

## ABSTRACTS



### Summaries of papers and posters presented at the Eighth Hellenic Phytopathological Congress Heraklion, Crete, Greece, October 22-24, 1998

The 8th National Phytopathological Congress, organized every two years by the Hellenic Phytopathological Society (HPS), was held in Heraklion, Crete, October 22-24, 1998 and it was attended by 400 participants. At this meeting, 44 oral presentations and 55 posters were presented dealing with plant diseases caused by fungi, bacteria, viruses, non-parasitic disorders and disease control. Moreover, two round table discussions were held. The first was on “Modern Methods for Disease Diagnosis and Identification of Plant Pathogens” and the second on “New Groups of Fungicides. Advantages over Older Compounds”. Also, a special session was held for review and discussion about the poster presentations. Abstracts of the papers and posters of this congress are presented in this issue.

#### Fungal diseases

**Metabolites of *Phoma sepium* f. sp. *arvensis* responsible for pathogenesis in *Convolvulus arvensis*.** M. CHRYSAYI-TOKOUSBALIDES. *Agricultural University of Athens, Pesticide Science Laboratory, 75 Iera Odos, 118 55 Athens, Greece.*

Seedlings of *Convolvulus arvensis* inoculated with a spore suspension of *Phoma sepium* f. sp. *arvensis* develop severe stem dieback and pinpoint spots on the leaves. Leafy stem cuttings of *C. arvensis* show similar symptoms of phytotoxicity after being half-immersed in filtrates or extracts from cultures of the fungus. More than one metabolite produced in culture seems to be responsible for the production of the phytotoxicity symptoms. The phytotoxic metabolites are of polar nature, high molecular weight and rather selective to species of *Convolvulus*. Stem cuttings of other weed or crop species exposed to filtrates from cultures of the fungus did not develop any symptoms of phytotoxicity.

**Phytotoxic metabolites produced by *Alternaria alternata* and their mode of action in sunflower pathogenesis.** A.L. LAGOPODI<sup>1</sup>, C.C. THANASSOULOPOULOS<sup>1</sup> and M. LIAKOPOULOU-KYRIAKIDOU<sup>2</sup>. <sup>1</sup>*Aristotelian University, Faculty of Agriculture, Plant Pathology Laboratory, P.O.Box 269, 540 06 Thessaloniki, Greece.* <sup>2</sup>*Aristotelian University, Faculty of Chemical Engineering, 540 06 Thessaloniki, Greece.*

*Alternaria alternata* produces host specific or non-host specific toxins in sunflower, the role of which has not been clarified yet. Phytotoxic metabolites from *A. alternata* produced in Richard's medium were extracted by a series of chromatographic methods and were divided into two categories. To the first category, water soluble substances which caused a strong phytotoxic effect on plant tissues at concentrations of Richard's medium were assigned. These substances, mainly of low molecular weight, remained in the main body of Richard's solution even after extraction with various organic solvents. Phytotoxic metabolites extracted with organic solvents such as ethyl acetate were in the second category. These substances show their toxicity after concentration of the organ-

ic layer to a minimum volume. Two phytotoxic metabolites from the second category were isolated by various chromatographic procedures. One of them was found to be the tetrapeptide Ser-Val-Gly-Glu to which the name AS-I (Alternaria Sun-toxin I) was given. The fact that these two metabolites at the concentration tested were toxic only to sunflower and not to other plants tested suggested that they were host-specific toxins.

**Growth rate of *Botrytis cinerea* (Pers.) under low O<sub>2</sub> and high CO<sub>2</sub> concentrations at temperatures 0 and 10°C, *in vitro* on PDA and *in vivo* on kiwi-fruit.** N. ΝΙΚΛΙΣ<sup>1</sup>, C. ΘΑΝΑΣΣΟΥΛΟΠΟΥΛΟΣ<sup>2</sup> and E. ΣΦΑΚΙΟΤΑΚΙΣ<sup>3</sup>. <sup>1</sup>*Regional Center of Plant Protection and Quality Control, Thessaloniki.* <sup>2</sup>*Aristotelian University, Laboratory of Plant Pathology, 540 06 Thessaloniki, Greece.* <sup>3</sup>*Aristotelian University, Laboratory of Pomology, 540 06 Thessaloniki, Greece.*

A major problem in post-harvest handling of kiwi fruits is infection with *Botrytis cinerea* (grey mold). In the present work the influence of low O<sub>2</sub> and CO<sub>2</sub> short-term exposure to fungus growth was studied. Petri dishes with PDA were inoculated with *Botrytis* and placed in the following atmospheres: 21% O<sub>2</sub> (control), 0.5% O<sub>2</sub>, 0.25% O<sub>2</sub>, 100% N<sub>2</sub> and 30% CO<sub>2</sub> at temperatures of 0 and 10°C for 2, 4, 8 and 12 days. Dishes were then transferred to normal air conditions at 18°C for 10 days. Kiwi fruits were punctured and inoculated at the stem end with mycelium disks of the fungus on PDA and placed under the same conditions. Immediately after inoculation the kiwi fruits were transferred to 0°C for a number of days, so that the total growth period was 30 days. The *in vitro* growth of the fungal colony in 100% N<sub>2</sub> and 30% CO<sub>2</sub> was reduced by 33 and 66% respectively, while kiwi fruit infection was reduced by 80 and 65% respectively. Under the same conditions of low O<sub>2</sub> and high CO<sub>2</sub> at 10°C, fungal growth *in vitro* was reduced by 33 and 0% respectively. The concentrations of 0.25 and 0.5% O<sub>2</sub> had no significant effect on fungal growth *in vivo* or *in vitro*.

**Damping-off and stunting of sugar beet seedlings caused by *Aphanomyces cochlioides*.** D. LASCARIS<sup>1</sup> and K. DOULIAS<sup>2</sup>. <sup>1</sup>*Benaki Phytopathological Institute, 8 S. Delta Str., 145 61 Kifissia, Athens, Greece.* <sup>2</sup>*Hellenic Sugar Industry, Factory of Orestias, Orestias, Greece.*

Diseased sugar beet seedlings from the area of Serres and Evros in Northern Greece, showing damping-off or stunting symptoms, with black discolouration and collapse of the hypocotylar cortex, were examined in order to detect the cause of the damage. The presence of oospores, belonging to a phytomycetous fungus, was observed during the microscopic examination of the damaged plant tissues. However, the fungus was not isolated with common techniques, due to the rapid growth of other fungi, especially those belonging to *Fusarium* spp. When the diseased tissues were incubated in sterile distilled water, sporangia with masses of encysted zoospores, characteristic of the genus *Aphanomyces*, were formed. The fungus was isolated in a semi-selective medium containing thiophanate methyl, iprodione, chloramphenicol and metalaxyl and was identified as *A. cochlioides* on the

basis of morphological characteristics. Sugar beet seedlings, grown in perlite in the glasshouse and inoculated with a suspension of motile zoospores of the isolated fungus, showed damping-off symptoms or severe hypocotyl damage. *A. cochlioides* was re-isolated from the hypocotyls and roots of these seedlings. Soil samples from six fields from the Evros area, naturally infested with *A. cochlioides*, were sown with sugar beet seeds and kept saturated in the glasshouse at 25-27°C. *A. cochlioides* was isolated from diseased seedlings grown in all the above six soil samples. Although this is the first record of *A. cochlioides* in Greece, it seems that the fungus is widespread and probably has been present in Greek soils for several years, since characteristic symptoms of the disease have been observed in several areas in the past. However, due to isolation difficulties, the fungus had not been detected so far. Four sugar beet cultivars, Rizor, Maribo Ultramono, Kaweduca and Turbo were susceptible to the pathogen when tested in the glasshouse.

**Studies on pathogens causing damping-off of sugar-beet seedlings and on the effectiveness of fungicides as seed coats.** K. DOULIAS<sup>1</sup>, I. VLOUTOGLOU<sup>2</sup>, K. ELENA<sup>2</sup>, E.J. PAPLOMATAS<sup>2</sup> and D. LASCARIS<sup>2</sup>. <sup>1</sup>*Hellenic Sugar Industry, Factory of Orestias, Orestias, Greece.* <sup>2</sup>*Benaki Phytopathological Institute, 8 S. Delta Str., 145 61 Kifissia, Athens, Greece.*

Damping-off of sugar-beet seedlings, one of the most serious fungal diseases of sugar-beet crops in Greece, appears in two plant growth stages: a) early, as seed rotting or damping-off of the emerged seedlings, and b) later (growth stage 2-8 leaves), as a crown dry rot resulting in a characteristic shrivelling of the hypocotyledon. Although in this latter case several plants survive, they appear stunted. A great number of diseased plant specimens, originating from areas grown with sugar-beet in Evros district (North East Greece), were examined in the Benaki Phytopathological Institute during 1995 and 1996 for identification of the causal pathogens. Results showed that the damping-off symptoms were mainly caused by fungi belonging to *Pythium*, *Rhizoctonia*, *Fusarium*, *Alternaria* and *Aphanomyces*. At the same time, an extended net of experimental trials was established by the Hellenic Sugar Industry in order to study the effectiveness of the following fungicides applied as a seed coat: hymexazol, tolclofos-methyl, fludioxonil, PCNB, iprodione, thiram and metalaxyl+mancozeb. Based on the results of these experiments, the most effective fungicides for the control of sugar-beet damping-off are iprodione, hymexazol and PCNB, when they are applied in mixtures with fungicides already in use (i.e. thiram, metalaxyl+mancozeb). Furthermore, thiram proved to be the most effective fungicide in clay soils and the mixture metalaxyl+mancozeb in light soils.

**Species spectrum of powdery mildew on *Abelmoschus esculentus* in Greece.** D.J. VAKALOUNAKIS and E.K. KLIRONOMOY. *National Agricultural Research Foundation, Plant Protection Institute, 711 10 Heraklion, Crete, Greece.*

Powdery mildew is one of the main fungal diseases of okra (*Abelmoschus esculentus* (L.) Moench) worldwide. However, the identity of the pathogens has not been elucidated so far.

To identify the powdery mildew species infecting okra, a survey was carried out in Crete during 1994-95. Samples of diseased plants were collected from 35 different field crops. Monoconidial isolates of powdery mildew (one per sample) were generated and preserved on detached cotyledons of the cucumber cv. Knossos placed on a culture medium in Petri dishes under axenic conditions. For powdery mildew species identification, both the asexual and the sexual stages of the pathogens were used. To produce the sexual stage and determine the mating type, the monoconidial isolates were placed in pairs in all possible combinations on detached cotyledons in Petri dishes with conidia of one isolate smeared in a row opposite a similar line of inoculum of the other isolate and incubated at 21 °C under aseptically conditions. If the two isolates belonged to the same species but to opposite mating types, cleistothecia were produced after about 12 days of incubation. Of the 35 monoconidial isolates of powdery mildew three species were identified: *Sphaerotheca fusca* (Fr.) Blumer emend. U. Braun (= *S. fulginea* (Schlecht. ex Fr.) Poll.) (26 isolates), *Erysiphe orontii* Cast. emend. U. Braun (= *E. cichoracearum* DC. sensu lato) (eight isolates) and *Leveillula taurica* (Lév.) Arnaud (one isolate). In cross-inoculation experiments, using young plants at the four true-leaf stage, monoconidial isolates of *S. fusca* from okra infected okra and cucumber, while monoconidial isolates from cucumber infected cucumber, but not okra. This indicates that *S. fusca* isolates from cucumber and okra belong to different pathotypes. Cross-inoculation experiments with monoconidial isolates of *E. orontii* from okra and cucumber are in progress. All 26 and eight monoconidial isolates of *S. fusca* and *E. orontii* respectively fell into two mating types, indicating that these two pathogens were bipolarly heterothallic.

**Influence of pH of adjusted media on growth of *Rhizoctonia solani* Kühn isolates belonging to different anastomosis groups.** P.N. ROZA. *Aristotelian University, Faculty of Agriculture, Plant Pathology Laboratory, P.O.Box 269, 540 06 Thessaloniki, Greece.*

Isolates of *Rhizoctonia solani* Kühn assigned to different anastomosis groups vary in both morphological and physiological characteristics. This led to the study of the impact of pH of the media on the growth of isolates of the fungus belonging to different anastomosis groups. Three isolates from each of the anastomosis groups AG-2-1, AG-3 and AG-4 were tested on two different media: the liquid medium of Ogoshi & Ui, and solid V-8 agar. Media were adjusted to pH 4.5, 5.5, 6.5 and 7.5 with 1N KOH. As a criterion of growth on the liquid medium the dry weight of the produced colony was used, while on solid medium it was used the radial growth. The dry weight was measured after 7, 14 and 21 days of incubation at 25 °C in the dark, while the radial growth was measured every 24 hours. Three replicates were used and the experiment was repeated twice. The results showed that the growth of isolates belonging to AG-4 group was not affected by the pH of the media, while the growth of those belonging to AG-3 group showed variations on both solid and liquid media. Moreover, in the latter case there was a positive correlation

between pH values and colony growth, with almost no growth at pH 4.5. Isolates of AG-2-1 showed a small increase in colony growth as pH was increased.

**Pyricularia leaf spot of ctenanthe, a new disease of Marantaceae.** A.C. PAPPAS<sup>1</sup> and E.J. PAPLOMATAS<sup>2</sup>. <sup>1</sup>*University of Thessaly, Laboratory of Plant Pathology, Pedion Areos, 383 34 Volos, Greece.* <sup>2</sup>*Benaki Phytopathological Institute, 8 S. Delta Str., 145 61 Kifissia, Athens, Greece.*

In June 1995 in a glasshouse of Magnissia county, a new leaf spot on young plants of *Ctenanthe oppenheimiana* and *C. setosa* imported into Greece from the Netherlands, but originating in Brasil, was observed. The disease was observed again on adult plants of *C. oppenheimiana* in a glasshouse of Chania county in May 1996. The primary symptoms on young leaves consisted of individual circular to slightly irregular pinpoint spots with white necrotic centres surrounded by brown-yellow halos. On mature leaves extended necrotic areas were formed usually concentrated on the periphery of the leaf blade and resembling those caused by phytotoxicity. From the diseased spots the fungus *Pyricularia oryzae* Cav. was isolated on PDA. Artificial inoculations on leaves of 14 different plant species of the family Marantaceae were made to evaluate the pathogenicity and host range of fungal isolates from ctenanthe and rice plants. Spore suspensions ( $5 \times 10^4$  ml<sup>-1</sup>) from 13-day-old cultures on PDA were used as inoculum. *Ctenanthe oppenheimiana*, *C. setosa*, *Stromanthe sanguinea*, *S. amabilis*, *Maranta leuconeura* and *Calathea ornata* were found to be very susceptible to infection by *P. oryzae* isolated from *C. oppenheimiana*. By contrast, the fungal isolates from rice exhibited reduced virulence on the plant species tested. Analysis of esterase and lactate dehydrogenase isozymes showed different banding patterns between the rice and ctenanthe isolates of *P. oryzae*. This suggests the presence of races with physiological specificity that cause serious damage to various plants within the family Marantaceae.

