mixture of *V. dahliae* ms with a diameter greater than 71 µm and at a density of 15 ms/2 mm^3 was applied in a 2-mm-thick water-agar film as a supportive medium. The boxes were slanted so as to ensure contact between the roots and the agar layer. Light penetration through the transparent wall was prevented. The soil was drenched twice (five-day interval) with a suspension of bacterial strain K-165 (*Paenibacillus* sp.*)* or *Bacillus* strain 5-102 at a concentration of 10^7 cfu/ml. Five days after the second soil-drenching, the root tips and the roots 2 cm from the root tip (zone of root elongation) were collected. In a binocular microscope we calculated the percentage of germinated ms, the number of hyphae of each germinated ms and the hyphal length. Bacterial strain K-165 drastically reduced the germination of ms by about 40–50% compared with the controls but the number and length of hyphae of germinated ms were not affected. By contrast, isolate 5-102 did not exhibit any effect at all. To examine the effect of immunization with K-165 on ms germination, one eggplant seedling cv. Black Beauty at the stage of six fully developed leaves was transplanted to each box and the root system split into two equal parts. Roots were drenched with a bacterial suspension of K-165 (10^8 cfu/ml). Four days after application of the bacterial strain, a mixture of 3 ms/2 mm^3 and the water agar medium was applied to the non-bacterized side. Lastly, ms were collected from the same part of the root system and the percentage of germinated ms, number of germinated ms and hyphal length calculated. K-165 reduced ms germination by about 15% compared with the controls, while the number and length of hyphae were not affected. This study is a promising new approach to evaluate the antagonistic activity of bacterial strains such as K-165 against *V. dahliae* ms. The method also showed for the first time that an antagonistic *Paenibacillus* restricts ms germination *in situ* either directly or by induced systemic resistance.

Effect of olive-oil mill wastes on soil-borne phytopathogenic fungi. G. Argeiti, C. Ehaliotis, P. Katsaris, G. Zervakis and K. Papadopoulou. *National Agricultural Research Foundation, Institute of Kalamata, 85 Lakonikis St., 241 00 Kalamata, Greece.*

Olive-mill wastes (OMW) have antimicrobiotic activity, which has been attributed to the phenolic compounds, long-chain fatty acids and volatile acids they contain. Agricultural soils are prime receptors for OMW, which improves their fertility and structure. However very little information exists on the direct and/or indirect effect of OMW on soil-borne fungi. In the present work, the effect of OMW on two taxonomically different soilborne phytopathogenic fungi was studied: an isolate of the genus *Phytophthora* and an isolate of *Fusarium oxysporum* f. sp. *radicis-lycopersici*. The two isolates differed not only in their physiology and ecology but also in the means employed to control them. Crude or neutralized OMW (pH 6.5–7.0) was used in different concentrations to investigate the effect of OMW on fungal mycelium growth *in vitro*. The *Phytophthora* isolate was considerably more sensitive even to low concentrations of OMW than the *Fusarium* isolate. The pH was not a significant factor in the growth inhibition observed. However, total phenolics purified from OMW seem to play a role in OMW toxicity.

The possibility to control *Botrytis* on greenhouse tomato by the use of ionized water in the spraying solution. V.A. Bourbos and E.A. Barbopoulou. *National Agricultural Research Foundation, Institute of Subtropical Plants and Olive Trees of Chania, Plant Pathology Laboratory, Agrokipio, 731 00 Chania, Crete, Greece.*

Botrytis cinerea Pers. is a serious disease of tomato in unheated greenhouses. The purpose of this experiment was to examine the possibility of controlling this pathogen on greenhouse tomato (Baya hybrid) with ionized water in the spraying solution. A specific ionizator of the Superior Aqua Systems Company was used for water ionization. The average Cu ion content of the ionized water was 1 ppm. The widely used fungicide Sumico WP containing diethofencarb 25% +

carbendazim 25% was chosen as the reference product, applied at 100 g/hl water. The same product was used at 100, 50 and 25 g/hl water in combination with ionized water. The effectiveness of the fungicide+natural water combination ranged from 83.5 to 86.7%. When the fungicide was used with ionized water at 100, 50 and 25 g/hl, percent control of the fungi was, respectively, 97.4–100, 82.5–83.5 and 72.9–75.9%. Under the conditions of the experiment, ionized water in the spraying solution increased fungicide effectiveness. Particularly, the possibility of reducing fungicide recommended dose by 50% is of practical interest.

Control of powdery mildew of greenhouse cucumber using reduced dose of fungicides in combination with essential vegetable oils. V.A. Bourbos[1] and K. Georgiadis[2]. [1]*National Agricultural Research Foundation, Institute of Subtropical Plants and Olive Trees of Chania, Plant Pathology Laboratory, Agrokipio,731 00, Chania, Crete, Greece.* [2]*Geovet Hellas LTD, Veria, Greece.*

Powdery mildew of cucumber is attributed mainly to the fungus *Sphaerotheca fuliginea* (Schlechtend:Fr.) Pollacci and, under certain conditions causes severe damage to greenhouse cucumber crops. In the present work, we studied the possibility of controlling this pathogen by using a reduced dose of fungicide in combination with essential vegetable oils. We tested the fungicides pyrifenox and penconazole marketed as Dorado 20 EC and Topas, 100 EC at the normal and half the normal dose (Dorado 20 EC, 20 and 10 ml/hl water; Topas 100 EC 30 and 15 ml/hl water) in combination with a mixture of essential vegetable oils (marketed as Vivere-fyt) at 0.2 l/hl water. Vivere–fyt alone reduced the pathogen by 74.32%. When combined with pyrifenox and penconazole effectiveness increased to 97.3–98.0% for normal and 96.6–97.3% for half doses.

Biological control of *Fusarium oxysporum* f. sp. *lycopersici* on tomato grown in unheated plastic greenhouses. V.A. Bourbos[1], G. Michalopoulos[2] and E.A. Barbopoulou[1]. [1]*National Agricultural Research Foundation, Institute of Subtropical Plants and Olive Trees of Chania, Plant Pathology Laboratory, Agrokipio,731 00 Chania, Crete, Greece.* [2]*ZENECA HELLAS SA, Syngrou 231, 17121 Athens, Greece.*

Fusarium oxysporum Schl. f. sp. *lycopersici* (Sacc.) Snyder and Hansen under certain conditions causes damage to greenhouse tomatoes in various parts of Crete. The control of this pathogen with a biological product (trade name, Promot) containing the antagonists *Trichoderma harzianum* Rifai and *T. koningii* Oudem. applied at 0.15 g/m^2 was examined. Trials took place during two consecutive cultivation periods in unheated plastic greenhouses where disease risk was high. The experimental protocol included soil with four weeks solarization during summer and non solarized soil. The cv. Early pack no. 7, sensitive to Fusarium wilt of tomato, was used and the fungicide quintozene (46.8%) + etridiazol (11.5%), (trade name Terraclor super X) was used as a reference product at 35 g/hl water. The product was applied by irrigation and spraying of the soil surface immediately after transplantation and again after one, three and four months. Evaluation of product effectiveness was based on the amount of pathogen in soil, the number of wilted plants, the percentage of plants with burnished stem vessels and the yield per plant. In the non-solarized experimental plots receiving biological product and fungicide the amount of pathogen was reduced by 83.0–93.1 and 98.6–98.8% respectively. Similar reduction were obtained with the following treatments: solarized soil (89.4–90.3%), solarized soil + Promot (97.7–98.7%) and solarized soil + fungicide (99.0–99.3%). By contrast, in the control soil where solarization was not applied, pathogen inoculum increased by 37.5–100%. The percentage of wilted plants in each experimental plot was 0.0–0.2% for the treatments in solarized soil with biological products and 1.7% in the non-solarized control soil. The percentage of plants with burnished stem vessels ranged from 0.2 to 1.3% (biological product) and from 0.2–1.6% (fungicide), while it was 16.5% with the controls. Yield per plant was greater on the solarized experimental plots (7.56–7.86 kg) than on the non-solarized plots (7.29–7.32 kg). With the non-solarized control yield per plant did not exceed 4.75 kg.

Control of citrus dry root rot with soil solarization and chitin. V.A. Bourbos[1], K. Venetis[2] and E.A. Barbopoulou[1]. [1]*National Agricultural Research Foundation, Institute of Subtropical Plants and Olive Trees of Chania, Plant Pathology Laboratory, Agrokipio, 731 00 Chania, Crete, Greece.* [2]*Intrachem Hellas LTD, Kifissia Ave, 115 23 Athens, Greece.*

Citrus dry root rot caused by the fungi *Fusarium proliferatum* (Matsushima) Niremberg, *F. sambucinum* Fuckel and *F. solani* (Mart.) Sacc. has been responsible for serious damage to Greek citrus orchards in the last few years. Symptoms consist of progressive drying, hemiplegia and apoplexy depending on the biotic and abiotic factors prevailing in the soil. The aim of this trial was to study the control of pathogens by soil solarization in combination with the use of chitin. Soil solarization was performed for 6 weeks in summer using 100-μm-thick transparent polyethylene film. Chitin was incorporated into the soil at a rate of 420 g/m^2 of the commercial product Clandosan just before the soil was covered with polyethylene. The amount of pathogen was significantly reducted by 97.5–98% (*F. solani*), 96.5–96.7% (*F. sambucinum*) and 96.4–97% (*F. proliferatum*) in tests on solarized soil with chitin incorporation. These low levels continued for 3 consecutive years. By contrast, pathogen inoculum increased by 16.3–67% in control soil.

