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ABSTRACTS

Summaries of papers and posters presented at the Ninth Hellenic Phytopathological Congress Athens, Greece, 20-22 October 1998

The Ninth National Phytopathological Congress, organised every two years by the Hellenic Phytopathological Society (HPS), was held in Athens, Greece on October 20-22, 1998, and was attended by 400 participants. Thirty-seven oral presentations and 52 posters were presented at the meeting, dealing with plant diseases caused by fungi, bacteria, viruses and non-parasitic disorders, and with disease control. Moreover, one round-table discussion was held on "Re-emerging diseases". Abstracts of the papers and posters of the congress are presented in this issue.

Fungal diseases

Downy mildew of carnation in greenhouses. A.C. Grigoriou and D.V. Bata. *Agricultural University of Athens, Department of Plant Pathology, 75 Iera Odos, 118 55 Athens, Greece.*

In February 1998, flowering carnation plants variety Simona were found to be severely infected by *Peronospora dianthicola* Barthelet in greenhouses of Aspropyrgos (Attiki). Shoot infection was mainly on the tips, where yellow-green spots appeared. Subsequently, the infected tissues became brown and soft and, in most cases, were covered by a black mould, due to secondary infection by *Stemphylium* sp. Usually, the shoots bent at the infection site. The leaves had initially a light green color, but later they became yellow-green. Curling of the older leaves was also observed, whereas the younger leaves dried out. The flowers were infected more on sepals than on petals. Initially, a green-yellow discoloration of the infected parts of the flowers was noticed but later the flowers dried out. Once more *Stemphylium* sp. grew saprophytically on the infected tissues. It must be noted that before the appearance of any symptoms, due to the high relative humidity in the greenhouse, conidiophores and conidia of the pathogen covered the infected parts of the plants, forming an abundant white mould. Conidiophores (270–340x7–10 µm) were dichotomously branched. Conidia were ovoid [14–29x12.5–20.5 µm (av. 23.5x18 µm)]. Inside the infected tissues the fungus produced many oospores, 40µm in diameter, heavily verrucose with yellow-brown walls.

A comparative study on pathogenicity of *Armillaria* species. P. Tsopelas. *National Agricultural Research Foundation, Institute of Mediterranean Forest Ecosystems, Terma Alkmanos, 115 28 Athens, Greece.*

Inoculation trials with forest and cultivated plants were carried out using four out of five *Armillaria* species recorded in Greece. Both *A. mellea* and *A. ostoyae* showed high virulence on black pine [*Pinus nigra* Arn. subsp. *pallasiana* (Lamb.) Holmboe], although they did not differ significantly in virulence among themselves. *A. gallica* showed lower virulence to black pine in comparison to *A. mellea* and *A. ostoyae*, while *A. tabescens* was not pathogenic to this tree species. In inoculation trials with fir (*Abies cephalonica* Loud.), *A. mellea* showed significantly higher

virulence in comparison with *A. gallica*. No other *Armillaria* species were tested on this host. Artificial inoculations of almond and olive trees with three *Armillaria* species (*A. mellea*, *A. gallica* and *A. tabescens*) were also carried out. Almond was the most susceptible host to all three *Armillaria* species. Of these,however, *A. mellea* showed significantly higher virulence, killing all inoculated almond trees within short time. *A. tabescens* was the least virulent, while *A. gallica* showed intermediate virulence. Olive trees, on the other hand, showed higher resistance to *Armillaria* infection. *A. mellea* was the only species that caused serious infections and tree mortality. *A. gallica* caused only some light infections, mainly on the dead wood of the rootstock. Finally, *A. tabescens* was not pathogenic on olive trees.

***Sphaeropsis* sp. A new pathogen of cypress for Greece.** S. Xenopoulos and P. Tsopelas. *National Agricultural Research Foundation, Institute of Mediterranean Forest Ecosystems, Terma Alkmanos, 115 28 Athens, Greece.*

In spring of 1995, dead branches and stem cankers on a few ramets of one selected cypress clone, caused by a fungus of the genus *Sphaeropsis*, were observed for the first time in the experimental plot of Manolada (Peloponnese). The cankers were similar to those caused by *Seiridium cardinale*, a pathogen causing resin exudation and bark discoloration impregnated with resin. However, the cankers by *Sphaeropsis* sp. differed by a characteristic fissuring and a light discoloration of the wood underneath the cankered area. Gradually, the fungus spread to other cypress clones. In a survey carried out in 1998, among 33 dead or cankered branches of several cypress clones, *Sphaerospis* sp. was isolated from 13, whereas *S. cardinale* was isolated from the other 20. The pathogenicity of *Sphaerospis* sp. was proved by artificial inoculations on ramets of certain resistant and susceptible to *S. cardinale* cypress clones. The mean canker length on susceptible to *S. cardinale* clones was significantly larger in comparison with that on resistant ones. Based on these results, it seems that the resistance reaction of the cypress clones to both fungi is similar. The spore size and the cultural characteristics on PDA were identical to those of the fungus *Sphaeropsis sapinea* f.sp. *cupressi* (syn. *Diplodia pinea* f.sp. *cupressi*) described in Israel, which is different from the pine pathogen *Sphaeropsis sapinea* (syn. *Diplodia pinea*). In artificial inoculations on *Pinus halepensis* and *P. pinea*, *Sphaeropsis* sp. was not pathogenic to these hosts. On the other hand, three isolates of *S. sapinea* from Italy were pathogenic to both pine species but not to cypress seedlings.

Characterisation and pathogenicity of anastomosis groups of *Rhizoctonia solani* isolated from Greek sugarbeet fields. D. Gilpathi[1], D. Lascaris[1] and K. Doulias[2]. *[1]Benaki Phytopathological Institute, Plant Pathology Department, 8, S. Delta Street, 145 61 Kifissia, Athens, Greece. [2]Hellenic Sugar Industry, Factory of Orestias, P.O. Box 6, 682 00 Orestias, Greece.*

According to a survey conducted during 1995-97 on damping-off and root rot diseases in Greek sugarbeet fields, *Rhizoctonia solani* was commonly isolated from seedlings and older plants. One hundred and one isolates characterized as *R. solani* were classified in four anastomosis groups (AG): AG2-2 predominated and comprised 35% of isolates, followed by AG-4 (23%), AG-5 (2%) and AG-3 (1%). A high percentage of isolates (35%) did not anastomose with any of the tester cultures (AG-1 through AG-7 and AG-BI). The pathogenicity of 97 isolates on sugarbeet seedlings (*Beta vulgaris*) was tested under greenhouse conditions. Isolates belonging to anastomosis group AG-4 were highly pathogenic, followed by less pathogenic AG2-2, whereas AG-3 and AG-5 were not pathogenic. The non-grouped isolates varied in their pathogenicity from highly virulent to non-pathogenic. Nine isolates that represented the predominant AG groups and the non-grouped ones were tested for their pathogenicity on pea (*Pisum sativum*), bean (*Phaseolus vulgaris*), wheat (*Triticum aestivum*) and corn (*Zea mays*) seedlings. In most cases, AG-4 isolates were virulent on bean and pea, but were less or not virulent on corn and wheat. AG2-2 isolates were pathogenic only on bean seedlings. The pathogenicity of six characteristic isolates from the two predominant AG groups (3 from AG-4 and 3 from AG-2-2) was tested on roots of 14-week-old sugarbeet plants (cv. Maribo Ultramono and Rizor). The three AG-4 isolates were highly virulent on both cultivars. Two of the three AG2-2 isolates were less pathogenic and the third was highly virulent on both cultivars.

Development of a rapid method to evaluate the resistance of olive varieties and rootstocks to *Verticillium dahliae*. P.P. Antoniou and E.C. Tjamos. *Agricultural University of Athens, Department of Plant Pathology, 75 Iera Odos, 118 55 Athens, Greece.*

Resistance of olive rootstocks to Verticillium wilt is one of the most promising approaches in solving this problem worldwide. However, evaluation of resistance is a particularly time-consuming procedure that lacks rapidity and reliability. The development of a rapid method of evaluation, described in this study, was based on the known difference in susceptibility to wilt between two olive varieties. The varieties used were the highly susceptible "Amfissis" and the tolerant "Kalamon". The experimental study took place under controlled glasshouse conditions after acclimatization of 200 2-year-old olive trees of both varieties. Infection took place under the sites of ramification of branches or at the base of the branches by opening holes in the bark and injecting a conidial suspension of 100 ml (10^8 conidia/ml) of a very virulent olive isolate of *Verticillium dahliae*. The conidial suspension was poured into the holes (3 mm x 4 mm, diameter x depth) immediately after they were made. The wounds were sealed with vaseline. Disease symptom expression on infected trees was recorded by defining the minimum and maximum time needed for symptom development and the difference between tested varieties. The speed of symptom development, the severity and incidence of the disease on "Kalamon" variety were significantly slower than those on the susceptible "Amfissis" variety. The evaluation of this method ended three months after the artificial infection, when most of the tested olive

trees of the "Amfissis" variety showed intense symptoms (almost 99% of trees developed intermediate to very severe symptoms, dry leaves and wilted branches). By contrast, disease symptoms on the "Kalamon" variety were mild to absent, being present only on 9% of trees, and with symptoms below average, while most of the trees did not develop apparent symptoms at all. The data of this experimental approach support the application of the method for the evaluation of resistance of olive rootstocks.

Cleistothecia of *Uncinula necator* (Schw.) Burr. in Greece: preliminary studies for the determination of their role in the overwintering of the fungus. E. Kalogeropoulou[1], A. Kalamarakis[1,2], N. Petsikos-Panagiotarou[1,2] and S. Konstantinidou-Doltdinis[3]. [1]*Benaki Phytopathological Institute, Department of Pesticides Control and Phytopharmacy, Laboratory of Efficacy Evaluation of Pesticides, 8, S. Delta Street, 14561 Kifissia, Athens, Greece.* [2]*National, Agricultural Research Foundation, Athens, Greece.* [3] *National, Agricultural Research Foundation, Plant Protection Institute of Patras, 260 04 Patras, Greece.*

Uncinula necator, the causal agent of grape powdery mildew, is thought to overwinter as mycelium in dormant buds of vine. The role of cleistothecia in the epidemiology of the fungus was not considered to be of a great importance. Recent studies in Europe and the USA however showed that cleistothecia function as an additional source of primary inoculum with a significant contribution to the spread of the disease. In Greece, the source of primary inoculum and the role of cleistothecia in grape powdery mildew epidemics have never been investigated. Preliminary studies indicated that the fungus overwinters by both the above-mentioned structures in most wine-growing regions of Greece. Large numbers of cleistothecia were found on the leaves, canes and berries and in the bark of the vines. The percentage of viable ascocarps, obtained from the leaves in autumn, was found to be ca. 53% as tested by the fluorescent vital stain fluorescein diacetate. However, only dead ascocarps with degenerated ascospores were obtained from samples of bark (upper and lower trunk) collected the following spring, possibly due to the adverse environmental conditions.

New hosts of the fungus *Verticillium dahliae* for Greece and worldwide. E.K. Ligoxigakis[1], D.J. Vakalounakis[1] and C.C. Thanassoulopoulos[2]. [1]*National Agricultural Research Foundation, Plant Protection Institute, P.O. Box 1802, 711 10 Heraklion, Crete, Greece.* [2]*Aristotelian University of Thessaloniki, Faculty of Agriculture, Plant Pathology Laboratory, 540 06 Thessaloniki, Greece.*

In 1996-98, an extensive survey on Verticillium wilt of cultivated plants and weeds showing disease symptoms was carried out in Crete, with the aim of detecting new hosts of *Verticillium dahliae* Kleb. Eight new hosts of the fungus were found for Greece; they belonged to the families: Asteraceae, Brassicaceae and Geraniaceae. Two of the new hosts belonged to the following cultivated species: *Tagetes erecta* L. and *Brassica oleracea* L. var. *italica,* and six to the weed species: *Anthemis melanolepis* Boiss., *Lactuca serriola* L., *Sonchus oleraceus* L., *Raphanus raphanistrum* L., *Sinapis arvensis* L. and *Erodium* sp. Four species: *T. erecta, A. melanolepis,* L. *serriola* and *Erodium* sp. are here reported for the first time as new hosts of the pathogen worldwide. The fungus was also isolated from seven species: *Amaranthus* sp. *Pistacia vera* L., *Vitis vinifera* L. ssp. *vinifera, Senecio vulgaris* L., *Capsalla bursapastoris* L., *Chenopodium album* L. and *Malva sylvestris* L. which are reported for the first time as hosts of the pathogen in Crete.

Mycotoxin production by fungi causing post-harvest rot in pears. I.A. Laidou[1], C.C. Thanassoulopoulos[1] and M. Liakopoulou-Kyriakidou[2]. [1]*Aristotelian University of Thessaloniki, Faculty of Agriculture, Plant Pathology Laboratory, 540 06 Thessaloniki, Greece.* [2]*Aristotelian University of Thessaloniki, Department of Chemical Engineering, Organic Chemistry Laboratory, 540 06 Thessaloniki, Greece.*

The fungi *Penicillium expansum, Alternaria alternata, Aspergillus flavus* and *Stemphylium vesicarium* cause storage rot in pears of the cultivar Abate Fetel and produce a common secondary metabolite, the mycotoxin patulin, with mutagenic and probably carcinogenic properties. Pears with spots approximately 10 mm in diameter and 6-10 mm in depth were divided into four zones of 6 mm each, starting from the surface (zone 1) to the centre of the fruits (area around the carpophyll, zone 4), in order to trace the pathogens at several depths and perform a qualitative and quantitative analysis of the mycotoxin with thin-layer (TLC) and high-pressure liquid chromatography (HPLC). *P. expansum* was isolated from zones 1-4, the toxin from zones 1-3 and in amounts of 144-288 ppm, which is 58-115 times the maximum residue limit (MRL), which is 50 ng/g (50 ppb). *A. alternata* was isolated from zones 1-3 and the toxin from 1-2 in amounts of 36-144 ppm, or 14-58 times the MRL. *S. vesicarium* was isolated from zones 1-2, and the toxin from 1-4 in amounts of 36-72 ppm, or 14-29 times the MRL. Finally, *A. flavus* was isolated from zones 1-4 but with this fungus the toxin was not traced at any depth. In consequence, it appeared that the pathogens and the mycotoxin also occurred in healthy tissue and hence posed possible problems to public health.

***Alternaria solani*, an emerging threat to the tomato crop in Greece.** I. Vloutoglou and S.N. Kalogerakis. *Benaki Phytopathological Institute, Plant Pathology Department, 8, S. Delta Street, 145 61 Kifissia, Athens, Greece.*

During 1995-98, an increase in the incidence and severity of the disease caused by *Alternaria solani* on tomato crops in Greece has been observed. In some cases, the high disease severity forced farmers to abandon the crop within almost one week of the appearance of first symptoms. In the present study, the susceptibility of commercial tomato cultivars and hybrids as well as the effect of the duration of wet periods and that of the growth stage of the host on infection by *A. solani* was studied. Results showed that under the experimental conditions none of the cultivars or

hybrids tested was tolerant to infection by the pathogen. Most of the tomato cultivars were less susceptible than the hybrids tested. Only hybrids No. 1247 and 1251 showed some degree of tolerance (infection rates of 24 and 33% respectively), whereas the rest of the tomato hybrids and cultivars were susceptible (infection rate 57-80%) or highly susceptible (infection rate >80%). The minimum duration of a wet period to induce appearance of the first symptoms was 4 or 6 h, depending on the susceptibility of the host. Although all growth stages were susceptible to infection by *A. solani*, plants at the pre-flowering stages were less susceptible (infection rate 66-92%) than those that were at the flowering or post-flowering stage (infection rate100%).

The defoliating strain of *Verticillium dahliae* on cotton: first report for Greece. K. Elena and E.J. Paplomatas. *Benaki Phytopathological Institute, Plant Pathology Department, 8, S. Delta Street, 145 61 Kifissia, Athens, Greece.*

Vegetative compatibility groups (VCGs) of 71 Greek *Verticillium dahliae* Kleb. isolates obtained from cotton plants were tested. *Nit* mutants derived from these isolates were tested against tester strains of previously described VCGs from the USA and Greece. Most of the isolates were assigned to VCG2, where most of the non-defoliating strains of this fungus belong, except for isolates from Australia, which belonged to VCG4. Only two Greek strains were assigned to VCG4. Isolate V63 was assigned to VCG1, where the defoliating strains of the fungus are grouped according to previous studies. Moreover, the RAPD analysis of isolate V63 with five selected primers gave DNA profiles similar to those obtained from isolates T-9 and V-44 from the USA, which are known to belong to the defoliating strain of the pathogen. Since this was the first report of VCG1 in Greece, pathogenicity tests were performed with isolate V63 and strains that belonged to other VCGs. Isolate V63 was the most virulent killing all plants within 9-20 days from inoculation. Moreover, all plants were defoliated. The remaining strains were less virulent, causing moderate to severe symptoms on plants.

Differentiation of *Fusarium oxysporum* f. sp. *radicis-cucumerinum* isolates causing root and stem rot of cucumber by pathogenicity, vegetative compatibility and RAPD fingerprinting. J. Vakalounakis and G. Fragkiadakis. *National Agricultural Research Foundation, Plant Protection Institute, 711 10 Heraklion, Crete, Greece.*

Seventy-one isolates of *Fusarium oxysporum* Schlechtend.:Fr. obtained from infected cucumber plants showing typical root and stem rot symptoms were characterized for pathogenicity, vegetative compatibility and random amplified polymorphic DNA (RAPD). Twelve isolates of other formae speciales and races of *F. oxysporum* from cucurbit hosts, three avirulent isolates of *F. oxysporum* and four isolates of *Fusarium* spp. obtained from cucumber were included for comparison. Of the 71 isolates of *F. oxysporum* from cucumber, 68 were identified by pathogenicity as *F. oxysporum* f. sp. *radicis-cucumerinum* D. J. Vakalounakis (FORC), while the remaining three isolates were avirulent on cucumber. Sixty isolates of FORC belonged to vegetative compatibility group (VCG) 0260, five isolates to VCG 0261, while three isolates were vegetatively compatible with isolates from both VCGs 0260 and 0261 (bridging isolates). FORC isolates were vegetatively incompatible with *F. oxysporum* f. sp. *cucumerinum* J.H. Owen (FOC) and the other *Fusarium* isolates tested. All 68 isolates of FORC belonged to a single RAPD group. Vegetative compatibility and RAPD were effective in identifying isolates of FORC. Phylogenetic analysis of the RAPD data, generated by the unweighted pair-group method with arithmetic averages (UPGMA), indicated that FORC and FOC possibly have different ancestors.

Phylogenetic analysis of *Verticillium* spp. using RAPDs. E.J. Paplomatas and C. Lampropoulos. *Benaki Phytopathological Institute, Plant Pathology Department, 8, S. Delta Street, 145 61 Kifissia, Athens, Greece.*

The phylogenetic relation of 25 Greek isolates of *Verticillium dahliae* from several hosts and geographic areas was studied using RAPDs (random amplified polymorphic DNA). The cotton defoliating and non-defoliating strains and a cauliflower isolate, all from California, were also included in the analysis. From the other *Verticillium* species, five isolates of *V. tricorpus*, two isolates of *V. albo-atrum* and one isolate from each of *V. theobromae*, *V. nigrescens* and *V. lecanii* were studied. Selection of the five most appropriate primers among 30 random decamer oligonucleotides was based on their ability to produce multiple DNA bands in PCR reactions against two *V. dahliae* isolates. The DNA profiles that were produced after electrophoresis of the PCR products were used to calculate genetic distances for Dice's coefficient using the RAPDistance software package. Dendrograms were generated using UPGMA (unweighted pair-group method with arithmetic average) with the aid of Phylip 3.5 software. It was found that *V. dahliae* isolates were separated in two different RAPD groups. In the first group, isolates from olive, potato, melon, pistachio and dimorphotheca were included, while the second group contained isolates from watermelon, cucumber, pepper, mint, artichoke and chrysanthemum. However, isolates from tomato and cotton were spread in both groups with the exception of the defoliating strain that was placed on a separate branch of the dendrogram. The only Greek isolate of the fungus that was not placed in either of the above groups was one from the weed *Xanthium strumarium*. The cauliflower isolates that are considered as nearly diploid also assumed a separate branch. Finally, the isolates of *V. theobromae*, *V. nigrescens*, *V. lecanii* and *V. albo-atrum* were fully separated from each other. However, these species showed a closer phylogenetic relation with *V. dahliae* than with *V. tricorpus*, which, although it has a lot of common morphological characters with *V. dahliae*, was the most genetically distant group.