**Studies on pathogenicity of Greek isolates of *Verticillium dahliae* on cotton, tomato and watermelon.** E.J. PAPLOMATAS, E. VLOUTOGLOU and C. LAMPROPOULOS. *Benaki Phytopathological Institute, 8 S. Delta Str., 145 61 Kifissia, Athens, Greece.*

The pathogenicity of *Verticillium dahliae* isolates from diseased cotton, tomato and watermelon plant samples from Peloponnese, Sterea Hellas and Thessali was studied. Twenty-nine out of 78 cotton isolates and all tomato and watermelon isolates (19 and 17 respectively) were tested. The pathogenicity of isolates was tested not only on their host of origin but also on the other two hosts. Single-spore isolates were produced from all fungal cultures. Tomato cultivar Early Pak No. 7, susceptible to races 1 and 2 of *V. dahliae*, and cv. Ace 55 VF, resistant to race 1 of the fungus, cotton cv. Zeta 2 and watermelon cv. Sugar Baby, both susceptible to the pathogen, were used for the pathogenicity tests. Results showed that cotton isolates were very virulent on watermelon and cotton, while they were not pathogenic or caused moderate wilt symptoms on tomato. All tomato isolates were patho-

genic on susceptible tomato cv. Early Pak No. 7, while only five of them caused symptom development on resistant cv. Ace 55 VF. Moreover, they were all very virulent on watermelon and cotton. The reactions with the tomato isolates were similar to those of the watermelon isolates on tomato, watermelon and cotton. Specifically, all watermelon isolates tested were pathogenic on cv. Early Pak No. 7 but only six on resistant cv. Ace 55 VF. The presence of the pathogen was verified on artificially inoculated tomato plants with or without wilt symptoms after reisolation of the fungus from the plant stem at 5, 10 and 20 cm above ground. The pathogen was re-isolated from cv. Early Pak No. 7 at all three heights, while from cv. Ace 55 VF only at 5 and 10 cm. It is however noteworthy that one *V. dahliae* isolate belonging to race 2 and used as a positive control was re-isolated from a stem height of 20 cm as well.

**Relativeness of vegetative compatibility groups of *Verticillium dahliae* isolates from different hosts.** K. ELENA and E.J. PAPLOMATAS. *Benaki Phytopathological Institute, 8 S. Delta Str., 145 61 Kifissia, Athens, Greece.*

Thirty Greek isolates of *Verticillium dahliae* from different hosts and areas were tested through vegetative compatibility groups (VCGs). A relationship between VCGs and the taxonomic position of the host from which the strains were isolated (olive, tomato, cotton, pistachio, potato, melon, dimorphotheca and artichoke) was also investigated. Complementation tests among the Greek strains and between these strains and U.S.A. testers of four groups (1, 2, 3 and 4) were carried out. Nitrate non-utilizing (*nit*) mutants were generated from all isolates. The *nit* strains were identified as *nit1*, *nit3* or *NitM* depending on their ability to grow on nitrate, nitrite or hypoxanthine medium. Only a few *nit3* strains were obtained. Eighteen Greek isolates belonged to two different groups, the nondefoliating cotton strains of *V. dahliae* were classified in group 2 (U.S.A.) while strains from solanaceous hosts were assigned to group 4 (U.S.A.). The isolates 2V, 5V, 6V, 13V, 15V, 16V, 18V, 23V, 24V, 27V and 29V did not form heterokaryons with the U.S.A. testers or with any Greek isolate or among one another. The 21V isolate did not give *NitM* mutants and the *nit1* mutants did not form heterokaryons with the testers. In this study the *V. dahliae* isolates from the same host did not belong to the same VCG but groups 2 and 4 included strains from different hosts. This study showed that *V. dahliae* is characterized by a wide genetic diversity.

**A study of a Greek population of the fungus *Fusarium oxysporum* f. sp. *dianthi* using molecular markers.** E.J. PAPLOMATAS and K. ELENA. *Benaki Phytopathological Institute, 8 S. Delta Str., 145 61 Kifissia, Athens, Greece.*

*Fusarium* wilt caused by the fungus *Fusarium oxysporum* f. sp. *dianthi* is the most serious disease of carnation. Since there is no effective chemical control of the disease, *Fusarium* wilt of carnation is managed in practice by soil fumigation and by using healthy planting material and resistant varieties. Since several races of the pathogen, able to attack various carnation cultivars, have been recognized, knowl-

edge of the population structure of the fungus is necessary in order to develop control strategies of the disease. In a previous research effort, we studied the genetic relatedness of a number of *Fusarium oxysporum* f. sp. *dianthi* isolates using vegetative compatibility groups (VCGs). It was found that the Greek population of the fungus belonged to only one VCG known as 0021 worldwide. The goal of this research was to further study this population using molecular markers. For this purpose we obtained from the Plant Protection Institute at Wageningen, the Netherlands (IPO-DLO), a DNA probe able to differentiate between the various VCGs of *Fusarium oxysporum* f. sp. *dianthi* by restriction fragment length polymorphism (RFLP) analysis. Total genomic DNA from representative isolates of the fungus was isolated and after digestion with the endonuclease *Hind* III and electrophoresis on an agarose gel, was transferred to nylon membranes (Southern blotting). Hybridization with the above probe revealed a similar DNA profile for all the isolates. Moreover, this profile was identical with the RFLP profile characteristic of VCG 0021. The fungal population was further studied using random amplified polymorphic DNA (RAPD) analysis. For this purpose, 10 oligonucleotide decamers were evaluated as random primers for the polymerase chain reaction (RAPD-PCR). Among them, a primer with the DNA sequence 5'-ACAGGGCTCA-3' produced a polymorphic banding pattern when the *Fusarium oxysporum* f. sp. *dianthi* isolates were analyzed by RAPD-PCR. This analysis showed that all the isolates tested had an identical electrophoretic DNA profile. From all the above it is evident that the Greek population of the fungus of *Fusarium oxysporum* f. sp. *dianthi* is homogeneous and belongs to only one group.

**Screening of several chestnut provenances for resistance to attack by *Cryphonectria (Endothia) parasitica*.** S.G. XENOPOULOS. *National Agricultural Research Foundation, Institute of Mediterranean Forest Ecosystems, Terma Alkmanos, 115 28 Athens, Greece.*

A study to find chestnut clones resistant to the fungus *Cryphonectria (Endothia) parasitica* (Murr.) Barr. was made by artificial inoculation of the fungus on 500 five year-old seedlings of *Castanea sativa* in the Pillion region, originating from 13 different Greek provenances. Some seedlings of *C. mollissima* and a hybrid of *C. mollissima* × *C. dentata* from America were included for comparison. The results show that all the Greek provenances were susceptible to the fungus. A high percentage of the inoculated trees died in the 5 months after inoculation, and the rest in late spring/early summer of the following year. The seedlings of *C. mollissima* and the hybrid were resistant, since they developed smaller cankers most of which closed within 1 or 2 years. Among the Greek provenances there was small differentiation as regards their degree of susceptibility. The provenances of Lamia, Mitilini, Arnea and Karpenissi were less susceptible from the others, since they developed smaller cankers and had also a smaller percentage of dead trees 5 months after inoculation. A correlation was observed between the percentage of dead trees, the stem diameter at the point of inoculation and mean canker length. Bigger diameters and smaller mean canker length were related to a smaller proportion of dead trees. The high

percentage of dead trees in the short time of 5 months after inoculation is not due to a high susceptibility of the trees, but to favourable conditions for disease development and to unfavourable conditions for seedling development (tree age, exceptionally hot and dry summer), which gave the seedlings no chance to react to infection. These results show that the resistant reaction in most of the naturally infected chestnut coppices, by forming superficial cankers with callus tissue, is not genetic, but is due to an ontogenic predisposition for resistance in the trees during infection.

**Linkage between *Fr1* (*Fusarium oxysporum* f. sp. *radicis-lycopersici* resistance) and *Tm-2* (tobacco mosaic virus resistance-2) loci in tomato (*Lycopersicon esculentum*).** D.J. VAKALOUNAKIS<sup>1</sup>, H. LATERROT<sup>2</sup>, A. MORETTI<sup>2</sup>, E.K. LIGOXIGAKIS<sup>1</sup> and S. SMARDAS<sup>3</sup>. <sup>1</sup>National Agricultural Research Foundation, Plant Protection Institute, 711 10 Heraklion, Crete, Greece. <sup>2</sup>INRA-Avignon, Station d'Amélioration des Plantes Maraîchères, 841 43 Montfavet, France. <sup>3</sup>National Agricultural Research Foundation, Institute of Viticulture and Vegetable Crops, 710 10 Heraklion, Crete, Greece.

Because several tomato (*Lycopersicon esculentum* Miller) lines with either the *Tm-2* gene or the *Tm-2<sup>2</sup>* gene (resistance to several races of tobacco mosaic virus, TMV) are also resistant to *Fusarium oxysporum* Schlechtend.: Fr. f. sp. *radicis-lycopersici* W.R. Javris & Shoemaker, it appears that the *Tm-2* and *Fr1* (resistance to *F. oxysporum* f. sp. *radicis-lycopersici*) loci may be linked. To study this linkage relationship further, a proper genetic analysis was carried out. For this purpose the tomato inbred Motelle, which is susceptible to both pathogens (*[Fr1<sup>+</sup>/Fr1<sup>+</sup>, Tm-2<sup>-</sup>/Tm-2<sup>-</sup>]*) was crossed with the inbred line IRB-301-31, which is resistant to *F. oxysporum* f. sp. *radicis-lycopersici* and certain races of TMV (*[Fr1/Fr1, Tm-2/Tm-2]*). When 222 F<sub>2</sub> plants were self-pollinated, and from each resulting F<sub>3</sub> family different groups of 15-60 seedlings each were tested for resistance to either *Fusarium oxysporum* f. sp. *radicis-lycopersici* or TMV race 0, segregation data indicated a very tight linkage between *Fr1* and *Tm-2*, equal to 2.2±0.67 map units. The evidence for the very tight linkage between *Fr1* and *Tm-2* will be very useful in breeding for resistance, because selection for resistance to one pathogen will simultaneously give resistance to the other pathogen with a very high probability and without a selection procedure.

**Contribution to research on the sexual reproduction of *Pleurotus ostreatus* wild isolates. I.** THEOCHARI-ATHANASIOU. National Agricultural Research Foundation, Fodder crops and Pastures Institute, 411 10 Larissa, Greece.

Sexual reproduction of *Pleurotus ostreatus* is of great interest, as it is the only method used in breeding programmes in order to obtain desirable propagating material of the above mushroom. In our work we studied seven natural populations of *P. ostreatus*, derived from basidiomata, which had been collected from different areas in Greece, and from different tree species. Single-spore cultures obtained from each of the wild isolates, fell into four classes according to their mating type. This proves that mating type is a tetrapolar

heterothallic character. Pairings between homokaryotic progenies of all the wild isolates revealed the existence of many mating-type alleles, for each of the two genes, which control the incompatibility system in the genus *Pleurotus*. The allele distribution is very large so considerable heterogeneity is presumed in our biological material. Such heterogenic material assures a high outbreeding efficiency and is very useful for further breeding work.

**Diurnal and seasonal periodicity in dispersal of *Alternaria linicola* conidia - Spatial and temporal spread of the disease.** I. VLOUTOGLOU<sup>1</sup>, B.D.L. FITT<sup>2</sup> and J.A. LUCAS<sup>3</sup>. <sup>1</sup>Benaki Phytopathological Institute, 8 S. Delta Str., 145 61 Kifissia, Athens, Greece. <sup>2</sup>BBSRC-Rothamsted, Harpenden, Herts AL5 2JQ, UK. <sup>3</sup>IACR-Long Ashton Research Station, Department of Agricultural Sciences, University of Bristol, Long Ashton, Bristol, BS18 9AF, UK.

Dispersal of *Alternaria linicola* Groves & Skolko conidia, a serious pathogen of linseed (*Linum usitatissimum* L.), followed diurnal and seasonal periodicities influenced by weather conditions and the percentage of crop infection. The number of conidia collected by the Burkard spore sampler increased between 8:00 and 12:00 hours, a period which coincided with an increase in temperature and wind speed and a decrease in relative humidity. The greatest number of conidia was collected between 12:00 and 13:00 hours. Liberation and dispersal of conidia occurred only during the day time and after periods of rain or high relative humidity, especially between flowering and harvest (July - September). The number of dispersed conidia within the crop increased with decreasing height above ground and with decreasing distance from the source of their production. The spread of the disease from two different in geometry inoculum sources was studied during the growing season and it was found out that: a) the percentage of infected plants decreased with increasing distance from the inoculum source and b) by the end of the growing season and irrespective of the direction of the prevailing wind, the disease had spread in all directions, but at relatively short distances from the inoculum source (60 m). The spatial and temporal spread of the disease was described by two different mathematical models depending on the geometry of the inoculum source, the weather conditions, the cultural practices and the presence or not of other sources of primary inoculum.

## Virus diseases

**Transmission of grapevine leafroll-associated virus 3 (GLRaV-3) by two mealybug species, *Planococcus ficus* and *P. citri*.** N. IOANNOU, A. HADJINIKOLIS and ARTEMIS HADJINIKOLI. Agricultural Research Institute, Nicosia, Cyprus.

Leafroll is the most widespread virus disease of grapevines in Cyprus, having been detected in virtually all introduced and traditional varieties with an average incidence of about 80% and 45% respectively. Although its etiology is still not fully established, a number of different closteroviruses have been associated with the disease in recent years. In Cyprus,

four different types of GLRaV, designated 1, 2, 3 and 4, have been identified. Of these, the most prevalent appears to be GLRaV-3. The widespread distribution of GLRaV-3 led us to investigate the possibility of its natural spread by an efficient air-borne vector. During 1991-95, virus incidence was monitored by ELISA in four variety collections grown in different areas. Results so far clearly indicate that GLRaV-3 is capable of spreading by means other than infected propagating material and that its rate of spread varies widely, depending on the site, grapevine cultivar and year. The highest rate of spread was noted at Zygi, where average virus incidence increased from 19.7% in 1991 to 67.4% in 1995. Virus incidence was much higher in vinifera varieties than in American rootstocks. The most important source of primary infection appeared to be the greenhouse used for the production of rooted cuttings, which were heavily infested with *Planococcus ficus* and *P. citri* mealybugs. Lighter mealybug infestation was also noted in the field. Transmission tests in the laboratory showed that GLRaV-3 was readily transmitted to healthy grapevine plants by both mealybugs species, but *P. ficus* was a more efficient vector than *P. citri*. The levels of transmission recorded in American rootstock varieties were much lower than those in vinifera varieties. This varietal difference is being pursued further along with other matters regarding virus transmission by mealybugs.

**Production of transgenic tobacco plants resistant to tobacco rattle tobavirus.** F. BEM<sup>1</sup>, D.J. ROBINSON<sup>2</sup>, H. BARKER<sup>2</sup>, B. REAVY<sup>2</sup> and V. PLASTIRA<sup>1</sup>. <sup>1</sup>Benaki Phytopathological Institute, 8 S. Delta Str., 145 61 Kifissia, Athens, Greece. <sup>2</sup>Scottish Crop Research Institute, Dundee, UK.

A construct containing a cDNA copy of the tobacco rattle virus (TRV) 59K polymerase read-through gene, together with a selectable gene for kanamycin resistance, in a binary Ti-based plasmid was prepared and introduced into a disarmed strain of *Agrobacterium tumefaciens*. This construct was used in five attempts to transform Samsun tobacco. In the first two transformation experiments, no satisfactory transformants were obtained. Although a few plants survived selection on kanamycin medium, they were phenotypically abnormal and could not be propagated. In two further attempts, kanamycin resistant plants of normal appearance were obtained. Independent transformed lines were tested, but none was resistant to infection with TRV by mechanical inoculation. Moreover, PCR and Southern blot tests showed that, although the plants contained the kanamycin-resistant gene, they did not contain the TRV 59K gene. In the last transformation experiments, only 5% of the transformants proved to be resistant to infection by mechanical inoculation of TRV and to contain the 59K gene. Very low frequency of transformation was also obtained in other research centres by using comparable constructs, suggesting that this may be a general problem with this class of constructs. Seed of these transformed tobacco lines has been produced by self-pollination, and will be used for testing their resistance to infection by a range of TRV strains and by both mechanical and nematode-mediated inoculation under glasshouse and field conditions.