Biological control of sugarbeet and cucumber damping-off with bacterial and fungal strains. D.G. Georgakopoulos[1], C. Leifert[2], P. Fiddaman [3] and N.E. Malathrakis[1]. [1]*Technological Educational Institute of Crete, School of Agricultural Technology, 715 00 Heraklion, Crete, Greece.* [2]*Aberdeen University, Dept. of Plant and Soil Science, Aberdeen AB24 3UU, Scotland, UK.* [3]*Microbio Ltd., IACR Rothamsted, Bawden Building, Harpenden, Herts AL5 2QJ, UK.*

Five selected bacterial strains belonging to the species *Bacillus subtilis*, *Pseudomonas fluorescens* and *P. corrugata* and

two fungal strains belonging to the species *Trichoderma viride* and *Gliocladium virens* were evaluated for their efficacy in controlling sugarbeet and cucumber damping-off caused by *Pythium ultimum*. The antagonistic activity of bacteria was evaluated *in vitro* from the inhibition zone of various *Pythium* sp. strains grown in dual cultures in various media. Generally, *Pseudomonas* strains exhibited better inhibition of the pathogen than *Bacillus* strains. The possibility of antagonist combinations was also evaluated, based on antagonist growth in broth previously used by other antagonists. All possible dual combinations of antagonists were examined and three compatible pairs of bacterial strains were selected. Only bacteria were used for biocontrol of sugarbeet damping-off *in vivo*, whereas all antagonists were used with cucumber. Bacterial antagonists were applied by soaking seed in bacterial suspension for a final concentration of 10^5 cells/seed. In cucumber, bacterial antagonists were also applied by mixing a bacterial suspension in soil and by seed coating with bacteria in a peat carrier. Soil was artificially infected by incorporating homogenised *P. ultimum* mycelia from a plate culture. Satisfactory control of seedling damping-off was achieved with *Pseudomonas* strains; in cucumber, bacteria were efficient only when applied in soil or as a peat seed coat.

Effect of essential oils on soilborne tomato pathogens. F.T. Gravanis, S. Xifilidou, D. Karadimos and N.S. Efstathiou. *Technological Educational Institute of Larissa, Department of Plant Production, 411 10 Larissa, Greece.*

Six essential oils extracted from oregano (*Origanum vulgare*), mint (*Mentha piperita*), thyme (*Thymus vulgaris*), salvia (*Salvia officinalis*), lavender (*Lavandula officinalis*) and dittany (*Origanum dictamus*), were tested for their efficacy in controlling soilborne tomato diseases. Fungal isolates of *Fusarium oxysporum* f. sp. *lycopersici* and *Verticillium dahliae*, together with bacterial strains of *Clavibacter michiganensis* pv. *michiganensis* and *Pseudomonas syringae* were used in this study. In preliminary *in vitro* tests, all six essential oils showed a fungistatic and bacteriostatic effect against all four soilborne pathogens. *In vivo* tests, 20-day-old tomato seedlings were transplanted to 9-cm-diameter pots filled with a mixture of sand and peat (50% v:v). At transplanting time, the seedlings were inoculated with the pathogens and the essential oils were applied at three concentrations (40 µl, 20 µl and 10 µl per plant,) by watering the soil. All possible combinations of pathogen and essential oil were tested in 10 replicates per combination. The plants were kept in a greenhouse and 21 days after transplanting plant height, net weight, dry weight, stem diameter and the overall appearance of the plants was recorded. At concentrations of 40 and 20 µl all essential oils, especially those of thyme and oregano, showed a statistically significant toxic effect against all pathogens compared with the control. At a concentration of 10 µl all essential oils showed a statistically significant effect against all pathogens except *C. michiganensis*. Essential oil of dittony was more effective than that of lavender, whereas the remaining essential oils did not differ significantly either among each other or with lavender and dittany.

The avenacin biosynthetic pathway. K. Haralampidis, K. Papadopoulou and A.E. Osbourn. *Sainsbury Laboratory, John Innes Centre, Norwich NR4 7UH, UK.*

Saponins are glycosylated secondary plant metabolites found in many plants. Many saponins are antimicrobial and so act as pre-formed chemical barriers to pathogen attack. This project involved the isolation of the genes required for the synthesis of antifungal triterpenoid avenacin saponins from the diploid oat species *Avena strigosa*. We isolated saponin-deficient (*sad*) mutants of *A. strigosa* that were compromised in their resistance to a range of fungal pathogens. Although very little is known about the biosynthetic pathway for avenacins or indeed about saponin biosynthesis in plants in general, these molecules were synthesised from 2,3-oxidosqualene via the triterpenoid biosynthetic pathway. Mutants 610 and 109, representing different mutant alleles at the *sad1* locus, both lacked detectable levels of beta-amyrin. We cloned the cDNAs and the genomic regions predicted to encode beta-amyrin synthase and cycloartenol synthase and confirmed their identity by functional expression in yeast. Computational analysis of the regulatory sequences revealed several putative binding sites for plant transcription factors. Among them were the Dof and PBF single zinc finger transcription factor, the *myb*-like phenylpropanoid biosynthetic gene regulator and the *myb*-homologous P maize activator of flavonoid biosynthetic genes. Northern blot analysis showed that the transcript levels of the beta-amyrin synthase gene were substantially reduced in *sad1* mutants, while cycloartenol synthase activity and mRNA levels were wild-type. The southern blot hybridization pattern indicated that beta-amyrin was a single copy gene, while cycloartenol was most likely represented in two copies in the oat genome. Since a number of other enzymes are likely to be involved in the transformation of beta-amyrin into avenacins, complementary molecular and biochemical approaches are being used to identify more genes of the avenacin biosynthetic pathway. A number of sequences predicted to encode enzymes of the appropriate classes have already been identified. A variety of strategies will be used to confirm the function of candidate genes/cDNAs, including analysis of expression in wild-type and mutant oat roots, heterologous expression in yeast or other appropriate systems, and complementation of mutants by transient expression. This will enable us to clarify and understand better this secondary metabolite pathway in plants.

Plant row covers for vegetable protection against whitefly and aphid-transmitted viruses. N. Ioannou, M. Ioannou and C. Poullis. *Agricultural Research Institute, 1516 Nicosia, 22016 Cyprus.*

The whitefly/virus complex is a major limiting factor for vegetable cultivation in Cyprus. *Tomato yellow leaf curl virus* (TYLCV), in particular, causes severe damage to tomato crops, especially in summer and early autumn, when the whitefly vector *(Bemisia tabaci)* attains its greatest numbers. Comparable virus problems exist in the cucurbits, where aphid-transmitted viruses such as *Zucchini yellow mosaic* (ZYMV), *Cucumber mosaic* (CMV) and *Watermelon mosaic* (WMV-1 and WMV-2), are the most important. In greenhouse crops, virus diseases are effectively controlled

through an IPM program, which involves fitting insect-proof nets on doors and windows of greenhouses. In order to assess the effectiveness of this approach for vegetables in the open field, a series of 11 trials were carried out on tomato and three on zucchini squash. Various insect-proof materials were evaluated as plant-row covering, from time of transplanting to the beginning of fruit setting (5–6 wk). In tomato, all materials tested provided effective protection from TYLCV, especially when combined with chemical control of whiteflies. The use of healthy transplants was also a necessary prerequisite. The following materials were selected on the basis of their effectiveness, durability and convenience of application: a) spunbonded, non-woven fabrics of polypropylene (Lutrasil) or polyester (Lutradur), both weighing about 20–30 g/m^2, and b) coarse-mesh plastic nets (Agronet type) of 15–22 g/m^2 weight. All these materials can be placed directly on the plants without any support. They also provided effective protection of zucchini squash and increased yield by 50%, even though in this case the crop became partly infected by aphid-borne viruses after it was uncovered.

Evaluation of yeasts as biocontrol agents against *Botrytis cinerea* of tomatoes and cucumbers. S. Kalogiannis, A. Stergiou and E.C. Tjamos. *Agricultural University of Athens, Department of Plant Pathology, 75 Iera Odos, 118 55 Votanikos, Athens, Greece.*

The ability of 30 epiphytic yeasts to prevent infection of tomato leaves by *Botrytis cinerea* was evaluated *in planta*. Detached tomato and cucumber leaves were incubated at 20°C with a 12-h day in Petri dishes containing a solid manitol sucrose agar medium. Suspensions of yeasts (10^7/ml) were applied one day before *B. cinerea*. After wounding, detached leaves were inoculated with a *B. cinerea* conidial suspension (30 µl containing 10^5 conidia/ml). Symptoms of infected leaves were scored on a 0–4 scale where: 0 = symtomless leaf tissue; 1 = rot extending 1–5mm around wound; 2 = rot extending 6–10mm around wound; 3 = rot extending 11–15 mm around wound; 4 = rot extending more than 15 mm around wound. Detached leaves were incubated for 8 days and disease assessment started 48 h after inoculation. Most yeasts significantly reduced disease severity and sporulation. The mean disease index of the leaves treated with an effective yeast was 1 while that of the the untreated control leaves was 4. Of the nine yeasts tested four were most effective in a number of experiments. The best was yeast 44, which was identified as *Rhodotorula glutinis*. Tomato stems co-inoculated with yeasts and *B. cinerea* and incubated at 15°C and 95% RH developed less *B. cinerea* stem rot than stems inoculated with *B. cinerea* alone. Plants were inoculated at wound sites with 5 µl of a 10^5/ml *B. cinerea* spore suspension. Stem wounds were inoculated by cutting leaves adjacent to the stems already spread with 10^{7}spores/ml of yeast cells. *B. cinerea* lesions developed by the end of the first week or earlier. Plants were considered infected when lesions exceed 5 mm in diameter after two weeks. Infection was finally assessed 15 days after inoculation. The antagonistic yeasts significantly delayed the development of decay and reduced the proportion of infected wounds compared with the control. The yeasts reduced infection by 70–90% while infection of control stems ranged from 70–85%. *Rhodotorula glutinis* isolate Y-44 was the most effective in preventing *B. cinerea* infection. Yeasts delayed conidia germination but none inhibited fungal growth. Yeasts did not produce *in vitro* antibiotics or other toxic substances, inhibiting the growth of *B. cinerea*.