Differentiation of six phytopathogenic *Verticillium* species based on an intron region of the histone H4 gene. V.K. Karapapa and M.A. Typas. *University of Athens,*

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PCR amplification products of H4 DNA segments of genomic DNA of 12 different species of *Verticillium*: *V. dahliae, V. longisporum, V. albo-atrum, V. nigrescens, V. tricorpus, V. nubilum, V. lecanii, V. chlamydosporium, V. lamellicola, V. psalliotae, V. catenulatum* and *V. rexianum*, using one set of primers designed to amplify a DNA region that spans the intron 2 in H4 gene of *Neurospora crassa*, were sequenced to determine intraspecific diversity within the genus *Verticillium*. A distinct similar size PCR product of approximately 250 bp was produced from the first six phytopathogenic species, while the other non-phytopathogenic species produced a PCR product of approximately 200 bp. Sequence analyses of the amplified 250 and 200 bp products revealed intron-containing genes and intron-less genes at conserved positions respectively 49 bp from the H4-1a primer and 142 bp from H4-1b primer with GT A and YAG 5' and 3' intron boundaries. Heterology was higher in the intron sequences than in the coding regions and comparative sequence analyses of the introns revealed a high degree of divergence and distinguished the phytopathogenic species.

Competitive PCR assay to quantify *Verticillium dahliae* on resistant and susceptible tomato hybrids. I. Aspromougos and E. Schlösser. [1]*Benaki Phytopathological Institute, Plant Pathology Department, 8, S. Delta Street, 145 61 Kifissia, Athens, Greece.* [2]*Institut für Phytopathologie und Angewandte Zoologie, Bismarckstraße 16, D-35390 Gießen, Germany.*

Monitoring the fungal growth in the host plant, especially in the case of fungal wilt diseases such as Verticillium wilt during the important latent stage of symptom development, is a useful tool for many purposes such as breeding, collection of epidemiological data or research of host-parasite interactions. In the present work the specificity and sensitivity of competitive PCR was used to quantify disease development in one resistant and one susceptible tomato hybrid artificially inoculated with *Verticillium dahliae*. The design of the primers was based on the differences of the ITS1 and ITS2 regions for *Verticillium* spp. ribosomal DNA sequences of the 5.8S RNA. A homologous truncated DNA template was used as competitor to the target fungal DNA template. The PCR-product was quantified by HPLC.

Ligase Chain Reaction-based detection of the potato pathogen *Phytophthora infestans*. E. Hatziloukas[1], P.W. Tooley[2], and M.M. Carras[2]. [1]*Aristotelian University of Thessaloniki, Plant Pathology Laboratory, Faculty of Agriculture, P.O. Box 269, 540 06 Thessaloniki, Greece.* [2]*USDA/ARS/Foreign Disease-Weed Science Research Unit, 1301 Ditto Ave., Fort Detrick, MD 21702, USA.*

Phytophthora infestans is the causal agent of late blight of potato. The recent establishment of new pathogen genotypes, in combination with the time-consuming and insensitive detection practice of visual symptom inspection, make necessary the development of a quick, reliable, sensitive and specific detection technique. The polymerase chain reaction (PCR) allows the timely detection of the pathogen (four days post-inoculation in potato tubers), with high sensitivity (1-10 pg of purified DNA) and specificity (discriminates between late blight, caused by *P. infestans*, and pink rot, caused by *P. erythroseptica*). A PCR primer pair, derived from the pathogen's Internal Transcribed Spacer Region 2 (ITS2), amplifies the corresponding regions from five more *Phytophthora* species. The severity of the disease requires the development of primers that are as specific as possible. Since LCR has theoretically a higher specificity potential than PCR, we investigated its applicability to this case. For this purpose we determined the nucleotide sequence from 20 different *Phytophthora* species and designed specific LCR primers. These primers showed lower sensitivity (detection threshold, 1 ng DNA), but higher specificity, because they used as template DNA from *P. infestans, P. mirabilis* and *P. phaseoli* only. In order to improve the sensitivity limits of our LCR assay, we combined a PCR round, with a subsequent LCR one, thus increasing the sensitivity level of the assay up to 100 times compared to PCR alone. This procedure allowed us to detect up to 10 fg of input DNA, or 400 mg tissue from artificially infected tubers.

Pathogenicity of *Phytophthora nicotianae* isolates from different hosts on tobacco and tomato plants. K. Elena and T. Tsimplis. *Benaki Phytopathological Institute, Plant Pathology Department, 8, S. Delta Street, 145 61 Kifissia Athens, Greece.*

The pathogenicity of 61 isolates of *Phytophthora nicotianae* Breda de Haan (syn. *Phytophthora parasitica* Dastur) from different hosts was tested on tobacco and tomato seedlings. All isolates were maintained on corn meal agar (CMA) slants. Each isolate was subcultured on 9-cm CMA plates and incubated at 22°C for 7 days. The inoculum for each isolate was prepared by blending the contents of 14 inoculated plates in a litre of deionized water. Fifty to 100-day-old tobacco plants and 15-day-old tomato plants were inoculated in four experiments for tobacco and two experiments for tomato. Seven plants of tobacco and seven of tomato, transplanted in 9-cm diameter plastic pots with compost, were inoculated with each *Phytophthora* isolate. The isolates of *P. nicotianae* from tobacco were pathogenic to tobacco plants but none of them was pathogenic to tomato. The isolates from tobacco constituted a separate group causing typical black-shank disease symptoms on tobacco plants. Only 19 out of the 61 *P. nicotianae* isolates, mostly isolated from tomato, were virulent to tomato plants. The rest of the isolates failed to cause symptoms on tomato.

Evaluation of resistance of stone fruit rootstocks to artificial inoculation with *Phytophthora* species. K. Elena[1], K. Tsipouridis[2] and A. Issaakidis[2]. [1] *Benaki Phytopathological Institute, Plant Pathology Department, 8, S. Delta Street, 145 61 Kifissia Athens, Greece.* [2]*National Agricultural Research Foundation, Pomology Institute, 592 00 Naoussa, Greece.*

New stone fruit rootstocks screened for many traits were evaluated for two years (1996-97) for their susceptibility to

two isolates of *Phytophthora cactorum* (Leb. and Cohn) Schroeter isolated from the bark of *Prunus dulcis* (Miller) D.A. Webb and *Malus domestica* Borkh., one isolate of *P. citrophthora* (R.E. Smith and E.H. Smith) Leonian, and one isolate of *P. megasperma* Drechsler from *Prunus dulcis*. Two-year-old seedlings of stone fruit rootstocks were inoculated with a 5-mm in diameter mycelial plug that was inserted between the bark and the cambium. *P. megasperma* was not virulent to any rootstock. During the first year of field experimentation at Naoussa, the rootstocks Tsukuba 9, J1 and St Julien 655/2 were the most tolerant to all strains of *Phytophthora*, while Myrandier 617 and Italian x Nemaguard were the most susceptible. In the following year, the Tsukuba 9, St. Julien 655/2 and Tsukuba 5 were tolerant, while Myrandier 617 was susceptible. Among the rootstocks tested with *P. citrophthora* only, Damas 1869 was the most tolerant. At Macryhori (Veria), all pathogens except *P. megasperma* were virulent to GF 677 and Stylianidis roostocks.

Differentiation of *Fusarium oxysporum* f.sp. *cucumerinum* isolates causing Fusarium wilt of cucumber, by pathogenicity, vegetative compatibility and RAPD fingerprinting. G. Fragkiadakis and D.J. Vakalounakis. *National Agricultural Research Foundation, Plant Protection Institute, 711 10 Heraklion, Crete, Greece.*

Thirty-four isolates of *Fusarium oxysporum* Schlechtend.:Fr., obtained from diseased cucumber plants showing Fusarium wilt symptoms, were characterized for pathogenicity, vegetative compatibility and random amplified polymorphic DNA (RAPD). Twelve isolates of other formae speciales and races of *F. oxysporum* from cucurbit hosts, three avirulent isolates of *F. oxysporum* and four isolates of *Fusarium* spp. obtained from cucumber were included for comparison. Of the 34 isolates of *F. oxysporum* from cucumber, 32 were identified by pathogenicity as *F. oxysporum* f.sp. *cucumerinum* J.H. Owen (FOC), while the remaining two isolates were avirulent on cucumber. Eighteen isolates of FOC were assigned to vegetative compatibility group (VCG) 0180, four to a second group named VCG 0181, two to a third group named VCG 0182, two to a fourth group named VCG 0183, four (each comprising a single-membered VCG) to an artificial group named VCG 018-, while two were heterokaryon self-incompatible (018-HIS). FOC isolates were vegetatively incompatible with *F. oxysporum* f.sp. *radicis-cucumerinum* D.J. Vakalounakis (FORC) (root and stem rot of cucumber) and the other *Fusarium* isolates tested. All FOC isolates of VCG 0180 belonged to a single RAPD group, while isolates of VCGs 0181, 0182, 0183, 018- and 018-HIS belonged to a second RAPD group. Vegetative compatibility and RAPD were effective in identifying isolates of FOC.

Wood-inhabiting macrofungi from Mt. Aroania. Z. Gonou-Zagou and P. Delivorias. *University of Athens, Department of Biology, Section of Ecology and Systematics, Panepistimiopolis, 157 84 Athens, Greece.*

The present work is part of an overall study of the macrofungi in Mt. Aroania and part of the research activity of our laboratory to determine the diversity of macrofungi in Greece. The wood-inhabiting macrofungi are a group of specific ecological interest as they are mainly responsible for wood decay and various types of wood rot. These fungi are mostly saprotrophs, but a few species are parasitic on living trees. Material was collected from several sites on the southeastern slopes of Mt. Aroania (altitude 1130-1320 m), close to the border between the prefectures of Achaia and Korinthia, from autumn 1996 to spring 1998. This area consists of a mixed coniferous forest composed of *Abies cephalonica* and *Pinus nigra*, with *A. cephalonica* predominating. Specimens were collected from various substrates, such as stumps, fallen trunks and branches, cones, and the base of living trees. In total, 30 species were identified, belonging to 24 genera of Basidiomycetes and one genus of Ascomycetes. All specimens are deposited at the mycological herbarium of the University of Athens (ATHU-M).

Apple canker caused by *Botryosphaeria stevensii* Shoemaker. A.C. Grigoriou. *Agricultural University of Athens, Department of Plant Pathology, 75 Iera Odos, 118 55 Athens, Greece.*

During 1994-98, a severe infection of a large number of apple trees of the cv. Delicious Pilafa and Starking caused by *Botryosphaeria stevensii* [anamorph *Diplodia mutila* (Fr.) Mont.] was observed in the area of Tegea, Arkadia. The fungus attacked the branches and the fruit. The bark of infected branches became brown and fissured both vertically and parallel to the axis of the branch. As the canker grew, the bark began to detach itself and the wood was left uncovered, especially on the bigger branches. When the canker completely girdled the branch, the leaves above the canker turned yellow, wilted and dried out. On the fruit, infection usually started from the calyx. Initially a small, brown spot appeared and grew until it covered the whole fruit. Affected tissues became soft, dehydrated and finally shriveled. Pycnidia are black, globose, subcutaneous, 400-500 µm in diameter. Conidia are ellipsoid or cylindrical, initially hyaline and one-celled, later becoming black, two-celled, 22-30x12-16 µm. The teleomorph of the fungus was not observed.

***Pleospora herbarum* (Pers.: Fr.) Rabenh. on tomato fruit.** A.C. Grigoriou. *Agricultural University of Athens, Department of Plant Pathology, 75 Iera Odos, 118 55 Athens, Greece.*

During the winter months of 1995-98, a severe infection of tomato fruit cv. Scala, by *Pleospora herbarum* (Pers.: Fr.) Rabenh. (anamorph *Stemphylium botryosum* Wallr.) was observed in greenhouses on the island of Syros. The initial symptoms of the disease were noticed mainly on mature fruit around the pedicel attachment, rarely at the stylar end of the fruit. The symptoms were small, green spots of a hard texture. As these spots increased in size they became soft and spread to a large part of the fruit. In section, the tissues showed brown rot. On the spots surface, conidia and conidiophores of the anamorph of the fungus formed an abundant black mould. Both the anamorph and the teleo-

morph of the fungus formed on PDA medium. Conidia were dictyospores, brown or olivaceous, oblong, oval or almost globose, echinulate, 18-40x12-24 µm (average 28.5x19.0 µm). Ascospores were dictyospores, yellow-brown, ellipsoid, 24-46x12-18 µm.

Widespread occurrence of race 2 of *Fusarium oxysporum* f.sp. *niveum* in watermelon crops in Cyprus and its control by means of resistant rootstocks. N. Ioannou[1], C. Poullis[2] and J. B. Heale[3]. [1]*Agricultural Research Institute, Nicosia, Cyprus.* [2]*Department of Agriculture, Nicosia, Cyprus.* [3]*King´s College, University of London, UK.*

Fusarium wilt of watermelon, caused by *Fusarium oxysporum* f. sp. *niveum* (FON), is a limiting factor for watermelon production in Cyprus. Trials with reportedly resistant cultivars showed that all were susceptible to the local isolates of FON. In order to identify the prevailing races in Cyprus, pathogencity tests were carried out on three differential cultivars, using 20 local isolates of unknown race designation, in comparison with isolates from USA and Israel, known to belong to races 0,1 and 2. The majority of local isolates belonged to race 2, a highly aggressive race able to attack all resistant cultivars produced so far. Efforts to control the disease were therefore directed towards the use of resistant rootstocks on which susceptible watermelon cultivars could be grafted. Several endemic cucurbit species and imported commercial rootstocks were evaluated in the laboratory for resistance to FON and for compatibility with the main watermelon cultivars. Ten prospective rootstocks were preselected and subsequently evaluated in four field trials, with the following results: a) All rootstocks provided 100% protection from FON, but *Cucurbita maxima* (local) and *C. ficifolia* (imported) were susceptible to *Rhizoctonia solani* and *Pythium* spp. b) The growth and yield of grafted plants were more than double that of the non-grafted controls, while fruit quality was not affected by grafting. c) The most promising rootstocks were the introduced RS841 F1 and Early M F1 as well as the endemic *Lagenaria siceraria* "clavata" and *Cucurbita pepo* "melopepo".

Molecular characterization of the plant pathogen *Verticillium longisporum* based on the 18S-rRNA gene. V.K. Karapapa and M.A. Typas. *University of Athens, Division of Genentics and Biotechnology, Department of Biology, Panepistemiopolis, Kouponia, 157 01 Athens, Greece.*

DNA from 40 isolates of *Verticillium longisporum* from different cruciferous hosts and locations, and 20 isolates of *V. dahliae* from different genera of host plants, as well as at least two isolates each of 16 different *Verticillium* species (*V. albo-atrum, V. tricorpus, V. nigrescens, V. nubilum, V. theobromae, V. cephalosporium, V. chlamydosporium, V. catenulatum, V. psalliotae, V. lamellicola, V. lecanii, V. fungicola, V. rexianum, V. epiphytum, V. hemipterigenum* and *V. insectorum*) was amplified using one set of primers designed to amplify the 18S-rRNA gene. With the exception of two recombinant isolates, a PCR product of approximately 2600 bp was amplified from all isolates of *V. longisporum*. All the other phytopathogenic and non-phytopathogenic species produced a PCR product of approximately 1780 bp. The sequencing of the 18S-rRNA gene product of one typical isolate of *V. longisporum* is in progress and its comparison with the corresponding region of *V. dahliae* is expected to reveal the nature of this size difference.

***Alternaria dauci* found to produce chlamydospores.** A.L. Lagopodi, G.V. Bloemberg, I. H.M. Mulders, and B.J.J. Lugtenberg. *Leiden University, Institute of Molecular Plant Sciences, Wassenaarseweg 64, 2333 AL Leiden, The Netherlands.*

Alternaria dauci (Kühn) Groves and Skolko, the causal agent of carrot leaf blight, was found to produce chlamydospores *in vitro*. Chlamydospores in long chains were produced on PDA as well as on Malt agar at 28°C from neighbouring cells of hyphae of the submerged mycelium. Formation of chlamydospores was initiated started within the first week of growth and was completed in a multiple-stage process, including swelling of cells, development of secondary septa, rounding up of cells and cell wall thickening. Mature chlamydospores were observed after four weeks of growth, when the cultures started to dry out. In old cultures, chlamydospores were released from the mycelium singly, in pairs, in short or long chains, or in clusters. They were globose to ellipsoid, smooth or verrucose, pale brown in colour, and averaged 13.87x11.37 µm (length x width). Formation of chlamydospores by *A. dauci* has not been reported in the literature so far.

Pathogenicity of *Verticillium dahliae* isolates belonging to races 1 and 2 and obtained from new and common hosts on various cultivated plant species. E.K. Ligoxigakis[1], D.J. Vakalounakis[1] and C.C. Thanassoulopoulos[2]. [1]*National Agricultural Research Foundation, Plant Protection Institute, P.O. Box 1802, 711 10 Heraklion, Crete, Greece.* [2]*Aristotelian University of Thessaloniki, Faculty of Agriculture, Plant Pathology Laboratory, 540 06 Thessaloniki, Greece.*

The pathogenicity of 13 isolates of *Verticillium dahliae*, obtained from 11 cultivated plant species: *Lactuca sativa* L. var *longifolia* Lam., *Raphanus sativus* L., *Beta vulgaris* L. var *sicula, Citrullus vulgaris* Schrad., *Cucurbita pepo* L., *Cicer arietinum* L., *Vicia faba* L., *Abelmoschus esculentus* (L.) Moench., *Lycopersicon esculentum* Mill. (with and without the *Ve* gene), *Olea europea* L. and *Vitis vinifera* L. ssp. *vinifera*, plus two weed species: *Cardaria draba* (L). Desv. and *Solanum nigrum* L., originating in several parts of Crete, was checked in artificial inoculation tests on 20 cultivated plant species. Of the 13 isolates, six, from: *L. sativa* var. *longifolia, C. pepo, C. arietinum, O. europea, L. esculentum* (with the *Ve* gene) and *S. nigrum* belonged to race 2 of the fungus, while the remaining seven belonged to race 1. Of the 20 species tested, 15: *L. sativa* var. *longifolia, Foeniculum vulgare* L. ssp. *vulgare, Cichorium endivia* L., *C. intybus* L., *Brassica oleracea* L. var *botrytis, B. oleracea* L. var. *capitata, R. sativus, Sinapis arvensis, C. pepo, B. vulgaris* var. *sicula, C. arietinum, Lathyrus ochrus* L., *Lens culinaris L., Pisum sativum* L., and *Vicia sativa* L. are new hosts of the fungus in

Greece and/or worldwide, while the remaining five species: *C. vulgaris, Vicia faba* L., *Phaseolus vulgaris* L., *A. esculentus* and *L. esculentum* are previously known hosts. The pathogenicity tests revealed differences in virulence among the isolates tested. Plant species tested were inoculated with variable number of isolates. The number of isolates was determined on the base of the virulence of the isolates and on the hosts from which the isolates derived and the race of the pathogen. The fungus was re-isolated from the vascular tissues of all the plants showing disease symptoms.

Characterization of isolates of *Fusarium proliferatum* from asparagus with RAPDs and VCGs. E.J. Paplomatas and K. Elena. *Benaki Phytopathological Institute, Plant Pathology Department, 8, S. Delta Street, 145 61 Kifissia, Athens, Greece.*

The most serious pathogens causing root and crown rot of asparagus are the soilborne fungi *Fusarium proliferatum* (Matsusima) Nirenberg and *F. oxysporum* f.sp. *asparagi* Cohen. In a survey of a large number of infected plant samples from the most important areas of asparagus cultivation in the country, *F. proliferatum* was mostly isolated. Due to the severity of the disease but also to the fact that asparagus cultivation is economically important in Greece, the genetic and molecular variation of a collection of 20 isolates of the fungus from several geographic areas was studied with RAPD and vegetative compatibility group (VCG) analyses. The primer used was a synthetic decamer oligonucleotide that was capable of producing polymorphic DNA bands in RAPD-PCR reactions with genomic *F. oxysporum* DNA. After electrophoresis of the PCR products, the profile of the DNA bands was recorded for each isolate. The data were used to calculate genetic distances with the aid of the RAPDistance software package. Based on the genetic distances, a cladogram was generated using the Phylip 3.5 software. It was found that the isolates fell into four different groups. These same isolates were found to belong to VCGs by crossing stains resistant to chlorates and unable to use nitrates as a sole nitrogen source (*nit* mutants). Using this method, all the isolates that could be grouped fell into four groups that were, however, different from the RAPDs groupings. It is worth noticing that using the above primer or the VCG method, 20 isolates of *F. oxysporum* f. sp. *dianthi*, the carnation wilt pathogen, fell into one group. It is deduced that *F. proliferatum* has a wide genetic diversity in Greece, something that has also been found worldwide.