**Detection of plum pox potyvirus by the molecular immunocapture-polymerase chain reaction technique.** C. VARVERI and K. BOUTSIKA. Benaki Phytopathological Institute, 8 S. Delta Str., 156 61 Kifissia, Athens, Greece.

The highly sensitive immunocapture-polymerase chain reaction (IC-PCR) technique was applied to plum pox potyvirus (PPV) detection. This technique consist of a virus immunocapture step for its purification and concentration before a reverse transcription-polymerase chain reaction amplification. It gives rise to a 243 bp product corresponding to the carboxy terminal region of the coat protein gene, which is a rather conserved part of the genome, thus allowing the detection of a wide range of isolates. The application of IC-PCR with greenhouse samples (GF 305 indicator) allowed a 10,000 fold gain in sensitivity over the ELISA test. To validate IC-PCR effectiveness under field conditions and further compare it with ELISA, 70 leaves not showing PPV symptoms collected from an infected apricot tree were simultaneously tested by both techniques. ELISA gave positive results for 22 samples (31.4%) and IC-PCR for 37 samples (52.8%). In a second application, out of 56 apricot mother-trees giving ELISA readings around the detection threshold, 13 (23.2%) were found infected by IC-PCR. When IC-PCR was combined with RFLP analysis and thus applied as an analytical method to determine the virus group to which 24 Greek isolates belong, it was shown that they were all M-type strains, except one peach isolate from central Greece (Larissa), which gave the characteristic pattern of a D-type strain.

**Occurrence of eggplant mottled dwarf nucleorhabdovirus (EMDV) in tobacco and cucumber crops in Greece.** N.I. KATIS<sup>1</sup>, E.K. CHATZIVASSILIOU<sup>1</sup>, C. CLAY<sup>2</sup>, A. AVGELIS<sup>3</sup>, I. MANOUSSOPOULOS<sup>4</sup> and H. LECOQ<sup>5</sup>. <sup>1</sup>Aristotelian University, Faculty of Agriculture, Plant Pathology Laboratory, 540 06 Thessaloniki, Greece. <sup>2</sup>Horticulture Research International, Warwick, Wellesbourne CV 35 9EF, UK <sup>3</sup>Plant Protection Institute, National Agricultural Research Foundation, 711 10 Heraklion, Crete, Greece. <sup>4</sup>Institute of Molecular Biology and Biotechnology, 711 10 Heraklion, Crete, Greece. <sup>5</sup>INRA, Station de Pathologie Végétale, B.P. 94, 841 43 Montfavet Cedex, France.

In 1993, unusual virus-like symptoms were observed in tobacco plants (*Nicotiana tabacum* L.) of the cv. Myrodata Agriniou in the area of Agrinio (central Greece). In 1994 and 1995, similar symptoms were noticed in the cv. Virginia in the area of Kilkis (Macedonia). Similar symptoms were also recorded in cucumber crops (hybrids Kamaron and Sandra) in Preveza (Epirus) and in Vassilika (Macedonia) in 1993 and 1994 respectively. The main symptoms were severe stunting, leaf curling and crinkling and chlorotic to yellow mottling of the leaves. In cucumbers, fruit deformation was also observed. Diseased plants were mainly in the field margins and the disease incidence was fairly low (0.5-1.5%). Virus identification was based on mechanical inoculation on indicator plants, serology, electron microscopy (decoration against EMDV, cytopathology) and western blot

analysis. The virus was transmitted mechanically from field tobacco and cucumber to a number of test plants: *N. tabacum* L. cv. Samsun, *N. rustica* L., *N. glutinosa* L., *N. clelandii* Gray, *Petunia hybrida* Vilm., *Capsicum annuum* L. cv. Florinis and *Lycopersicon esculentum* Mill cv. Dombó that reacted with chlorotic local lesions followed by systemic vein clearing and leaf crinkling. *Gomphrena globosa* reacted only with reddish local lesions. The host range was similar to that reported for EMDV. Electron microscopy observations of leaf dips revealed bacilliform particles which were decorated with an EMDV antiserum. In ultra thin sections of infected leaves, numerous bacilliform and bullet-shaped particles were observed to accumulate in perinuclear spaces and also in cytoplasmic vesicles. Serological immunodiffusion tests confirmed the identification of our isolates as EMDV. Western blot analysis revealed that our isolates had a pattern similar to that of two German EMDV isolates from eggplant and tomato respectively. Field symptoms were reproduced in tobacco (cv. Myrodata Agrinui and Virginia) and cucumber plants after mechanical inoculation.

**Characterization of tospoviruses in Greece.** E.K. CHATZIVASSILIOU<sup>1</sup>, N.I. KATIS<sup>1</sup>, R.J. WEEKES<sup>2</sup>, J. MORRIS<sup>3</sup>, K.R. WOOD<sup>2</sup> and I. BARKER<sup>3</sup>. <sup>1</sup>Aristotelian University, Faculty of Agriculture, Plant Pathology Laboratory, 540 06 Thessaloniki, Greece. <sup>2</sup>School of Biological Sciences, University of Birmingham, Edgbaston, Birmingham, B15 2TT, UK. <sup>3</sup>Central Science Laboratory, MAFF, Hatching Green, Harpenden, Hertfordshire, AL5 2BD, UK.

Tospoviruses are widespread in Greece, but taxonomical studies are rather limited. In this study, characterization of tospoviruses was based on mechanical inoculation to indicator plants, ELISA and PCR tests. Tospovirus isolates were identified as tomato spotted wilt virus (TSWV), on the basis of serological techniques, using ELISA tests with polyclonal and monoclonal antibodies against the N protein of the virus. Among isolates several differences were observed, which were possibly derived from the existence of various epitopes on the N protein of the virus. Isolates from Drama, Katerini and Xanthi are possibly consisted of different strains of the virus. ELISA results were confirmed by PCR tests of some isolates, using a pair of universal primers, capable of detecting all tospoviruses, and two pairs of TSWV specific primers (TSWV-L, TSWV-CP). Only one isolate, from Kilkis, identified as TSWV in ELISA, reacted very weakly in PCR. The host range of the isolates, that were differentiated in the ELISA and PCR tests was similar to that of typical TSWV isolates. No isolate reacted with antibodies prepared against *Impatiens necrotic spot virus* (INSV). Electron microscopy observation revealed typical spherical particles (diameter 80-110 nm).

**Frequency of transmission of Artichoke Italian latent nepovirus by *Longidorus fasciatus* (Nematoda: Longidoridae) from artichoke fields in the Iria region of Argolis, Peloponnesus, Greece.** D.J.F. BROWN<sup>1</sup>, P.E. KYR-

IAKOPOULOU<sup>2</sup> and W.M. ROBERTSON<sup>1</sup>. <sup>1</sup>Scottish Crops Research Institute, Invergowrie, Dundee, DD2 5DA, Scotland, UK. <sup>2</sup>Agricultural University of Athens, Department of Plant Pathology, Iera Odos 75, 118 55 Athens, Greece.

Artichoke Italian latent nepovirus (AILV), transmitted by *Longidorus fasciatus* is a causative agent of artichoke patchy chlorotic stunting (APCS), a serious disease of artichoke in Argolis, North East Peloponnesus, the main artichoke center in Greece. Populations of *L. fasciatus*, collected from Iria and Candia in Argolis, were used in laboratory experiments to determine the frequency and efficiency of transmission of the virus by its natural vector. One tenth to almost one half of *L. fasciatus* specimens recovered from soil collected in two artichoke fields showing APCS, transmitted AILV. Giving nematodes access for four weeks to herbaceous plants mechanically infected with AILV increased the number of individual specimens able to transmit the virus. However, the total number of specimens transmitting the virus in an experiment did not exceed fifty percent of the individuals tested. Virus-like particles were only observed adsorbed to the inner surface of the odontostyle, and it is suggested that the high frequency of transmission of AILV by *L. fasciatus* is a result of efficient dissociation of virus particles from the specific sites of retention of the virus.

## Biological and integrated control

**Combined control of citrus mal secco by sprays and removal of infected branches.** I. VLOUTOGLOU<sup>1</sup>, E.I. PAPONOMATAS<sup>1</sup>, A. CHITZANIDIS<sup>1</sup> and E.C. TJAMOS<sup>2</sup>. <sup>1</sup>Benaki Phytopathological Institute, 8 S. Delta Str., 145 61 Kifissia, Athens, Greece. <sup>2</sup>Agricultural University of Athens, Plant Pathology Laboratory, 75 Iera Odos, 118 55 Votanikos, Athens, Greece.

Fungicide sprays were applied to lemon trees in combination with removal of infected branches for the control of mal secco disease caused by *Phoma tracheiphila*. The experiment was carried out at the Horticultural Station of Xylocastro (northern Peloponnesus) on lemon trees of the cultivars Maglino and Adamopoulou grafted on sour orange and *Citrus volkameriana*. At the beginning of the experiment the trees were five years old and appeared healthy, whereas those of neighbouring orchards showed disease symptoms. Six sprays were applied between October and May for three consecutive years. The fungicides used were copper oxychloride (0.18% Cu), captafol (0.073% a.i.), imazalil (0.008% a.i.) and maneb (0.21% a.i.) + thiophanate methyl (0.05% a.i.). In July of each year, all the infected branches were removed and the disease intensity was recorded. The number of diseased trees, the number of infected branches per tree and the length of their discoloured sections were assessed. The results showed that there were significant differences among the fungicides used, with copper oxychloride being the most effective, also differing from the unsprayed control. The disease intensity varied significantly between years, being higher on the cv. Maglino and the rootstock *C. volkameriana*.

**Evaluation of Greek isolates of the antagonistic fungi *Talaromyces flavus* and *T. helicus* in controlling *Verticillium* wilt of eggplants.** A. MILA and E.C. TJAMOS. *Agricultural University of Athens, Plant Pathology Laboratory, 75 Iera Odos, 118 55 Votanikos, Athens, Greece.*

The biological control of *Verticillium* wilt of eggplants by the antagonistic activity of a collection of *Talaromyces* spp. isolates, including the well known antagonist *T. flavus* was evaluated. The collection of isolates came from tomato root tips grown in soils where soil solarization had been applied and the species were identified. Fifteen of the isolates belonged to the species *T. helicus*, while another 6 belonged to an unidentified species of the genus *Talaromyces*. All isolates showed inhibitory activity against the pathogenic fungus *Verticillium dahliae* in dual cultures. The strains tested differed in the extent of the inhibition in solid medium and in the ability of inhibition of the pathogen in potato extract liquid medium amended with dextrose, fructose, galactose or sucrose. As for the reduction of disease symptom development from artificially prepared inocula of microsclerotia of the fungus (50 microsclerotia/g soil) incorporated into the soil, the antagonist fungi applied as drench to the roots of eggplants ( $10^6$  ascospores/ml) in greenhouse experiments showed various levels of antagonistic activity. The percentages of final infection ranged from 25-50% for isolates of *T. flavus*, from 25-62.5% for *T. helicus* and from 25-50% for isolates of *Talaromyces* sp. compared with 100% of the control. As for the percentage of diseased leaves some isolates of *T. flavus* (T-1 and T-10), *Talaromyces* sp. (T-4 and T-20) and *T. helicus* (T-2, T-11, T-13 and T-22), were considered particularly effective against *V. dahliae* since they reduced symptoms down to 20% in comparison with 100% for the control. Further evaluation of the selected isolates will be continued in plastic-house experiments.

**A strategy for the biological control of the fungus *Verticillium dahliae*.** E. VELLIOS, E.C. TJAMOS and C.G. PANAGOPOULOS. *Agricultural University of Athens, Plant Pathology Laboratory, 75 Iera Odos, 118 55 Votanikos, Athens, Greece.*

Methods for the biological control of *Verticillium dahliae* were studied. The methods concentrated on the inhibition of microsclerotia formation, the reduction in variability of already formed microsclerotia, or the reduction in germination of microsclerotia at the root tips of eggplants grown in a soil with fungal or bacterial antagonists. Stems of cotton or eggplants from diseased plants, which had been sprayed with a suspension of ascospores of the antagonistic fungus *Talaromyces flavus* or with a bacterial suspension of an isolate from microsclerotia which had lost their viability were used. The stems were incorporated into the soil and maintained for various periods of time under greenhouse or field conditions. Eggplants were grown in special pots for direct observation of microsclerotia germination at a distance of 1 mm from the eggplant root tips. It was shown that, although the antagonists used

remained alive at the tissues (3-15 months after spraying), they did not inhibit microsclerotia formation either on the surface or on the phloem or in the vessels of infected plants. The effects of the antagonists on the germination of already formed microsclerotia were a lower percentage of germination and reduction in the diameter of the colonies of microsclerotia sampled from eggplant stems. In cotton stems only the colony size was affected by the antagonists. As for the effect of the antagonists on the germination of microsclerotia, in preliminary experiments it was shown that *T. flavus* reduced germination by 15-40% in comparison with the control, whereas an isolate of *Bacillus* sp. reduced germination by up to 50-60%.

**Control of *Fusarium oxysporum* f. sp. *cucumerinum* and *Clavibacter michiganensis* subsp. *michiganensis* with low doses of methyl bromide, alone or in combination with soil solarization and impermeable plastic sheets.** P.P. ANTONIOU, E.C. TJAMOS and C.G. PANAGOPOULOS. *Agricultural University of Athens, Plant Pathology Laboratory, 75 Iera Odos, 118 55 Votanikos, Athens, Greece.*

The effectiveness of soil disinfestation with low doses of methyl bromide (20 or 30 g/m<sup>2</sup>) or with short-term soil solarization (15, 30 days) alone or in combination with low doses of methyl bromide and impermeable plastic sheets (polyamide sheet in between of two transparent polyethylene sheets) was evaluated. Experiments were carried out in tomato and cucumber greenhouses at Preveza county during the summers of 1994 and 1995. Experimental data gave the following results:

1. artificial inocula (chlamydospores) of *Fusarium oxysporum* f.sp. *cucumerinum* (F.o.f.sp.c.) were reduced significantly in treatments where impermeable plastic sheets were used. However, no reduction was observed in the inocula of *Clavibacter michiganensis* subsp. *michiganensis*, incorporated into the soil, by low doses of methyl bromide. On the contrary, bacterial population was reduced by 2-3 logarithmic units when soil solarization was applied.
2. The reduction in chlamydospores population of F.o.f.sp.c. by 2 logarithmic units resulted not only in the suppression of disease symptoms but also in the reduction of disease incidence from 83% (control) to 8-10% in treatments where 35 g/m<sup>2</sup> methyl bromide in combination with impermeable sheets were used. The combination of soil solarization for 15 days with low doses of methyl bromide and impermeable plastic sheets was the most successful method and gave similar results to those achieved with soil solarization for 50 days (6-7%). Yield was equally affected. The reduction in the percentage of plants infected by *Clavibacter michiganensis* subsp. *michiganensis* from 47-55% (control) to 32% when soil solarization was applied for 15 days in combination with low dose of methyl bromide (35g/m<sup>2</sup>) was not sufficient. On the contrary, soil solarization for 30 days alone or in combination with 35 g/m<sup>2</sup> methyl bromide controlled the disease almost completely (10%). Data on two-year experimentation suggest that the use of low doses of methyl

bromide in combination with impermeable plastic sheets can effectively control soil borne fungal pathogens, whereas soil solarization is needed to control bacterial canker of tomato.