Effectiveness of the plant extract *Reynoutria sachalinensis* against powdery mildew (*Sphaerotheca fuliginea*) and grey mould (*Botrytis cinerea*) in cucumber. S. Konstantinidou-Doltsinis[1], A. Markellou[2,3], A. Kalamarakis[2,3], K. Tzebelikou[1], and N. Petsikos-Panagiotarou[2,3]. [1]*National, Agricultural Research Foundation, Plant Protection Institute, 260 04 Patras, Greece.* [2]*Benaki Phytopathological Institute, 8 S. Delta St., 145 61 Kifissia, Athens, Greece.* [3]*National, Agricultural Research Foundation, Athens, Greece.*

In the framework of an EU-funded research project (FAIR-CT98-4413) the liquid formulation Milsana® (VP-1999, an extract of the plant *Reynoutria sachalinensis*) was tested in small and large-scale trials against *Sphaerotheca fuliginea* (Schlecht) Poll. and *Botrytis cinerea* Pers.:Fr. in cucumber plants. Results from two small-scale trials showed that Milsana® (0.5% v:v) was highly effective against *S. fuliginea* regardless of the time of application (preventively 1 to 4 days before or curatively 1 to 4 days after artificial inoculation) or the susceptibility of the cultivar to the pathogen (3 cvs.). Its effectiveness was equal to that of the reference fungicides triforine and dinocap and significantly different from the control. Data from a large-scale naturally infected greenhouse experiment showed that weekly applications of Milsana® at 0.5% v:v resulted in: a) significant disease reduction in the leaves equal to that of the fungicides regularly used and b) an increase in the number and weight of harvested fruits/plant. Between Milsana and the fungicides no statistically significant differences were found, in the leaf area covered by powdery mildew, or in yield. Milsana® was not effective against *B. cinerea* on cucumber leaves or fruits in the small-scale trials.

Olive mill wastewater in the soil inhibits the plant pathogen *Rhizoctonia solani*. M. Kotsou[1], I. Mari[2], S. Tasiopoulou[1], A. Kiriakou[1] and K. Balis[1]. [1]*Harokopio University of Athens, Department of Microbiology, 70 El. Venizelou St., 176 71 Athens, Greece.* [2]*Agricultural University of Athens, Department of Mechanics and Natural Resources, 75 Iera Odos, 118 55 Athens, Greece.*

Olive mill wastewater (OMW) has a high organic content and is rich in fertilizing substances such as potassium, phosphorus, magnesium and others. It also exhibits phytotoxic properties, mainly due to a high C/N ratio, the presence of polyphenols and fatty acids and high levels of mineral salts. Polyphenols and fatty acids are responsible for the antimicrobial properties of OMW against several microbial species. In order to study this effect of OMW against the plant pathogen *Rhizoctonia solani*, soil repeatedly treated with this waste was used, while soil treated with water was used as a control. After allowing a sufficient time (45 days) for the elimination of OMW phytotoxicity (during that period both treat-

ments received only water), the soil was artificially infected with the pathogen. The inoculum potential of the fungus was determined on plants sensitive to infection planted at different time intervals after soil infection (lettuce seeds planted at 0, 5, 10 and 15 days after soil infection). The results showed that the percentage of infected plants was significantly lower (P<0.05) in the soil treated with the waste than in the control soil during the whole period tested. This effect was attributed to microbial activity, as measured by the respirometric activity of the soil. Microbial activity was significantly increased by OMW treatment as compared with the control during the whole period (P<0.05). Bacteria that created inhibition zones against the fungus in Petri dishes were isolated from both OMW and water-treated soil. In OMW-treated soil the bacteria isolated belonged to the genera *Bacillus* and *Alcaligenes* while in the water-treated soil the bacteria belonged to the genera *Bacillus, Alcaligenes* and *Streptomyces*. The population of antagonistic bacteria with the OMW treatment was much greater than with the water treatment. Lastly, the colony radial growth rate was measured on solid substrates containing various concentrations of OMW. It was revealed that OMW itself did not inhibit fungus growth. It appears that the inhibitory effect of soil treated with OMW is due to the selection of an antagonistic microflora which uses the waste ingredients as a substrate to increase its own population.

Effect of volatile compounds from grapes on the pathogenicity of *Botrytis cinerea*. E.K. Koulakiotou[1], C.C. Thanassoulopoulos[1] and E.M. Sfakiotakis[2]. [1]*Aristotelian University of Thessaloniki, Faculty of Agriculture, Plant Pathology Laboratory, 540 06 Thessaloniki, Greece.* [2]*Aristotelian University of Thessaloniki, Faculty of Agriculture, Laboratory of Pomology, 540 06 Thessaloniki, Greece.*

With the exception of sulphur dioxide treatment of table grapes, there are no commercially acceptable or safe postharvest treatments to prevent disease during storage of grapes. The objective of this research was to evaluate *in vivo* the effect of natural volatile compounds of grapes on the pathogenicity of *Botrytis cinerea* Pers. in a resistant variety of grape. The experiment was carried out at different stages of maturity of the berry: semi-ripe, ripe and over-ripe. *Botrytis*-inoculated berries in proportions of 6, 10 and 20% of the entire berry were placed at the bottom of 27 micro-chambers which were connected by a closed Mariotte system in a such way that the concentration of oxygen was stable but there was no exchange of gases. There were 100 berries per micro-chamber. Nine micro-chambers contained berries resistant to the pathogens, nine contained susceptible berries and nine had a mixture of both susceptible and resistant berries, of which only the susceptible ones were inoculated. The system was placed in a chamber at 21ºC and berries were examined after 7 days. The number of infected berries were counted as well as those in which conidia of the fungus were observed. When berries of the resistant variety were semi-ripe, the natural volatile compounds prevented *B. cinerea* from displaying its pathogenicity in both resistant and susceptible varieties when the latter were inoculated and in presence of the resistant variety. With ripe and over-ripe fruits of the resistant variety, pathogenicity of the fungus was inhibited only in the resistant variety.

Effect of volatile compounds of grapes on the growth of *Botrytis cinerea*. E.K. Koulakiotou[1], C.C. Thanassoulopoulos[1] and E.M. Sfakiotakis[2]. [1]*Aristotelian University of Thessaloniki, Faculty of Agriculture, Plant Pathology Laboratory, 540 06 Thessaloniki, Greece.* [2]*Aristotelian University of Thessaloniki, Faculty of Agriculture, Laboratory of Pomology, 540 06 Thessaloniki, Greece.*

Natural volatile compounds with an *in vitro* antifungal activity may be useful as fumigants for the control of postharvest pathogens. The aim of this experiment was to study *in vitro* the effect of natural volatile compounds of grapes on the growth of *Botrytis cinerea* produced by a resistant grape variety. Twelve sets each of five Petri dishes containing potato dextrose agar were inoculated with *B. cinerea* and each set was transferred to one of 12 micro-chambers. Ripe and unripe grapes of the resistant and susceptible varieties were placed at the bottom of the micro-chambers which were connected by a closed Mariotte system and kept at 21ºC. The conidia and sclerotia per Petri dish were counted after 15 days. The natural volatile compounds of the resistant grape variety inhibited the formation of sclerotia irrespective of the stage of maturity of the fruits. In the unripe fruits, conidia were not produced.

Programme to control C*itrus tristeza virus* in Cyprus. A. Kyriakou, T. Kapari-Isaia and N. Ioannou. *Agricultural Research Institute, 1516 Nicosia, 22016 Cyprus.*

Citrus tristeza virus (CTV) is a major threat to Cyprus citriculture, due to the widespread use of the susceptible sour orange rootstock. A programme to control CTV was initiated in 1992, with the following main objectives: a) a survey of all citrus groves to determine CTV incidence; b) removal of infected trees or groves, where feasible, with compensation to growers; c) production and distribution of healthy propagating material for the establishment of new groves. The survey is being conducted by indexing 10–20% of the trees of each grove in the five citrus-producing districts of Cyprus, using the enzyme-linked immunosorbent assay (ELISA). So far, a total of 601 groves with about 212,200 trees have been surveyed. Infection with CTV was found in 152 groves (incidence 25.3%) and in 2,483 trees out of 50,750 trees indexed by ELISA (average incidence 4.9%). Incidence of infection in different districts ranged from 2.7 to 18.3%. The highest proportion of infected trees (18.3%) and groves (71%) was in the district of Ammochostos, where it was decided that eradication of tristeza is no longer feasible. Eradication in this district is therefore limited to trees infected with severe strains of the virus, while a legal regulation was issued which forbids the transport of citrus planting material from this district to other areas. In the other four districts (Nicosia, Limassol, Larnaca and Paphos) all infected trees, including seven entire groves, have been removed with compensation to the growers. In addition, a National Budwood Certification Scheme, implemented since 1995, has resulted in the production of virus-free propagating material for establishing new groves.