Differentiation of *Pyricularia oryzae* isolates from ctenanthe and rice using molecular markers. E.J. Paplomatas[1] and A.C. Pappas[2]. *[1]Benaki Phytopathological Institute, Plant Pathology Department, 8, S. Delta Street, 145 61 Kifissia, Athens, Greece [2]University of Thessalia, School of Agriculture Crop, Plant and Animal Production, Laboratory of Plant Pathology, Pedion Areos, 383 34 Volos, Greece.*

It was found that a case of severe leaf spotting observed for the first time on young ctenanthe plants (*Ctenanthe oppenheimiana* and *C. setosa*) in a glasshouse in Magnissia county in May 1995, was due to infection by the fungus *Pyricularia oryzae*. Pathogenicity tests showed that, while isolates of the fungus originating from ctenanthe plants were highly pathogenic on various plant species within the family Marantaceae, isolates obtained from rice caused either hypersensitive or immune response symptoms in various members of this family. Moreover, zymograms of esterase and lactate dehydrogenase showed different profiles for isolates of the pathogen from the two different hosts. The aim of the present work was to study the phylogenetic relation of fungal isolates from ctenanthe or rice plants with the aid of molecular markers. Specifically, besides the above two enzyme systems, isozyme analysis of malate, isocitrate dehydrogenase and sorbitol as well as RAPD analysis with five different primers were performed. Data from the zymograms and the DNA profiles after the electrophoresis of the PCR products were used to generate dendrograms using the Phylip 3.5 software package. It was found that both molecular markers differentiated the fungal isolates from each host, placing them in two different groups. Moreover, isolate variation was evident within each group, while the dendrogram from the RAPD analysis showed higher variation than that from the isozymes.

Development of appressoria of *Colletotrichum acutatum* in connection with the non-staining cells of strawberry stolons. A. Pattas and T.R. Swinburne. *Olive Protection Fund, 241 00 Kalamata, Greece.*

The appressoria of the fungus *Colletotrichum acutatum*, incitant of the disease "black spot" of strawberry, practically infected only certain cells on the surface of the strawberry stolons, which appeared to have different staining qualities than normal epidermal cells. However, no statistically significant differences in the number of such cells could be found between stolons of three varieties and stolons of different age. The results contribute to the understanding of the infection process of the vegetative parts of strawberry by *C. acutatum* and suggest that appressoria penetration alone is not adequate for lesions to appear and therefore cannot explain differences in varietal resistance to black spot.

Identification of *Armillaria* species occurring in Greece on the basis of morphological characteristics of vegetative mycelium in culture. P. Tsopelas. *National Agricultural Research Foundation, Institute of Mediterranean Forest Ecosystems & Forest Products Technology, Terma Alkmanos, 115 28 Ilissia, Athens, Greece.*

Five *Armillaria* species have been recorded in Greece: *A. mellea, A. gallica, A. tabescens, A. ostoyae* and *A. cepistipes*. The identification of most *Armillaria* species is possible by the use of the morphological characteristics of the vegetative mycelium on malt extract agar (MEA) and carrot agar (CA). The species *A. gallica*, and *A. cepistipes* cannot be thus distinguished, but cultural characters can be used to distinguish these two species from other *Armillaria* species, since *A. gallica*, and *A. cepistipes* form cylindrical rhizomorphs which on CA grow on the surface of the medium. Moreover, these two fungi are the only ones that form aerial rhizomorphs when they are cultured on wood species. *A. cepistipes* occurs at high altitudes (>1400 m) in the moun-

tains of northern Greece, and this mostly enables it to be distinguished from *A. gallica*, which usually occurs in lower altitudes and is very common in most parts of Greece. *A. ostoyae* forms flattened rhizomorphs and an abundance of crustose mycelium on MEA, while on CA, rhizomorphs are absent or scarce and the colony has a circular margin and a smooth surface. The other species do not form a circular colony margin. *A. mellea* forms thick flattened rhizomorphs on both media. On CA, *A. tabescens* usually does not form crustose areas and its rhizomorphs are numerous, thin and curly.

Effects of biotic and abiotic factors on sugarbeet infection by *Cercospora beticola*. I. Vloutoglou and E. Birkou. *Benaki Phytopathological Institute, Plant Pathology Department, 8, S. Delta Street, 145 61 Kifissia, Athens, Greece.*

The effect of inoculum density and leaf wetness duration on sugarbeet infection by *Cercospora beticola* were studied under controlled environmental conditions. Two strains of the pathogen, one with low sensitivity to benomyl (ED_{50} 0.85 µg/ml) and one more sensitive (ED_{50} 0.03 µg/ml), and two sugarbeet cultivars, Ultramono (susceptible to *C. beticola*) and Vergina (tolerant) at the growth stage of six true leaves, were tested. Results showed that: a) the pathogenicity of the strain with low sensitivity to benomyl was higher than that of the sensitive strain, irrespective of cultivar susceptibility, inoculum density, or the duration of wetness; b) disease incidence and severity on both cultivars increased with increasing inoculum density. With inoculum densities lower than $1x10^4$ conidia/ml, there were significant differences (P=0.05) in disease severity between cultivars, whereas for higher inoculum densities (≥$1x10^4$ conidia/ml), the percentage of infection did not differ significantly between the cultivars tested (P=0.05). Moreover, there was a linear relationship between inoculum density and disease severity (R^2>0.85, depending on the pathogen strain and cv. combination); c) no symptoms appeared on plants when the leaf wetness duration was ≤6 h. However, increasing the wetness duration from 6 to 72 h, led to an increase in the disease severity from 10 to 98%, depending on the pathogenicity of the strain and the susceptibility of the cultivar.

Three species of the genus *Juniperus* as new hosts of the pathogen *Seiridium cardinale*, the causal agent of Cypress canker. S. Xenopoulos. *National Agricultural Research Foundation, Institute of Mediterranean Forest Ecosystems and Forest Products Technology, Terma Alkmanos, 115 28 Ilissia, Athens, Greece.*

In 1997, a serious disease causing the death of branches, tips or whole trees, due to *Seiridium cardinale*, was observed for the first time in the natural forest of *Juniperus foetidissima* and *Juniperus excelsa* in Psarades at Prespes lakes. This fungus is known to attack species of the genus *Cupressus*, causing serious losses in California, New Zealand, Africa and the Mediterranean countries. Recently, dead branches killed by the same fungus were also found on *J. oxycedrus* in the above area. The fungus was readily isolated from cankered bark tissue from the three above-mentioned *Juniperus* species. Acervula with the spores of the fungus were found on cankers of infected *J. foetidissima* and *J. excelsa* trees. These two species seemed to be more susceptible to the pathogen (higher disease severity) than *J. oxycedrus*, on which only dead branches were found. The symptoms of the disease on *Juniperus* species were similar to those found on cypress. The fungus attacks bark tissue causing typical cankers with resin exudation which is characteristic of the disease. This is the first record of *S. cardinale* on the above three *Juniperus* species worldwide.

Bacterial diseases

Use of essential oils as antibacterial agents against ice nucleation active bacteria. K.I. Karamanoli[1], O.I. Menkissoglu[2], and H.A. Constantinidou[1]. [1]*Aristotelian University of Thessaloniki, Department of Agriculture, Laboratory of Agricultural Chemistry, 540 06 Thessaloniki, Greece.* [2]*Aristotelian University of Thessaloniki, Department of Agriculture, Laboratory of Pesticides, 540 06 Thessaloniki, Greece.*

At relatively mild frost temperatures (-2 to -5°C), the most important ice nuclei that cause injury to frost-sensitive plants are ice nucleation active (INA) bacteria. Among plant species, the most highly distributed INA bacteria are strains of *Pseudomonas syringae* van Hall and *Erwinia herbicola* (Lohnis) Dye. Control of these bacteria is achieved rather satisfactorily by chemical and other frost-management methods. In this presentation the use of oregano and eucalyptus essential oils as antibacterial agents against *P. syringae* and *E. herbicola*, was evaluated on bean plants under greenhouse conditions. Results indicated that the essential oils were fairly effective in inhibiting growth of INA bacteria. The degree and duration of bacterial growth reduction depended on the chemical structure of the essential oil constituents and the experimental conditions.

The ring rot disease of potato caused by *Clavibacter michiganensis* subsp. *sepedonicus*. Present status in Crete. D.E. Goumas[1], A.K. Chatzaki[1], J. Troulakis[2], K. Louskas[3] and J. Giannoulis[3]. [1]*National Agricultural Research Foundation, Plant Protection Institute, P.O. Box 2228, 710 03 Heraklion, Crete, Greece.* [2]*Regional Center of Plant Protection and Quality Control of Heraklion, Crete.* [3] *Plant Protection Science, Ministry of Agriculture, Athens, Greece.*

The quarantine bacterium *Clavibacter michiganensis* subsp. *sepedonicus* was detected for the first time in 1997 on the Lasithi plateau (Crete). The bacterium was isolated from infected potato tubers (cv. Spunda and Kennebec) showing visual symptoms (discoloration and ring rot) and was identified using the method approved by the European Union. A survey undertaken in 1997 and 1998 confirmed the presence and the distribution of the bacterium throughout the Lasithi and Heraklion provinces. The bacterium was detected in three out where six samples of volunteer potato plants collected from a field of the first record occurred. Moreover, the bacterium was detected on four out of 30 potato tuber samples collected from the surrounding fields. This year, 99 out of 459 samples of ware potatoes (21%) tested on the La-

sithi plateau were infected. During 1998, *C. michiganensis* subsp. *sepedonicus* was recovered from 18 out of 155 samples collected from different potato fields in Heraklion province. Finally two samples of seed potatoes amongst 39 tested, one from France and one from Italy, were found to be infected and were rejected.

Preliminary evaluation of resistance of Pomaceous trees to the bacterium *Erwinia amylovora*. J. Tsiantos[1] and P. Psallidas[2]. [1]*National Agricultural Research Foundation, Plant Protection Institute, 380 01 Volos, Greece.* [2]*Benaki Phytopathological Institute, Plant Pathology Department, 8, S. Delta Street, 145 61 Kifissia, Athens, Greece.*

The resistance of different pomoidae cultivars of Greek, Italian, Spanish and French origin to *Erwinia amylovora* was preliminarily evaluated under Mediterranean climatic conditions in 1997-98. The trees were planted in an experimental field at Velestino (Magnesia) in 1993-94. Four cultivars of loquat trees (*Eriobotrya japonica*), 13 of apple trees and 37 of pear trees were evaluated. With natural infection, the only cultivars that showed high susceptibility were those of cider apple (mostly flower infections) and the pear cv. Santa Maria (flowers and shoots infected). With artificial inoculation, 20 growing shoots, 10-20 cm long, located in 10 trees, were employed. The inoculum consisted of a water suspension of 10^8 cfu/ml of five strains of Greek origin. The inoculation was performed at the tips of shoots by a hypodermic syringe and the severity of the disease was evaluated 40 days later by calculating the mean percentage of shoot infection. Depending on the mean degree of infection, the assessed cultivars were grouped in six classes ranging from very low shoot infection (<10%) to very high (>60%). The susceptibility of the apple and pear cvs varied in all six classes. All the loquat cultivars were highly susceptible. The results showed significant variation and the experiments will be continued.

Detection of *Bacillus* biocontrol isolates in soil. A.S. Venieraki[1,2], I. Tsitsigiannis[1], S.E. Tjamos[1], E.K. Tjamos[1] and P. Katinakis[2]. [1]*Agricultural University of Athens, Department of Plant Pathology, 75 Iera Odos, 118 55 Athens, Greece.* [2]*Agricultural University of Athens, Department of Agricultural Biotechnology, 75 Iera Odos, 118 55 Athens, Greece.*

The development of biological methods for controlling plant pathogens that cause vascular mycosis is based on the evaluation and use of antagonistic bacteria from the rhizosphere. The work presented here concerns the ecology of two endorhizocal *Bacillus* strains (5-127, K-165) which antagonize *in vitro* and *in planta* the fungus *Verticillium dahliae*. Besides the classical microbiological and phytopathological techniques for the identification and characterization of bacteria in the soil and *in planta*, the PCR technique has been shown to be faster and more accurate for the identification and characterization of these bacteria. In order to identify the aforementioned strains in the soil and *in planta*, we cloned and sequenced the ribosomal intergenic spacer region between the 23S and 16S RNA. The sequence alignment between the two strains and among other *Bacillus* strains yielded non-conserved and unique areas. Thus specific primers for these isolates can be designed and used in PCR experiments. Moreover, we developed a method for extracting total genomic DNA from soil infected with a known concentration of bacterial cells. Preliminary results demonstrated that the PCR technique could amplify bacterial DNA up to 10^5-10^3 cells/g of soil.

Possible aetiology of watermelon rind necrosis. D.E. Goumas and A.K. Chatzaki. *National Agricultural Research Foundation, Plant Protection Institute, P.O. Box 2228, 710 03 Heraklion, Crete, Greece.*

Symptoms of watermelon rind necrosis were observed in watermelon fruits at different cultivated areas on the island of Crete. Symptoms included a brown, corky, dry and firm necrosis of the interior part of the rind. Infection began as small water-soaked lesions with a yellow-brown centre. Lesions coalesced to form large necrotic areas restricted to the rind. Necrosis extended into the flesh of watermelon only in a few severely affected and unripe fruits. These were misshapen and often showed brown spots on the epidermis, frequently at the same place as the rind lesions. Affected fruits were unmarketable when sliced. Comparative physiological, biochemical and pathological analyses conducted by classical bacteriological tests and by API 20E and API CHE commercial strips on 10 selected isolatesfrom 32 infected watermelon fruits were very similar to those obtained with the reference strains of *Pantoea ananatis* (LMG 2665, 2667 and 5342). *P. ananatis* is a member of the Enterobacteriaceae family. The isolates elicited a hypersensitive reaction in tobacco and in some cases infected the tobacco plant, producing symptoms similar to those of *P. ananatis* from pineapple fruits. In cross-inoculations on unripe detached fruits, the reproduced symptoms were similar to those that developed after natural infections on watermelon and pineapple fruits. This preliminary study demonstrated that the most frequently isolated bacterium from diseased watermelon fruits in Crete possessed features identical to strains of *P. ananatis* (*Erwinia ananas*), which is a member of the Enterobacteriaceae family and is possibly the causal agent of the disease in Crete.

Virus diseases

Water stress and spread of *Barley yellow dwarf virus* (BYDV). I.N. Smirnioudis[1,2], R. Harrington[1], N.I. Katis[2] and S.J. Clark[3]. [1]*IACR-Rothamsted, Department of Entomology and Nematology, Harpenden, Herts AL5 2JQ, UK.* [2]*Aristotelian University of Thessaloniki, Faculty of Agriculture, Plant Pathology Laboratory, 540 06 Thessaloniki, Greece.* [3]*IACR-Rothamsted, Department of Statistics, Harpenden, Herts AL5 2JQ, UK.*

The effect of drought-stress and temperature on the dispersal of the wingless aphid *Rhopalosiphum padi* (L.) and the pattern of spread of BYDV within wheat plants was quantified in controlled environment chambers. A combination of three different levels of drought-stress, high, moderate, and an unstressed control, and three different temperatures, 15±1, 10±1 and 5±1°C were investigated. With increased water stress and temperature there was an increase in the

average distance of aphid movement and in the number of plants being visited and infected. Drought-stress had no effect on aphid movement or on virus dispersal at 10 and 5°C. When plants were drought-stressed, the aphids' movement was greater at 15°C than at 10 or 5°C. There were no differences between treatments in the number of aphids recovered at the end of the experiment. It is concluded that drought-stress is of considerable importance in aphid dispersal and virus spread, but only at high temperatures.

A closterovirus (Family: Closteroviridae) isolated from tobacco crops in northern Greece (Macedonia). E.K. Chatzivassiliou[1], M. Tchomguia[2], D. Peters[2] and N.I. Katis[1]. [1]*Aristotelian University of Thessaloniki, Faculty of Agriculture, Plant Pathology Laboratory, 540 06 Thessaloniki, Greece.* [2]*Wageningen Agricultural University, Laboratory of Virology, 6709 PD, Wageningen, The Netherlands.*

In 1997, virus-like symptoms similar to those caused by *Potato Y potyvirus* (PVY) were observed on tobacco crops cv. Basmas in the area of Drama in Northern Greece. Diseased plants were showing severe stunting, necrosis of the petioles, vein clearing and necrosis of the main veins. Serological tests using polyclonal antibodies prepared against PVY, *Cucumber mosaic cucumovirus* (CMV), *Alfalfa mosaic alfamovirus* (AMV) and *Tomato spotted wilt tospovirus* (TSWV) gave negative results. The virus was mechanically transmitted from infected field tobacco plants to a number of test plants of the genus *Nicotiana*, such as *N. tabacum* cv. White Burley, Samsun NN, Xanthi, Christie, Basmas, *N. benthamiana, N. clevelandii, N. glutinosa, N. roselata* and *N. rustica*. These test plants generally reacted with chlorotic local lesions followed by systemic vein clearing, often vein necrosis, leaf curling and severe dwarfing. Similar symptoms were produced on *Petunia hybrida*. Other indicator plants such as, beet, faba beans, lettuce, tomato and watermelon were immune to the virus. Aphid transmission studies using *Myzus persicae* showed that the virus is transmitted in a semi-persistent manner. Electron microscope observations of leaf squash preparations from infected plants revealed the presence of long filamentous virus particles of 1600-1800 nm length, typical of the family Closteroviridae. The virus was purified in a process in which the extract was treated with Triton, followed by two cycles of high speed centrifugation on a 20-40-60% sucrose cushion and incubation at 4°C for some days between Triton treatment and centrifugation steps. On view of these results we consider this virus the first virus of the genus *Closterovirus* (family: Closteroviridae) that has been isolated from tobacco crops. Research studies on virus purification for the production of antibodies and its relationship to other members of the genus *Closterovirus* are in progress.

Clonal and sanitary selection of traditional grapevine varieties in Cyprus. N. Ioannou[1], N. Roumbos[2], A. Emmanuel[2] and A. Hadjinicolis[1]. [1]*Agricultural Research Institute, Nicosia, Cyprus.* [2]*Viticulture and Oenology Section, Department of Agriculture, Lemesos, Cyprus.*

A research project on grapevine viruses and production of healthy propagating material of grapevines was initiated in Cyprus in the 1980s. The first phase of the project centred on the identification of the main virus and virus-like diseases of grapevine, based on symptomatology, on the reaction of virus indicators, including certain grapevine species/varieties and herbaceous test plants, and on the use of laboratory diagnostic techniques, in particular the enzyme-linked immunosorbent assay (ELISA). Ten different diseases were identified, of which the most important were the Infectious Degeneration complex (Fanleaf, Yellow Mosaic, Vein Banding), the Leafroll complex, and Rugose Wood. Other, less important diseases identified were Fleck, Corky Bark, Vein Necrosis and Yellow Speckle. Leafroll was the most widespread disease with about 80% average incidence in introduced varieties and 45% in traditional varieties. For about 60 introduced varieties the problem of virus diseases was resolved by re-introducing of healthy propagating stocks from reliable foreign sources. For the local and other traditional varieties, a program of clonal selection, thermotherapy, and phytosanitary controls (symptomatology, bioindexing and ELISA) was initiated in 1987. So far about 30 healthy clones of 10 traditional varieties have been selected. Prebasic stocks of these clones are maintained in an insect-proof screenhouse to protect them against mealybugs, which in Cyprus are efficient vectors of grapevine leafroll-associated closterovirus. Basic and mother-plantations have also been established for the production of certified propagating material of grapevine on a commercial scale.

Viroids of cultivated and wild pear in Greece. P.E. Kyriakopoulou[1,2] and A. Hadidi[1]. [1]*Fruit Laboratory, ARS-USDA, Beltsville, MD 20705, USA.* [2]*Agricultural University of Athens, Laboratory of Phytopathology, 75 Iera Odos, 118 55 Athens, Greece.*

Three viroids have been found in cultivated (*Pyrus communis* cv. Kontoula and others) and wild (*Pyrus amygdaliformis*) pears in Greece: *Apple scar skin viroid* (ASSVd), *Pear blister canker viroid* (PBCVd) and *Peach latent mosaic viroid* (PLMVd). Hybridization experiments with total nucleic acids (TNA) extracted from trees with ASSVd and PBCVd symptomatology using c-RNA probes of ASSVd, PBCVd and PLMVd labelled with DIG-11, showed the presence of all these three viroids. RT-PCR, using ASSVd and PLMVd primers, amplified ds-DNAs of the expected sizes (ASSVd: 330 bp, PLMVd: 336-337 bp). Cloning of the PLMVd primer product gave a nucleotide sequence coinciding with that of PLMVd. The same sequence was also found in a pear specimen of cv. Passa Crassana from Bologna (L. Giunchendi). These data show that the host range of PLMVd does not only include peach, as was thought for many years, and three additional *Prunus* species (plum, cherry and apricot) as was shown recently, but also other species of the genus *Pyrus*. This indicates that the above host range of six species may not becomplete and that further research may give more natural hosts. For ASSVd, on the other hand, the above data show two new natural host species, the NE Mediterranean as a new area of origin besides NE Asia, and indicate wild pear as the second known spontaneous species to host viroids. The occurrence of all three viroids tested shows the existence of addition-

al viroids in *Pyrus*, while the high frequency of these viroids (ASSVd 12/17=70.5%, PLMVd 15/17=88%, PBCVd 16/17=95%) indicates their wide spread throughout Greece. A phylogenetic view of these data indicates the possible origin of ASSVd, PLMVd and PBCVd in the NE Mediterranean from wild pear, a wild, thorny, inaccessible shrub or a tree growing in the mountain slopes. Wild pear has traditionally been the rootstock for cultivated pear and other pome fruits in Greece and possibly other Mediterranean countries, and we believe that traditional pear germplasm in Greece has been widely infected by the three viroids by grafting and vegetative propagation. Lastly, the serious symptoms on fruits (ASSVd) and stems (PBCVd), which are probably due to these viroids but possibly to additional viroids or other pathogens also wide-spread in Greece in these two pear species and apple, underscores the importance of the sanitation of pome fruit propagation material.