**Biological control of *Verticillium dahliae* with antagonistic endorhizosphere bacteria of the genus *Bacillus*.** D.I. TSITSIGIANNIS, E.C. TJAMOS and C.G. PANAGOPOULOS. *Agricultural University of Athens, Plant Pathology Laboratory, 75 Iera Odos, 118 55 Votanikos, Athens, Greece.*

*Verticillium* wilt caused by *Verticillium dahliae* has a considerable economical impact on many annual and perennial crops due to the fact that its control is extremely difficult. In the present study a new approach to control *V. dahliae*, *in vitro* and *in planta*, was investigated, using antagonistic endorhizosphere bacteria able to occupy the rhizosphere and prevent entrance of the pathogen through the root tips. The bacteria were isolated from the endorhizosphere of the root tips of tomato plants that were cultivated on soils of different origin. A total of 435 bacterial isolates were examined to find out if they prevented growth of *V. dahliae* *in vitro*. Fifty-three isolates (12.18%), which formed an antagonistic zone of variable diameter were found to be antagonistic against the pathogen. The majority of antagonistic bacteria came from soils that had been subjected to solarization. Finally, 20 bacteria were chosen for *in vivo* evaluation against the pathogen with the following composition: 17 isolates of endorhizosphere bacteria with intense and unimpaired antagonistic action against *V. dahliae*, two bacterial antagonists that originated from random contamination of *V. dahliae* cultures forming intense antagonistic zones against the fungus and one endorhizosphere bacterium that did not present any antagonistic action against the pathogen. All selected isolates were Gram-positive and belonged to the genus *Bacillus*. *V. dahliae* microsclerotia used for the inoculation of eggplants were produced in a liquid culture medium (SSN). These microsclerotia proved to be the most effective in causing acute symptoms of the disease. During three glasshouse evaluations, all antagonistic bacteria used significantly reduced disease to levels lower than those in the control plants. All applications with antagonistic bacteria caused development of a low percentage of diseased leaves during the first 50 days after inoculation, varying between 0-50% of the total while disease symptoms on the control plants reached 100% within the first 35 days. Three bacterial isolates, designated as 5-127, K-165 and K-160, were particularly effective against *V. dahliae*. The reduction in the levels of infection reached 40-70% compared to the untreated controls two months after inoculation, while the growth of the plants was significantly accelerated. The use of the three antagonistic bacteria chosen as most effective, limited symptom expression of the disease to a low percentage of plants and resulted in a significant delay in the appearance of the first symptoms in comparison with the control. Further evaluation of the most effective bacterial antagonists is continuing in field experiments.

**The effect of garlic (*Allium sativum*) extract on tomato plant pathogens, *in vitro*.** F.T. GRAVANIS and I.D. SALIGKARIAS. *Technological Education Institute Larissa, Department of Plant Production, Plant Protection Laboratory, 411 10 Larissa, Greece.*

In the present study, the *in vitro* effect of aqueous garlic extract prepared from cooking garlic powder over five tomato plant pathogens, has been tested. Non-sterile aqueous garlic extract, when incorporated into growth medium (PDA) at the concentration of 25 mg·ml<sup>-1</sup>, decreased the radial colony growth of *Fusarium oxysporum* f. sp. *lycopersici*, *Verticillium dahliae* and *Phytophthora parasitica* up to 42, 48 and 100%, respectively. The minimum inhibitory concentration (MIC) for *V. dahliae* was 30 mg·ml<sup>-1</sup> after 24, 48 and 72 h of incubation. The MIC for *P. parasitica* was 12.5, 12.5 and 25 mg·ml<sup>-1</sup>, respectively, after the same hours of incubation. For *F. oxysporum* f. sp. *lycopersici* no MIC was recorded at all concentrations tested. It has also been observed a decrease in the number of spores produced by *F. oxysporum* f. sp. *lycopersici* and *V. dahliae*. The bacteria species *Clavibacter michiganensis* subsp. *michiganense* and *Pseudomonas syringae* p.v. *tomato* showed MIC of 30 mg·ml<sup>-1</sup>, after 72 h of incubation. Sterilized aqueous garlic extract lost every fungistatic and bacteriostatic effect.

**Control of *Botrytis cinerea* Pers. on greenhouse tomato with the essential oils of *Salvia fruticosa* and *Origanum vulgare* ssp. *hirtum*.** T.L.J. MARCOVIC<sup>1</sup>, V.A. BOURBOS<sup>2</sup> and M.T. SKOUDRIDAKIS<sup>2</sup>. <sup>1</sup>*Mediterranean Agronomic Institute of Chania, Department of Natural Products: Aromatic and Medicinal Plants, 731 00 Chania, Crete, Greece.* <sup>2</sup>*National Agricultural Research Foundation, Subtropical Plants and Olive Trees Institute of Chania, Laboratory of Plant Pathology, 731 00 Chania, Crete, Greece.*

The activity of the essential oils of *Salvia fruticosa* (sage) and *Origanum vulgare* ssp. *hirtum* (oregano) against *Botrytis cinerea* Pers. was studied in the tomato cultivar Early Pak 7 in a plastic non heated greenhouse. The artificial inoculation of the fruits of the plants was done by using four strains of the pathogen with different level of resistance to benzimidazole and dicarboximide fungicides. More specifically the strains RbRd (resistant to benzimidazoles and dicarboximides), RbSd (resistant to benzimidazoles and sensitive to dicarboximides), SbRd (sensitive to benzimidazoles and resistant to dicarboximides), SbSd (sensitive to both groups of the fungicides) were used. Two doses of essential oils were tested against each strain of the fungus. The first dose was determined on the basis of results of *in vitro* tests and represented a concentration of essential oils of sage and oregano which gave 100% inhibition of spore germination of the pathogen. The second dose was double the first. The evaluation of the effectiveness of the essential oils used was based on counting the infected fruit. The essential oils of sage and oregano of the first dose showed an effectiveness that varied from 76.92% (SbRd) to 92.59% (SbSd) and from 66.67% (SbRd) to 75.76% (RbSd and SbSd) respectively. The double dose completely (100%) controlled all the fungal strains. The above doses of sage and oregano essential oils did not cause phytotoxicity under the conditions of the trial.

## Bacterial and non-parasitic diseases

**Evaluation of the "Firescreens" warning system for predicting fire blight epidemics in Greece.** J. TSIANTOS<sup>1</sup> and P. PSALLIDAS<sup>2</sup>. <sup>1</sup>*Plant Protection Institute of Volos, 380 01 Volos, Greece.* <sup>2</sup>*Benaki Phytopathological Institute, 8 S. Delta Str., 145 61 Kifissia, Athens, Greece.*

The French computerized "Firescreens" warning system for predicting fire blight epidemics was evaluated for the first time in Greece, for pears and apples in the years 1994-1996. Experimental orchards were divided into three parts. In the first part the trees were sprayed three times during bloom, as recommended. In the second part the trees were sprayed according to the prediction of the system. In the third part the trees received no sprays and were used as controls. The results showed that at least under the conditions of the experiment - low inoculum potential, few rains - in most cases, the recommended three sprays were not necessary since disease symptoms did not appear in all the experimental plots. The sprays demanded by the system were one or two or, in most cases, none. So an economy of up to three sprays is achieved, with all the advantages that it means. Evaluation of the system must continue in order to be tested under more favourable disease conditions and it should also be compared with other warning systems.

***Agrobacterium vitis*: current status in Crete. Effect of hot water treatment on grapevine propagated materials.** D.E. GOUMAS<sup>1</sup>, A.K. CHATZAKI<sup>1</sup>, and E.M. ARGYRAKIS<sup>2</sup>. <sup>1</sup>*National Agricultural Research Foundation, Plant Protection Institute, 711 10 Heraklion, Crete, Greece.* <sup>2</sup>*National Agricultural Research Foundation, Institute of Viticulture Vegetable Crops and Horticulture, 711 10 Heraklion, Crete, Greece.*

Crown gall disease of grapevine caused by *Agrobacterium vitis* was rare and localised in some Cretan vineyards before 1980, without any economic importance. However, severe infections in newly replanted vineyards ranging from 2 to 33% have been recorded in Crete during the last decade, suggesting that new propagation material is the inoculum source. In the present study the effectiveness of hot water treatment (50°C for 40 min) was assessed on propagating material of 50 local (Greek) and introduced varieties. It was concluded that:

1. There was variation in thermosensitivity among the used strains of *Agrobacterium vitis* but these were more sensitive than strains of *Agrobacterium tumefaciens* (biovar 1 or 2).
2. Treating dormant cuttings with hot water (50°C for 40 min) did not affect survival or growth of the primary buds or the success of the grafting of sultanina on rootstocks R110 or 41B.
3. The treatment significantly reduced the appearance of tumors on propagated material but did not eliminate the bacterium.

**Characteristics of Greek isolates of *Clavibacter michiganensis* subsp. *michiganensis*.** A.S. ALIVIZATOS, C.D. CARAFLA, P.E. GLYNOS and M.C. CHOLEVA. *Benaki Phytopathological Institute, S. Delta Str. 8, 145 61 Kifissia, Greece.*

The variability among 30 Hellenic isolates of *Clavibacter michiganensis* subsp. *michiganensis* (Cmm), originating in

11 different geographic areas of Greece, was checked by a comparative study of the morphological, physiological, biochemical, serological, pathogenic, enzymatic and genetical characters, protein profiles and sensitivity to 5 disinfectants. All isolates showed typical and identical results in the morphological and in most physiological, serological (IF, ELISA, Slide agglutination) and enzymatic [oxidase, amylase, polygalacturonase, endocellulase (with 2 exceptions)] tests. However variability was detected in the behaviour of isolates to the following tests: pathogenicity, susceptibility of tomato varieties, biochemical tests, cell protein profiles, genetic tests (differences in plasmids, PCR, DNA product) and sensitivity to temperatures and 5 disinfectants.

**Variability of Greek isolates of *Agrobacterium vitis*.** A.S. ALIVIZATOS and G.P. KONTOSFIRIS. *Benaki Phytopathological Institute, S. Delta Str. 8, 145 61 Kifissia, Greece.*

Thirty isolates of *Agrobacterium vitis* from 7 geographic areas of Greece were studied with reference to the morphological, physiological and biochemical characteristics, growth on various culture media, pathogenicity on 16 plant species, serological properties (using slide agglutination, ELISA and IF test), protein profiles, genetic and enzymatic characteristics and the production and use of opines.

The results showed uniformity among isolates on the basis of the morphological and biochemical tests, growth on selective and non-selective culture media (NA, RS, Kerr, Schroth), serological reactions and the enzymatic (oxidase, polygalacturonase) and genetic (PCR product) properties. All isolates also gave the same results on the production and use of opines (octopine, nopaline, vitopine) with both the biochemical tests and PCR. Variability was however found among isolates on the basis of protein profiles and pathogenicity on 19 host plants, among which the best host plant indicator was *Phaseolus vulgaris* cv. Zargana for the formation of galls.

**Cases of necrotic symptoms on Greek crop plants. Is air pollution a cause for many of them? A case study at Gomfi, Trikala, Thessaly.** D. VELISSARIOU<sup>1</sup>, J. VENOUZIOU<sup>2</sup> and K. PAPANAS<sup>2</sup>. <sup>1</sup>*Benaki Phytopathological Institute, 8 S. Delta Str., 145 61 Kifissia, Athens, Greece.* <sup>2</sup>*Ministry of Agriculture, Local Office, Trikala.*

There is evidence that over the last decade a large number of non-parasitic problems of crop plants are due to toxic effects by air pollutants. Misuse of agrochemicals or mismanagement of resources are the main reasons for the most of these problems. However, in many cases, the cause remains unknown. The damage appear to be persistent in particular agricultural areas and time periods, affecting many different kind of crop plants. In such cases the effects of air pollution must not be ignored, even if there is no apparent pollution source in the vicinity. In the Mediterranean basin, and in particular in Greece, the problem of photochemical air pollution is very well known. The most important phytotoxic air pollutant is ozone, and its toxic effects on sensitive crop plants or natural flora have been reported in Greece. In July 1995, in a study carried out in collaboration with the Local Office of the Ministry of

Agriculture at Trikala, Thessaly, in the agricultural area of Gomfi, necrotic symptoms on crop-plant of unknown cause, was recognised as being a characteristic visible symptom of ozone toxicity. The most severely affected plant was muskmelon and in many cases its productivity was dramatically reduced due to extensive foliar damages. Typical ozone symptoms were also recorded at nearby farms of watermelon and bean, which are considered to be ozone bioindicators. Similar symptoms were also observed in the vicinity on onion, maize and parsley. Ozone bioindicators (Bel-W3 tobacco plants) exposed in the area confirmed the presence of the pollutant not only in Gomfi but also in the city of Trikala. In June 1996, identical symptoms were recorded in the area on the same crop plants but to a much wider extent. Similar symptoms were also recorded on cotton plants. Photochemical pollution problems were recorded for the first time in the vicinity of the Thessaly plain. Intensive monitoring and research are needed to verify the phenomenon. Further studies are also required to investigate the spatial dispersal of ozone in the greater agricultural area of Thessaly, as well as any possible long term effects (premature senescence, crop losses) on important crop plants such as cotton, which very rarely develops visible symptoms.

**Factors affecting tree mortality in the natural fir-forests of Greece.** P. TSOPELAS<sup>1</sup>, S. XENOPOULOS<sup>1</sup> and D. VELISSARIOU<sup>2</sup>. <sup>1</sup>National Agricultural Research Foundation, Institute of Mediterranean Forest Ecosystems, Terma Alkmanos, 115 28 Athens, Greece. <sup>2</sup>Benaki Phytopathological Institute, 8 S. Delta Str., 145 61 Kifissia, Greece.

Tree mortality is a very common phenomenon in the fir-forests of Greece, especially in those located in southern Greece. Extensive fir-tree mortality usually occurs during long periods of drought, when plants suffer from water deficiency. However, mortality of fir-trees occurs also during years of high rainfall. During the last three years (1994-1996), although rainfall was fairly high in the National Park of Parnis north of Athens, thousands of fir-trees died there annually. It became evident that this type of tree mortality in the fir-forests was a complicated phenomenon, and the result of interaction between biotic and abiotic factors. Dead and dying trees have been found in their majority to be infested by bark beetles. The most common species of these insects are: *Phaenops knoteki* Reitt., *Pityokteines spinidens*, Reitt. *Pityokteines curvidens* Germ., and *Cryphalus piceae* Rtzb. Bark beetles usually attack and kill trees already stressed by other factors. It is thus possible that under certain conditions (especially during periods of drought), when the bark beetle population is increased significantly, insects become the primary cause of mortality. Pathogenic root fungi are considered stress factors and also killing agents of fir-trees. The most common root rot agent in the fir forests of Greece is *Heterobasidion annosum* (Fr.) Bref. Infections have also been observed by *Armillaria mellea* (Vahl.: Fr.) Kummer, *A. ostoyae* (Rom.) Herink, and *A. gallica* Marx. & Rom. In certain fir-forests, the mistletoe *Viscum album* L. has been found to infect large numbers of trees, also acting as a stress factor. Air pollution is one of the abiotic factors that contribute to the weakening of forest trees and predispose them to insects and fungal pathogens. The presence of photo-

chemical air pollutants (mainly O<sub>3</sub>) in Greece is well known; however, no detailed studies to determine their effects on the forests of the country have been carried out. In the area of Mount Parnis, the presence of high levels of O<sub>3</sub> has been ascertained and toxic symptoms of this oxidant have been observed on trees of fir and Aleppo pine.