Visualisation of interactions between *Pseudomonas* biocontrol bacteria and *Fusarium oxysporum* f. sp. *radicis-lycopersici* on tomato roots using autofluorescent proteins. A.L. Lagopodi, G.V. Bloemberg, A.F.J. Ram, A.H.M. Wijfjes, G.E.M. Lamers, C.A.M. J. J. Van den Hondel and B.J.J. Lugtenberg. *Institute of Molecular Plant Sciences, Leiden University, Wassenaarseweg 64, 2333 AL, Leiden, The Netherlands.*

The biological control of soil-borne plant pathogenic fungi with rhizobacteria is an alternative to chemical pesticides. Crown and root rot of tomato caused by the fungus *Fusarium oxysporun* f. sp. *radicis-lycopersici* (*Forl*) has been reported to be suppressed by two *Pseudomonas* biocontrol strains, *P. fluorescens* WCS365 and *P. chlororaphis* PCL1391. Artificial inoculations of tomato with *Forl* and biocontrol treatments with *Pseudomonas* bacteria were reproduced in a gnotobiotic sand system. *Forl* was transformed with *GFP* (green fluorescent protein). *GFP*-labeling allowed visualisation of the colonisation and infection of tomato roots by *Forl* with confocal scanning laser microscopy. Various autofluorescent proteins like GFP and CFP (cyan fluorescent protein) were used to label the bacteria. Microscopic analysis showed that the pathogen and the biocontrol bacteria competed in colonisation of specific sites on the host roots and that the bacteria interacted directly with the fungus by colonising the hyphae. Unravelling the interactions between *Pseudomonas* and *Forl* on tomato roots will contribute to a deeper knowledge of the nature of the biocontrol effect, and to the development of more efficient methods for biocontrol. On the other hand, the combination of various autofluorescent proteins in labelling different microbial communities, such as pathogenic fungi and biocontrol bacteria, opens new perspectives for rhizosphere studies *in vivo*.

Pre-formed secondary metabolites with antifungal activity and their effect on symbiotic arbuscular mycorrhizal fungi. K. Papadopoulou. *National Agricultural Research Foundation, Institute of Kalamata, 85 Lakonikis St, 241 00 Kalamata, Greece.*

Saponins are glycosylated secondary metabolites that occur constitutively in healthy plants and represent unbuilt chemical barriers to infection. Many saponins have been implicated in the protection against a wide range of potential pathogens and they exhibit strong antifungal activity. Saponins consists of triterpenoid, steroid or steroidal glycoalkaloid molecules bearing one or more sugar chains. The fungitoxic effects of saponins have been attributed to their ability to interact with membrane sterols by means of free 3b-hydroxyl groups. Successful pathogens of saponin-producing plants must circumvent the antimicrobial activity of these molecules. Tolerance to saponins can arise either from membrane characteristics of the fungus or from fungus ability to enzymatically detoxify the saponin. Arbuscular mycorrhizal fungi (AMF) represent the most common root endosymbiotic association and they develop between the roots of most higher plants and fungi belonging to the order Glomales. AMF have to penetrate the root epidermis and ramify in the root cortex and are likely to encounter saponins at concentrations found to be inhibitory to microbes *in vitro*. In this study, the toxic effects of saponins on these beneficial fungi and the mechanisms of resistance employed by the latter were investigated. AMF species differing in the sterol content of their spores were germinated in the presence of purified saponins from oats (avenacins) and tomato (a-tomatine). The correlation between the degree of colonization in oat and tomato and sensitivity to saponins was also assessed. RT-PCR was employed to detect the presence of saponinase-like genes expressed during the AMF-plant interaction.

Allelopathic control of root and stem rot of cucumber *(Fusarium oxysporum* f. sp. *radicis-cucumerinum)* with *Lactuca sativa* incorporated into the soil prior to the main cucumber crop. G.C. Pavlou[1] and D.J. Vakalounakis[2]. [1]*National Agricultural Research Foundation, Agricultural Research Station, 722 00 Ierapetra, Crete, Greece.* [2]*National Agricultural Research Foundation, Plant Protection Institute, 711 03 Heraklion, Crete, Greece.*

The possibility of allelopathic control of *Fusarium oxysporum* f. sp. *radicis-cucumerinum*, which causes root and stem rot of cucumber, was investigated in an unheated plastic greenhouse of the Ierapetra Agricultural Research Station in the crop season 1997–98 (October–May). Lettuce (*Lactuca sativa)* plants were incorporated into soil that was disinfected with methyl bromide, and then artificially infected with conidia of *F. oxysporum* f. sp. *radicis-cucumerinum* before being planted with cucumber (Brunex F_1). The following four soil treatments were applied: (a) lettuce plants, grown in the experimental soil were not harvested but incorporated *in situ*, into the soil at the maturation stage; (b) lettuce plants cut at the maturation stage and transferred to experimental soil were chopped up and incorporated into the soil; (c) *F. oxysporum* f. sp. *radicis-cucumerinum* infected soil without lettuce amendment (control); and (d) *F. oxysporum* f.sp. *radicis-cucumerinum* non-infected soil without lettuce amendment (control). No significant difference in cucumber yield was found between treatments (a) and (b), but there was a significant 35% increase in yield with these treatments over treatment (c). However, yield with (a) and (b) was 65% less than with treatment (d). Disease incidence with treatments (a, b), (c), and (d): 2.5, 10 and 0% respectively two months after transplanting, 25, 50 and 2% after four months, 84, 100 and 2% after six months and 96, 100 and 4% after eight months.

Preliminary study on possible biological control of root and stem rot of cucumber (*Fusarium oxysporum* f. sp. *radicis-cucumerinum*) with *Burkholderia cepacia*. G.C. Pavlou[1] and D.J. Vakalounakis[2]. [1]*National Agricultural Research Foundation, Agricultural Research Station, 722 00 Ierapetra, Crete, Greece.* [2]*National Agricultural Research Foundation, Plant Protection Institute, 711 03 Heraklion, Crete, Greece.*

To investigate whether *Burkholderia (Pseudomonas) cepacia* type Wisconsin isolate J82 (*DENY*®) (commercially used to control various *Fusarium* spp.), can be used to control *Fusarium oxysporum* f. sp. *radicis-cucumerinum*, which causes root and stem rot of cucumber, a preliminary experiment

was conducted on soil disinfected with methyl bromide in an unheated greenhouse at the Ierapetra Agricultural Research Station in the crop season 1998–99 (October–May). Three types of soil were tested: (a) soil artificially infected with *F. oxysporum* f. sp. *radicis-cucumerinum* + *DENY* (applied according to manufacturer' specifications); (b) soil artificially infected with *F. oxysporum* f. sp. *radicis-cucumerinum* (control); and (c) soil not infected with *F. oxysporum* f. sp. *radicis-cucumerinum* (control). Cucumber seedlings grown on type (a) soil were irrigated with a *DENY* suspension (125 ml/100 l of water) one week prior to transplanting, and on the day of transplanting, each plant was watered with 300 ml of *DENY* suspension. Thereafter *DENY* was applied every 15 days via drip irrigation system at a rate of 150 ml *DENY*/1000 m^2 of soil (0.1 ml preparation/l of water/plant). The results (plant development, crop yield, and disease incidence) showed that *DENY* is not effective against root and stem rot of cucumber.

Greenhouse experiments on the biological control of cucumber mosaic virus in tomato using benign Japanese isolates of CARNA 5. A.P. Sklavounos[1], H. Sayama[2], M. Kominato[2], J.M. Kaper[3] and P.E. Kyriakopoulou[1]. [1]*Agricultural University of Athens, Department of Plant Pathology, 75 Iera Odos, 118 55 Votanikos, Athens, Greece.* [2]*Nippon del Monte Co. Japan.* [3]*Retired, USDA, ARS, Beltsville, MD, U.S.A.*

In the mid 1980s, a serious disease of open field tomatoes arose in Greece, in processing and table tomatoes. It was caused by *Cucumber mosaic cucumovirus* (CMV), both alone and with its satellite CARNA 5, and had already been common in various Mediterranean countries such as France, Spain and Italy, for 2–3 decades. The disease led to the elimination of tomato cultivation from various areas of Greece, where it had become traditional such as Argolis where the industrial unit of KYKNOS closed, Zaharo *etc.* In other areas where tomato was grown, the disease has also become common as on processed tomatoes in large parts of Amalias. There has been extensive investigation internationally into the disease using various control methods. Biological control based on cross-protection, by pre-inoculating plants with benign CMV-CARNA 5 has been extensively studied in France, China, Japan, USA and Italy, and widely applied to protect tomatoes in the field in China and Japan since the early 1990s. In view of the seriousness of the disease in Greece, it was considered useful to test this method on tomatoes in Greece. The experiment was performed in a temperature and light-controlled greenhouse of the Agricultural University of Athens, using as protectors the tomato hybrid Barbara and two Japanese benign CMV strains carrying benign CARNA 5 (390 nt), and as challengers strains NDM 1 and KO3 of the Nippon Del Monte Co., both belonging to CMV group 1a, and four strongly pathogenic Greek CMV isolates, CMV-Mar, CMV-Sav, CMV-Ist and CMV-Tir; the latter carrying necrogenic CARNA 5. Ten plants were used in each of 30 treatments: 2 tomato hybrids x preimmunization (blank, NDM1, KO3) x challenge inoculation (Blank, CMV-Mar, CMV-Tir, CMV-Sav CMV-Ist). Tomato seedlings were preimmunized at the cotyledon stage. After 10 days, and when the protecting 390 nt CARNA 5 was detected by 5% PAGE in all preimmunized plants, they were challenge-inoculated with the pathogenic isolates. The non-immunized (unprotected) plants reacted with the expected severe symptoms, but none of the preimmunized (protected) plants showed any symptoms. All preimmunized plants contained CARNA 5 (by 5% PAGE) and CMV (by DAS ELISA) from the "vaccine", NDM 1 or KO3. The experimental tomatoes were kept in the greenhouse for 2 months, and throughout this period the protection picture remained unchanged. The protected and challenged plants grew normally like the unvaccinated unchallenged controls, with an insignificant reduction in growth. The unvaccinated challenged plants showed severe dwarfing, malformation, necrosis etc. The results are encouraging for further field experiments.