Pea early browning virus: particle distribution in tissues and cells of root tips and leaves of *Nicotiana benthamiana*. E.C. Vellios, S.A. Macfarlane, I.M. Roberts, G.H. Duncan and D.J.F. Brown. *Scottish Crop Research Institute, Invergowrie, Dundee DD2 5DA, Scotland, UK.*

Tobraviruses (*Tobacco rattle virus*, TRV, *Pea early-browning virus*, PEBV and *Pepper ring-spot virus*, PRV) are acquired from and transmitted to plant roots during the feeding of Trichodorid nematodes. It was claimed that TRV was not present in the root epidermal cells 14 days after mechanical inoculation of *Nicotiana benthamiana* plants with the virus, but only in the stele. The absence of virus particles from the epidermal cells was considered the reason for the erratic acquisition of the virus by trichodorid nematodes. The mechanisms determining tobravirus-vector recognition, occurrence, distribution and spread of tobraviruses in plant roots are examined. Preliminary results revealed that tobravirus particles are transported from mechanically inoculated leaves via the phloem to the root-tip meristem and columella, which form the root cap. Sloughed cells were observed to contain tobravirus particles, and epidermal cells behind the meristem region were also infected by PEBV. It was found that there is a difference in the occurrence of the wild type of PEBV and a mutant, which does not produce the two non-structural proteins involved in the transmission of the virus. In particular, the number of particles of the mutant that reached the root meristem was much lower than that of the wild type. It was also found that the particles of PEBV were aggregated in the meristematic and epidermal cells either in crystalline aggregates or in an arrangement lying lengthwise to one another forming long chains. Finally, blister-like attachments observed on the surface of epidermal cells were filled with PEBV particles.

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

The onion thrip, *Thrips tabaci* Lind., is the main vector of *Tomato spotted wilt tospovirus* (TSWV) in tobacco grown in Eastern Europe, where this virus causes significant crop losses. However, its ability to transmit the virus has been questioned, due to conflicting laboratory findings. To elucidate the capacity of this insect to transmit TSWV, a study was undertaken to test the transmission efficiency of different *T. tabaci* populations — an arrhenotokous population derived from leek and tobacco, and thelytokous population derived from leek — using the petunia leaf disc method. The results showed that none of the thelytokous leek populations transmitted TSWV or infested tobacco plants. The arrhenotokous leek populations proved to be poor vectors (up to 7.9%) and showed a low preference for tobacco. Males of these populations were considerably more efficient at transmitting TSWV than females. Natural arrhenotokous populations, collected from heavily infected tobacco fields, appeared to be very efficient transmitters (12.0-48.5%). Males and females from these populations, when grown on tobacco populations exhibited high transmission rates (up to 66.7%). However, when tobacco populations were continuously grown on leek, the transmission efficiency and the ability to feed on tobacco were affected. The results of this study showed that some populations of *T. tabaci* are very efficient vectors of TSWV, but they also suggest that adaptation to a host affects the vectoring ability.

Occurrence of virus yellows of sugarbeet (*Beet yellows closterovirus*, BYV) in Central Macedonia (Greece) and its correlation with attacks by aphids. P.M. Ioannides. *Hellenic Sugar Industry S.A., Plant Protection and Research Service, 590 32 Platy Imathia, Greece.*

During 1996-98, typical symptoms of virus yellows occured in sugarbeet crops in Central Macedonia. The incidence of the disease differed depending on the growing region. At the same time, serious attacks by the aphids *Myzus persicae* and *Aphis fabae* were observed, mainly between the end of May-beginning of June. The serious and extensive attacks by the aphids observed in 1996, 1997 and 1998 were accompanied by a widespread appearance of virus yellows in the crop. In the present study, the population of aphids was estimated from captures by yellow water traps (Moericke type) and weekly plant samplings. In Greece so far, *Beet yellows virus* (BYV) is the only virus causing virus yellows in sugarbeet compared with the northern European countries where *Beet mild yellowing luteovirus* (BMYV) is the most abundant. In addition to field observations and plant samplings, the virus yellows was identified in the lab by the ELISA technique using the Adgen diagnostic systems protocol. The results from the valley of Imathia showed that 40% of the collected plant samples were infected. From the infected plants, 14% showed mild symptoms, whereas 26% of them exhibited severe ones. For each sample, two tests were performed with the ELISA technique. The results in the lab were in agreement with those of the disease incidence in the field.

Comparative study of ELISA methods for the detection of *Cucumber mosaic virus*. I.E. Tzanetakis[1], A. Sklavounos[1], K. Makkouk[2] and P.E. Kyriakopoulou[1]. [1]*Agricultural University of Athens, Department of Phytopathology, Iera Odos 75, 118 55 Athens, Greece.* [2]*ICARDA, Aleppo, Syria.*

In the present work, the sensitivity of four techniques of the ELISA method were compared using dilution of antiserum or of IgG as factors of comparison. Of the four techniques, DAC-ELISA, dot-blot ELISA and tissue-blot ELISA were compared with the classical DAS-ELISA. Pure cultures on tobacco of two highly virulent isolates of *Cucumber mosaic virus* (CMV), from tomatoes of Eleia and Euboea counties respectively, were used. Except for tissue blot, for which sap dilution has no meaning, in all other methods a constant sap dilution 1:20 was used; sensitivity was measured with serial dilutions of antibodies. Alkaline phosphatase (Alp) was used as the enzyme of the conjugate with all ELISA techniques, but with DAC-ELISA penicillinase (Pen) was also used. The results showed that after 1 hour incubation with the substrate, dot blot and tissue blot ELISA had a sensitivity of 0.5 ng antibodies/ml, 8 times as much as DAC with Alp (4 ng/ml), 40 times as much as DAS with Alp (20 ng/ml) and 200 times as much as DAC with Pen (100 ng/ml). Therefore the great diagnostic value of dot-blot ELISA and tissue-blot ELISA was confirmed, using antibody dilutions in the present work. These techniques, besides their higher sensitivity, have the advantage of speed (results in 1 day compared to the 2 days for DAS-ELISA), economy (multiple use of the same antibody solution and other reagents) and convenience due to the possibility of storing the blotted membranes for long periods or mailing them for later laboratory processing. Between these two methods, tissue-blot ELISA has the additional advantage that blotting in the field is possible.

Identification of the serological and molecular characteristics of *Cucumber mosaic cucumovirus* isolates. C. Varveri and K. Boutsika. *Benaki Phytopathological Institute, Plant Pathology Department, 8, S. Delta Street, 145 61 Kifissia, Athens, Greece.*

Cucumber mosaic cucumovirus (CMV) is responsible for serious epidemics with various symptoms, especially on tomato. To elucidate the symptomatology, 40 local CMV isolates of different origin and symptoms in the field were analysed for subgroup identification and satellite RNA presence. All isolates reacted positively with monoclonal antibodies towards the DTL serotype and were thus classified to subgroup I of CMV strains. Most isolates also gave the characteristic *Msp* 1 and *Eco* R1 restriction profiles of this subgroup for amplicons from part of the coat protein gene. Five isolates, however, showed supplementary restriction sites and the profiles obtained could be confused with those of subgroup II. This showed that restriction enzyme analysis of amplified products used for strain identification needs to be treated with caution, especially in the case of viruses like CMV showing genomic heterogeneity. The immunocapture-polymerase chain reaction method was also developed for satellite RNA detection and results coincided with those of molecular hybridization with a dig-DNA probe. Seventy-seven per cent of the isolates were found to carry satellite RNAs, which are known to differentiate between virus symptoms.

Differentiation of two Closteroviruses causing yellowing symptoms on cucurbits by RT-PCR and elucidation of the termini of CYSDV HSP70 homologue gene by a modified RACE-PCR protocol. I.C. Livieratos[1], N.I. Katis[2] and R.H.A. Coutts[1]. [1]*Imperial College of Science, Technology and Medicine, Biology Department, Prince Consort Road, London SW7 2BB, UK.* [2]*Aristotelian University of Thessaloniki, Faculty of Agriculture, Plant Pathology Laboratory, 540 06 Thessaloniki, Greece.*

Beet pseudo-yellows closterovirus (BPYV) and *Cucurbit yellow stunting disorder* (CYSDV) are two whitefly-transmitted closteroviruses that elicit indistinguishable yellowing symptoms in a number of common cucurbit species. In order to differentiate the two viruses, oligonucleotide primers derived from the HSP70 homologue genes of both BPYV and CYSDV were used in RT-PCR assays. RT-PCR-amplified products for each virus were subsequently cloned and used as probes in northern analysis of dsRNA species extracted from infected cucumber and melon leaves collected from whitefly-infested greenhouses in Greece and Spain respectively. In order to obtain the 5' and 3' termini of the CYSDV HSP70 homologue gene, a modified RACE-PCR protocol was applied. Viral specific dsRNA was denatured using methyl mercury hydroxide and specific oligonucleotide primers initiated the production of cDNA that served as template in PCR assays with the combination of the same primer and a random hexamer. Amplified products with molecular size 2.3 and 12 kbp included the 5' and 3' termini of the CYSDV homologue gene providing the complete nucleotide sequence of the gene. Comparison of the amino-acid sequence of the complete CYSDV HSP70 homologue with equivalent proteins of other closteroviruses showed highest levels of conservation amongst the whitefly-transmitted BPYV, tomato infectious chlorosis virus (TICV) and lettuce infectious yellows virus (LIYV), tentative members of the newly proposed genus of Crinivirus.

Use of the polymerase chain reaction method for the reliable detection of garlic viruses. C.I. Dovas[1], E. Hatziloukas[1], R. Salomon[2], Y. Shiboleth[2] and N.I. Katis[1]. [1]*Aristotelian University of Thessaloniki, Faculty of Agriculture, Plant Pathology Laboratory, 540 06 Thessaloniki, Greece.* [2]*ARO Volcani Center, Department of Virology, P.O. Box 6, Bet Dagan 50-250, Israel.*

Onion yellow dwarf potyvirus (OYDV) and *Leek yellow stripe potyvirus* (LYSV) are economically the most important viral pathogens in garlic crops. Since meristem-tip culture is the only way to obtain virus-free plants, and virus titer is usually very low, a sensitive detection method, such as PCR, is essential, all the more so as, the indexing of garlic bulbs with ELISA is not reliable. We therefore valuated optimal conditions for RT-PCR, IC-PCR and the use of other sample treatments and compared the specificity and sensitivity of these methods to each other and to DAS-ELISA. Se-

quences of two PCR primer pairs were derived from highly conserved regions in the coat protein ORF of OYDV and LYSV isolates from different countries, in order to amplify a 283 bp fragment of OYDV and a 304 bp fragment of LYSV. Garlic samples, all infected with OYDV and LYSV, were tested by RT-PCR and IC-PCR using the above primers. All three procedures (IC-PCR, RT-PCR with crude extracts, and RT-PCR with total RNA) gave very similar sensitivity thresholds, approximately 1000 times lower, than that obtained with ELISA. A single tube IC-RT-PCR was developed using crude garlic leaf extract and found to be 100 times more sensitive than ELISA, and more convenient for the detection of garlic potyviruses. Tests on garlic bulbs indicated that RT-PCR using crude extracts was 100 times as sensitive as ELISA. Furthermore, PCR primers designed at the ARO Volcani Center for *Garlic common latent virus* (GCLV) and the Allexviruses were also effective in detecting of Greek virus isolates.

The role of non-structural genes of TRV RNA1 in transmission of virus by vector nematodes. N. Vassilakos, D.J.F. Brown and S. 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*. Vector transmissibility is highly specific and is determined by components of the smaller RNA2 segment of the tobravirus genome. Specifically for PEBV, the virus coat protein and at least two other viral proteins are involved in the transmission process, while for the PpK20 isolate of TRV, which has the nematode *P. pachydermus* as its vector, probably only the coat protein and one other protein are necessary for transmission. For further investigation of tobravirus transmission, the RNA2 of a new isolate of TRV (PaY4) was studied. This isolate has as vectors the nematodes *P. anemones* and *P. pachydermus*. TRV PaY4 RNA2 was found to be 3,926 nucleotides in length and contains three open reading frames encoding the coat protein and two other proteins, 2b and 2c. Mutations were introduced in the full-length cDNA clone of PaY4 in the 2b and 2c genes. Transcripts were synthesized from the mutant clones, mixed with wild-type TRV RNA1 and inoculated to *N. benthamiana* plants. The recovered stable mutant viruses were tested for transmission by the two nematode vectors of isolate PaY4. The results from these experiments showed that mutation of the 2b gene abolished transmission of the virus whereas the mutations of the 2c gene had no effect on the efficiency of transmission of the virus by either vector. Current experiments aim to identify how TRV isolates PaY4 and PpK20 maintain a specific interaction with the shared vector nematode *P. pachydermus*, while only the PaY4 isolate can interact with *P. anemones*.

Occurrence of the disease "Watermelon chlorotic stunt" in Greece. F. Bem and E.J. Paplomatas. *Benaki Phytopathological Institute, Plant Pathology Department, 8, S. Delta Street, 145 61 Kifissia, Athens, Greece.*

In June 1996, symptoms of a new disease in watermelon cultivations were observed in the Elia and Trikala prefectures. The infection rate ranged from 2 to 5%. Infected watermelon plants showed severe stunting, small leaves and little fruit or fruitlessness. The leaves showed impressive mottling and curling, whilst the small fruits had chlorotic lesions on their surface. In the laboratory, the disease could be transmitted to healthy watermelon plants by grafting but not by infected sap or aphids. The above symptoms as well as the means of disease transmission are typical of "watermelon chlorotic stunt" first reported in 1985 from Yemen, which is believed to be caused by the very little studied Geminivirus, *Watermelon chlorotic stunt virus* (WCSV). It has been reported that this virus is transmitted only by grafting and through the whitefly *Bemisia tabaci*. Application of PCR, using degenerate universal primers (kindly provided by R.L. Gilbertson) that amplify a segment of the A component of Geminiviruses, failed to detect this particular virus. This was probably because the primers used had been designed following DNA sequences of Geminiviruses of a "New World" origin. Our research will be continued by cloning the virus and preparing a specific probe. On account of its manner of transmission, this disease is considered to be a threat for watermelon cultivation in Greece.

Classification of Greek isolates of *Cucumber mosaic virus* from tomato and other plants. A.P. Sklavounos, G. Vidalakis and P.E. Kyriakopoulou. *Agricultural University of Athens, Department of Phytopathology, Iera Odos 75, 118 55 Athens, Greece.*

Cucumber mosaic virus (CMV) is a cosmopolitan virus, and a very common and serious pathogen in tomato, cucurbits, and other crops. It exhibits very high biodiversity. A number of CMV strains have been internationally characterized. Various attempts have been made to classify the strains and isolates of CMV, and two groups have been identified, DTL or WT or 1, and ToRS or S or 2, based on their serological affinities. This classification work is useful, especially when the biological control of the virus and its satellite RNA is to be applied, using mild virus and satellite strains or transgenic plants. The frequency and very high severity of CMV infections in Greece, especially on open-field, processing and table tomatoes, its emergence in new disease forms in the Mediterranean over the last three decades, and its known high tendency for genetic change prompted us to study its biodiversity and the methodology for its strain or isolate characterization. Thirty-five isolates of CMV from industrial tomato in Eleia county near Ancient Olympia, one from table tomato in Istiaia on Euboea island, and one from tobacco in Attica (field of the Agricultural University of Athens at Votanikos) were characterized biologically (in indicator plants), electrophoretically, for the mobility of their particles in the electrical field in agarose gel, and serologically for their specialization using DAS-ELISA. As standards, known strains of CMV, K, Q and S were used. The 35 isolates from Eleia County showed uniformity in their biological reactions in the indicators, whereas the other two were different from the 35 and from each other. In the electrical field, all 37 isolates coincided with K and Q, but they differed from S. Serologically, they showed a strong

tendency to fall into one of the two serological groups, DTL or WT or 1 and ToRS or S or 2, with a slight preference for the first. The semipurified preparations showed a lower tendency towards serological differentiation than the crude sap.

Ornamental plants as hosts of *Tomato spotted wilt tospovirus* (TSWV) and its thrips vectors in Greece. E.K. Chatzivassiliou[1], I.C. Livieratos[1], G. Jenser[2] and N.I. Katis[1]. [1]*Aristotelian University of Thessaloniki, Faculty of Agriculture, Plant Pathology Laboratory, 54006 Thessaloniki, Greece.* [2]*Plant Protection Institute, Hungarian Academy of Sciences, 1525 Budapest II, Herman Otto' ut 15 Pf 102, Hungary.*

This survey was carried out in order to record the ornamental plants that are hosts of *Tomato spotted wilt tospovirus* (TSWV) in Greece. Samples were collected from plants showing symptoms typical of tospovirus infection. Polyclonal antibodies were prepared against the nucleocapsid (N) protein of a Greek isolate of TSWV isolated from *Gerbera* sp. (GR-34). The samples were tested by enzyme-linked immunosorbent assay (ELISA) using polyclonal antibodies against the N protein of TSWV (GR-34 and BR-01) and of impatiens necrotic spot tospovirus (INSV) (NL-07). Subsequently positive samples were mechanically inoculated on *Petunia hybrida, Nicotiana rustica* and *N. benthamiana*. TSWV was present in the following 39 species: *Anemone* sp., *Antirrhinum majous, Aralia japonica, Aster* sp., *Astromeria* sp., *Begonia* sp., *Beloporone guttata, Calendula officinallis, Callistephus chinensis, Celosia cristata, Coleus* sp., *Cineraria nana hybrida, Chrysanthemum* sp., *Dahlia hybrida, Dianthus sinensis, Diephenbachia* sp., *Dimorphotheca sinuata, Fuchsia* sp., *Gazania* sp., *Geranium* sp., *Gerbera jamesonii, Impatiens* sp., *Iris* sp., *Mathiola incana, Ocimum basilicum, Pelargonium* sp., *Portulaca grandiflora, Petunia hybrida, Ranunculus* sp., *Saintpaulia ionantha, Salvia splendens, Santendescia* sp., *Solanum capsicastrum, Stephanotis floribunta, Tagetes erecta, Tropaeulum majus, Viola tricolor, Vinca rosea* and *Zinia elegans*. None of the samples reacted with INSV. Thrips collected from infected plants were in most cases identified as *Frankliniella occidentalis* except on *Chrysanthemum* sp. and *Dianthus* sp. where *Thrips tabaci* was also found.

Sampling conditions for reliable detection of two garlic Potyviruses using ELISA. C.I. Dovas[1], A.P. Mamolos[2], N. Dalli 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 potyvirus (OYDV) and *Leek yellow stripe potyvirus* (LYSV) are the most important viral pathogens of garlic worldwide. Enzyme-linked immunosorbent assay (ELISA) is the main diagnostic tool used for large-scale routine testing. Reliable viral diagnosis is negatively affected by factors such as the position of infected leaf tissue on the plant, the sampling period and storage conditions of collected samples until testing. However, this information is not known for these viruses. For this reason, optimal conditions of sampling (position of the infected leaf tissue, period of sampling) were investigated during the growing period on samples of leaves from ten garlic plants grown under field conditions. The effect of storage conditions at different temperatures (5 and -30°C) was also studied. Results indicated uneven distribution of both viruses in the garlic plant. Tips of the two youngest apical leaves showed the highest levels of both viruses, while virus content in older leaves levelled off. Leaf tips had higher virus concentrations than the middle and basal sections of the youngest leaves, but in older (mature) leaves the situation was reversed or there were no differences. Virus concentrations in the plants were higher until March but reliable detection was possible until the beginning of May. Leaf samples were stored at 5 and -30°C in order to evaluate the maximum storage period for reliable viral detection. The results showed that detection of both viruses in each treatment was reliable until the 12th day at 5°C and until the 4th month at -30°C. Finally, our results indicate that the part of plant tissue taken is the most important factor for reliable detection of both viruses.