**Activity of two new strobilurin fungicides on a mixed benomyl resistant/sensitive population of *Sphaerotheca fuliginea* (Schlecht.) Pollacci.** N. PETSİKOS-PANAYOTAROU<sup>1,2</sup>, A. KALAMARAKIS<sup>1,2</sup> and D. KYRIAKOPOULOU<sup>1</sup>. <sup>1</sup>Benaki Phytopathological Institute, 8 S. Delta Str., 145 61 Kifissia, Athens, Greece. <sup>2</sup>National Agricultural Research Foundation, Athens, Greece.

The alternation of fungicides with different modes of action is a common practice in resistance management strategies for disease control. Strobilurins are naturally occurring derivatives of β-methoxyacrylic acid, which are regarded as a new class of chemicals with a novel mode of action and very interesting biological properties. In the present study the activity of two antifungal agents of this class, azoxystrobin and kresoxim methyl, was examined on a benomyl resistant/sensitive (R/S) population of *Sphaerotheca fuliginea*, the main agent of cucumber powdery mildew. Systemic action of azoxystrobin and kresoxim methyl, following root application to young cucumber plants at concentrations of 0.5 to 20 µg a.i./ml was studied over a root uptake period of 1 to 5 days. Evaluation was based on a) microscopic observations of spore germination and hyphal development and elongation, and b) assessment of the leaf infection percentage 48 h and 7 days after artificial inoculation respectively. Azoxystrobin, even at the lowest concentration used (0.5 µg a.i./ml), moved rapidly upwards and in less than 24 h reached the leaves at concentrations sufficient to cause complete inhibition of hyphal development and disease control. By contrast, kresoxim methyl, even at the highest concentration used (20 µg a.i./ml) and the longest root uptake period (5 days), had little apoplastic movement, which was insufficient to inhibit fungal development. Disease control by this compound was therefore very poor (70% leaf infection compared to 100% in the untreated plants). The systemic fungicides benomyl and triforine gave insufficient control of powdery mildew as well. The preventive and curative action of azoxystrobin and kresoxim methyl was studied by foliar application, at concentrations ranging from 50 to 200 µg a.i./ml, to cucumber plants artificially inoculated with the *S. fuliginea* population 1 to 7 days before or after spraying. In these tests both strobilurin fungicides showed excellent preventive and curative action. Benomyl and triforine applied similarly, on the other hand, did not give satisfactory control of the disease and an explanation for that is discussed.

**Alternative respiration as a biochemical mechanism of resistance to methoxyacrylates in *Septoria tritici*.** B.N. ZIOGAS. Agricultural University of Athens, Laboratory of Phytopathology, 118 55 Votanikos, Athens, Greece.

The mechanism of resistance of a *Septoria tritici* mutant, grown in the laboratory by UV irradiation, to ICIA5504 was investigated. The level of resistance (RI) was approximately

10 when measured against spore germination or growth. Glucose oxidation in whole cells was inhibited in the wild type (80% inhibition at  $0.1 \mu\text{g ml}^{-1}$ ), whereas in the resistant mutant oxygen uptake was stimulated (50% stimulation at  $0.1 \mu\text{g ml}^{-1}$ ). This respiration of the wild type was inhibited by antimycin A and cyanide; in contrast, respiration of the mutant was unaffected by these respiratory inhibitors. The alternative respiratory pathway in the mutant was inhibited by the addition of 2 mM salicylhydroxamate (SHAM). These results were confirmed using mitochondria isolated from both strains. Antimycin A and ICIA5504 did not completely inhibit NADH oxidation in both strains. The addition of SHAM inhibited part of the antimycin- and ICIA5504-insensitive oxygen uptake only in mutant mitochondria. For complete inhibition of oxygen reduction, SHAM and cyanide need to be present. Thus three systems of electron transfer from exogenous NADH to oxygen are present in *S. tritici* mitochondria: the cytochrome pathway which is sensitive to ICIA5504 and antimycin A inhibition in both strains; the system of NADH-cytochrome *c* reductase which bypasses the methoxyacrylate inhibition at the cytochrome *bc<sub>1</sub>* complex; and the alternative oxidase which is inhibited by SHAM, and which is only present in the ICIA5504 resistant mutant. When the *S. tritici* isolates were tested for their sensitivity to ICIA5504 on wheat, the resistant strain was controlled better than the wild type. This indicates that decreased ATP formation by the alternative pathway of respiration was inadequate for efficient parasitic growth on the host.

**Evaluation of fungicides for control of sour rot caused by *Geotrichum candidum* on citrus fruit.** B.N. ZIOGAS and E.N. KALOGEROPOULOU. *Agricultural University of Athens, Laboratory of Phytopathology, 118 55 Votanikos, Athens, Greece.*

The efficacy of 13 fungicides for the control of sour rot in lemon fruit caused by *Geotrichum candidum* was investigated in the present work. In a potato dextrose agar (PDA) medium, growth was unaffected by the fungicides SOPP, benomyl, TBZ or imazalil. The fungitoxicity of flusilazol, triflumizole, pyrifenoxy, tebuconazole, flutriafol and fenpropimorph was weak (MICs  $20\text{--}100 \mu\text{g ml}^{-1}$ ). High fungitoxicity was found in the case of iminoctadine, guazatine and propiconazole with MICs 0.07, 2.5 and  $1.5 \mu\text{g/ml}^{-1}$  respectively. When the last three fungicides were applied to lemons 6, 24 and 48 hours after inoculation with *Geotrichum candidum*, iminoctadine was more effective than the others. The development of disease was completely inhibited at concentrations of 1 and  $10 \mu\text{g ml}^{-1}$  after 6 and 48 h respectively. In the case of guazatine, disease was completely inhibited at concentrations of 50 and  $250 \mu\text{g/ml}^{-1}$  after 6 and 24 hours, respectively. Propiconazole was less effective than the other two fungicides. Study of the resistance risk for these three fungicides showed that iminoctadine and guazatine are low risk since it was impossible to obtain resistant mutants with UV mutagenesis. Mutants of *Geotrichum candidum* with low (Rf 13-20) or moderate (Rf 50) resistance to propiconazole were isolated with a frequency of  $8 \times 10^{-5}$ . Fitness-determining characteristics such as rate of mycelial growth, sporulation, germination and pathogenicity were found to be reduced signifi-

cantly in the mutant strains. Cross resistance studies showed that propiconazole-resistant mutants slightly reduced sensitivity to iminoctadine and guazatine (Rf 2-4) only when the evaluation was based on ED<sub>50</sub> values. Their sensitivity was unaffected when the evaluation was based on MIC values (Rf1).

**Study of mechanism of expression of mutations for resistance to morpholines and a piperidine in *Ustilago maydis*.** A.N. MARKOGLIOU and B.N. ZIOGAS. *Agricultural University of Athens, Laboratory of Phytopathology, Iera Odos 75, 118 55 Votanikos, Athens, Greece.*

Mutants of *Ustilago maydis* carrying major-gene (*U/fpm-1A*, *U/fpd-1*) or minor-gene (*U/fpm-1B*, *U/tdm-2*) mutations for resistance to the morpholines fenpropimorph and tridemorph and to the piperidine fenpropidin were investigated with regard to the mechanism of resistance. Gas-liquid chromatography analysis of nonsaponifiable lipids from control sporidia of both wild-type and mutant strains showed that ergosterol is the predominant sterol in the fungal membranes of wild-type as well as of mutant strains. Treatment of mutant strains with fenpropimorph or tridemorph caused the accumulation of  $\delta^8$ -sterols and the  $\delta^{8,14}$ -sterols. The  $\delta^{8,14}$ -sterols, mainly ignosterol, accumulated at higher fenpropimorph concentrations. No differences between wild-type and mutant strains were observed, indicating that sterol biosynthesis follows the same pathway in both sensitive and resistant isolates. Treatment of sporidia with the enzymatic preparation Novozym 235 resulted in protoplast formation in both type and mutant strains, but the cell wall lysis of the resistant sporidia required more time than that of the sensitive ones. Study of protoplast sensitivity to morpholines showed that resistance was provable only in intact sporidia, but not in the protoplast stage. From the above results it may be concluded that a structural change in cell wall region is possibly responsible for morpholine and piperidine resistance coded by both major and minor-gene mutations.

**Field isolates of *Cercospora beticola* Sacc. with reduced sensitivity to sterol-inhibiting fungicides.** G.S. KARAOGLANIDIS<sup>1</sup>, F.I. IOANNIDIS<sup>2</sup> and C.C. THANASSOULOPOULOS<sup>1</sup>. <sup>1</sup>*Aristotelian University, Faculty of Agriculture, Plant Pathology Laboratory, P.O.Box 269, 540 06 Thessaloniki, Greece.* <sup>2</sup>*Hellenic Sugar Industry S.A., Plant Protection Division, Platy, Greece.*

Over the last 15 years, sterol-inhibiting fungicides (SBIs) have been used by the Hellenic Sugar Industry, for controlling sugarbeet leaf-spot caused by *Cercospora beticola* Sacc. An extensive survey was carried out in order to study the sensitivity patterns of the pathogen to the 14C demethylation-inhibiting fungicides bitertanol (Baycor 25% WP) and flutriafol (Impact 12.5% SC). Single-lesion isolates of the pathogen were obtained from commercial sugarbeet fields in three areas with different levels SBI use. In Amyndeon, SBIs had never been used, whereas in Imathia they had been used at a moderate rate and in Serres at a more intensive rate. Fungal isolates were grown on ACM growth medium, fungicide-free and fungicide-amended, and the EC<sub>50</sub> of 100

isolates from each area was estimated. The sensitivity distribution of the Amyndeon population was similar to that of the whole population of northern Greece in the early 80's. In the other two areas a small decrease in population sensitivity was noticed, with greater decrease in the area of Serres. The mean EC<sub>50</sub> values for flutriafol in the populations of Amyndeon, Imathia and Serres were 0.5, 0.73, and 1.05 g ml<sup>-1</sup> respectively, and for bitertanol 0.47, 0.81 and 0.72 g ml<sup>-1</sup> respectively. Isolates with a high degree of resistance to flutriafol were also resistant to bitertanol. These results suggest that attention must be paid to ensure the continued usefulness of the SBIs. Probably a reduction of selection pressure by using SBIs only during periods of high risk is the best solution.

**Fungicide sensitivity of *Botrytis cinerea* isolates obtained from natural and agricultural ecosystems.** E.K. KULAKIOTU<sup>1</sup> and S.A. ARCHER<sup>2</sup>. <sup>1</sup>Aristotelian University, Faculty of Agriculture, Plant Pathology Laboratory, P.O.Box 269, 540 06 Thessaloniki, Greece. <sup>2</sup>Imperial College of Science, Technology and Medicine, University of London, Department of Biology, Ascot, Berkshire, SL5 7PY, UK.

*Botrytis cinerea* is a ubiquitous pathogen causing grey mould diseases of great economic importance, especially on grapes. Development of resistance to commonly used fungicides like the benzimidazoles (e.g. benomyl) has made the control of this pathogen difficult. The aim of this study was to detect the presence of such resistant isolates in different ecosystems. One of the important findings was the widespread occurrence of benomyl and iprodione-resistant strains both in agricultural and natural ecosystems. Double resistance to benomyl and iprodione was noted in the majority of isolates. EC<sub>50</sub> values for benomyl were found to be greater than 100 ppm, whilst values for iprodione were less than 6 ppm. A major finding was the non-existence of a boundary between agricultural and natural ecosystems in terms of the presence of resistant isolates.

## Posters

**Parasitism of the fungus *Sclerotinia sclerotiorum* (De Bary) by four species of the genus *Trichoderma*.** M.D. AGGELAKI and K. TZAVELLA-KLONARI. Aristotelian University, Faculty of Agriculture, Plant Pathology Laboratory, P.O.Box 269, 540 06 Thessaloniki, Greece.

Parasitic action of the species: *Trichoderma koningii*, *T. harzianum*, *T. hamatum* and *T. viride* against *Sclerotinia sclerotiorum* was studied *in vitro* in dual cultures on PDA. In three-day-old dual cultures of *S. sclerotiorum* with *T. koningii* a zone of mycelial contact was observed. There was no further growth of *S. sclerotiorum* beyond the zone of mycelial contact, whereas *T. koningii* continued to grow over the half of the plate that had been covered by *S. sclerotiorum*. Sclerotia of *S. sclerotiorum* were observed only at the edges of the zone of mycelial contact. In dual cultures of *S. sclerotiorum* with each of the species *T. harzianum*, *T. hamatum* or *T. viride*, inhibition of the growth of *S. sclerotiorum* was

observed, while no previous contact between the hyphae of parasite and pathogen existed. In all cases, the presence of species of the genus *Trichoderma* caused signs of degeneration in the hyphae of *S. sclerotiorum*, such as, vacuolation, granulation of cytoplasm and bursting of hyphae. In microscopic observations of the zone of mycelial contact between *S. sclerotiorum* and *T. koningii*, the penetration of hyphae of *T. koningii* into hyphae of *S. sclerotiorum* from points near the septa was observed. In addition, development of appressoria at the end of hyphae of *T. koningii* at the point of contact with hyphae of *S. sclerotiorum* and development of chlamydospores of *T. koningii* inside the hyphae of *S. sclerotiorum* were observed. It is obvious that the parasitic action of *T. koningii* is expressed mainly as hyperparasitism, while the other three species act by the secretion of inhibitory substances.

**Anthracnose of weeping-willow (*Salix babylonica*), a new disease in Greece.** M.D. AGGELAKI, D.A. KARADIMOS and K. TZAVELLA-KLONARI. Aristotelian University, Faculty of Agriculture, Plant Pathology Laboratory, P.O.Box 269, 540 06 Thessaloniki, Greece.

A disease on weeping-willow trees in home gardens was observed in the Chalkidiki region during spring of 1995 and 1996. Small purplish brown or black necrotic spots appeared on the leaves, often on the veins, causing leaf deformation and defoliation. Elongated, slightly sunken cankers with a dark brown rim and paler centre appeared on the young shoots. Whitish acervuli belonging to the fungus *Marssonina salicicola* (Bresad) Magnus covered the cankers and the leafspots. The conidia in the acervuli were hyaline, clavate to pyriform, two-celled (the two cells were of different sizes), with dimensions 14-19x4-7 µm. According to the literature, the perfect stage (teleomorph) of the fungus is the ascomycetes *Drepanopeziza sphaeroides* (Pers.) Hohnel. The disease is known in many European countries (Italy, England, Germany), also in America, Egypt and New Zealand and as far as we know this is the first report of the disease in Greece.

**Occurrence of the disease "Internal deterioration of watermelon fruit" in watermelon cultivations in Greece.** F.P. BEM and N. VASSILAKOS. Benaki Phytopathological Institute, Laboratory of Virology, 8 S. Delta Str., 145 61 Kifissia, Athens, Greece.