Induction of resistance of cucumbers, eggplants and *Arabidopsis* against Verticillium wilt by means of a rhizosphere *Paenibacillus*. S.E. Tjamos[1], C. Arambatzis[1], P. Katinakis[2] and E.C. Tjamos[1]. [1]*Agricultural University of Athens, Department of Plant Pathology, 75 Iera Odos, 118 55 Votanikos, Athens, Greece.* [2]*Agricultural University of Athens, Department of Biotechnology, 75 Iera Odos, 118 55 Votanikos, Athens, Greece.*

A rhizosphere *Paenibacillus* designated as K-165, when applied as a seed coating preparation controlled *Verticillium dahliae* in potato field trials, significantly reducing symptom development and increasing yield. The strain exercised antibiotic activity *in vitro* against *V. dahliae*, produced chitinase in Luria Broth medium amended with glycol chitin and also produced indolebutyric acid. To study further the mode of action of this antagonist we tested its ability to induce resistance against *V. dahliae* in cucumber, eggplant and *Arabidopsis* plants. On cucumber and eggplant the split root system was used. A bacterial cell suspension of 10^8 cfu/ml or a bacterial preparation containing 10^8 cfu/gr talc was used. The root system was either drenched with the suspension during transplanting or treated with the bacterial preparation. In cucumber plants inoculated with a Verticillium spore suspension from a *V. dahliae* isolate from cucumber, disease symptoms appeared 7 days after bacterial application at the site opposite to the bacterized one demonstrating that K-165 retarded symptom expression and lowered final symptom development by 40–50% compared with the untreated control. Similar evidence was obtained using eggplants and an eggplant *V. dahliae* isolate. For the *Arabidopsis-Verticillium dahliae* bioassay the rock wool system described by Leeman *et al.* was used. The ecotype Columbia was tested thoroughly with plants inoculated with a *V. dahliae* isolate from *Raphanus sativus*. It was also demonstrated a 40–50% reduction in symptom development. Using the *Arabidopsis* mutants jar1, etr1, npr1 and NahG it was demonstrated that npr1 plays a crucial role in the expression of ISR.

Effect of triterpenoid avenacins on pathogenic fungi of the genus *Fusarium*. M. Tourna and K. Papadopoulou. *National Agricultural Research Foundation, Institute of Kalamata, 85 Lakonikis St., 241 00 Kalamata, Greece.*

Avenacins comprise a group of four triterpenoid saponins, which are produced in oat roots and exhibit strong antifungal activity. The successful pathogen of oats *Gaeumannomyces graminis* var. *avenae* enzymatically detoxifies the avenacins by producing the extracellular enzyme avenacinase, which removes the glucose molecules. In the present study the effect of avenacins on some species of the genus *Fusarium* with varying levels of pathogenicity to oats (*F. culmorum*, *F. graminearum*, *F. avenaceum*) was investigated. Mycelium growth of the three species in the presence of purified avenacins was monitored to determine their sensitivity to the avenacins. The ability of the fungi to infect oat mutants defective in the biosynthesis of avenacins was also determined. *F. avenaceum*, which normally infects oats, exhibited full resistance against avenacins. Partial purification and characterization of a glucosidase was achieved which detoxified the avenacins by removing a different sugar moiety than the avenacinase from *G. graminis avenae*. Growth of *F. culmorum*, a non-pathogen of oats, was inhibited *in vitro* by the avenacins. Two strains were transformed with the avenacinase gene using the *E. coli* hygromycin phosphotransferase gene (*hph*) as a selectable marker. Stable transformants resistant to the avenacins were obtained which infected wild-type oats. However, disease severity was less than with the natural pathogens of the plant.

The effect of *Trichoderma koningii* on the growth of tomato plants and the production of cytokinins by the biocontrol agent. P.C. Tsahouridou[1], M. Koukourikou-Petridou[2] and C.C. Thanassoulopoulos[1]. [1]*Aristotelian University of Thessaloniki, Faculty of Agriculture, Plant Pathology Laboratory, 540 06 Thessaloniki, Greece.* [2]*Aristotelian University of Thessaloniki, Faculty of Agriculture, Biology of Horticultural Plants Laboratory, 540 06 Thessaloniki, Greece.*

Increased plant growth was observed on tomato seedlings after seed treatment with *Trichoderma koningii*. The biocontrol agent also seemed to increase significantly the chlorophyll content in the leaves of 30-days-old seedlings. These facts suggest that there are higher levels of cytokinins in the plant cells or the growing medium. To test this supposition, *T. koningii* was cultured for 30 days on two synthetic media and the filtrates and fungal mycelia were tested for cytokinins. The presence of the cell-division factors was demonstrated with a cucumber cotyledon bioassay, which is specific for the detection of cytokinins.

Comparison of fire blight prediction systems. I. Tsiantos[1] and P. Psallidas [2]. [1]*National Agricultural Research Foundation, Plant Protection Institute of Volos, 380 01 Volos, Greece.* [2]*Benaki Phytopathological Institute, 8 S. Delta st., 145 61 Kifissia, Athens, Greece.*

Two prediction systems of fire blight epidemics, "Firescreens" (French) and "Maryblyt" (American) were evaluated and compared under Greek climatic conditions in 1999 and 2000. The experiments were carried out in pear orchards (7 in 1999, 9 in 2000) cvs. Krystalli, Santa Maria, Kontoula and Williams during the blooming period. In both years one or two sprayings were recommended with "Firescreens", none or one with "Maryblyt", compared with two or three that would have been carried out with conventional spraying instructions. The results showed that both systems made possible a reduction in the number of sprayings since there were no infections in most (14 out of 16) experimental orchards. However, in 2000 in one experimental orchard under heavy inoculum pressure there were more infections in the untreated trees than in the sprayed ones. When "Maryblyt" recommended a spray, "Firescreens" likewise recommended a spray the same day, although the latter system predicted more infection days. Furthermore "Firescreens" predicted the appearance of symptoms 1–2 days earlier, and "Maryblyt" 4–6 days later than the actual day. Although there is a saving in the number of sprayings required with these prediction systems, both of them still need improvement.

The effectiveness of plant extracts from *Reynoutria sachalinensis* (F. Schmidt) Nakai against powdery mildew of grapes (*Uncinula necator*). K. Tzembelikou[1], A. Markellou[2,3], A. Kalamarakis[2,3], N. Petsikos-Panagiotarou[2,3] and S. Konstantinidou-Doltsinis[1]. [1]*National, Agricultural Research Foundation, Plant Protection Institute, 260 04 Patras, Greece.* [2]*Benaki Phytopathological Institute, 8 S. Delta St., 145 61 Kifissia, Athens, Greece.* [3]*National Agricultural Research Foundation, Athens, Greece.*

In the framework of an EU-funded research project, the effectiveness of the liquid formulation Milsana® (VP-1999), produced from extracts of the plant *Reynoutria sachalinensis*, against *Uncinula necator* (Schw.) Burr., was studied in relation to the rate of application, spray intervals and cultivar treated. In small-scale trials, Milsana® was highly effective (disease reduction>95%) against leaf powdery mildew of grapes independently of spray interval (7, 10 or 14 days), dose (0.5 and 1% v:v) or cultivar treated (Soultanina, Black Corinth and Cabernet). Its effectiveness was not statistically different from that of the reference fungicide dinocap. In a small-scale field trial (cv. Roditis) where 1% Milsana® was applied every 7, 10 or 14 days, disease reduction on the berries differed significantly among treatments. Milsana® applied at 7-day intervals was the most effective (disease reduction 53.3%). Results from a large-scale field trial (cv. Black Corinth, organic farming) showed a significant disease reduction on the berries (23.5%) and an increase in yield (49.7%) on plots treated with 1% Milsana®.

Systemic resistance induced against *Fusarium oxysporum* f. sp *raphani* and *Pseudomonas syringae* pv. tomato in *Arabidopsis* plants by a rhizosphere *Paenibacillus*. A. Venieraki[1], P. Katinakis[2] and E.C. Tjamos[1]. [1]*Agricultural University of Athens, Department of Plant Pathology, 75 Iera Odos, 118 55 Votanikos, Athens, Greece.* [2]*Agricultural University of Athens, Department of Biotechnology, 75 Iera Odos, 118 55 Votanikos, Athens, Greece.*

The resistance induced by a rhizosphere bacterial strain, designated as K-165 and belonging to the genus *Paenibacillus* was studied. This isolate successfully antagonises physiological races of *Fusarium oxysporum* and *Verticillium dahliae ex planta* and *in planta*. In order to study how this strain

induces systemic resistance against various pathogens we used as a model plant ecotypes of *Arabidopsis thaliana*. To determine the capacity of the antagonistic strain to induce systemic resistance (ISR) against *F. oxysporum* f. sp. *raphani* (*For*), we grew the plants in a rock wool system and treated their roots with a bacterial suspension of 10^8 cfu/g in talc powder. Five days later the plants were inoculated with *For*. It was demonstrated that 30 days after infection K-165 reduced symptom expression by 50% compared with the control plants. Studies were also carried out on the genes involved in ISR. *Arabidopsis* plants were immunised by drenching their roots with a bacterial suspension of K-165 prior to the inoculation with a virulent strain of *Pseudomonas syringae* pv. *tomato* DC3000. The results showed significant differences between treated and untreated plants. cDNA fragments of the ISR involving genes PR1, PR2, PR5, Pal1, Vsp and Pdf1.2 have already been amplified with PCR, subcloned and sequenced. Our aim is to use them as probes to identify their temporal and spatial expression and their location in *Arabidopsis* plants.