Identification of viruses infecting garlic (*Allium sativum*) and wild *Allium* species in Greece. C.I Dovas[1], S. Mokiou[1], P. Bounda[1], S. Samaras[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, Agricultural Research Center of Makedonia and Thraki, Greek Gene Bank, 570 01 Thermi-Thessaloniki, Greece.*

A survey for viruses infecting garlic crops and wild *Allium* species was carried out. Virus identification was based on ELISA, the polymerase chain reaction (PCR) and decoration. Samples from 1300 garlic plants were collected from Evoia, Arkadia, Larissa, Thessaloniki and Evros. Moreover, 180 wild *Allium* plants, belonging to 21 different species were collected from all over Greece. The following eight viruses were identified: a) *Onion yellow dwarf potyvirus* (OYDV), b) *Leek yellow stripe potyvirus* (LYSV), c) *Garlic common latent carlavirus* (GCLV), d) *Shallot latent carlavirus* (SLV), e) *Garlic allexvirus-C* (GV-C), f) *Garlic miteborne mosaic allexvirus* (GMbmV), g) MbFV (allexvirus), and h) Mb 1146 (allexvirus). Out of the total number of tested samples, LYSV incidence was 98.7% and OYDV incidence 87.2%, and these were the most common garlic viruses in all regions. Allexvirus occurrence ranged from 62% to 71.4%. GCLV was found only in Arkadia and Evoia with 98 and 18% of samples respectively, while in Larissa it was detected in only one field (24%). SLV was found in Theva and Evros. In wild *Allium* plants only *A. ampeloprasum* ssp. *ampeloprasum* and *A. flavum* were LYSV infected and *A. sphaerocephalon* was infected by an unknown potyvirus.

Eradication of potyviruses and allexviruses from garlic plants by meristem-tip culture. C. Dovas[1], N. Leventakis[2], E. Hatziloukas[1] and N.I. Katis[1]. [1]*Aristotelian University of Thessaloniki, Faculty of Agriculture, Plant Pa-*

thology Laboratory, 540 06 Thessaloniki, Greece. [2]*Vitro Hellas, 593 00 Nisseli Imathias, Greece.*

Garlic is seriously infected by viral complexes including Poty- Carla- and Mite-borne viruses and this is due to its vegetative mode of propagation. The only effective way of combating viral diseases of garlic is to use virus-free propagative material produced by meristem-tip culture. Meristems were cultured on a medium containing: MS, 0.5 µM BAP, 0.1 ÌM FAP, 30 g/l sucrose, and 8 g/l agar (pH 5.8). The size range of the excised meristem tips was between 0.6 and 1.0 mm. Meristems were incubated for 30 days on the above medium, before grown plantlets were transferred to a hormone-free MS medium, containing 120 g/l sucrose, 5 g/l activated charcoal, and 4.2 g/l agar (pH 5.8). All transferred plantlets developed bulbs within 20 days of incubation. Virus identification was performed by enzyme-linked immunosorbent (ELISA) using polyclonal antibodies and polymerase chain reaction (PCR). Preliminary results showed that the efficiency of virus elimination differs between potyviruses and allexviruses. *Onion yellow dwarf potyvirus* (OYDV) was eliminated in 12 out of 14 plants and *Leek yellow stripe potyvirus* (LYSV) in all six out of six plants. On the contrary, the eradication of allexviruses using this method was less efficient, and eliminated the viruses in only three out of 10 cultured plants.

Nepovirus infecting onion crops in Korinthia (Greece). C.I. Dovas[1], E.K. Chatzivassiliou[1], J. Cohen[2], A. Kritzman[2] A. Gera[2], S. Konstandinidou-Doltsinis[3] and N.I. Katis[1]. [1]*Aristotelian University of Thessaloniki, Faculty of Agriculture, Plant Pathology Laboratory, 540 06 Thessaloniki, Greece.* [2] *ARO Volcani Center, Department of Virology, P.O. Box 6, Bet Dagan, 50-250, Israel.* [3] *Crop Protection Institute, National of Agricultural Research 260 04 Patras, Greece.*

In 1997, unusual virus-like symptoms such as dwarfing, striping and leaf yellowing appeared on onion crops in the Korinthia area (Northern Pelloponese). Samples from the affected crops did not react in ELISA to antisera prepared against *Onion yellow dwarf potyvirus* (OYDV), *Shallot latent carlavirus* (SLV), *Garlic common latent carlavirus* (GCLV), Allexviruses (four antisera), *Iris yellow spot tospovirus* (IYSV), *Tomato spotted wilt tospovirus* (TSWV), *Tomato black ring virus* (TBRV) or *Arabis mosaic virus* (ArMV). In indicator plants such as *Chenopodium amaranticolor* and *C. quinoa,* the virus caused chlorotic local lesions, epinasty of the inoculated leaves followed by systemic tip necrosis. In *Cucumis sativus* it induced systemic mosaic with leaf enations. In *Nicotiana* sp. it caused mosaic and ringspots, while newly developed leaves did not show any symptoms. Aphid transmission by *Myzus persicae* in the non-persistent manner was unsuccessful. Electron microscopy examination on thin sections of tissues of infected *N. benthamiana* leaves showed tubular structures containing rows of 20-30 isometric virus particles. Decoration tests using antisera of *Tomato ringspot virus (*ToRSV), *Tobacco ringspot virus* (TRSV), ArMV, TBRV, *Raspberry ringspot virus* (RRSV), *Tobacco necrosis virus* (TNV), and *Strawberry latent virus* (SLRSV) were also unsuccessful. In SDS-PAGE of partially purified virus, one major protein component with MW of 56,000 daltons was observed. These results strongly indicate that the causal agent of the above disease is a new nepovirus.

Characterization of *Zucchini yellow fleck virus* (ZYFV) in Greece. D. Karagiannidou[1], G. Karagiannidou[1], P. Loukos[1], C.I. Dovas[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 Virology Laboratory, 711 10 Heraklion, Crete, Greece.*

In this paper we studied the various characteristics of five isolates of *Zucchini yellow fleck potyvirus* (ZYFV), such as reaction of test plants, host range, aphid transmission and serological relations. We also investigated ZYFV incidence on various zucchini crops and arable weeds. The results showed that all isolates caused similar symptoms on *Cucumis melo, C. sativus, Cucurbita pepo* and *Citrullus lanatus,* whereas the species *Chenopodium quinoa, C. amaranticolor, Vigna sinensis, Gomprena globosa, Nicotiana benthamiana, Lyffa acutangula* and *Phaseolus vulgaris* were immune. Transmission efficiency of all isolates by *Myzus persicae* in the non-persistent manner was similar. No serological differentiation was observed between the five isolates in ELISA, using polyclonal antibodies prepared against a French isolate. In *C. pepo* virus concentration increased until the 28th day post-inoculation but later decreased. ZYFV incidence in zucchini crops ranged from 0 (Xanthi) to 85% (Crete). Testing of more than 400 samples of arable weeds such as *Convolvulus arvensis, Chenopodium album, Amaranthus retroflexus, Portulaca oleracea* and *Ecballium elaterium* revealed that only the last was a host of the virus. Finally, the possible application of PCR for virus detection was investigated and a single tube IC-RT-PCR was developed using anti-poty (monoclonal) and anti-ZYFV (polyclonal) antibodies. This method was found to be more reliable and less laborious than using RNA or crude plant extract. Sequences of the two PCR primers were derived from highly conserved regions in the coat protein ORF of potyviruses, in order to amplify a 409 bp fragment.

Tomato vein purpling, diagnostic symptom for early mass assessment of CMV spread in the field. P.E. Kyriakopoulou. *Agricultural University of Athens, Department of Phytopathology, Iera Odos 75, 118 55 Athens, Greece*

For more than 15 years, *Cucumber mosaic virus* (CMV) has been the most serious plant pathogen of tomato in Greece and other Mediterranean countries, as a very epidemic and serious pathogen, causing tomato shrinkage, tomato necrosis and tomato fruit necrosis. In an epidemiological study of CMV, the estimation of percentages of infected plants at a given moment and of the trend of infection rate during the growing season in a given field and area is neccessary. These data, however, require extended field sampling and laboratory examinations, which are usually very difficult to realize when only limited laboratory facilities are available. Tomato vein purpling, an early and characteristic CMV symptom on tomato, attracted our attention and interest during

the last years, as a possible practical *in situ* diagnostic method permitting the inexpensive, quick and large scale estimation of CMV infections of tomato at the initial stages of infection and especially, in areas like Eleia county, where CMV has been the single most important virus pathogen of tomato for more than 10 years. It allows the examination of about 1,000 tomato plants per hour with no need for any consumables or other labour or expenses. It is very sensitive, especially during the initial stages of infection, while the infection is still localized or unevenly distributed within the plant beyond the inoculation sites. This method was tested for four consecutive years (1995-98, mainly in 1998) for fields of Eleia county. In randomly selected fields of early grown processing tomato, 3-4 randomly selected standard rows/field were examined for vein purpling symptoms and assessed for infection at about 15-day intervals. In comparative testing with DAS ELISA of 100 randomly selected leaves collected weekly in a few of these experimental fields, throughout the 4-years of the study, the infection rate trends were sigmoid and comparative for the two methods, the self-indexing method under discussion giving smoother curves. It was found that this method, exclusive to CMV infection of tomato, can safely and practically be applied in areas where CMV is epidemic. However, it requires experienced observers, and, in early transplanted crops, it can be applied after the early spring cold is over, since low temperatures on young transplanted tomatoes cause general vein purpling of the leaves masking the possible CMV vein purpling.

Two viral diseases of dimorphotheca (*Dimorphotheca* sp.) caused by *Lettuce mosaic potyvirus* (LMV) and *Tomato spotted wilt tospovirus* (TSWV). I.N. Manoussopoulos[1], E.K. Chatzivassiliou[2], I.N. Smyrnioudis[2,3] and N.I. Katis[2]. [1]*University of Thessalia, Faculty of Crop and Animal Huspandry, 333 34 Pedion Areos, Volos, Greece.* [2]*Aristotelian University of Thessaloniki, Faculty of Agriculture, Plant Pathology Laboratory, 540 06 Thessaloniki, Greece.* [3]*IACR-Rothamsted, Department of Entomology and Nematology, Harpenden, Herts AL5 2JQ, UK.*

In a greenhouse cultivation of dimorphotheca (*Dimorphotheca* sp.) near Heraklion (Crete), a large number of plants (25-30%) showed typical virus-like symptoms. These consisted of systemic chlorotic spots appearing mainly on the older leaves, and mild stunting. Mechanical inoculations with sap from symptomatic plants onto *Chenopodium amaranticolor* and *C. quinoa* caused chlorotic local lesions followed by systemic infection, whereas sap inoculation of healthy dimorphotheca plants caused symptoms similar to those previously observed in the field crop. Electron microscopy of leaf squash preparations revealed flexible filamentous particles about 720-750 nm in length, which were decorated with specific *Lettuce mosaic potyvirus* (LMV) antiserum. The virus was transmitted in a non-persistent manner by the aphid *Myzus persicae*. In Pieria (Macedonia), similar symptoms (mainly consisting of chlorotic rings) were observed on a large number of dimorphotheca plants. Following artificial inoculations onto indicator plants, *Petunia hybrida* reacted with necrotic local lesions, *Nicotiana benthamiana* developed systemic necrosis and *N. tabacum* cv. Samsun showed mosaic. Electron microscopy of leaf squash preparations from either naturally or artificially inoculated plants, revealed quasi-spherical particles about 80-110 nm in diameter. In ELISA tests, sap from diseased plants reacted with polyclonal antibodies against the nucleocapsid N protein of tomato *Spotted wilt tospovirus* (TSWV). On diseased dimorphotheca plants, colonies of the thrip vector *Frankliniella occidentalis* were identified.

Protection of squash plants from aphid-borne viruses using row covers. C. Varveri and I. Yannakopoulos. *Benaki Phytopathological Institute, Plant Pathology Department, 8, S. Delta Street, 145 61 Kifissia, Athens, Greece.*

The effectiveness of the use of row covers for the protection of squash plants from aphid-borne viruses was evaluated on July 2nd 1997 in an experimental plot established in Marathonas Attiki. The plot comprised three repetitions of blocks with 20 plants covered (Lutrasil Thermoselect) and 20 plants not covered. On August 19th the cover was removed for practical reasons. During the experiment the yield of symptomless fruit was recorded. Virus spread was determined by testing plants with ELISA at transplantation and thereafter at monthly intervals for *Cucumber mosaic virus* (CMV), *Zucchini yellow mosaic potyvirus* (ZYMV), *Watermelon mosaic potyvirus* 2 (WMV 2) and *Zucchini yellow fleck potyvirus* (ZYFV). The protection of plants during the time they were covered was adequate. Uncovered plants exhibited high infection rates. especially with WMV 2, revealing occurrence of high inoculum pressure conditions. These also resulted in immediate infection of the initially covered plants in 11 days after cover removal (WMV 2:45-100%, ZYMV: 47-85%). Finally, during this experiment no economical benefit was obtained, but it was shown that first row covers have to be kept as long as possible and that the growth conditions of plants under cover must be studied for further optimization.

Non-parasitic diseases

Phytotoxic ozone concentrations in the fir forest of the Parnis National Park. D. Velissariou and L. Skretis. *Benaki Phytopathological Institute, Plant Pathology Department, 8, S. Delta Street, 145 61 Kifissia, Athens, Greece.*

It is well known that the city of Athens is a permanent source of photochemical air pollutants. Amongst them, ozone is the most phytotoxic, affecting mainly semi-rural and rural ecosystems. Nowadays, tropospheric ozone is considered to be one of the main causes of forest decline, reducing the defense mechanisms of plants against diseases and other abiotic stresses. The Parnis fir forest (*Abies cephalonica* Loud.), the most important natural ecosystem near Athens, is considered to be in decline. In 1997, the presence of ozone in the fir forest at the National Park of Parnis mountain was studied by two methods: by direct monitoring using an ozone monitor set up at the Forest Service Station of Aghia Triada from 31/5/97 to 30/9/97, and with five consecutive exposures of ozone indicator plants (tobacco Bel-W3) at eight sites throughout the Park, for the same period of time. The monitoring data significantly exceeded the critical levels

for ozone for European forests, as established by UN-ECE: 10,000 ppb x h of ozone over 40 ppb from April to September (AOT40). In fact, on Parnis and only for the period June–September, the AOT40 was calculated to be 20,334 ppb x h and the ozone hourly mean hardly fell below 40 ppb. The average damage of the indicator plants was significant at all eight sites, damage fluctuating between 40 and 70% of leaf surface, and being always higher than the damage at the control site at Kifissia. The most severe damage was recorded at northern side of the mountain the opposite to the city (sites Mola, Kiafa, Kandalidi, Platovouni), where during dry summers, climatic conditions are more favourable to the trees. In conclusion, the strong presence of tropospheric ozone on Parnis Mountain should be considered as a potential cause of fir forest deterioration.

Dust deposition on leaves of grapevines and olive trees around the construction works of the new Athens airport in the agricultural area of Spata. D. Velissariou[1], B. Kyritsi[1], L. Skretis [1] and D. Panoutsos[2]. [1]*Benaki Phytopathological Institute, Plant Pathology Department, 8, S. Delta Street, 145 61 Kifissia, Athens, Greece.* [2]*Agricultural University of Athens, 75 Iera Odos, 118 55 Athens, Greece.*

The effect of dust deposition on the leaves of agricultural crops, due to industrial or construction activities is a problem studied worldwide. The aim of this study, funded by the Athens International Airport S.A., was to monitor the deposition of dust from airport construction work on leaves of grapevines and olive trees in the surrounding area. For that purpose, nine sampling sites were established around the works and samples of grapevine and olive tree leaves were collected every 10 days from 18/9/97 to 31/6/98. Four of the sampling sites were close to the four edges of the works area (within 50-100 m), four were about 1 km away from the first sites in the same direction so as to have an indication of dust dispersion. The ninth sampling site was the control at a long distance (about 10 km) from the works. Dust deposition, expressed as grams per unit leaf area (g m^{-2}), was measured by a laboratory technique described in the international literature. The results for the test period showed that at all eight sites mean values were higher than at the control site but they did not reach the "critical levels", as described in the international literature, considering that there are no international standards for this kind of assessment. The highest deposition values were measured at two of the nearest sites, in the SW and the SE that were , located downstream of the prevailing winds in the area. Moreover, olive leaves were found to retain more dust than grapevine leaves, obviously due to their more hairy surface.

Study of the role of the UV-b radiation on symptom expression on tomato plants infected by *Cucumber mosaic virus* (CMV). F. Bem, D. Velissariou, J. Panagopoulos and L. Skretis. *Benaki Phytopathological Institute, Plant Pathology Department, 8, S. Delta Street, 145 61 Kifissia, Athens, Greece.*

Since the mid '80s, tomato cultivation in Greece has suffered severe damage from *Cucumber mosaic virus* (CMV). In many cases, it was observed that the disease was more severe on tomatoes grown in the field than on those in glasshouses or in shaded locations in the same field. It is also known that since the '70s there has been a serious global environmental change, the destruction of the stratospheric ozone layer, that results in a continuous increase in ultraviolet radiation, especially ultraviolet radiation of biological effect (UV-b). In Greece, recent data have shown a greater than 10% increase in UV-b, over the last five years. The direct effect of the increased UV-b on plants or on plant-pathogen relationships have been studied over the last decade. The aim of the present study was a preliminary experimental approach to examine the possible role of solar radiation quality on symptom expression of tomato plants infected with CMV. Two tomato cultivars, cv. Packmore (table tomato) and Rio Royo (processed tomato) inoculated with a CMV isolate from the Elia prefecture (Peloponnese), were grown in a growth chamber under artificial light with or without additional UV-b radiation. The experimental treatments were: (+CMV +UV-b), (+CMV -UV-b), (-CMV +UV-b) and (-CMV -UV-b). The severity of symptoms was rated in four classes. The results showed a clear tendency for an increase of symptom severity in tomato cultivars both infected by CMV and exposed to UV-b (treatment: +CMV +UV-b). It seems that changes in light quality, and especially increased UV-b radiation, must be taken into account along with other factors when explaining the serious damage caused during the last few years by some viroses in Greece.

Accumulation of nitrates in lettuce leaves due to nitrogen fertilization. D. Velissariou, L. Skretis, E. Paspatis and A. Alexandrakis. *Benaki Phytopathological Institute, 8, S. Delta Street, 145 61 Kifissia, Athens, Greece.*

The accumulation of nitrates in crop plants, and mostly in leaf vegetables (lettuce, spinach, celery, etc.), is a problem that has excercised scientists and the public over the last two decades. Amongst the causes contributing to nitrate accumulation in plant tissues, nitrogen fertilizers are the most important. Excessive nitrate fertilization, usually applied by growers, force plants to take up more nitrate they can assimilate, resulting in nitrate accumulation. In order to study this problem under field conditions in Greece, a 600 m^2 experimental plot of lettuce (cv. Romana) was set up at the agricultural area of Livanates in the Fthiotis prefecture, designed as a fully randomized block. The farming practices for the experimental plot were typical common practices of the local lettuce growers. Four fertilization treatments in four replicates each were applied: 25, 50, 75 and 100 kg of ammonium nitrate (35-0-0) per 1000 m^2. The fertilization treatments were applied at the stage of lettuce "leaf change". The experimental plants were sampled in three intervals until harvest (early April). Fresh and dry weights were recorded and the nitrate levels in plant tissues were measured using HPLC. The results showed a modest trend of mean dry weight increase with the treatment of 75 kg per 1,000 m^2 and no significant increase with the 100 kg per 1000 m^2. Nitrate levels on the other hand showed a significant increase at both the 75 and the 100 kg

per 1000 m^2, reaching the highest and, according to the EU directive for the winter crop, critical levels of nitrates (=4,500 mg/kg in fresh weight).

Calcium levels in peach fruits showing symptoms of the disorder known as "blossom end rot". A. Assimakopoulou[1], D. Velissariou[1] and V. Georgopoulos[2]. *[1]Benaki Phytopathological Institute, Plant Pathology Department, 8, S. Delta Street, 145 61 Kifissia, Athens, Greece. [2]PEGEAL, 204 00 Xilokastro, Korinthia, Greece.*

Peach fruits showing severe symptoms of the non parasitic disease known as "blossom end rot", were examined at the Laboratory of Non Parasitic Diseases in the Benaki Phytopathological Institute, since it is a serious problem resulting in the loss of commercial value of peaches in the agricultural area of Velvendos (Kozani). In the literature, this disorder is associated only with fruit size in years of low productivity, although "blossom end rot" is generally associated with calcium (Ca) deficiency in fruits . For that reason, fruits from the above-mentioned agricultural area, with and without visible "blossom end rot" symptoms, were dry-weighed and their concentrations of calcium, nitrogen, phosphorus, potassium, magnesium, iron, manganese, zinc and boron analysed. Moreover, the allocation of inorganic elements at the upper (stem end), mid and lower (blossom end) part of the fruit was determined. For fruits with "blossom end rot" symptoms, the dry weights were higher and the concentrations of calcium, potassium and magnesium were lower than those of fruits without visible symptoms. Calcium concentration gradually decreased from the stem end to the blossom end of the fruit for both fruit categories, but levels were always lower in fruits with symptoms. The rest of the analysed parameters did not differ significantly. The results showed that fruit size is important for the occurrence of the disorder. According to our findings, the large fruit size is due to the dilution of calcium into the fruit tissues; this is considered as the main cause of "blossom end rot".