In July 1995, a serious disease of watermelon occurred for the first time in Greece in watermelon cultivations in many regions of the Iliia, Messinia and Trikala prefectures. The disease symptoms characteristically appeared only in mature fruits of grafted watermelon plants. The pedicel of infected fruits showed brown epidermal necrotic streaks which coalesced and covered the whole surface of the pedicel. The interior of the fully-grown fruits showed an increase in yellow fibers and contained regions with crescent-shaped crevices. The inner pulp of the mature fruits when cut open appeared to be water-soaked and dirty red. Moreover, apparently healthy fruits that were harvested in the above prefectures were unsuitable for marketing. Serological testing by ELISA of a large number of fruit samples exhibiting

the above symptoms revealed the presence of the cucumber green mottle mosaic tobamovirus (CGMMV). The same virus was also detected in samples of bottleground (*Lagenaria siceraria*) and of some cucurbitaceous hybrids grown by the farmers of these districts for seed production of watermelon rootstocks. However, the virus was not detected in samples of watermelon seeds. This explained why CGMMV occurred only in grafted watermelon plants. The symptoms in watermelon, the host range and symptoms on the indicator plants, the means of transmission and the results of the serological tests, all proved to be typical of the watermelon strain of CGMMV. This strain has been reported to cause the "konnyaku" disease affecting watermelon cultivations in Japan, India, Pakistan and Korea. In 1979, a cucumber strain of CGMMV was reported by A. Avgelis from cucumber cultivations in the Ierapetra region of Crete. The fact that this watermelon disease is transmitted only by infected seeds and contaminated grafting tools and was unknown before the rootstock technique was introduced to watermelon cultivation in Greece, leads to the conclusion that it was introduced to Greece through rootstock seed imported from the Far East.

**Control of soil borne diseases of cultivated plants by mycorrhizae.** V.A. BOURBOS and M.T. SKOUDRIDAKIS. *National Agricultural Research Foundation, Subtropical Plants and Olive Trees Institute of Chania, Laboratory of Plant Pathology, 731 00 Chania, Crete, Greece.*

The mycorrhizae, a symbiosis of fungi and roots, are found in almost all cultivated species. Depending on their manner of growth they are classified in ectomycorrhizae or ectotrophic, endomycorrhizae or endotrophic; ectendomycorrhizae or ectenotrophic. Mycorrhizae, and especially the vesicular arbuscular mycorrhizae (VAM), play an important role in plant growth and health. For example their presence on plant roots facilitates the uptake of macro-, micronutrients and water, plant prosperity in alkaline soils, the avoidance of toxicity caused by microelement excess, their better adaptation to temperature variations in the soil environment and their protection from various pests and diseases. Indeed it has been ascertained that a decrease of many nematodes of the genus *Meloidogyne* and various bacterioses is due to the pathogens *Pseudomonas solanacearum*, *Agrobacterium rhizogenes*, *Erwinia carotovora* and *Pseudomonas syringae*. The importance of mycorrhizae is due mainly to the protection they give to the root system of plants against soil borne diseases. This protection is expressed mechanically by the alteration of the physicochemical and biological mycorrhizal environment, by the production of allelochemicals from symbiotics, by the parasitism of the symbiotic fungus on pathogens, by the stimulation of the defense system of the plant and by the improvement of nutrition. Many researchers have reported a significant decrease in attacks on plants from various soil fungi belonging to the genera *Cylindrocarpus*, *Aphanomyces*, *Gaeumannomyces*, *Thielaviopsis*, *Phytophthora*, *Pythium*, *Rhizoctonia*, *Pyrenochaeta* and *Sclerotium* due to the presence of mycorrhizae. The production of biological products based on mycorrhizae has already begun. However, more and deeper research is need-

ed, especially in the area of mass production of symbiotic organisms with the plant root system fungi. Particular attention should be paid to this approach because, depending on the circumstances, the use of mycorrhizae will contribute to the limitation or replacement of soil disinfectants.

**Control of *Alternaria alternata* (Fr.) Keissler on stored fruits of *Actinidia chinensis*.** V.A. BOURBOS and M.T. SKOUDRIDAKIS. *National Agricultural Research Foundation, Subtropical Plants and Olive Trees Institute of Chania, Laboratory of Plant Pathology, 731 00 Chania, Crete, Greece.*

*Alternaria alternata* is isolated with high frequency from the leaves, blossoms and fruit of *Actinidia chinensis*. Under certain conditions the fungus causes serious damage to the fruit stored in refrigerators. In our study the possibility to control the pathogen with fungicide sprays before harvesting was investigated. The fungicides used were iprodione at the rate of 50 g hl<sup>-1</sup> and mancozeb at the rate of 180 g hl<sup>-1</sup>. The sprays were applied to fruit of the cultivar Monty 10 days before harvesting. Sprayed fruit was stored for four months in the refrigerator at 0-3°C. The amount of pathogen on the surface of sprayed before harvesting showed decreases which varied from 97.83% (mancozeb) to 99.57% (iprodione). On unsprayed fruit the pathogen showed an increase of 56.76%. Based on fruits which showed soft rot symptoms, the effectiveness of the fungicides varied from 77.53% (mancozeb) to 97.63% (iprodione). According to these results protection of stored fruit against *A. alternata* is possible with the application of a single spray of iprodione 10 days before harvest.

**Reaction of tomato line PS 55289 and *Capsicum chinense* PI 152225 and C00493 to Greek isolates of tomato spotted wilt tospovirus (TSWV).** E.K. CHATZIVASSILIOU and N.I. KATIS. *Aristotelian University, Faculty of Agriculture, Plant Pathology Laboratory, 540 06 Thessaloniki, Greece.*

In glasshouse tests the reaction of resistant tomato cultivar PS 55289 and sensitive *Capsicum chinense* Jacquin lines PI 152225 and AVRDL C00943 to tomato spotted wilt tospovirus (TSWV) was recorded. The tomato was inoculated at the two-true-leaf stage with ten isolates of TSWV from tomato, originating from different areas, and virus concentration was estimated by ELISA every ten days. Although all isolates produced local necrotic lesions on the inoculated leaves, some induced systemic infection and sudden death of the plant. In particular, two isolates from northern Greece (Kavala, Chalkidiki) infected all tested plants systemically, three infected only some of the inoculated plants, whereas the tomato cultivar was completely resistant to only two isolates. Systemic infection of tomato cultivar PS 55289 by TSWV was detected 10 days later than in the control plants. Moreover, TSWV concentration in tomato cultivar was lower compared to that in the control plants and it often resulted in the death of plants. On the contrary, the susceptible control plants, although exhibiting severe symptoms, survived for many weeks. In further

tests, *C. chinense* was inoculated at the four-true-leaf stage with four isolates from pepper. The line PI 152225 produced systemically necrotic lesions, whereas the line AVRDL C00943 was resistant to three isolates, producing only necrotic lesions. However, the latter line was susceptible to one isolate exhibiting chlorotic lesions on systematically infected leaves.

**Activity of the fungicides pencycuron and diclomezine against isolates of *Rhizoctonia solani*.** M. CHRYSAYI-TOKOUSBALIDES and M. KASTANIAS. *Agricultural University of Athens, Pesticide Science Laboratory, 75 Iera Odos, 118 55 Athens, Greece.*

The fungicides pencycuron and diclomezine were evaluated against isolates of the fungus *Rhizoctonia solani* representing different anastomosis groups (AGs). All isolates were highly virulent to bean, cotton and corn and grew readily on culture media. Diclomezine was effective against all isolates, with no variability in sensitivity levels ( $ED_{50}$  values 0.1 to 0.3  $\mu\text{g ml}^{-1}$ ). Tolclofos-methyl, which was used as a reference fungicide, was equally effective against all isolates ( $ED_{50}$  values 0.1 to 0.4  $\mu\text{g ml}^{-1}$ ). Pencycuron on the other hand, showed strong activity against some isolates but was ineffective against others, with  $ED_{50}$  values ranging from 0.1 to  $>20 \mu\text{g ml}^{-1}$ . In the presence of pencycuron abnormal branching was observed in pencycuron-sensitive, but not in the resistant isolates. Abnormal branching was not observed in the presence of diclomezine with any of the isolates tested.

**Grapevine viruses in Nemea, Greece. Preliminary data.** D. DIMOU<sup>1</sup> and I. DROSPOULOU<sup>2</sup>. <sup>1</sup>Ministry of Agriculture, Argolis Agricultural Direction, 211 00 Nafplion, Greece. <sup>2</sup>Control Station for Vegetative Planting Material, 193 00 Aspropyrgos, Attica, Greece.

Grapevine is of basic agricultural importance in Greece. Nemea, a semi-mountainous area in the Corinthia prefecture, North East Peloponnesus, is one of the most important viticultural areas in Greece. The famous export wine "Mavro Nemeas" comes from the homonymous grapevine variety, the most widely grown in this area. The antiphylloxeric replacement with American rootstocks started in the late 1970s using rootstocks mainly from state nurseries. The import of propagative material is limited, and refers to cutting or rooted rootstocks either plain or grafted with French varieties. Following some symptomatological observations in vineyards in Nemea, where Grapevine Leaf Roll (GLRV) symptoms were observed, an attempt was made to test with ELISA samples of various grapevine varieties. The samples were tested for Grapevine Fanleaf virus (GFLV), Grapevine Leaf Roll virus (GLRV) type I and III, Grapevine fleck virus (GFkV) and Grapevine virus A. All five viruses were detected in the samples, and some samples showed mixed infection by two or more viruses. Very severe GLRV symptoms occurred in both young (5-10 years old) and old plantations of the variety Mavro Nemeas (Black of Nemea), as well as in table varieties introduced for cultivar testing. We also noticed the occurrence of grapevine

stem pitting. Vines with symptoms of grapevine Flavescence dorée or Mediterranean yellows were also observed both on old and young vineyards. The above data by showing the presence of five grapevine viruses and two virus or virus-like diseases in the vineyards of Nemea, mainly on vines on American rootstocks points out the urgent need for grapevine virus research in Nemea and for using healthy propagative material produced under a well organized national certification programme.

**Stemphylium leaf spot or "summer burning" of asparagus.** K. ELENA. *Benaki Phytopathological Institute, 8 S. Delta Str., 145 61 Kifissia, Athens, Greece.*

During the summer of 1992, the *Stemphylium* leaf spot or "summer burning" disease of asparagus was first observed in Greece in the Orestias area. In 1995, the disease was also observed in the Pella, Kavala and Orestias areas (northern Greece), after periods of heavy rainfall. The symptoms were brown lesions with dark purple margins on the main stems, branches and cladophylls of the affected ferns, on which *Stemphylium* spores were observed. The plants turned yellow or brown and the fields looked burned. The fungus *Stemphylium botryosum* Wallr. was isolated from diseased tissues. The identification was based on the following characteristics: conidia 19-29x14-23  $\mu\text{m}$ , with a length:width ratio 1.2:1.4. In pathogenicity tests on asparagus variety UC157 under greenhouse conditions, plants developed symptoms similar to those observed in the field. In Greece the disease reduces the photosynthetic ability of plants, resulting in a reduction of the number of spears the following year. No symptoms on asparagus spears were observed since in Greece these are harvested before they emerge from the planting beds.

**Phytophthora boehmeriae on cotton. A new species for Europe.** K. ELENA and E.J. PAPLOMATAS. *Benaki Phytopathological Institute, 8 S. Delta Str., 145 61 Kifissia, Athens, Greece.*

A boll decay was observed on cotton (*Gossypium hirsutum* L.) in Larissa and Volos counties (Greece) in August 1993. In 1995 the same disease was observed in the Tricala and Phitotis areas. Localized spots appeared on the bolls that progressively tended to join and cover the whole boll surface, mostly on the lower parts of the plants. Fruiting bodies (sporangia and oospores) of fungi of the genus *Phytophthora* were found in the infected tissues. The fungus was isolated and the morphological characteristics were as follows: the mean size of sporangia was 32-48x29-35  $\mu\text{m}$  with a ratio of 1.2:1.5, or 47-59 x 34-49  $\mu\text{m}$  with a ratio of 1.1:1.4. The diameter of oogonia ranged from 32-38  $\mu\text{m}$  or from 24-32  $\mu\text{m}$ . Antheridia were in general amphigynous. The maximum growth temperature on CMA (corn-meal-agar) ranged from 32.5-33°C. On the basis of those characters the isolated *Phytophthora* species were assigned to *Phytophthora boehmeriae* Sawada. Moreover, analysis of  $\alpha$ -esterase isozymes showed that Greek isolates of *P. boehmeriae* shared a similar pattern that was clearly different from the isozyme profile of *P. cactorum* or *P. parasitica*. During the 1993 grow-

ing season the diseased crops were irrigated 2 to 3 days before a heavy rainfall. These conditions favoured the formation of fruiting bodies, and then this inoculum was splash-dispersed from the soil to the lower bolls.

**A new *Phytophthora* species for pear in Greece.** K. ELENA<sup>1</sup>, E.J. PAPLOMATAS<sup>1</sup> and A. ASSIMAKOPOULOU<sup>2</sup>. <sup>1</sup>*Benaki Phytopathological Institute, 8 S. Delta Str., 145 61 Kifissia, Athens, Greece.* <sup>2</sup>*National Agricultural Research Foundation, District Laboratory of Agricultural Advisory Service and Fertilizer Analysis, Xilokastro, Greece.*

Pear trees of the kontoula variety on quince rootstock with typical symptoms of *Phytophthora* collar rot were observed in the Korinthia area in February and March 1996. The symptoms started from the grafting point and extended to the branching of the trees. The disease was very serious after a prolonged period of rain. A decay of lemon fruits was observed in the same field. The strains of the fungus isolated from pear formed papillate sporangia 54-89x29-41 µm with a ratio of 1.5:1.8 and a few chlamydospores; oospores were not formed. The maximum growth temperature on corn-meal-agar (CMA) was 33°C and the culture was slightly radiate. The morphological and physiological characters of the fungus were like those of *Phytophthora palmivora*. The strains isolated from lemon fruits formed papillate sporangia 38-65x30-39 µm and hyphal swellings but no oospores. The maximum growth temperature on CMA was 25°C. This strain belonged to the *Phytophthora syringae* species and was different from the strain isolated from the pear trees. In pathogenicity tests, the pear isolates on two-year old kontoula variety trees, caused collar rot symptoms similar to those on *P. cactorum*. The fungus was reisolated from inoculated trees. Esterase and lactate dehydrogenase isozyme analysis showed that the fungal isolates from pear had an electrophoretic profile similar to that of *P. palmivora* isolates. However, since they did not differ from the zymogramme of *P. citrophthora* isolates, the comparison of the isolates will be continued using molecular markers.

**First report of *Pseudomonas savastanoi* pv. *savastanoi* on *Myrtus communis* sp.** D.E. GOUMAS<sup>1</sup>, N.E. MALATHRAKIS<sup>2</sup> and A.K. CHATZAKI<sup>1</sup>. <sup>1</sup>*National Agricultural Research Institute, Plant Protection Institute, 711 10 Heraklion, Crete, Greece.* <sup>2</sup>*Technological Education Institute, Stavromenos, Crete, Greece.*

Myrtle (*Myrtus communis* L.) is a perennial, long-lived bush 1-3 m high and is part of natural flora of the Mediterranean countries. In March 1995 symptoms of a new disease were observed on naturally grown myrtle plants in areas of Zaros, Crete and on the island of Paros, Greece. The main symptoms were tumorous overgrowths, mainly on the shoots, twigs, branches and trunks. Roots and crowns of the bushes were rarely affected. Young tumors resembling olive-knots are green-tan coloured and have internally a soft, spongy consistency with pockets of bacteria in the form of water-soaked, glossy and sticky areas similar to those described for olive knot disease. Also, the overgrowths resembled the "Myrtidanon" which was described by Dioskouridis in the

1st century as an "epiphysis" (outgrowth); that is, an abnormal, uniformly coloured, likewise hands overgrowth developed on the myrtle bush. Severely affected twigs appear stunted and in heavy infections eventually die. Infections occur through wounds, and particularly through leaf scars. A fluorescent bacterium was consistently isolated from the internal water-soaked pockets of galls on King's B medium. Ten representative isolates showed morphological, physiological and biochemical behaviour similar to reference strains of *Pseudomonas savastanoi* pv. *savastanoi* from olive. They were typical of group 1b of green-fluorescent *Pseudomonads* according to the LOPAT scheme. Pathogenicity of all isolates was confirmed by inoculation onto 3 month-old myrtle, and olive shoots. Accordingly to our knowledge, this is the first report of myrtle as a natural host of the bacterium.