Chemical control

Trial results of iprovalicarb mixtures in Greece. I. Arvanitis, K. Bloukidis, D. Theodosiou and I. Athanasopoulos. *Bayer Hellas AG, Technical Department, Akakion 54A, 151 25 Polydroso-Amarousion, Greece.*

Iprovalicarb is a new active substance with specific activity against oomycetes. In Greece, iprovalicarb has been developed and tested as mixture with: a) mancozeb, under the trade name Melody Med (6+60) WP for grapes and tobacco b) propineb, as Melody Duo (5.5+61.25) WP for potato and c) copper, as Melody Compact (4.2+20.3) WP for grapes and potato. From 1996 to 1999, 11 field trials were carried out to assess the efficacy of these mixtures against downy mildew in grapes, tobacco and potato. The trials included preventive sprayings until disease appearance. All trials were performed according to EPPO guidelines and the results were statistically analyzed with Duncan's test. According to the results, the efficacy of iprovalicarb mixtures in late potato blight was 80–90%, while in untreated plots infection reached 100%. Iprovalicarb-mixtures showed better results than the reference product mancozeb+metalaxyl (56+5.5) WP. In tobacco and grapes, the efficacy of iprovalicarb mixtures was high (80–100%) even with high disease pressure. The trial results from Greece, as from other EU countries, proved the high efficacy of iprovalicarb mixtures against the oomycetes *Plasmopara, Peronospora* and *Phytophthora*.

Comparative fungitoxicity of the natural anthraquinone cynodontin and three synthetic anthraquinones. M. Chrysayi-Tokousbalides, M. Kastanias, A. Valakas and I. Sklavos. *Agricultural University of Athens, Pesticide Science Laboratory, 75 Iera Odos, 118 55 Votanikos, Athens, Greece.*

A red pigment was isolated from cultures of a pathotype of *Drechslera avenae* and was identified as cynodontin (3-methyl-1,4,5,8-tetrahydroxy-anthraquinone). This particular anthraquinone is a product of secondary metabolism and is produced in cultures of the fungus, reaching yields as high as 0.1 mg/ml. Tricyclazole, an inhibitor of fungal melanin biosynthesis did not inhibit cynodontin biosynthesis but rather accelerated its production and increased the overall yield. The effect of natural cynodontin on *in vitro* growth of *Sclerotinia minor, S. sclerotiorum, Botrytis cinerea* and *Verticillium dahliae* was studied and compared with the fungitoxicity of emodin (1,3,8-trihydroxy-6-methyl-9,10-anthracenedione), chrysophanol (1,8-dihydroxy-3-methyl-9,10-anthracenedione) and anthraquinone (9,10-anthracenedione), which are synthetic anthraquinones. Test results indicate that the number and position of the substituents of the anthraquinone moiety may play a role in the bioactivity of such compounds, since only cynodontin exhibited interesting fungitoxicity while the others, synthetic anthraquinones, did not.

Iprovalicarb – a novel systemic fungicide for the control of downy mildew and late blight. S. Dutzmann. *Bayer AG, Agrochemicals Division, Development Fungicides, Agricultural Center Monheim, D-51368 Leverkusen, Germany.*

The systemic oomycete fungicide iprovalicarb of the chemical class of amino acid amide-carbamates is an ideal answer to the many demands made by farmers for efficient control of downy mildew and late blight caused by the fungal genera *Peronospora, Pseudoperonospora, Bremia*, and *Phytophthora*. The compound exhibits a favourable toxicological and ecotoxicological profile and affects all important stages of the fungal infection chain by protective, curative, and eradicative effects. Although the biochemical action of iprovalicarb still remains to be elucidated, it must be different from all market-established compounds, since it does not have a direct effect on respiration or on nucleic acid or lipid metabolism. Iprovalicarb is thus thought to have a novel biochemical mode of action, which is probably specific. The good performance of iprovalicarb in economically important crops such as grapevine, potatoes, vegetables, and tobacco is described.

Strategies for managing the resistance of grape powdery mildew (*Uncinula necator*) to DMI fungicides. A. Kalamarakis[1,2], A. Markellou[1,2], A. Tsigas[3], B. Ziogas[4] and N. Petsikos-Panagiotarou[1,2]. *[1]Benaki Phytopathological Institute, 8 S. Delta St., 145 61 Kifissia, Athens, Greece. [2]National Agricultural Research Foundation, Athens, Greece. [3]Novartis (Hellas), Leoforos Anthousas, 15344 Anthousa Attiki Greece. [4]Agricultural University of Athens, Department of Plant Pathology, 75 Iera Odos, 118 55 Votanikos, Athens, Greece.*

A reduced sensitivity of grape powdery mildew to the DMI fungicide triadimenol was first observed in 1997 in our laboratory. Since then, further studies have confirmed our initial report on the occurence of triadimenol-resistant powdery mildew phenotypes in Greece (separate announcement). The purpose of this study was to compare different spray programmes in relation to their selective effect on powdery mildew populations and their effectiveness against

the disease. Evaluation of the different strategies was conducted in the field (percentage of infected clusters) and in the laboratory (EC_{50} values and percentage of resistant conidia for each population). Field experiments were carried out and a total of six sprays was applied from the stage of inflorescence emergence to that of completeness of berry touch. DMIs were applied with lowered frequency (two to four applications) at different times during the growing season. Applications schedules, where triadimenol was applied three or four times, following instructions at the beginning and/or end of the season, led to the highest levels of disease in the field and to the highest percentage of resistant phenotypes in the population. The most promising results were obtained when applications of triadimenol were further reduced to two, one each at fruit setting and berry touch completion. The effectiveness of other strategies where fungicides from different chemical groups are applied in alternation is also discussed.

Developed resistance of the phytopathogenic fungus *Gibberella fujikuroi* to the imidazole fungicide triflumizole. E. KALOGEROPOULOU and B.N. ZIOGAS. *Agricultural University of Athens, Department of Plant Pathology, 75 Iera Odos, 118 55 Votanikos, Athens, Greece.*

The plant pathogen *Gibberella fujikuroi* (anamorph *F. moniliforme*) is responsible for losses in both quality and yield to many economically important plants and produces mycotoxins harmful to humans and animals. An investigation into the fungitoxicity *in vitro* of a large number of inhibitors of this pathogen revealed the following order of effectiveness: triflumizole > flutriafol > fluazinam > triadimenol > azoxystrobin > fenpropimorph > fenpropidin > tridemorph > fludioxonil > fenpiclonil > iminoctadine. The imidazole fungicide triflumizole completely inhibited wild-type strains 3125 and 3120 at concentrations of 0.5 µg/ml and 3 µg/ml respectively. Mutants of *G. fujikuroi* resistant to triflumizole were isolated after UV mutagenesis at a high mutation frequency of $3.1\text{-}5.6 \times 10^{-3}$. The level of resistance of the mutants was low (Rf_{MIC}: 3–6, Rf_{ED50}: 3–5) or moderate (Rf_{MIC}: 20–40, Rf_{ED50}: 13–17). The mutations conferming resistance on triflumizole seemed to affect mating ability and the rate of growth and sporulation of *G. fujikuroi*, but not conidial germination. Pathogenicity seemed to be affected in resistant isolates from WT-3125 but not in those from WT-3120. Cross-resistance studies showed positive cross-resistance of triflumizole with the triazole inhibitors flutriafol and triadimenol. Negative cross resistance was found with the phenylopyrrole fludioxonil and iminoctadine. Some of the mutants showed higher sensitivity to the strobilurin azoxystrobin than the wild-type strain. When examining the cross-resistance pattern of triflumizole with the morpholine fungicides fenpropimorph and tridemorph, the piperidine fenpropidin and the phenylopyridinamine fluazinam, three categories of mutant strains were recognized: those with sensitivity higher or lower than or similar to that of the wild type strains. Preliminary studies on the genetic control of resistance of *G. fujikuroi* to triflumizole with seven representative mutants showed the occurrence of two or more chromosomal genes.

Influence of fungicide spray combinations on the sensitivity of *Cercospora beticola* to the sterol demethylation-inhibiting fungicide flutriafol. G.S. KARAOGLANIDIS[1], P.M. IOANNIDIS[2] and C.C. THANASSOULOPOULOS[1]. *[1]Aristotelian University of Thessaloniki, Faculty of Agriculture, Plant Pathology Laboratory, 540 06 Thessaloniki, Greece. [2]Hellenic Sugar Industry S.A., Plant Protection Department, 590 32 Plati Imathia, Greece.*

Field experiments were conducted over a 3-year period (1997–1999) in order to study the effect of several fungicide spray-schedules on the sensitivity of *Cercospora beticola* populations to the DMI fungicide flutriafol. The effectiveness of the spray programs in controlling sugar beet leaf-spot was also evaluated. Spray programs included applications of flutriafol, alone at the recommended dose, alone at a reduced dose, or in a mixture with maneb, and applications of flutriafol, alone or mixed with maneb, alternating with a tank mixture of fentin acetate and maneb. Sprays of flutriafol at the recommended dose were significantly more effective than all the other treatments, while sprays of flutriafol alternating with the tank mixture of fentin acetate and maneb were less effective than the other flutriafol treatments. However, fungal populations from plots continuously treated with flutriafol, whether alone at full dose or at a reduced dose mixed with maneb, were less sensitive to flutriafol than populations sprayed with flutriafol in alternation with the tank mixture fentin acetate/maneb. The results indicated that successive applications of flutriafol, both at full and at reduced strength, favored the selection of highly resistant strains. Since applications of flutriafol in alternation with the tank mixture of fentin acetate and maneb were not very effective the best available anti-resistance strategy seems to be to restrict the number of DMIs treatments, applying them only when environmental conditions are particularly favorable for disease development and using other fungicides during the rest of the growing season.