Measurement and biomonitoring of ozone levels in a rural area in central Peloponnese – Greece. C. A. Saitanis and M.G. Karandinos**.** *Agricultural University of Athens, Laboratory of Ecology and Environmental Sciences, 75 Iera Odos, 118 55 Athens, Greece.*

We present the results of 38 days (28 May-5 July 1996) of continuous measurement of ozone (O_3) concentration, carried out instrumentally at a remote site in central Peloponnese (Pournaria - Arcadia, 21º56´ N, 37º47´ W, altitude ~ 520 m), and its biomonitoring using tobacco potted plants (*Nicotiana tabacum* L. var. Bel-W3 and var. Zichnomirodata - KK6/5) placed at that site during the same period. The maximum recorded O_3 concentration was 61.5 ppb, while mean values of 26.3, 31.7 and 34.6 ppb respectively were obtained at 24, 12 and 7 h. During the monitoring period (total 912 hours) the thresholds of 30, 40 and 50 ppb was exceeded for 361, 189 and 59 hours respectively. The diurnal pattern consisted of 1) a period of ozone increase during the morning hours (07:00 - 11:00); 2) a 7-hour period (11:00-18:00) of relatively constant concentrations where O_3 peaked, and 3) a period of O_3 decrease from late afternoon till the following morning. The O_3-induced necrotic spots characteristic and specific for each variety ("weather flecks") developed on the leaves of the exposed plants. Visible leaf injury increased with increasing duration of exposure. The present study showed that ozone levels that can affect at least sensitive species or varieties of plants after chronic exposure may occur in rural areas of Greece.

Chemical Control

Evaluation of the fungicide CGA 329351 against Oomycetes in field trials with various crops in Greece. I. Papageorgiou, A. Tsigas and V. Vaiopoulos. *Novartis Hellas S.A., Anthousa Avenue, 153 44 Anthousa Attikis, Greece.*

CGA 329351 is a systemic fungicide active against Oomycetes. It is the R-enantiomer of metalaxyl, which belongs to the chemical group of the phenylamides. CGA 329351 is used to control diseases caused by *Phytophthora, Pythium, Plasmopara, Peronospora* and *Pseudoperonospora* in various crops. During the period 1996-98, more than 30 trials were carried out in many areas of Greece to evaluate the effectiveness of CGA 329351 against pathogens of the following crops: potato (*Phytophthora infestans*), tomato (*Phytophthora infestans*), tobacco (*Peronospora tabacina* and *Phytophthora parasitica*), grapevine (*Plasmopara viticola*), cucumber (*Pseudoperonospora cubensis*) and cotton (*Pythium* spp. and *Phytophthora* spp.). Applications were as foliar spray, drench or seed treatment, depending on the crop and the pathogen. Six formulations containing CGA 329351, alone or in combination with other compounds, such as mancozeb, copper, folpet and CGA 245704, were tested. In all trials, CGA 329351 gave good disease control and was comparable to metalaxyl at half the rate of active ingredient.

Control of damping-off pathogens in tobacco and tomato seedling-nurseries with reduced doses of the product Laisol 40 SL (metham sodium 40%). E.J. Paplomatas[1], F. Legaki[2], N.D. Tsimboukis[2] and S. Spilioti[2]. *[1]Benaki Phytopathological Institute, Plant Pathology Department, 8, S. Delta Street, 145 61 Kifissia, Athens, Greece. [2]Hellafarm S.A., 15, Fleming Street, 151 23 Maroussi, Athens, Greece.*

The aim of the work was to evaluate the effectiveness of the soil disinfectant Laisol 40 SL (metham sodium 40% w/v) in controlling soil-borne fungal pathogens in seedling-nurseries of tomato and tobacco with doses lower than those recommended. Two doses of 0.75 and 0.90 l/10 kg water were applied to a soil surface of 10 m^2, while Hellapam 32.7 AS (metham sodium 32.7% w/v) was used as a reference compound at 1.5 l/10 kg water for 10 m^2 soil surface. The pathogen-targets were the soil-borne fungi *Rhizoctonia solani* and *Phytophthora nicotianae*. The pathogenicity of isolates

on tomato and tobacco was tested in preliminary greenhouse experiments. For *R. solani*, an isolate pathogenic to both hosts was used, while for *P. nicotianae* two isolates were used, one for each host. For inoculum preparation, pathogens were grown on a medium containing peat/sand/straw/carrot (6:1:1:1 per volume). In the growing medium of *R. solani*, 93 ml/l of 10% malt extract was substituted for carrot. After three weeks of incubation, inoculum was dispersed in plastic cups (10 cm in diameter) applying 1 l/3500 cm^2 surface of sterilized soil mix. Seeds were sown 25 days after the chemical application. There were five replications (cups) for each treatment with 10 tomato or 15 tobacco plants per replication. It was found that Laisol 40 SL at the higher dose (0.90 l/10 m^2) was equally effective as Hellapam against damping-off caused by *P. nicotianae*, and differed significantly from the positive control. In tomato, Laisol 40 SL was effective at both doses without statistic differences from the reference compound or the negative control. In soil infected with *R. solani*, both Laisol 40 SL at the high dose and Hellapam were effective for tobacco, while for tomato there were no significant differences between treatments. This might be due to the limited ability of *R. solani* to survive in soil depleted of nutrients for 25 days between the application of chemicals and sowing.

Effect of azoxystrobin and kresoxim methyl on diverse growth stages of *Sphaerotheca fuliginea* (Schlecht.) Pollacci. N. Petsikos-Panagiotarou[1,2], A. Kalamarakis[1,2] and E Kalogeropoulou[1]. [1]*Benaki Phytopathological Institute, Department of Pesticides Control and Phytopharmacy, Laboratory of Efficacy Evaluation of Pesticides, 8, S. Delta Street, 145 61 Kifissia, Athens, Greece.* [2]*National Agricultural Research Foundation, Athens, Greece.*

Azoxystrobin and kresoxim methyl are two new fungicides which belong to the chemical class of the strobilurins. These compounds were earlier found to be very effective against *Sphaerotheca fuliginea* (unpublished data), which is the main causal agent of cucurbit powdery mildew in Greece. A knowledge of the effect of these fungicides on the pathogen is considered to be important for determining the best timing of fungicide application for successful control of the pathogen. An experiment was carried out to study the effect of azoxystrobin and kresoxim methyl on diverse growth stages of *S. fuliginea*. The preventive and curative action of these fungicides was tested on cucumber plants. Foliar application of the fungicides (concentration of 50 µg a.i/ml) was carried out 24 h prior to and 2 and 4 days after artificial inoculation. It was found that azoxystrobin and kresoxim methyl, when applied preventively, inhibited conidial germination by 50% compared to the control. A considerable percentage of germinated conidia did not continue growth beyond germ tube formation, and those that did, formed primary and secondary hyphae with reduced length and fewer cells. Preventive applications of azoxystrobin and kresoxim methyl on cucumber cotyledons resulted in either inhibition of haustoria formation or reduction of the number of haustoria per conidium. Both fungicides significantly reduced the production of conidia when applied curatively 4 days after artificial inoculation. The results of this study showed the effect of strobilurin fungicides on different growth stages of *S. fuliginea* and explained their good effectiveness against this pathogen.

Evaluation of fungicides for control of green mould caused by *Penicillium digitatum* on citrus fruit. B.N. Ziogas and A.B. Kambotiati. *Agricultural University of Athens, Laboratory of Phytopathology, 75 Iera Odos, 118 55 Athens, Greece.*

In the present work, the effectiveness of 13 fungicides against green mould of citrus caused by *Penicillium digitatum* was investigated. Experiments *in vitro* showed that iminoctadine (quanidine), benomyl (benzimidazole), propiconazole, tebuconazole, fluzilazole (EBIs-triazole) and triflumizole (TBZ-imidazole) were highly fungitoxic to the linear growth of the pathogen (MICs 0.06-0.25). Lower fungitoxicity was observed for the fungicides thiabendazole (TBZ-benzimidazole), imazalil (EBIs-imidazole), flutriafol (EBIs-triazole) and fenpropimorph (EBIs-morpholine), (MICs 0.5-2.0). Weak fungitoxicity was found for the fungicides quazatine (quanidine), pyrifenox (EBIs-pyridine) and SOPP (AHDs) with MICs 2.0, 4.0 and 45.0, respectively. Experiments *in vivo* on oranges (var. Merlin) using Befran (iminoactidine), Benlate (benomyl), Tilt (propiconazole) and Punch (flusilazole) showed that Befran was the most effective to control green mould of citrus. Complete inhibition of the disease was achieved when Befran, Benlate and Tilt were applied 6 h after inoculation at concentrations 1, 10 and 100 µg/ml, respectively. When they were applied 24 h after inoculation at the concentrations of 1 µg/ml, the protection was 80, 20 and 30%, respectively. The treatment of 24 h before inoculation was not effective for any of the fungicides tested. Punch was not effective for controlling green mould in all treatments before and after inoculation with *P. digitatum*. Study of the resistance risk for iminoctadine and propiconazole showed that iminoctadine is a low-risk fungicide, since it was impossible to obtain resistant mutants after chemical mutagenesis (MNNG). Mutants of *P. digitatum* with low (RF:2-10) resistance to propiconazole were isolated with a frequency of $6x10^{-5}$. Fitness-determining characteristics such as mycelial growth, sporulation, germination and pathogenicity were found to be significantly reduced in the mutant strains.

Relative fitness of DMI-resistant and -sensitive isolates of *Cercospora beticola*. G.S. Karaoglanidis and C.C. Thanassoulopoulos. *Aristotelian University of Thessaloniki, Department of Agriculture, Plant Pathology Laboratory, P.O. Box 269, 540 06 Thessaloniki, Greece.*

Isolates of *Cercospora beticola* Sacc. resistant to triazole fungicides were collected from sugarbeet fields in Northern Greece. The fitness of these isolates was compared with that of isolates having wild-type sensitivity to DMIs. The fitness parameters studied were growth rate, germination of conidia, growth of germ tubes, viability of conidiaat low temperatures, virulence, sporulation and incubation period. Nine DMI-resistant isolates were compared to 12 wild-type isolates and 10 isolates with mean population sensitivity. For four out of seven parameters measured, the mean values were not statistically different (growth rate, germination of co-

nidia, viability of conidia, incubation period and sporulation). The mean values of the other two fitness parameters were slightly lower for DMI-resistant isolates than for the wild-type isolates. However, within groups, no relation existed between the degree of resistance and the degree of fitness. It is concluded that the hypothesis that resistance to DMIs is unlikely to develop under practical conditions because of the decreased fitness of DMI-resistant strains does not seem to hold for *C. beticola*.

Study of the fungicidal action of fludioxanil and of the risk for resistance development to phenylpyrrole fungicides. B.N. Ziogas and M.S. Girgis. *Agricultural University of Athens, Laboratory of Phytopathology, 75 Iera Odos, 118 55 Athens, Greece.*

The fungicidal action of fludioxanil (CGA-173505) against *Botrytis cinerea, Ustilago maydis* and a heterozygous diploid of non-pathogenic *Aspergillus nidulans* was studied in the present work. Fludioxanil showed high fungitoxicity for *B. cinerea* and *U. maydis*. The fungicide concentrations for 50% (ED_{50}) and 100% (MIC) inhibition of growth were found at values of 0.005/0.045 and 0.05/0.25 μg/ml, respectively. A very effective control of grey mould on cucumber seedlings was achieved by fludioxanil. Complete inhibition of the disease development was found at 10 μg a.i./ml. Cross resistance studies showed that fludioxanil is highly effective for controlling strains of *B. cinerea* resistant to benzimidazoles and to the mixtures of benzimidazoles/phenylcarbamates but not to strains resistant to aromatic hydrocarbons and dicarboximides (AHDs), such as tolclofos methyl, PCNB, tecnazene, chloroneb, iprodione, procymidone and chlozolinate. Genetic analysis in *U. maydis* identified mutations at three chromosomal loci (*Sa*1, *Sa*2 and *Sa*3), responsible for high resistance to fludioxanil, fenpiclonil and AHDs. Studies with the heterozygous diploid strain of *A. nidulans* showed that fludioxanil also increased mitotic recombination, similarly with the other AHDs. Study of resistance risk showed that the phenylpyrrole fungicides are at risk for the following reasons: (a) resistant mutants were isolated from both *B. cinerea* and *U. maydis* at high frequencies ($1x10^{-5}$ and $2x10^{-3}$). (b) The mutants from both *B. cinerea* and *U. maydis* were highly resistant to fludioxanil (RF: 3000 and 400, respectively). (c) Fitness-determining characteristics such as rate of growth, conidial production and pathogenicity, except a higher sensitivity to osmotic pressure, were not affected in the mutant strains. (d) Fludioxanil did not control grey mould when cucumber cotyledons were infected by a mutant strain.

Reduced sensitivity to DMIs in *Uncinula necator* (Schw) Burr. populations. N. Petsikos-Panagiotarou[1,2], A. Kalamarakis[1,2], E. Kalogeropoulou[1] and B.N. Ziogas[3]. [1]*Benaki Phytopathological Institute, Department of Pesticides Control and Phytopharmacy, Laboratory of Efficacy Evaluation of Pesticides, 8 S. Delta Street, 14561 Kifissia, Athens, Greece.* [2]*National, Agricultural Research Foundation, Athens, Greece.* [3]*Agricultural University of Athens, Laboratory of Plant Pathology, 75 Iera Odos, 118 55 Athens, Greece.*

The appearance of resistant strains of the fungus *Uncinula necator*, the casual agent of grape powdery mildew, to DMI fungicides in Europe and the USA, has led to the present research programme that aimed to study the current state of grape powdery mildew in Greece and to evaluate the effectiveness against this disease of the widely used DMI fungicides. The following actions were taken: a) collection of data from different grape-producing areas in Greece and creation of a database, b) development of a sampling method of powdery mildew infected plant material, c) isolation and maintenance of different grape powdery mildew populations separately to avoid cross-contamination on young grape plants under controlled conditions and d) development of a bioassay in order to monitor the sensitivity to DMIs of the different powdery mildew populations. Data obtained so far indicate that a) grape powdery mildew is a serious problem in Greece, b) the fungus *U. necator* overwinters as mycelium in dormant infected buds and as cleistothecia on plant tissues, and c) reduced sensitivity of powdery mildew to DMIs was recorded in the regions of Korinthia, Ahaia, Kavala, Halkidiki, Crete (Helaklion) and Attiki, which are the main grape-producing areas in Greece. Powdery mildew populations isolated from the regions of Likovrisi-Attiki (K1), Ilioupoli-Attiki (K2) and Heraklion-Crete (K3) showed variable sensitivity to DMIs, decreasing as follows: K3>K2>K1. The population isolated from Crete (K3) was found significantly less sensitive to DMIs than the wild type.

Effect of cell wall on the sensitivity to morpholines and related piperidine fungicides in *Ustilago maydis*. N. Markoglou and N.N. Ziogas. *Agricultural University of Athens, Laboratory of Phytopathology, 75 Iera Odos, 118 55 Athens, Greece.*

The possible effect of the cell wall on the sensitivity to morpholines and related piperidine fungicides in the phytopathogenic Basidiomycete *Ustilago maydis* was studied in the present work. Previous studies concerning the resistance of the pathogen to the above inhibitors showed that the reduced sensitivity of the mutant strains is due to major (*U/fpm*-1A, *U/fpm*-2 and *U/fpd*-1) or minor (*U/fpm*-1B, *U/tdm*-1 and *U/tdm*-2) unlinked chromosomal gene mutations. It was suggested that the above mutations coded different structural changes of the sporidial cell wall, resulting in a different binding capacity for the morpholine and piperidine fungicides and therefore reduced entrance into the cell and accumulation of toxic molecules. Cross-resistance studies have shown that these major and minor gene mutations for resistance to morpholines and piperidines also reduce sensitivity to polyoxins, which are the specific inhibitors of "chitin synthase". The major-gene mutations, which are responsible for high resistance to fenpropimorph and fenpropidin, also confered high resistance on polyoxins (Rf: 100-125 at the sporidial level and 50-75 in the protoplasts). The minor-gene mutations also coded for low resistance to polyoxins (Rf: 25 at the sporidial level and 10 in the protoplasts). Isolation of polyoxin resistant strains (Rf: 100-125, on the basis of MICs) after UV light irradiation, resulted in two mutant phenotypes with a mutation frequency of $8x10^{-7}$: a) strains with resistance only to polyoxins, and b) strains that also have reduced sensitivity to the morpholines fenpropimorph

(Rf: 75-100) and tridemorph (Rf: 10-25) and to piperidine fenpropidin (Rf: 15-25). None of the above classes showed reduced sensitivity to other SBIs such as DMIs: triadimefon, triadimenol, propiconazole, flusilazole, fenarimol, pyrifenox, triflumizole, imazalil and the allylamine terbinafine. It seems that the changes in the structure and the function of "chitin synthase", which reduce the sensitivity to polyoxins, may also reduce the accumulation of other unrelated fungicides, by increased binding to the modified cell wall.

Study on sensitivity of Greek isolates of *Botrytis cinerea* to the fungicides anilinopyrimidine and phenylpyridinamine. N. Petsikos-Panagiotarou[1,2], A. Kalamarakis[1,2] and B. Mavroidis[1]. [1]*Benaki Phytopathological Institute, Department of Pesticides Control and Phytopharmacy, Laboratory of Efficacy Evaluation of Pesticides, 8, S. Delta Street, 145 61 Kifissia, Athens, Greece.* [2]*National Agricultural Research Foundation, Athens, Greece.*

One of the most serious problems in controlling *Botrytis cinerea* in greenhouse vegetable crops, is the extensive appearance of resistant strains to selective fungicides. In Greece, resistance to the extensively used benzimidazole, dicarboximide and N-phenylcarbamate botryticides has been detected and accounts for control failures of grey mould; therefore, the alternation of fungicides with others of a different mode of action is recommended as anti-resistance strategy. The present study aimed to evaluate the effectiveness of cyprodinil/pyrimethanil and fluazinam, belonging to the novel group of anilinopyrimidine and phenylpyridinamine fungicides respectively, against Greek isolates of *B. cinerea* resistant to the benzimidazoles and/or dicarboximides and to the mixture carbedazim+diethofencarb. The above-mentioned fungicides were effective *in vitro* against all the resistant strains tested. However, in preliminary experiments *in vivo*, this effect was not obvious in some cases (anilinopyrimidines) and further investigation is needed. No cross-resistance was observed between these new fungicides and the extensively used benzimidazoles, dicarboximides or the mixture carbendazim+diethofencarb.

Dielectric and calorimetric properties of the fungicide captan ($C_9H_9Cl_3NO_2S$). N.D. Papadimitropoulos[1] and J.C. Papaioannou. *University of Athens, Department of Chemistry, 157 71 Athens, Greece.*

The fungicidal properties of captan (N-trichloromethylthio)cyclohex-4-ene-1,2-dicarboximide) were described by A.R. Kittleston. This substance forms colourless crystals (m.p. 178°C) and its solubility in water is 3.3 mg/l H_2O. It is unstable under alkaline conditions and decomposes near its melting point. Dielectric spectroscopy is widely used for the determination of the mechanisms of biological systems. In the presentstudy, the dielectric behaviour of a powder sample of captan was investigated in the frequency range 0-1000kHz and a temperature range 150K-320K. The real part (ε') of the dielectric constant remains constant in the temperature range 150K-225K for every frequency f<1kHz. For temperatures T>225K, the ε' increases exponentially with temperature with a rate that decreases as the frequency increases. In the higher frequencies of 50-100kHz no variations were observed. In a similar way the imaginary part (ε") varies with temperature and frequency. The plots of ε', ε" vs frequency have a sigmoid and a bell-like shape correspondingly, for T<225K, which indicates Debye behavior with a single relaxation time. For T>225K the above plots are affected strongly by the ionic conductivity (-dispersion), which determines the biological activity of the sample. The differential Scanning Calorimetry investigation, with rates 40K min 20K min and 10K min, did not show any endothermic or exothermic transitions, which means that the sample did not undergo any phase changes.