**Record of *Pseudomonas syringae* pv. *mori* on mulberry in Crete.** D.E. GOUMAS<sup>1</sup>, N.E. MALATHRAKIS<sup>2</sup> and A.K. CHATZAKI<sup>1</sup>. <sup>1</sup>*National Agricultural Research Institute, Plant Protection Institute, 711 10 Heraklion, Crete, Greece.* <sup>2</sup>*Technological Education Institute, Stavromenos, Crete, Greece.*

*Pseudomonas syringae* pv. *mori* was recorded in Crete (Greece) on mulberry trees in areas of Peliada, Lasithi and the town of Heraklion. Initially small, water-soaked leaf spots appear that enlarge, turn black or brown with yellow halo and usually coalesce. In some cases affected leaves become distorted. Translucent longitudinal stripes appear on young shoots, becoming dark and sunken with translucent margins, and a yellowish bacterial ooze may be extruded. Infection of bark and wood was also observed in some cases. Bacteria were consistently isolated from the infected tissues in pure culture. These were identified as members of group 1a of LOPAT tests. Koch's postulates were successfully completed on young mulberry shoots and leaves.

**Fungal species isolated from greenhouse soils in the Larissa region.** F.T. GRAVANIS and S. XIFILIDOU. *Technological Education Institute Larissa, Department of Plant Production, Plant Protection Laboratory, 411 10 Larissa, Greece.*

Forty-five (45) fungal species were isolated from soil samples collected from seven greenhouses in the Larissa region. Thirty-two isolates belonging to different fungal genera were isolated on PDA plates, employing the soil dilution method. In particular, the following species were isolated and identified: *Fusarium oxysporum*, *F. oxysporum* var. *redolens*, *F. solani*, *F. graminearum*, *F. equiseti*, *F. scirpi*, *F. sporotrichioides*, *F. culmorum*, *F. avenaceum*, *Verticillium dahliae*, *V. nigrescens*, *Pythium ultimum*, *P. butleri*, *P. rostratum*, *Phytophthora parasitica*, *Trichoderma viride*, *T. hamatum*, two isolates of *Gliocladium* sp., *Aspergillus* sp., *A. niger*, two isolates of *Penicillium* sp., *Cladosporium cladosporioides*, *Mucor* sp., *Humicola insolens*, *Torula* sp., *Cylindrocarpum* sp., *Stachybotrys* sp., *Aureobasidium* sp., *Scopulariopsis chartarum*, *Trichocladium opacum*. Subsequently, the presence of the following species was investigated on their respective selective media: *Fusarium* spp., *Verticillium dahliae*, *Pythium ultimum*, *P. aphanidermatum* and *P. oligandrum*, *Phoma betae* and *P. macdonaldii*, *Thielaviopsis basicola* and

*Sclerotinia minor*. Seven *Fusarium* spp. were isolated, predominantly of *Fusarium oxysporum*. All the above mentioned species were isolated on their selective media tested, except *Thielaviopsis basicola* and *Sclerotinia minor*. Apart from the above mentioned species, the following fungi were also isolated and identified: *Verticillium lecanii*, *Eupenicillium lapidosum*, *Acremonium* sp., *Botryotrichum piluliferum*, *Chalara* sp., *Rhizopus* sp., *Cochliobolus geniculatus*, *Aspergillus eurotium* and *Alternaria* sp.

**The combined effect of the mycorrhizal fungus *Glomus mosseae* and *Verticillium dahliae* on vegetative growth and nutrient uptake of tomato and eggplant.** N. KARAGIANNIDIS<sup>1</sup> and F. BLETSOS<sup>2</sup>. <sup>1</sup>National Agricultural Research Foundation, Soil Science Institute of Thessaloniki, 551 10 Thessaloniki, Greece. <sup>2</sup>National Agricultural Research Foundation, Agricultural Research Center of Macedonia, Thraki, 570 01 Thermi, Greece.

The combined effect of the mycorrhizal fungi *Glomus mosseae* and *Verticillium dahliae* on the vegetative growth and nutrient uptake of tomato and eggplant was studied. The experiment was carried out during spring 1996. The plants were grown for 7 weeks in pots containing soil poor in nutrients. Phosphorous inorganic fertilization in non-soluble form was supplied in order to study the response of tomato and eggplant to the mycorrhizal fungus. The fresh weight, dry weight and plant height of tomato and eggplant increased by 116, 158 and 44% and by 147, 175 and 72% respectively, due to mycorrhizal fungus inoculation. Inoculation with *Verticillium* alone decreased the fresh weight, dry weight and plant height of tomato and eggplant by 38, 52 and 25% and 44, 65 and 33% respectively. The combination of the two fungi increased the fresh weight, dry weight and plant height of tomato and eggplant by 43, 41 and 32% and 48, 39 and 37% respectively. This means that the beneficial effect of the mycorrhizal fungus overcame the *Verticillium* effect. Phosphorous uptake increased about threefold in plants inoculated with the mycorrhizal fungus *G. mosseae*, while no differences in phosphorous uptake were observed in plants inoculated with *V. dahliae*. No significant differences in K, Ca and Mg uptake were found between treatments. Root colonization by *G. mosseae* was more extensive in plants treated with the mycorrhizal fungus only, and it was double the amount achieved with the combined mycorrhizal - *Verticillium* treatment. In addition, root colonization was much stronger in eggplant than in tomato.

**Infection of castorbean (*Ricinus communis* L.) by *Alternaria ricini* (Yoshii) Hansford, a new disease in Greece.** D.A. KARADIMOS, M.D. AGGELAKI and K. TZAVELLA-KLONARI. *Aristotelian University, Faculty of Agriculture, Plant Pathology Laboratory, P.O.Box 269, 540 06 Thessaloniki, Greece.*

A severe disease causing seedling blight, spotting and necrosis of cotyledons, appeared in experimental fields of castorbean cultivation at the University farm and in the Loudia Imathias region during spring and summer of 1995 and 1996. Chlorotic lesions appeared on the leaves, starting from the

oldest ones. The lesions soon turned brown and necrotic. Defoliation began from the lower part of the plant. Seed pods became purple dark brown and rotted. The fungus *Alternaria ricini* (Yoshii) Hansford was isolated from all the infected parts. Conidiophores arose singly or in groups, conidia were single, reddish brown, smooth, sometimes constricted at the septa, measuring 70-170 µm in length (beak included) and 13-27 µm in width. Cultures on PDA showed an abundant whitish-grey aerial mycelium, while the bottom of the culture turned green-black. The optimum temperature for linear growth was between 20-25°C. Sporulation *in vitro* was poor but some conidia formed after 25 days in 20°C. In pathogenicity tests, spots appeared on the leaves 4 days after spraying with a conidial suspension at 25°C. Infection of castorbean by *A. ricini* is the most important fungal disease of this plant. It has been reported from N. America, India, Italy, Russia and recently Yugoslavia and as far as we know, this is the first report of the disease in Greece.

**The response of *Cercospora beticola* Sacc. to benomyl.** D.A. KARADIMOS<sup>1</sup>, F.I. IOANNIDIS<sup>2</sup> and C.C. THANASSOULOPOULOS<sup>1</sup>. <sup>1</sup>*Aristotelian University, Faculty of Agriculture, Plant Pathology Laboratory, P.O.Box 269, 540 06 Thessaloniki, Greece.* <sup>2</sup>*Hellenic Sugar Industry S.A., Plant Protection Division, 411 10 Larissa, Greece.*

In 1972, development of resistance to benomyl by *Cercospora beticola* Sacc. was reported for the first time. Due to this fact the use of benomyl against the pathogen was stopped. Laboratory tests of fungal isolates in the following 13 years have shown that the percentage of resistant strains remained constant (almost 85%). However during field experiments by the Hellenic Sugar Industry for fungicide evaluation in the summer of 1995, it was noticed that benomyl was again effective against *C. beticola*. The sensitivity of more than 500 isolates of *C. beticola* from three different areas in northern Greece was studied in the laboratory. The differentiation between resistant and sensitive isolates was tested by 1 ppm a.i. of benomyl. The results showed that only 25% of the isolates were still resistant. The results also showed differences in the percentage of resistant strains among the three areas, which were probably due to the different spray programmes applied.

**Dissipation of the fungicide tetraconazole in greenhouse cucumbers.** S. KHALFALLAH, O. MENKISSOGLU<sup>1</sup> and H. CONSTANTINIDOU<sup>2</sup>. <sup>1</sup>*Aristotelian University, Plant Pathology Laboratory, 540 06 Thessaloniki, Greece.* <sup>2</sup>*Aristotelian University, Pesticide Laboratory, Plant Protection Division, Department of Agriculture, 540 06 Thessaloniki, Greece.*

Dissipation of residues of the fungicide tetraconazole was studied in an experiment on sprayed greenhouse cucumbers. Tetraconazole is a broad spectrum systemic triazole fungicide effective against major diseases of some important crops. The fungicide (DOMARK 10 EC) was applied to greenhouse grown cucumbers three times at ten days intervals, at rates of 40 mg a.i. l<sup>-1</sup> (low recommended dose, LRD) or 80 mg a.i. l<sup>-1</sup> (high recommended dose, HRD). Samples were collected before each spraying and 1 h, 1, 3, 7, 10 and 15 days after the

third application. Residues were detected with a suitably modified method, using gas chromatography with a nitrogen detector. The recovery of tetraconazole was found to be 96-103% and the limit of detection 0.01 mg kg<sup>-1</sup>. Tetraconazole residues were found to dissipate relatively rapidly and a linear decline of tetraconazole was observed with the two doses.

**Occurrence of cucumber mosaic cucumovirus and bean common mosaic potyvirus in *Phaseolus* species in Greece.** G.M. KOKINIS and N.I. KATIS. *Aristotelian University, Faculty of Agriculture, Plant Pathology Laboratory, 540 06 Thessaloniki, Greece.*

During 1994-96 more than three thousand samples of *Phaseolus vulgaris* L., *P. coccineus* and *P. multiflorus* were collected from various regions throughout Greece and tested for the presence of cucumber mosaic cucumovirus (CMV), and a small number was tested for bean common mosaic potyvirus (BCMV) using enzyme-linked immunosorbent assay (ELISA). Leaf samples with virus-like symptoms were collected, stored in deep freeze (-20°C) and tested. The infection level in *Phaseolus vulgaris* samples varied throughout the different regions and ranged from 0 to 100% for CMV and from 0-8% for BCMV. In *Phaseolus coccineus* or *P. multiflorus* infection level varied from 35 to 36% for the population "Gigantes" and 4% for the population "Elefantes". Five seed lots of different varieties (1000 seeds/variety) of *P. vulgaris* and *P. coccineus* or *P. multiflorus* were also tested for the presence of CMV and BCMV. Virus incidence ranged from 0-1% and 0.8-2% for CMV, respectively whereas BCMV was not detected. The host range of both viruses was also studied.

**Fluazinam: a new protectant fungicide.** M. KOULIZAKIS<sup>1</sup>, L. KARATSALOS<sup>2</sup>, K. KALOGEROPOULOS<sup>2</sup> and M. ANTONAKOU<sup>3</sup>. <sup>1</sup>Marketing and Sales Department of Hellafarm SA, 15 Fleming Str., 151 23 Maroussi, Athens, Greece. <sup>2</sup>2 Iofontos St., Pagrati, Athens, Greece. <sup>3</sup>Hellafarm's SA Field Trial Unit, 15 Fleming Str., 151 23 Maroussi, Athens, Greece.

Fluazinam, a product of Ishihara Sangyo Kaisha Japan, is a protectant fungicide of the dinitroaniline group with penetrant action. It has a broad spectrum of activity against crop diseases caused by fungi of the genera *Botrytis*, *Sclerotinia*, *Colletotrichum*, *Phytophthora*, *Pseudoperonospora* and *Venturia*. To test fluazinam's efficacy under Greek conditions, field trials were carried out against *Venturia inaequalis* on apples, *Botrytis cinerea* on vines, and *Phytophthora infestans* on potatoes, in various regions in Greece during the period 1993-95. All applications were conducted by foliage spraying. The field trials for potatoes and vines extended until 1996. In all cases fluazinam's effectiveness was equivalent to or surpassed (statistically significant) that of the reference compounds (both systemic and protective). An important advantage of fluazinam is that its effectiveness was obtained using relatively low rates of active ingredient. Moreover, its efficacy against strains of *B. cinerea* resistant to benzimidazole may make it a useful tool for resistance management.

**Cucumber mosaic virus in industrial tomato of Eleia county and the new threat from tomato necrosis.** P.E. KYRIAKOPOULOU<sup>1</sup>, N. VASSILAKOS<sup>2</sup>, F.P. BEM<sup>2</sup>, C. VARVERI<sup>2</sup>, M.S. GIRKIS<sup>1</sup> and N.G. SKOPELITIS<sup>1</sup>. <sup>1</sup>Agricultural University of Athens, Department of Plant Pathology, 75 Iera Odos, 118 55 Athens, Greece. <sup>2</sup>Benaki Phytopathological Institute, Laboratory of Virology, 8 S. Delta Str., 145 61 Kifissia, Athens, Greece.

Cucumber mosaic virus (CMV) is very widely distributed in Greece, infecting mainly vegetables, ornamentals, and weeds. During the last 10-15 years, it has caused severe problems in open field tomato crops, throughout Greece, mainly in the form of tomato shrinkage. Eleia county, one of the main tomato growing areas in Greece, is one of the most affected areas; due to this problem, open field tomato cultivation has been stopped in whole areas of Eleia county, like Zaharo, famous for the late table tomato, and the villages of Hanakia and Vounargo near Pyrgos where the industrial tomato used to be extensively grown. During the last years, the extent of this damage appears to be reduced. Nevertheless, problems are still occurring in many fields: in 1996, many tomato crops suffered severe damage, and some of them have been abandoned by their growers, and in the previous year, 1995, in two isolated crops of industrial tomato in Vounargo, extended necrotic to semilethal symptoms were observed on shoots, leaves and fruits of all plants, reminiscent of "tomato necrosis", known to be due to CMV hosting a necrotic form of a 5th, satellite, RNA molecule (CARNA-5). These two crops, despite the desperate efforts of growers, were destroyed by the disease and had to be abandoned by the grower. The virus isolated showed the properties of CMV on indicator plants, and caused the field symptomatology of tomato necrosis on tomato indicators, whereas electrophoresis of its purified preparation showed that the virus particles contained a 5th RNA of the expected size for pathogenic CARNA 5. A similar case was noticed and documented in the village of Alfeioussa about 10 km to the south. It seems that in the area around Pyrgos, the capital of Eleia, where these villages are located, severe strains of CMV and/or its satellite CARNA-5 reside which do not allow the cultivation of open field tomato. This area is geographically isolated from the area of Amalias, and it is hoped that due to this isolation the dangerous transfer of necrotic CMV inocula to the second area, north of Pyrgos, will not take place; otherwise the industrial tomato area of Amalias will be in great danger. In connection with this eventuality, the case of Argolis should be kept in mind: a lethal form of CMV-CARNA 5 has, since 1989, eliminated the cultivation of industrial tomato from Argolis with the consequence of closing the tomato factory unit there. Within the Amalias area, the main growing area of industrial tomato in Eleia, CMV was found to be hosted by about forty wild and several cultivated plant species, which could serve as primary sources of tomato infection through aphids.