Residues of azoxystrobin from grapes and raisins cv. Tompson seedless and a seed-producing clone. C. LENTZA-RIZOS and K. KOKKINAKI. *National Agricultural Research Foundation, 1 S. Venizelou St., 141 23 Lycovrisi Attiki, Greece.*

Azoxystrobin is a systemic fungicide of the strobilurine group with protective action, which has recently been admitted for use in the European Union. In Greece it is registered to control *Plasmopara viticola* and *Uncinula necator* in grapevine. It consists of a mixture of two isomers (E and Z at a 3:1 ratio). On the basis of critical GAP in Europe, that refers to uses in northern European countries, the value of 2 mg/kg was set as the MRL for grapes and the residue definition was E-isomer only. Trials carried out by the manufacturer on the grape cv. Black Korinth have shown that residue concentrations are higher in raisins than in the fresh fruit. The aim of this work was to determine the amount of azoxystrobin residues on fresh grapes of the typical cv. Thomson seedless, on a seed producing clone and on raisins after processing (immersion for 3 minutes in an

aqueous solution of 3% K_2CO_3 and 1% ethyl oleate, and drying for 15 days under direct sunlight). The commercial product QUADRIS® 25% SC was applied according to GAP. Samples were collected 15 days after application. The method used for analysis of residues was gas-chromatography with ECD detection; this yielded 86±12.5% recovery for grapes and 90±5.9% for raisins. The residues in grapes at the PHI of 15 days were 1.26 and 0.64 mg/kg for the seedless variety and seed-producing clone respectively. Those in raisins were 0.91 and 0.75 mg/kg respectively.

Genetic control of resistance to the piperidine fungicides fenpropidin and piperalin in *Ustilago maydis*. A.N. Markoglou and B.N. Ziogas. *Agricultural University of Athens Department of Plant Pathology, 75 Iera Odos, 118 55 Votanikos, Athens, Greece.*

Mutants of *Ustilago maydis* (DC.) Corda resistant to the piperidine fungicides fenpropidin and piperalin were isolated in the laboratory at high mutation frequencies of 3.2×10^{-5} and 2.4×10^{-5} respectively after UV-light irradiation and selection on media containing fenpropidin (75 µg/ml) or piperalin (50 µg/ml). Random analysis of a large number of progeny with 15 fenpropidin-resistant mutant isolates resulted in the identification of two unlinked chromosomal loci, *U/fpd*-1 and *U/fpd*-2, producing moderate resistance to fenpropidin and piperalin (Rf: 15 and 25 respectively, based on MIC values). A study with 15 piperalin-resistant strains led to the identification of two independent chromosomal genes, the *U/ppl*-1 locus with two alleles (*U/ppl*-1A and *U/ppl*-1B), and the *U/ppl*-2 locus. The mutant genes *U/ppl*-1A and *U/ppl*-2 caused moderate resistance to piperalin (Rf: 25–35) and fenpropidin (Rf: 15–20), while the *U/ppl*-1B mutation caused only a small reduction (Rf: 5 and 10 respectively) in fenpropidin and piperalin sensitivity. Crosses between mutants carrying one of the *U/fpd* or *U/ppl* genes with compatible isolates carrying the *U/fpm* or *U/tdm* mutations, which had been identified in previous studies on fenpropimorph and tridemorph resistance, showed that the *U/fpd*-1, *U/ppl*, *U/fpm* and *U/tdm* mutations were not allelic. All crosses between mutant strains carrying the above genes yielded a large number of recombinants with wild-type sensitivity. Conversely, progeny with wild-type sensitivity to fenpropimorph and fenpropidin was not produced by crosses containing mutants with the *U/fpd*-2 and *U/fpm*-2 mutations, indicating that the *U/fpd*-2 and the *U/fpm*-2 were alleles of the same locus, encoding different resistant levels. A cumulative gene effect was found between *U/ppl*-2, *U/ppl*-1B, *U/fpm*-1B and *U/tdm* mutations with haploid strains carrying two of these mutations exhibiting higher resistance to piperidine and related morpholine fungicides. Cross-resistance studies with other SBIs showed that piperidine-resistant strains exhibited positive cross-resistance towards the morpholines fenpropimorph (Rf: 10–100, based on MICs) and tridemorph (Rf: 5, based on MICs), but not to the inhibitors of steps of ergosterol biosynthesis preceding the Δ^{14}-reductase (DMIs and the allylamine terbinafine). A fitness study of the *U. maydis* mutants showed that the above mutations, with the exception of *U/ppl*-1B, had no obvious effect on the characteristics of phytopathogenic fitness such as the growth rate in liquid culture, virulence and rate of disease development.

Evaluation of fungicides as seed protectants against cotton seedling damping-off caused by *Pythium ultimum*. E.J. Paplomatas[1], J. Papageorgiou[2] and G. Koutroumpakis[1]. [1]*Benaki Phytopathological Institute, 8 S. Delta St., 145 61 Kifissia, Athens, Greece.* [2]*Novartis (Hellas) S.A., Anthousas Ave., 153 44 Anthousa-Attici, Greece.*

In greenhouse experiments, the effectiveness of fungicides as seed protectants, alone or in mixture, against cotton seed and seedling damping-off caused by the soilborne fungus *Pythium ultimum* Trow was evaluated. Apron (metalaxyl) at 60 g/100 kg cottonseed was used as a reference compound. The treatments compared were the following: Apron XL 350 ES (350 g/l metalaxyl-m, CGA 329351) at 30, 40, and 50 ml/100 kg seed and Maxim 035 FS (2.5 g/l fludioxonil, CGA 173506+1 g/l metalaxyl-m, CGA 329351) at 100 and 200 ml/100 kg seed. The pathogen was incorporated into the soil mix at a density of 60 propagules per g soil and the seeds (cv. ETH.I.AG.E. No1) were sown in plastic flats, six rows per flat and 20 seeds per row. The experiment was repeated three times. All the treatments differed statistically ($P<0.05$) from the positive controls (untreated seed sown in infected soil). No treatment differed statistically from the reference compound. Only the treatment with Apron XL 40 ml/100 kg seed differed from the treatment with Maxim 100 ml/100 kg seed; all the other treatments did not differ among each other. Seedling survival in the negative controls (untreated seed sown in uninfected soil) differed statistically from all the treatments given.

Differences in sensitivity to the DMI fungicide triadimenol among populations of *Uncinula necator* in Greece. N. Petsikos-Panagiotarou[1,2], A. Markellou[1,2], A. Kalamarakis[1,2], S. Konstantinidou-Doltsinis[3] and B.N. Ziogas[4]. [1]*Benaki Phytopathological Institute, 8 S. Delta St., 145 61 Kifissia, Athens, Greece.* [2]*National, Agricultural Research Foundation, Athens, Greece.* [3]*National, Agricultural Research Foundation, Plant Protection Institute, 260 04 Patras, Greece.* [4]*Agricultural University of Athens, Department of Plant Pathology, 75 Iera Odos, 118 55 Botanikos, Athens, Greece.*

The programme monitoring sensitivity of powdery mildew of grapevine [*Uncinula necator* (Schw.) Burr.] to DMI fungicides in Greece, initiated in 1997 in our laboratory in the framework of the National Research Project DIMITRA 95 and funded mainly by N.AG.RE.F., was continued in 1998 and 1999. In those years the sensitivity of *U. necator* populations from the regions of Attiki, Korinthia, Ahaia, Heraklion, Kilkis, Thiva and Ioannina to triadimenol, and various anti-resistant strategies to maintain control of powdery mildew (separate announcement), were investigated. The sensitivity of the above mentioned populations was evaluated by measuring the length of primary hyphae 3 days after artificial inoculation of grape leaf disks treated with a range of concentrations of triadimenol (0.1–20µg/ml). If the hyphal length exceeded 240 µm the fungus was taken

to be resistant. The EC_{50} values were determined using probit analysis. The percentage of resistant conidia in each population was also calculated. Different resistance factors (Rf) ranging from 1.7 to 10.1 were found in different sampling areas. The most resistant populations were found in the areas of Kilkis (Rf = 8.1) and Heraklion (Rf = 10.1) where DMIs have been extensively used. The proportion of resistant spores differed among populations, possibly because a variety of spray programs had been applied in each grape producing area to control powdery mildew. The results obtained showed that the resistance of *U. necator* populations to DMI fungicides is low and varies from low to moderate in different regions of Greece.

Biological activity of strobilurin fungicides against *Pyricularia oryzae* isolates. M. TSILIOPOULOU, R. BONTAROUDI and A.C. PAPPAS. *University of Thessaly, Faculty of Agriculture Crop and Animal Protection, Plant Pathology Laboratory, Pedion Areos, 383 34 Volos, Greece.*

The effect of the two new strobilurin fungicides, azoxystrobin and kresoxim-methyl, on various biological activities of *Pyricularia oryzae* Cavara was studied *in vitro*. Two fungal isolates from the ornamental plant ctenanthe and one from rice were used. The inhibitory effect on mycelial growth and sporulation was analyzed on cultures grown on potato-dextrose-agar supplemented with chloramphenicol (50 mg/l) and various fungicide concentrations, at 25°C in the dark for 7 d (mycelial growth) and 14 d (sporulation). The effect on spore germination and appressorium formation was examined by placing drops of a spore suspension (10^4/ml) containing various fungicide concentrations on glass slides coated with a thin layer of copolymerized tetrafluroethylene with perfluoroalkoxy groups (Teflon). The mode of conidia germination was recorded after 18–20 h incubation in a moist chamber at 25°C. In all tests aliquots of fungicide solutions in dimethyl sulfoxide, were prepared. The final concentration of the solvent in the medium did not exceed 1%. The results revealed remarkable differences in the sensitivity to strobilurins among isolates of *P. oryzae*. The ED_{50s} for mycelial growth and spore germination were ranged from 0.005 to >10 mg/l for each isolate/fungicide. Azoxystrobin was always more effective than kresoxim-methyl while fungal isolates from ctenanthe were much more sensitive to both fungicides than the isolate from rice. Fungicide concentrations sublethal for mycelial growth had no inhibitory effect on sporulation. However, at the highest concentration of 0.001 mg/ml, which had no effect on mycelial growth and spore germination, the percentage of conidia that formed appressoria was only 26 to 64% of the controls. From these results it seems likely that the appressorium formation is the main biological target of action of strobilurin fungicides, in *P. oryzae*.