Control of *Botrytis cinerea* on tomato in greenhouse by the fungicide Switch 62.5 WG (A-9219). V.A. Bourbos[1], I. Papageorgiou[2] and A. Tsigas[2]. [1]*National Agricultural Research Foundation, Institute of Subtropical Plants and Olive Trees of Chania, Laboratory of Phytopathology, Agrokipio, 731 00 Chania, Crete, Greece.* [2]*NOVARTIS (Hellas) AEVE, Department of Phytoprotection, Anthousa Avenue, 153 44 Anthousa, Attiki, Greece.*

The possibility of controlling *Botrytis cinerea* Pers. with the new fungicide cyprodinil 37.5% + fludioxonil 25%, also known as A-9219 62.5 WG or Switch 62.5 WG, was studied in a plastic unheated greenhouse,. The study was carried out during two successive growing periods on tomato cv. Early Pack No. 7. The fungicide under study was applied at doses of 80 and 100 g/hl. As a standard fungicide, diethofencarb 25%+carbendazim 25% at a dose of 100 g/hl of the commercial product Sumico WP was used. Sprays began after the appearance of the first symptoms. Three sprays were performed at 12-day intervals. The strains of the pathogen, which were predominant on the crop were resistant to the benzimidazol and dicarboximide fungicides. Estimation of fungicide efficacy was based on the measurement of infected fruits, leaflets and the number of infections on stems per plant. Switch controlled the pathogen with an efficacy level of 93.5 (fruits), 88.2 (leaflets) and 85% (stems) at a dose of 80 g/hl and 98.2, 98.0, and 100% control at a dose of 100 g/hl. At a dose of 100 g/hl the fungicide under study did not differ significantly in efficacy from the standard product.

Sodium bicarbonate for the control of *Erysiphe polygoni* in greenhouse tomato. V.A. Bourbos, M.T. Skoundridakis and E. Barbopoulou. *National Agricultural Research Foundation, Institute of Subtropical Plants and Olive Trees of Chania, Laboratory of Phytopathology, Agrokipio, 731 00 Chania, Crete, Greece.*

During the last few years, an infection of open-field and greenhouse tomato crops by the fungus *Erysiphe polygoni* D.C. has been observed. Sodium bicarbonate, used alone or in combination with the wetting agent Agral 90 was tested against this pathogen. Sodium bicarbonate and the wetting agent were used at doses of 200 g/hl and 25 ml/hl respectively. The fungicide pyrifenox, at a dose of 20 ml/hl of the commercial product Dorado 20 EC, was used as the reference product. The experiments lasted for two successive

growing periods in the greenhouse where the tomato cultivar Early Pack No.7 was grown. Each experimental unit included 20 plants. Four sprays were performed every 12 days with a low-pressure sprayer. The efficiency of sodium bicarbonate in the experimental plots where it was used alone ranged from 79.6 to 80.6%. The infection was reduced by 88.7% in the experimental plots, when sprays of the combination of sodium bicarbonate with the wetting agent performed, while there was no statistically significant difference from the reference product (88.6%). By contrast, an increases in the number of lesions per plant ranging from 9.93 to 89.84% and from 10.02 to 75.49% was observed on plants sprayed with pure water alone or with the wetting agent alone respectively.

Evaluation of fungicides for control of *Cladosporium echinulatum*. M. Chrysayi - Tokousbalides and M. Aliferis. *Agricultural University of Athens, Pesticide Science Laboratory, 75 Iera Odos, 118 55 Athens, Greece.*

In spore germination tests to control *Cladosporium echinulatum* isolated from diseased carnation (*Dianthus caryophyllus* L.) cultivated under cover, chlorothalonil was the most effective among the fungicides used. This fungicide at concentration of 1µg/ml strongly affected (95% inhibition) the germination of conidia. The fungicide carbendazim at concentrations of 1 and 4 µg/ml inhibited spore germination by 35 and 60%, respectively. Dicloran at 10 µg/ml reduced conidia germination by 28%. The fungicides bitertanol and bromuconazole at concentrations up to 10 µg/ml had no effect on spore germination. Carnation plants that had been inoculated with a conidial suspension of the fungus were sprayed with formulated products of chlorothalonil or carbendazim at rates of 2.2 and 1.6 g a.i./l respectively. Both fungicides, applied 1-2 h after inoculation, inhibited the development of the disease without any side effects on the plants. On the unsprayed plants (control) disease symptoms were observed 3-4 days after inoculation.

Study on the residues of the fungicide chlozolinate on table grapes. C. Lentza-Rizos, E. Avramides, A. Argyropoulou, V. Papadimitriou and K. Kokkinaki. *National Agricultural Research Foundation, 1 S. Venizelou Street., 141 23 Lykovrissi, Attiki, Greece.*

Trials with the dicarboximide fungicide chlozolinate were carried out in Greece (region of Athens) for two consecutive years on two varieties of table grapes ("Cardinal" and "Victoria"). Three sprays in 1996 and four in 1997 were applied at the recommended growth stages and rates (the lower rate, 0.075 kg a.s./hl, applied as 0.75 kg a.s./ha, in 1996, and the higher rate, 0.10 kg a.s./hl, applied as 1.00 kg a.s./ha, in 1997) by a knapsack sprayer in 1996 and by a tractor with a single-nozzle sprayer in 1997. Grapes samples were collected 0, 4, 8, 14, 21 (recommended PHI) and 28 days after first application. For the detection of chlozolinate residues, a multiresidue gas-chromatographic method was used, with an ECD detector yielding 94% recovery of the parent compound with a LOD of 0.004 mg/kg, while the main metabolite S1 was determined semiquantitatively (53% recovery). The dissipation constants were estimated on both varieties (higher on "Cardinal"). It was found that chlozolinate was a moderately persistent compound. The average residue concentrations at the recommended PHI were 0.74 and 3.41 mg/kg (1996 and 1997 respectively with "Cardinal") and 1.54 and 4.23 mg/kg (1996 and 1997 respectively with "Victoria"). These values are lower than those proposed by the European Commission MRL. Grape washing removed a considerable amount of the fungicide.

Evaluation of the fungicide Chorus 50 WG against apple scab (*Venturia inaequalis*) in field trials. A. Tsigas, V. Vaiopoulos and I. Papageorgiou. *Novartis Hellas S.A., Anthousa Avenue, 153 44 Anthousa Attikis, Greece.*

Chorus is a systemic fungicide that controls *Venturia, Alternaria* and *Monilinia* in top and stone fruits and also gives a good side effect against *Podosphaera* and *Sphaerotheca*. Chorus contains the active ingredient cyprodinil, which belongs to the new chemical group of the pyrimidinamines. It is systemic with an acropetal movement. Cyprodinil controls diseases of deciduous fruit and vegetables caused by *Venturia, Alternaria , Monilinia* and *Botrytis* spp. In cereals, cyprodinil has good activity against *Pseudocercosporella herpotrichoides, Erysiphe graminis, Pyrenophora teres, Rhynchosporium secalis* and *Septoria nodorum*. Cyprodinil has a new mode of action different from that of other chemical groups. It inhibits penetration of the pathogen into the leaf and the growth of mycelium inside plant tissues. During 1994-96, the efficacy of Chorus against apple scab was evaluated in field trials in the region of Magnissia. Chorus 50 WG at the rate of 15 and 20 g a.i./hl gave good control of apple scab on leaves and fruits and was equivalent to the standard product. Applications were started preventively before flowering every 7-10 days until fruit set and thereafter every 10-15 days. Chorus can be used in IPM programs.

Effectiveness of fungicides against *Cercospora beticola* Sacc. in relation to pathogenicity and host susceptibility. I. Vloutoglou and E. Birkou. *Benaki Phytopathological Institute, Plant Pathology Department, 8, S. Delta Street, 145 61 Kifissia, Athens, Greece.*

The effectiveness of flutriafol, difenoconazole, fentin acetate, benomyl and maneb in controlling *Cercospora beticola* was studied under controlled environmental conditions. Fungicides were applied to sugarbeet plants of the cv. Ultramono (susceptible to *C. beticola*) and Vergina (tolerant) protectively (three days prior to inoculation) and curatively (one and four days after inoculation). Plants at the growth stage of six true leaves were spray-inoculated with a conidial suspension of $2x10^4$ conidia/ml. Two strains of *C. beticola* were tested, one with reduced resistance to benomyl (ED_{50} 0.85 µg/ml) and one sensitive (ED_{50} 0.03 µg/ml). Results showed that the effectiveness of the fungicides depended on the time of application (protectively or curatively), the strain of the pathogen and host susceptibility. Flutriafol (0.01% a.i.) and benomyl (0.03% a.i.) showed the greatest protectant and curative activity (inhibition of infection 83-

100% and 60-100% respectively compared with the unsprayed control). Difenoconazole (0.015% a.i.) showed very good curative activity irrespective of the pathogen strain and cultivar combination used (inhibition of infection 77-100%). However, its protective activity on the susceptible cultivar was significantly (P=0.05) lower (inhibition of infection 23-50%) than on the tolerant cultivar (inhibition of infection 100%). Fentin acetate, applied four days after inoculation, inhibited infection in both cultivars, but only when the strain of the pathogen sensitive to benomyl was used (inhibition of infection 78-96%). Maneb, when applied one day after inoculation, showed very good curative activity (inhibition of infection 90-100%), irrespective of the pathogen strain and the cultivar combination tested.

Biological and Integrated Control

Study of the possibility to control *Phytophthora infestans* on potato with organic extracts. S.E. Patsaki[1], V.A. Bourbos[1] and K. Balis[2]. *[1]National Agricultural Research Foundation, Institute of Subtropical Plants and Olive Trees of Chania, Laboratory of Phytopathology, Agrokipio, 731 00 Chania, Crete, Greece. [2]Agricultural University of Athens, Laboratory of Agricultural Microbiology, 75 Iera Odos, 118 55 Athens, Greece.*

The possibility of controlling potato late blight, caused by the Phycomycete *Phytophthora infestans* (Mont.) de Bary was studied *in vitro* and *in planta* with the aid of Liochoumos and Biosol extracts. Liochoumos is the result of co-composting of olive mill wastes. Biosol is a product of *Penicillium* sp. The *in vitro* trials showed that Liochoumos inhibited the germination of sporangia and mycelia. In contrast to Liochoumos, Biosol inhibited sporangia germination, but had no effect on mycelial growth. In field experiments, these two products were applied 3 days and just before artificial inoculation, at two replications 9 days apart and at rates of 10 and 20% (Liochoumos) and 1% (Biosol). As a standard fungicide, metalaxyl 7.5%+mancozeb 56% at the rate of 300 g/1000m^2 of the commercial product Ridomil MZ 63.5 WP was used. The efficacy of extracts fluctuated from 43.49% (infected leaves extract) to 83.56% (infected tubers extract), from 63.63 (Liochoumos 10%) to 88.50% (Liochoumos 20%), and from 32.42 to 91.9% for Biosol. Liochoumos extracts satisfactorily controlled the pathogen when applied just before artificial inoculation. By contrast, Biosol controlled the pathogen more effectively when applied 3 days before artificial inoculation. It was shown that Liochoumos 20% and Biosol 1% organic extracts, not having any significant statistical differences from the standard fungicide (99.59 and 88.54%), may have an active role in potato late blight control programs.

Efficacy of plant extracts on powdery mildew in cucumber. S. Konstantinidou-Doltsinis and K. Tzempelikou. *National Agricultural Research Foundation, Institute of Plant Protection, 260 04 Patras, Greece.*

The effiectiveness of alcoholic extracts from 118 different plant species, mainly indigenous, against powdery mildew on young cucumber plants was studied in a series of experiments under controlled conditions. Plant extracts at a concentration of 2.5% d.w/v were sprayed on the first true leaf of young cucumber plants two or three days before their inoculation with a conidial suspension of the pathogen. Approximately 12 days after inoculation, the percentage of leaf area infected was assessed. The results showed that extracts of the plant *Lampranthus roseus* in the family Aizoaceae and of flowers or pods from plants in two different taxa of the genus *Cassia* in the family Leguminosae decreased infection by over 90%. The above mentioned plants are not indigenous and are cultivated as ornamental plants. In supplementary trials the effectiveness of lower concentrations (1%, 0.1%) of these extracts was tested. The first experiments were carried out to determine the effect of plant extract application time on the disease in relation to inoculation time. The results showed that when the extracts were applied before or at the same time as the inoculum, extract effectiveness was higher than when they were applied after inoculation. The overall results suggested that an induced resistance mechanism in the plants maybe involved.

A phytotoxic metabolite of a *Drechslera avenae* isolate with host-specificity to *Avena sterilis*. M. Kastanias and M. Chrysayi–Tokoysbalides. *Agricultural University of Athens, Pesticide Science Laboratory, 75 Iera Odos, 118 55 Athens, Greece.*

In a research project aiming at the detection and exploitation of fungal bioactive metabolites as crop protection agents, the fungus *Drechslera avenae* was isolated for the first time from *Avena sterilis*. In nature, this fungus infects the plant at temperatures of 10 to 20ºC, causing leaf spotting. Under controlled conditions this isolate was pathogenic to *A. sterilis*, causing symptoms similar to the those observed in nature. The isolate was not pathogenic to seedlings of *A. fatua* or *A. sativa*. A phytotoxic metabolite was isolated from cultures of the fungus, purified by sequential chromatography systems and characterized by GC-MS, LC-MS, NMR, UV and IR spectroscopy. It was a cyclic polyketide and could be detected in agar cultures incubated for one week, but for maximum production one month's incubation was required. The highest yield was obtained at 15ºC. When bioassayed, it caused severe symptoms of toxicity to *A. sterilis* and *A. fatua*, but not to *A. sativa*. The type and expression of the symptoms depended on the mode of metabolite application. This *D. avenae* metabolite is the only known compound of this chemical nature which is toxic to wild oats.

Pathogenicity of *Verticillium dalhiae* and *V. nigrescens* and biological effect of *V. nigrescens* on Verticillium wilt of tomatoes. F. T. Gravanis and S. Xifilidou. *T.E.I. of Larissa, Department of Plant Production, 411 10 Larissa, Greece.*

Verticillium dahliae and *V. nigrescens*, isolated from greenhouse soil, were shown to be aggressive and moderate patho-

gens respectively, causing wilt on tomato seedlings. Pathogenicity tests were carried out on 15- and 35-day-old tomato seedlings grown in pots. The plants were inoculated at the time of transplanting into pots. Wilt disease severity, plant height, stem diameter, fresh weight and the dry weights of inoculated plants were assessed at the end of the experiment. Plants of both ages (15 and 35-days old) were inoculated with both organisms on the same day and at 4-day intervals, in all combinations. Fifteen-day-old plants inoculated with *V. nigrescens* 4 days prior to inoculation with *V. dahliae* did not differ statistically from control plants in disease severity, stem diameter, fresh and dry weight. Fifteen- and 35-day-old plants treated in the same way did not differ statistically from control plants in disease severity, but they differed from all other treatments. All the plants inoculated on the same day with *V. nigrescens* before *V. dahliae* differed statistically from the controls and from all other treatments.

Wilt disease caused by isolates of *Fusarium oxysporum* and *F. oxysporum* var. *redolens* on tomatoes and biological effect of *Trichoderma viride* and *T. hamatum* on disease severity. S. Xifilidou[1], F.T. Gravanis[1] and A. Brugger[2]. [1]*T.E.I. of Larissa, Department of Plant Production, 411 10 Larissa, Greece.* [2]*Institut Supérieur d' Agriculture de Lille, Rue du Port, Lille, France.*

Fusarium oxysporum f.sp. *lycopersici* was isolated from the soil of a greenhouse in which wilt symptoms had been observed in the past. *F. oxysporum* var. *redolens* was isolated from the soil of another greenhouse in which no wilt symptoms had been observed. *Trichoderma viride* and *T. hamatum* were also isolated from the latter greenhouse. Tests *in vitro* showed that the two *Trichoderma* species delayed the growth of *F. oxysporum* var. *redolens* on PDA plates. In tests with potted tomato seedlings artificially inoculated with both *Fusaria*, spore suspensions of *T. viride* and *T. hamatum*, singly or in combination, were added simultaneously to the soil of the pots. Results showed that the presence of both *Trichoderma* species and especially that of *T. hamatum* caused lower disease incidence expressed with milder wilt symptoms, higher values of stem diameter and greater fresh and dry weight of inoculated plants.

Nematoctonous fungi from Greek soils. D. Dimou. *Agricultural University of Athens, Laboratory of General and Agricultural Microbiology, 75 Iera Odos, 118 55 Athens, Greece.*

Nematoctonous fungi infect or trap nematodes and feed on them. Although they comprise an ecologically exciting group, especially the nematode-trapping ones, and their study dates internationally from the 30's, they have so far not received enough attention from Greek mycologists. In examining different Greek soil samples in the Laboratory of General and Agricultural Microbiology of the Agricultural University of Athens, 22 nematochthonous species were observed and identified. Six of them were endoparasites (*Haptoglossa heterospora, Harposporium anguillulae, H. helicoides, Cephalosporium balanoides, Meria contospora* and *Meristacrum asterospermum*) and 16 were nematode trapping fungi (*Arthrobotrys arthrobotryoides, A. cladodes* var. *macroides, A. conoides, A. dactyloides, A. flagrans, musiformis, A. oligospora, Dactylaria brochopaga, D. vermicola, Dactylella leptospora, Monacrosporium doedycoides, M. eudermatum, M. gephyropaagum, M. phymatopagum, Stylopage grandis* and *S. hadra*). Eighteen of these species are reported for the first time in Greek soils, while 4 others, along with *Harposporium lilliputanum*, have already been reported by V.S. Kouyeas.

The effect of PCNB and *Trichoderma koningii* (Oud.) on the viability of sclerotia of *Sclerotinia sclerotiorum* (Lib.) de Bary. D. Aggelaki and K. Tzavella-Klonari. *Aristotelian University of Thessaloniki, Faculty of Agriculture, Plant Pathology Laboratory, P.O.B. 269, 540 06 Thessaloniki, Greece.*

The effect of PCNB alone and *Trichoderma koningii* alone or in combination with a low dosage of PCNB on the viability of sclerotia of *Sclerotinia sclerotiorum*, was assessed after 10- and 20-day incubation at 20°C. Nineteen percent of the sclerotia retained their viability 10 days after the combined application of *T. koningii* (6 g of wheat bran culture/50 sclerotia) and PCNB at the low dosage (1.5 g/l water). *T. koningii* alone, or PCNB alone at the proposed dosage (3 g/l water), significantly reduced the sclerotia viability by 53 and 47%, respectively. In comparison, when PCNB was applied at the low dosage, the sclerotia viability was not significantly reduced (17%). When the time of incubation was increased to 20 days, the combined application of *T. koningii* and low dosage of PCNB, as well as *T. koningii* alone, gave the best results, both reducing the sclerotia viability by 91%. For the same period of time the application of PCNB at the proposed and at the low dosage, significantly reduced the sclerotia viability by 59 and 25%, respectively.

Generation and analyses of transgenic Greek sugarbeet lines carrying genes that confer resistance to rhizomania. K. Kalantidis[1], A. Tsaftaris[1], E. Tsagris[2,3], I. Manoussopoulos[2], M. Providaki[2] and M. Tabler[2]. [1]*Aristotelian University of Thessaloniki, Department of Genetics and Plant Breeding, 540 06 Thessaloniki, Greece.* [2]*Institute of Molecular Biology and Biotechnology, Forth, Crete, Greece.* [3]*Department of Biology, University of Crete, Heraklion, Grete, Greece.*

Sugarbeet is a crop of great economic importance for Greece. Breeding the available varieties using all biotechnological advantages is essential to improve the competitiveness of Greek sugar production, to protect the agricultural environment, etc. One of the most important diseases of sugarbeet is rhizomania, caused by *Beet necrotic yellow vein virus* (BNVYY). Practically the only way to fight rhizomania is by generating resistant lines. Using genetic engineering methods for the development of resistant lines, three approaches were tried in our laboratories: a) genetic transformation of sugarbeet with a viral sequence (13K), aiming to increase plant resistance via co-suppression, b) genetic

transformation of sugarbeet with a gene encoding a ribozyme (RZ1) that specifically cleaves the viral (+) strand RNA, and c) genetic transformation of sugarbeet with a gene encoding a ribozyme (RZ3) that specifically cleaves the viral (-) strand RNA. All genetic transformations were conducted using the *Agrobacterium* co-cultivation method. All transgenic plants were tested for the presence of the novel sequences by PCR and southern hybridization, and for expression of the novel genes analysed by northern hybridization. Lastly, trangenic plants were tested for *in vivo* resistance to the virus.

Integrated control of powdery mildew of grapevine without damage to beneficial organisms. I.C. Roumbos[1], P. Papaioannou-Soulioti[2], D. Markogyannaki[2] and I. Adamopoulos[1]. [1]*National Agricultural Research Foundation, Plant Protection Institute, 380 01 Volos, Greece.* [2]*Benaki Phytopathological Institute, 8, S. Delta Street, 145 61 Kifissia, Athens, Greece.*

This work reports the results of trials carried out during the period 1995-97 in 10 different grapevine-growing areas of Greece on side effects of products widely used against powdery mildew of grapevine (*Uncinula necator*) particularly powdery sulphur, wettable sulphur and the triazole fungicide myclobutanil (Systhane) on predatory mites of the family Phytoseiidae and especially on *Phytoseius finitimus,* the most widespread mite in Greek vineyards. All trials were carried out over three successive years under field conditions in the grapevine-growing areas of Volos, Karditsa, Mantinia, Santorine, Lemnos and Samos and also for 1 or 2 years in the areas of Naoussa, Kavala, Nemea and Zitsa of Ioannina. Samplings were made at 2-week intervals before and after treatment during the period April to November. Recording of the phytoseiids was done on 100 leaves (25x4) for each treatment. Results showed that all three chemicals tested were harmless to populations of the phytoseiids.

Resistance to *Seiridium* canker in the Cupressaceae under controlled environmental conditions. K. Spanos[1] and S. Xenopoulos[2]. [1]*National Agricultural Research Foundation, Forest Research Institute, 570 06 Vassilika Thessaloniki, Greece.* [2]*National Agricultural Research Foundation, Institute of Mediterranean Forest Ecosystems & Forest Products Technology, Terma Alkmanos, 115 28 Ilissia, Athens, Greece.*

Artificial inoculations on young seedlings of the family Cupressaceae under greenhouse conditions showed that *Seiridium cardinale* was more pathogenic than *S. cupressi* and *S. unicorne*. *Cupressus macrocarpa* was highly susceptible to *Seiridium* canker, *C. sempervirens* very susceptible, while *C. torulosa* and *C. arizonica* were moderately susceptible. *Chamaecyparis lawsoniana* was highly resistant to *S. cardinale*, but very susceptible to *S. unicorne* and moderate susceptible *to S. cupressi*. Intraspecific variation in susceptibility to *S. cardinale* was found in *C. sempervirens*. *S. cupressi* was more pathogenic than *S. unicorne* on *C. macrocarpa*, *C. arizonica* and *C. torulosa*, while it was less pathogenic on *C. sempervirens*. Mature bark was more resistant to *Seridium* canker than young bark. It was found that *S. cardinale* had low variability in pathogenicity, as only one of eight isolates tested proved to be a weak pathogen.

Development of sugarbeet lines resistant to *Cercospora* by cloning genes encoding antioxidant enzymes. K. Tertivanidis, K. Goudoula, A. Giamoustaris and A. Tsaftaris. *Aristotelian University of Thessaloniki of Thessaloniki, Department of Genetics and Plant Breeding, School of Agriculture, 540 06 Thessaloniki, Greece.*

Sugarbeet is an important crop in Greece, but plant protection costs are high. One of the most important diseases affecting sugarbeet is caused by the fungus *Cercospora beticola* L. The deleterious effects of the disease are caused by cercosporine, a photo-induced toxin. This toxin leads to the formation of free oxygen radicals responsible for the destruction of cell macromolecules, resulting in cell death. Plants are protected from these radicals by antioxidant enzymatic systems such as superoxide dismutases (SODs), catalases (CATs) and peroxidases (pOXs). The aim of the present study was to develop genetically modified sugarbeet lines with genes encoding the above-mentioned enzymes. These enzymes can enhance the resistance of transgenic plants to oxidative stress damage and as a result also enhance resistance to *Cercospora*. The introduction and transfer of the SOD genes to sugarbeet was achieved by means of *Agrobacterium tumefaciens*. SOD genes from tomato, pea and tobacco, which express their activity in various cell compartments, were used for that purpose. The resulting putative transformants were checked for the presence of the introduced genes by PCR and southern hybridization analyses. Lastly, the genetically transformed plants were tested for their tolerance to a series of oxidative stresses and increased resistance was observed.

Parasitism of *Sclerotinia sclerotiorum* (Lib.) de Bary by *Coniothyrium minitans* (Campbell). D. Aggelaki[1], V. Tachmatzidou[2] and F. Eleftheriadou[2]. [1]*Aristotelian University of Thessaloniki, Faculty of Agriculture, Plant Pathology Laboratory, P.O. Box 269, 540 06 Thessaloniki, Greece.* [2]*Technological Educational Institution of Thessaloniki, Department of Plant Production, Laboratory of Phytopathology, P.O. Box 14561, 541 01 Thessaloniki, Greece.*

The fungus *Sclerotinia sclerotiorum* was isolated from carrots with soft rot symptoms. In cultures on PDA, as soon as sclerotia formed, they became covered with pycnidia belonging to the species *Coniothyrium minitans,* a well-known parasite of sclerotia, mainly those of the genus *Sclerotinia*. Pycnidia covered the surface of the sclerotia, while hyphae continued to grow into the rind and the medulla, causing plasmolysis. In dual cultures, sclerotia formed near the contact zone and, after a while, were parasitized by *C. minitans*. *S. sclerotiorum* continued to grow even though its hyphae were severely parasitized. Entrance and growth of *C. minitans* inside the hyphae of *S. sclerotio-*

rum, aggregation of the protoplast, vacuolation and degradation of cells were observed. *C. minitans* has been reported as a parasite of sclerotia in 29 countries. This is the first report of this biological relation in Greece.

The effect of benomyl and *Trichoderma koningii* (Oud.) on the post harvest infection of carrots by *Sclerotinia sclerotiorum* (Lib.) de Bary. D. Aggelaki and K. Tzavella-Klonari. *Aristotelian University of Thessaloniki, Faculty of Agriculture, Plant Pathology Laboratory, P.O. Box 269, 540 06 Thessaloniki, Greece.*

Sclerotinia sclerotiorum was assessed for its ability to infect carrots immersed in a solution of benomyl or in a suspension of the parasite *Trichoderma koningii* before storage at 10°C. When the carrots were immersed in a benomyl solution at a concentration of 0.5 g/500 ml water for 2 min, the percentage of infected carrots was only 22.4%, while that of the control was 88.3%. In low-dosage benomyl (0.25 g/500 ml water) or in 500 ml suspension of *T. koningii* at a concentration of 10^6 conidia/ml, protection was high, and growth of the pathogen was 56.2 and 64.2% respectively. When the carrots were first immersed in low-dosage benomyl and 1 h later in the suspension of *T. koningii*, the percentage of infection was 61%. The growth of the parasite was restricted only to the surface of the carrots and especially to the senescent parts.

The effect of carbendazim and *Trichoderma koningii* (Oud.) on infection of carrot seedlings by *Sclerotinia sclerotiorum* (Lib.) de Bary. D. Aggelaki and K. Tzavella-Klonari. *Aristotelian University of Thessaloniki, Faculty of Agriculture, Plant Pathology Laboratory, P.O.Box 269, 540 06 Thessaloniki, Greece.*

An isolate of *Trichoderma koningii* was evaluated for its ability to suppress infection of carrot seedlings by *Sclerotinia sclerotiorum* when applied alone or in combination with a low dosage of the fungicide carbendazim. Application of carbendazim at the recommended dose of 1 g/l water was the most effective, as percentage of infected seedlings was only 46%. However, at low dose (0.5 g/l water) this fungicide was not effective, as infection (84%) was close to that of the control (88%). When *T. koningii* was applied alone (2 g of wheat bran culture/25 seedlings) or in combination with a low dosage of carbendazim, infection rates were 72 and 76% respectively. This infection was significantly lower than the control (88%). Hyphae and spores of *T. koningii* were observed microscopically only on the surface of the seedlings. The application of *T. koningii* alone or in combination with a low dose of carbendazim reduced seedling infection by the pathogen.

Biological control of *Fusarium oxysporum* f.sp. *dianthi* using non-pathogenic strains of *F. oxysporum*. K. Elena, E.J. Paplomatas and C. Lampropoulos. *Benaki Phytopathological Institute, Plant Pathology Department, 8, S. Delta Street, 145 61 Kifissia Athens, Greece.*

Fusarium wilt of carnation, caused by *Fusarium oxysporum* f.sp. *dianthi* (*Fod*) (Prill. & Del.) Snyder & Hansen, is the most serious disease of carnation worldwide. The objective of this study was to test two non-pathogenic strains of *Fusarium oxysporum* (*Fo*) for the biological control of *Fod*. Two *Fod* isolates, F188 and F275 and two (*Fo*) strains, $F4_3$ and 618-12 B17 were used. Rooted cuttings of the carnation cv. White Sim, susceptible to the disease, Kastellaro 3038, semisusceptible, and Pallas 6056, resistant, were inoculated with *Fod*. The biological agents $F4_3$ and 618-12 B17 were added to the soil after planting, while the pathogens F188 and F275 were drenched 26 days later. To estimate the populations of the pathogen and antagonist, soil samples near the roots and plant samples were taken at the end of the experiment. Symptoms of the disease were also recorded during the experiment. Strain F188 had lost its virulence to carnation plants and did not cause symptoms on any of the three varieties tested. Strain F275 caused severe symptoms on the susceptible cv. White Sim 2002. Strain $F4_3$ suppressed the disease (only one wilted plant was observed) and reduced the pathogen's propagules by 50%. Strain 618-12 B17 caused the highest percentage of disease suppression (no symptoms on plants) and reduced *Fod* propagules by 86%. Similar results were obtained on the other cultivars. When both the pathogen and the biological factors were added, the colonization of soil or plant tissues by the pathogen was less than when the pathogen was applied alone.

Control of Fusarium wilt of watermelon in Cyprus with soil solarization in combination with ammonium fertilisers and low doses of methyl bromide fumigation. N. Ioannou[1], C. Poullis[2] and J.B. Heale[3]. *[1]Agricultural Research Institute, Nicosia, Cyprus. [2]Department of Agriculture, Nicosia, Cyprus. [3]King´s College, University of London, U.K.*

Fusarium wilt of watermelon, caused by *Fusarium oxysporum* f.sp. *niveum*, is a limiting factor for watermelon production in Cyprus. Efforts to control the disease by strip application of soil solarization or methyl bromide fumigation gave unsatisfactory results. The main objective of the present study was to improve the effectiveness of soil solarization through various modifications of the basic technique and combinations with other methods, including: a) increasing the width of the solarization strip from 1 to 2 m, b) solarization with a double rather than single polyethylene sheet, c) addition of ammonium fertilizers in the soil followed by solarization, and d) combined treatments of soil solarization in July-August, followed by methyl bromide fumigation in March, just before planting. The addition of ammonium fertilizers significantly improved the level of wilt control and increased yield by 40-140% over that obtained with solarization alone. A low dose of ammonium (120 kg/ha) was as effective as a high dose (240 kg/ha), but urea was ineffective. Similar results were also obtained with the combined soil solarization - methyl bromide fumigation treatments, which increased marketable yield by 42-90% over that of soil solarization alone. The half-dose of methyl bromide (40 g/m^2) was as effective as the normal rate used by growers (80 g/m^2).

A metabolite of *Drechslera avenae* with fungicidal action. M. Kastanias and M. Chrysayi-Tokousbalides. *Agricultural University of Athens, Pesticide Science Laboratory, 75 Iera Odos, 118 55 Athens, Greece.*

A pathotype of *Drechslera avenae* was isolated from naturally infected plants of *Avena sterilis*. It is a host-specific pathogen and in culture produces secondary metabolites with bioactivity. A fungitoxic metabolite was isolated from cultures of the fungus, purified through sequential chromatography systems and characterized by GC-MS, NMR, UV and IR spectroscopy. It was a red anthraquinone pigment and could be detected in agar cultures incubated for one week, but for maximum production 1-2 months were required. The highest yield was obtained at relatively high temperatures (22-27ºC). It inhibited the growth of *Sclerotinia minor* (ED_{50} 4.3 µg/ml), *S. sclerotiorum* (ED_{50} 5.5 µg/ml), *Verticillium dahliae* (ED_{50} 11.5 µg/ml) and *Botrytis cinerea* (ED_{50} 5.25 µg/ml). At concentrations up to 15 µg/ml, this compound, was not fungitoxic to *Ustilago maydis, Saccharomyces cerevisiae, Sclerotium rolfsii, Gibberella fujikuroi, Rhizoctonia solani* or *Rhizopus* sp. When bioassayed, this *D. avenae* metabolite was not phytotoxic to *A. sterilis*.

Chitin deacetylase gene cloning in sugarbeet for the development of sugarbeet lines resistant to the fungus *Cercospora beticola*. P. Madesis[1], A. Xristodoulidou,[2] N. Panopoulos[2] and A. Tsaftaris[1]. [1]*Aristotelian University of Thessaloniki of Thessaloniki, Department of Genetics and Plant Breeding, 540 06 Thessaloniki, Greece.* [2]*Institute of Molecular Biology, Heraklion, Crete, Greece.*

Greece is mainly an agricultural country, but diseases dramatically lower profit. Genetic engineering is a new technology that incorporates new genes from alien organisms into the plant genome. One of the strategies followed for the control of *Cercospora* and maybe other fungal diseases, is alien gene transfer into plants and with this the incorporation of the chitin deacetylase gene. Chitin deacetylase is the enzyme that catalyses the formation of chitosan from chitin by hydrolyzing the N-acetamido groups of N-acetyl-D-glucosamine residues in chitin. We suppose that this enzyme gives resistance to *Cercospora* and other fungi. Chitosan induces plant defense reactions, such as the formation of phytoalexins and lignin or the deposition of callose. This enzyme is also thought to add general resistance against organisms containing chitin in their body. The aim of this work was to transform plant explants via *Agrobacterium tumefaciens* with two chitin deacetylase enzymes. Chitin deacetylase genes were isolated from the fungus *Mucor ruxii* and from yeast *Saccharomyces cerevisae*.

The biological effect of three *Trichoderma* species on *Rhizoctonia solani*. C.T. Papagiannouli[1], F. T. Gravanis[1] and P. Jenkinson[2]. [1]*T.E.I. of Larissa, Department of Plant Production, 411 10 Larissa, Greece.* [2]*Harper Adams Agricultural College, Newport, Shropshire, TF10 8NB, UK.*

Three species of the genus *Trichoderma* and *Rhizoctonia solani* were cultured onto PDA in dual and volatile plates. Replicate plates were incubated at 5, 10, 15 and 20 °C. Five replicates per treatment were used. In dual culture, at 5 and 20°C, the growth rate of *R. solani* was retarded only by *Trichoderma harzianum*, but at 10 and 15°C it was retarded by all three *Trichoderma* species tested. In volatile culture, *T. harzianum* and *T. viride* retarded the growth rate of *R. solani* at all temperatures tested (5, 10, 15 and 20°C), but *T. polysporum* only at 15 and 20°C. The results indicate that *Trichoderma* spp. have a complex biological effect on *R. solani*, due to the production, even at low temperatures, of both soluble and volatile substances.

Effect of clear polyethylene mulch on grafted cucumber crop production and disease frequency caused by aerial infections of *Fusarium oxysporum* f. sp. *radicis-cucumerinum*. G.Ch. Pavlou. *National Agricultural Research Foundation, Agricultural Research Station, 722 00 Ierapetra, Crete, Greece.*

The present experiment was conducted during the 1997-98 crop season at the Agricultural Research Station of Ierapetra in an unheated plastic greenhouse on soil artificially contaminated with *Fusarium oxysporum* Schlechtend.: Fr. f. sp. *radicis-cucumerinum* D.J. Vakalounakis. Cucumber plants (Brunex F1) grafted on rootstock TZ-148 F1 *(Cucurbita maxima* x *C. moschata*, Tezier, France) were transplanted to be subjected to the following three soil treatments with four replications arranged in a complete randomized block design: (a) clear polyethylene mulch, (b) no mulch (control) and (c) no mulch, with the lower plants' stem and grafting union wrapped in a plastic tube 3 cm in diameter and 20 cm long to prevent contact with contaminated soil, including contact by the air-borne route. Results showed that: 1) total cucumber production of treatment (a) was significantly higher than that with treatment (c), while between treatments (a) and (b) there was no significant difference. 2) In April 1998 (a month before the end of the season), disease frequency caused by aerial infections was low (12.5%) with treatments (a) and (b), and high (56%) with treatment (c). The different results with treatment (c) was due to the infection of the aerial roots that developed on the wrapped cucumber stems above the graft union.

Preliminary study on the control of root and stem rot of cucumber (*Fusarium oxysporum* f.sp. *radicis-cucumerinum)* by lettuce cultivation and incorporation into the soil prior to cucumber cultivation (allelopathy). G.Ch. 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 00 Heraklion, Crete, Greece.*

To investigate whether allelopathy, by means of lettuce cultivation and incorporation into the soil prior to cucumber cultivation, could be used as a biological control method against root and stem rot of cucumber caused by *Fusarium oxysporum* Schlechtend.: Fr. f.sp. *radicis-cucumerinum* D.J. Vakalounakis (FORC), a preliminary experiment was conducted during the 1997-98 crop season at the Agricultural

Research Station of Ierapetra, Lasithi, Crete. Lettuce plants cv. Corsica, were grown in an unheated plastic greenhouse on soil previously disinfected with methyl bromide. When they were at the full development stage they were incorporated into the soil with a milling-machine and the soil was artificially contaminated with FORC. One day later, cucumber plants (Brunex F1) were transplanted to this soil. Four treatments were carried out in a complete randomized block design: (a) lettuce plus FORC, (b) only FORC, (c) only lettuce, and (d) no lettuce and no FORC. The results showed that lettuce cultivation and incorporation into the soil prior to cucumber cultivation reduced disease incidence in contaminated soil from 50 to 25% in the first four months of growth, with no effect on yield. It is concluded that allelopathy by means of lettuce cultivation and incorporation into the soil prior to cucumber cultivation may contribute to the control of root and stem rot of cucumber in an integrated disease management system.

Parasitism of *Sclerotium rolfsii* Sacc. sclerotia by the fungus *Trichoderma koningii* Rifai. P.C. Tsahouridou and C.C. Thanassoulopoulos. *Aristotelian University of Thessaloniki of Thessaloniki, Faculty of Agriculture, Plant Pathology Laboratory, P.O. Box 269, 540 06 Thessaloniki, Greece.*

From 24 isolates of various *Trichoderma* species, a particular *Trichoderma koningii* isolate, found to parasitize the mycelium of *Sclerotium rolfsii* and to produce metabolites that check mycelial growth of the latter *in vitro,* was chosen as a possible agent of colonization and degradation of pathogen sclerotia. Three different *Trichoderma* preparations were evaluated for their ability of *in vitro* colonization of *S. rolfsii* sclerotia: 1) conidial suspension, 2) mycelial fragment suspension, and 3) *Trichoderma*-colonized wheat bran. In all cases, sclerotium samples were periodically collected and examined for their viability and colonization by *T. koningii* on a selective PDA medium amended with kanamycin. Sections of sclerotia showing different stages of degradation were examined under the light microscope. Hyphae and chlamydospores of *T. koningii* were observed inside the sclerotia, and conidia on the outside. Degraded sclerotia were soft and empty, and disintegrated under slight pressure. The three different preparations of the biocontrol agent differed significantly in their effect on the germinability of *S. rolfsii* sclerotia. While the preparation with the *Trichoderma* wheat bran showed lower percentages of sclerotial colonization, it seemed to be the most effective in degrading sclerotia, evidently because of its higher production of hydrolytic enzymes, antibiotics and/or other toxic metabolites at the *Trichoderma* growth medium-sclerotium interface. Penetration ability though important is not the only property required for *Trichoderma* isolates to be efficient biocontrol agents.

Biological control of *Sclerotium rolfsii* Sacc. with *Trichoderma* spp. P.C. Tsahouridou and C.C. Thanassoulopoulos. *Aristotelian University of Thessaloniki of Thessaloniki, Faculty of Agriculture, Plant Pathology Laboratory, P.O.Box 269, 540 06 Thessaloniki, Greece.*

The activity *in vitro* of 24 isolates of *Trichoderma* spp. against *Sclerotium rolfsii* was studied in dual cultures. Similarities and differences in the antagonistic ability of various *Trichoderma* isolates were found to fit into one of the following three interactive categories: 1) the development of *Trichoderma* spp. beyond the contact zone and onto the colony of *S. rolfsii*, while growth of the latter was checked by the contact zone. In some cases growth of the pathogen was checked before any mycelial interaction between the two fungi occured. 2) *Trichoderma* spp. checked the mycelial growth of *S. rolfsii* but the pathogen produced aerial "rhizomorphs" that grew across *Trichoderma*. 3) the mycelium of *S. rolfsii* became dense and overgrew the *Trichoderma* mycelium. Depending on the *Trichoderma* isolate, there was deterioration of hyphal cells of the pathogen (plasmolysis, hyphae breaking up at septa, empty hyphal cells), coiling of *Trichoderma* spp. hyphae around *S. rolfsii* hyphae as well as hyperparasitism after direct hyphae penetration, evidently due to the action of extracellular enzymes of *Trichoderma* spp. and the digestion of the cell walls of *S. rolfsii*. In particular, one *T. koningii* isolate was found to form chlamydospores inside hyphae of *S. rolfsii*.

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