Karathane: product positioning in spraying programs to prevent development of resistance to powdery mildew in grapes. Selectivity on predatory mites and other benefits. S. VELUSCECK. *ALFA Agricultural Supplies S.A., 13 Tim. Filimona St. 115 21 Athens, Greece.*

The effectiveness of Karathane (dinocap) applications against early and late-season infections caused by populations of *Uncinula necator* some of which were sensitive and some resistant to the triazole fungicides, was studied in French and Italian trials. On early infections, Karathane alone or in mixture with sulphur at half dose, was more effective than sulphur alone. Later in the season, between flowering and fruit setting or after fruit setting of the vine, Karathane alone alternating with the triazoles at full dose, or 50–70% of Karathane's full dose in a mixture with triazoles, proved the Karathane effectiveness to prevent powery mildew resistant development to triazole based fungicides. Moreover, Karathane was selectively harmless to a great number of beneficial predators, such as *Typhlodromus pyri, Kampimodromus aberrans, Phytoseius finitimus, and T. exhilaratus*.

Reduced sensitivity to iprodione in *Alternaria alternata* on kiwifruit (*Actinidia chinensis*). I. VLOUTOGLOU and E. STAMELOU. *Benaki Phytopathological Institute, 8 S. Delta St., 145 61 Kifissia, Athens, Greece.*

Premature defoliation of *Actinidia chinensis* Planchon caused by *Alternaria alternata* (Fr.) Keissler has become one of the most serious problems in kiwifruit-producing areas in Greece. Attempts to control the disease in the area of Naoussa with iprodione, a fungicide mainly used for controlling *Botrytis cinerea* on kiwifruit, failed in some cases. Thirteen single-spore isolates of *A. alternata*, were collected in 1999 from 13 kiwifruit orchards in Naoussa. Another single-spore *A. alternata* isolate, recovered from a kiwifruit orchard in Larissa in which no fungicide had ever been applied, was also tested (wild-type). The radial-growth response of the isolates was tested on V8 agar amended with 0, 0.1, 0.2, 0.4, 0.8, 1, 5 and 10 µg/ml of iprodione (Rovral 50WP). Results showed that the majority of the isolates from Naoussa (9 out of 13) were sensitive to iprodione, with EC_{50} values from 0.51 to 0.86 µg/ml (the wild type 0.48 µg/ml). However, four of the isolates showed reduced sensitivity to iprodione, with EC_{50} values from 2.5 to 4.96 µg/ml. The occurence of *A. alternata* strains with reduced sensitivity to iprodione may indicate a population shift from sensitivity to resistance, which could have unfortunate consequences if this compound is used regularly to control *B. cinerea*.

Evaluation of fungicide sprays and their timing to control early blight of tomato (*Alternaria solani*). I. VLOUTOGLOU, A. DARRAS and E. STAMELOU. *Benaki Phytopathological Institute, 8 S. Delta St., 145 61 Kifissia, Athens, Greece.*

The activity of mancozeb (0.14% a.i., Trimanoc 75WG and 0.15% a.i,. Pennfluid 42SC), iprodione (0.075% a.i., Rovral 50WP), prochloraz (0.025% a.i., Octave 50WP), chlorothalonil (0.15% a.i., Daconil 75WP) and azoxystrobin (0.025% a.i., Quadris) against *Alternaria solani* Sorauer on tomato was studied under controlled environment conditions. Tomato plants (cv. Ace 55VF) were spray-inoculated with a conidial suspension of four *A. solani* isolates. In the first experiment, the fungicides were applied protectively (1 day

prior to inoculation) and curatively (1 and 2 days post inoculation). In the second experiment, the fungicides were applied only protectively 1, 3, 5 and 7 days prior to inoculation. Results showed that the protectant activity of fungicides was more effective than their curative activity. In the first experiment, when the fungicides were applied 1 day prior to inoculation disease severity was reduced by 91–100% and defoliation by 100% compared with the untreated control. Prochloraz, azoxystrobin and iprodione showed the greatest curative activity, especially when they were applied 1 day after inoculation (reduction in disease severity by 71–98%, in defoliation by 75–100%). In the second experiment the greatest protectant activity was achieved by chlorothalonil and prochloraz (mean reduction in disease severity by 98 and 92% respectively) followed by iprodione (87%), azoxystrobin (86%) and mancozeb (85 and 72% for Trimanoc 75WG and Pennfliud 42SC respectively). In all these cases protection was unaffected by the timing of application. Some phytotoxicity was evident with Pennfluid.

Genetic control of resistance to phenylpyrrole fungicide fenpiclonil in *Ustilago maydis*. B.N. Ziogas, V. Spyropoulou and A.N. Markoglou. *Agricultural University of Athens, Department of Plant Pathology, 75 Iera Odos, 118 55 Votanikos, Athens, Greece.*

Mutants of *Ustilago maydis* (DC.) Corda with low resistance to the phenylpyrrole fungicide fenpiclonil were isolated at a high mutation frequency 2.8×10^{-3}, after UV. irradiation. Random analysis of a large number of progeny showed that three unlinked chromosomal genes (*Ufpl*-1, *Ufpl*-2 and *Ufpl*-3) were responsible for reduced sensitivity to fenpiclonil and fludioxonil (Rf: 10 and 50 respectively, based on MIC values). A study of gene effect on the fitness of *U. maydis* showed that not all *U/fpl* mutations affected the growth rate in liquid culture, but the *U/fpl*-1 and *U/fpl*-2 loci increased the sensitivity to osmotic pressure. Haploid strains carrying two *U/fpl* mutations did not exhibit greater resistance to fenpiclonil, indicating on absence of gene interaction. Cross-resistance studies showed that the *U/fpl* mutations caused lower sensitivity to the dicarboxamide iprodione and the aromatic hydrocarbon dicloran (Rf: 11.3 and 10.6 respectively, based on MICs), but did not lower sensitivity to the morpholine fenpropimorph. Treatment of sporidia with the enzymatic preparation Novozym 238 resulted in protoplast formation in both wild-type and mutant isolates, at the same rate. Treatment of protoplasts with fenpiclonil showed that the mutant strains maintained their lower sensitivity at the protoplast stage. The results, indicate that the cell wall is not a factor in the mechanism of resistance to fenpiclonil.

Genetic control of resistance to strobilurin fungicides in *Ustilago maydis*. B.N. Ziogas, A. Tzima and A.N. Markoglou. *Agricultural University of Athens, Department of Plant Pathology, 75 Iera Odos, 118 55 Votanikos, Athens, Greece.*

Mutants of *Ustilago maydis* (DC.) Corda with a high resistance to azoxystrobin (Rf: 164–4714, based on EC_{50} values) were isolated with a mutation frequency of 2.3×10^{-7} after nitrosoguanidine mutagenesis and selection on media containing 1 µg/ml azoxystrobin and 0.5 mM salicylhydroxamate (SHAM). The lower sensitivity of the mutant isolates to azoxystrobin in the presence of SHAM, an inhibitor of alternative electron transfer, showed that alternative respiration was not the responsible biochemical mechanism causing resistance. Genetic analysis of nine such azoxystrobin-resistant mutant isolates showed that the progeny phenotypes did not follow mendelian segregation but satisfied the criteria of non-mendelian heredity: (a) uniparental inheritance; (b) vegetative segregation; and (c) intracellular selection. In crosses of three mutant isolates with wild-type strains sensitivity was inherited by the progeny from the wild-type parent strain (criterion a). In crosses of the wild-type strains with the six remaining mutants, there was a continuous distribution of sensitivity in the progeny (criterion b). The third criterion of cytoplasmic resistance was fulfilled by experiments on the stability of the resistance-phenotype: all but two mutants showed a lowering of resistance when they were grown on medium without azoxystrobin + SHAM. A study of the fitness of *U. maydis* mutants revealed that the above mutations reduced the rate of growth in liquid culture and the virulence in maize seedlings. Cross-resistance studies with other fungicides which inhibit electron flow through complex III of the respiratory chain, showed that the mutations bringing about resistance to azoxystrobin caused lowered sensitivity to kresoxim-methyl and antimycin-A. However, characteristic differences were observed between azoxystrobin and antimycin-A. All mutants but one (AZ-5009) showed a high resistance to azoxystrobin (Rf: 300–4700) and low resistance to antimycin-A (Rf: 10–20). Mutant AZ-5009 had low resistance to azoxystrobin (Rf: 164) and kresoxim-methyl (Rf: 18), but was highly resistant to antimycin-A (Rf: 305). The reason for these differences in the sensitivity of the mutant strains to strobilurins and antimycin-A, is obviously the activity of the first two in the Q_o, and that of the third in the Q_i center in complex III of the respiratory chain.

Abstracts text reviewed by the Hellenic Phytopathological Society.

ERRATA CORRIGE

Phytopathologia Mediterranea Vol. 39, April 2000, pag. 137: S. Tegli *et al.*, PCR-based assay for detection of *Phaeoacremonium* spp.

In the first paragraph of the section "PCR primer design and sensitivity" the indicated amounts of the four dNTPs and that of the specific primers and 2.5 U Taq DNA Polymerase were inadvertently misprinted and the passage should have read:

PCR primer design and sensitivity.

Two primer pairs, Pal1N-Pal2 and Pch1-Pch2,....., and containing 20 mM Tris-HCl (pH 8.0), 50 mM KCl, 1.5 mM $MgCl_2$, **50 μM** of each of the four dNTPs, **0.5 μM** of each of the two specific primers and 2.5 U Taq DNA Polymerase.