**Apple scar skin viroid (ASSVd) in *Pyrus communis* and *P. amygdaliformis* in Greece.** P.E. KYRIAKOPOULOU<sup>1</sup> and A. HADIDI<sup>2</sup>. <sup>1</sup>Agricultural University of Athens, Department of Plant Pathology, 75 Iera Odos, 118 55, Athens, Greece. <sup>2</sup>U.S. Department of Agriculture, Agricultural Research Service, National Germplasm Resources Laboratory, Beltsville, MD 20705, USA.

In early summer of 1994, several blemished pear fruits of the early Greek variety Kontoula (short) were brought to the attention of the first author by a concerned grower. The fruits were collected from an orchard located in Galatas village of Troizhnia county, North East Peloponnesus, about 15 km east of Ancient Epidaurus. Pear fruits showed severe russetting and cracking, or extended superficial russet spilling, the first rendering the fruits worthless and the second reducing their commercial value. These symptoms occurred on about 130 out of 375 (30%) trees in an 8-year-old orchard of Kontoula on quince rootstock, as well as on a large percentage of Kontoula on wild pear rootstock (*Pyrus amygdaliformis*) in a nearby abandoned orchard, whereas all pear trees, variety Kossia on quince rootstock, within the first orchard did not show these symptoms, or showed very mild symptoms. The grower had started his orchard with high enthusiasm, but since 1990, when his trees were 4 years old and entering production, he had noticed the above symptomatology which continued the following years. He had similar or even worse problems in 1995; so, in big disappointment, he uprooted his orchard and replaced it with other crops. The observed symptoms are similar to those of apple scar skin and pear rusty skin diseases which are caused by apple scar skin viroid (ASSVd). After these observations, the first author recalled that during her childhood in village near Ancient Olympia, West Peloponnesus, she had often observed this symptomatology in fruits of the widely growing wild pear (*Pyrus amygdaliformis*), the common traditional pear rootstock in Greece. So she visited the area and made a survey in the fall of 1994 and 1996, during which these symptoms were indeed found to be wide spread in the village, as well as in the whole mountainous area of Olympia Prefecture. Affected fruit samples examined by the second author were positive for ASSVd in RT-PCR and dot-spot molecular hybridization tests. This is the first report of ASSVd in pear in Greece and the first report of ASSVd in wild pear. ASSVd may pose a serious threat for pome fruits in Greece and therefore its possible inclusion in the certification programs of vegetative propagation material of these crops should be considered. The presence of ASSVd in wild pear trees on mountains away from human interference may indicate the natural coexistence of this viroid with wild pears for millenia in Greece, and possibly in the Mediterranean region.

**A thermophilic isolate of cucumber mosaic virus from tomato in Istiaia of Euboea island.** P.E. KYRIAKOPOULOU<sup>1</sup>, N.G. SKOPELITIS<sup>1</sup>, F.P. BEM<sup>2</sup> and C. VARVERI<sup>2</sup>. <sup>1</sup>*Agricultural University of Athens, Department of Plant Pathology, 75 Iera Odos, 118 55 Athens, Greece.* <sup>2</sup>*Benaki Phytopathological Institute, Laboratory of Virology, 8 S. Delta Str., 145 61 Kifissia, Athens, Greece.*

Cucumber mosaic virus (CMV) is an aphid-borne, non-persistent, spherical, tripartite virus, 30 nm in diameter, with a genome of single-stranded RNA divided into 4 pieces, in many cases it is accompanied by a foreign, fifth, satellite RNA, the so-called CMV-associated RNA 5 or CARNA-5. CMV shows great readiness to develop genetic variability. It is one of the oldest known viruses (1916), has an extremely wide host range, is transmitted by almost a hundred aphid spe-

cies, and its geographical distribution covers the whole world. It affects many crops, mainly vegetables and ornamentals, to which it causes problems. Especially severe are problems caused in open field tomato crops, both industrial and table tomato, during the last 15 or more, in Greece and other Mediterranean countries, where it causes the diseases named tomato shrinkage, tomato necrosis, and tomato fruit necrosis. In Greece, it has exterminated tomato from various traditional tomato growing areas. Such an area is Istiaia in the north of Euboea island, where since 1990 it has caused such disasters that it has brought the growers to despair. The symptomatology is typical of tomato shrinkage and, to a certain degree, of tomato necrosis, with the main consequence fruit uselessness. It occurs in the field in June, when the crop is about 30-45 days old, it intensifies in July when the fruits start to mature, and its intensity peaks in August when the disease destroys the crop. The destructiveness seems to increase parallel to the increase of the temperature. This phenomenon led us to study a pure isolate of CMV from affected tomato of Istiaia. The results showed that this isolate was extremely heat resistant, since it remained alive in crude juice of affected tissue even after exposing it to 95°C for 10', whereas the thermal inactivation point (TIP) of CMV is known not to exceed 75°C. In the same study, increasing the temperature to which the crude juice was subjected resulted in an increase of virus pathogenicity, as shown by extended necrotic local and systemic symptoms on squash indicator plants. This indicates the possibility of the emergence of thermophilic, strongly pathogenic populations of the virus during periods of high atmospheric heat and light intensity, which usually occur in July and August in Greece. This emergence, possibly due to the greater facility with which double stranded forms of viral or satellite RNA open, may be related to the above mentioned quick increase of symptom severity in tomato fields in the summer months in Istiaia. The CMV isolate studied was found to contain CARNA-5.

**Pesticide residues in Greek agricultural products.** C. LENTZA-RIZOS<sup>1</sup> and E. AVRAMIDES<sup>2</sup>. <sup>1</sup>*National Agricultural Research Foundation, Center of Agricultural Research, Athens, Greece.* <sup>2</sup>*Ministry of Agriculture, Athens, Greece.*

In order to comply with EU requirements for the systematic monitoring of pesticide residues in agricultural products, the pesticide residue laboratory of the Regional Center of Crop Protection and Quality Control of Piraeus has been set up in Lycovrissi, Attiki. The laboratory became functional in June 1995, with the signing of an agreement between the Ministry of Agriculture and the N.AG.RE.F. From this time until today (June 1996):

- 7 international residue detecting methods have been screened in accordance with quality control guidelines for their suitability in meeting the needs of the laboratory. Recovery tests have been carried out on 517 pesticide-crop combinations, with a minimum of three replicates for each.
- Analyses were carried out:
  - a) on 75 samples of local agricultural produce from the Athens Central Agricultural Market or from super markets. Two multiresidue methods (NPD, ECD) and one specialized method were used;

- b) on 93 imported potato samples for organochlorine pesticide residues;
- c) on 119 virgin oil samples for organophosphorus pesticide residues;
- d) on 22 baby food samples, at the request of the National Drug Organization.

Of the 168 samples of fruit and vegetables, 136 (81%) had no detectable residues, 19 (11%) contained residue concentrations below EU or FAO/WHO MRLs and 13 (8%) contained pesticide residue concentrations exceeding the MRL. Of the 119 virgin oil samples, 31 (26%) had no detectable residues; 71 contained pesticide residue concentrations below the MRL and 17 contained residues above the MRL. In the samples of baby food no detectable pesticide residues were found.

**New hosts of *Verticillium dahliae* in Greece and worldwide.** E.K. LIGOXIGAKIS and D.J. VAKALOUNAKIS. *National Agricultural Research Foundation, Plant Protection Institute, 711 10 Heraklion, Crete, Greece.*

Extensive sampling of several cultivated and weed species showing symptoms of *Verticillium* wilt, was carried out during the period 1994-97. The aim of this work was to detect new hosts of *Verticillium dahliae* among the greenhouse, open field, and weed species growing in Crete. Seven hosts, new in Greece, of the pathogen were detected. Of these hosts, which belonged to six families, five were cultivated species: Romaine lettuce (*Lactuca sativa* L. var. *longifolia* Lam.), ochrus pea [*Lathyrus ochrus* L. (DL.)], radish (*Raphanus sativus* L.), and busy Lissie (*Impatiens balsamica* L.), whereas the other two were weed species: ox tongue (*Helminthotheca echioides* L.), and the sun spurge (*Euphorbia helioscopia* L.). To the best of our knowledge, four of the above species: *L. sativa* L. var. *longifolia*, *L. ochrus*, *H. echioides*, and *E. helioscopia* are reported here for the first time as hosts of *V. dahliae* worldwide.

**Isoenzymic polymorphism among isolates of races 1 and 2 of *Verticillium dahliae*.** A.G. MANGANARIS<sup>1</sup>, E.K. LIGOXIGAKIS<sup>2</sup> and D.J. VAKALOUNAKIS<sup>2</sup>. <sup>1</sup>*National Agricultural Research Foundation, Pomology Institute, 592 00 Naoussa, Greece.* <sup>2</sup>*National Agricultural Research Foundation, Plant Protection Institute, 711 10 Heraklion, Crete, Greece.*

Four isolates of race 1 and six isolates of race 2 of *Verticillium dahliae* Kleb. were examined electrophoretically in an effort to differentiate the two races of the pathogen on the basis of isoenzymic polymorphism. Starch gels utilized for electrophoretic analysis of the enzymes: malic dehydrogenase (MDH), shikimate dehydrogenase (SKDH), and phosphoglucomutase (PGM). Acrylamide gels for the enzymes were: glutamate oxaloacetate transaminase (GOT), esterase (EST), acid phosphatase (ACP), peroxidase (PRX), superoxide dismutase (SOD), alcohol dehydrogenase (ADH), and diaphorase (DIA). The enzymes ACP, MDH, GOT, and PGM were found monomorphic, whereas EST, SOD, DIA, and PRX were polymorphic. EST and SOD showed six and five different electrophoretic phenotypes respectively, whereas DIA and SOD showed only two phenotypes each. However, the isolates of races 1 and 2 could not be distinguished using the

above zymograms. An interesting observation from the analysis concerns the isolates from the Lasithi plateau. These isolates showed an electrophoretic band EST which did not appear in the other isolates.

**Genetic control of resistance to morpholines and piperidines in *Ustilago maydis*.** A.N. MARKOGLIOU and B.N. ZIOGAS. *Agricultural University of Athens, Laboratory of Phytopathology, 118 55 Votanikos, Athens, Greece.*

After UV-mutagenesis, strains of the phytopathogenic Basidiomycete *Ustilago maydis* resistant to the morpholines fenpropimorph and tridemorph and to the piperidine fenpropidin were isolated at a frequency of  $10^{-5}$ . Genetic work identified mutations at 5 chromosomal loci (*U/fpm-1*, *U/fpm-2*, *U/fpd-1*, *U/tdm-1* and *U/tdm-2*), which gave various levels of resistance to fenpropimorph and to fenpropidin-related fungicides. The *U/fpm-1* and *U/fpm-2* loci had two allelic genes (*U/fpm-1A*, *-1B* and *U/fpm-2A*, *-2B*), each of which governed a different phenotype regarding fenpropimorph and fenpropidin sensitivity. The mutant genes *U/fpm-1A*, *U/fpm-2A*, *-2B* and *U/fpd-1* were responsible for high resistance to fenpropimorph (Rf 75-100) moderate resistance to fenpropidin (Rf 15-20), and low resistance to tridemorph (Rf 5). The mutations *U/fpm-1B*, *U/tdm-1* and *U/tdm-2* gave low resistance (Rf 5-15) to the above three fungicides. Only haploid recombinants carrying both mutant minor genes *U/tdm-1* and *U/tdm-2* exhibited higher resistance than the parent strains, indicating additivity of gene effects. Such positive interaction was not observed with the other mutant loci. Obviously, both major-gene and polygenic control of resistance to fenpropimorph and fenpropidin-related fungicides operate in *U. maydis*. Cross resistance studies showed that the major gene mutations *U/fpm-1A*, *U/fpm-2* and *U/fpd-1* did not affect sensitivity to inhibitors of C-14 demethylase (DMIs). Contrary to that, the minor genes (*U/fpm-1B* and *U/tdm-1*, *-2*) increased 2-10 fold the sensitivity to triazoles and to pyridine pyrifenoxy but not that to the other DMIs. Study of the gene-effect on phytopathogenic parameters of mutant strains showed that the major gene mutations had no obvious effect on phytopathogenic fitness, while the minor genes had adverse effects on the rate of growth, infection ability and the rate of disease development.

**Azoxystrobin - a fungicide with a novel mode of action, active against a broad range of diseases on a wide range of crops.** G. MICHALOPOULOS, E. ALEVRA, A. GHIOLBASANIS, S. BITIVANOS and A. KAZANTZIDOU. *ZENECA Hellas S.A., 231 Syngrou Ave., 171 21 Nea Smyrni, Athens, Greece.*

Azoxystrobin is a new fungicide derived from a natural substance isolated from the mushroom *Strobilurus tenacellus* (Pers. ex Fries, Singer). Its fungicidal activity is due to its inhibition of mitochondrial respiration in fungi, resulting from the prevention of electron transfer between cytochrome b and cytochrome c1. This novelty in the mode of action of azoxystrobin offers a new opportunity in resistance management.

Among the range of pathogens that Azoxystrobin controls are:

- *Plasmopara viticola*, *Uncinula necator* and *Phomopsis viticola* in grapevines,
- *Podosphaera leucotricha* and *Venturia inaequalis* in apples,
- *Pseudoperonospora cubensis*, *Sphaerotheca fuliginea* and *Erysiphe cichoracearum* in melons/cucumbers,
- *Phytophthora infestans*, *Phytophthora nicotianae*, *Alternaria solani*, *Leveillula taurica*, *Septoria lycopersici* and *Colletotrichum coccoides* in tomatoes,
- *Monilinia fructicola* and *Cladosporium carpophilum* in peaches,
- *Cercospora beticola* and *Erysiphe betae* in sugar beet,
- *Puccinia asparagi* and *Stemphylium spp.* in asparagus.

In addition, in countries of northern Europe Azoxystrobin is mostly useful in controlling a number of pathogens in wheat and barley. Azoxystrobin is mainly a protective fungicide, offering the bonus of some curative activity. It moves well translamarily and is diffused throughout the whole leaf, without being accumulated at the leaf margins. Finally, Azoxystrobin is highly suitable for use in integrated pest management programmes because it displays a benign ecotoxicological profile and is harmless to beneficial organisms at field application rates. In Greece, Azoxystrobin has been tested so far on grapevines, cucumbers, tomatoes, melons, sugarbeet and asparagus. The results of the 49 trials in the last 5 years show a very promising suitability for the Greek environment.

**PCR amplification and cloning of the coat protein gene of CMV.** E.J. PAPLOMATAS. *Benaki Phytopathological Institute, Laboratory of Virology, 8 S. Delta Str., 145 61 Kifissia, Athens, Greece.*

Cucumber mosaic virus (CMV) is at present the most serious problem of tomato cultivation in Greece. Since there are no therapeutic measures against virus diseases, CMV controlled is in practice by preventive measures. During the last years, advances in molecular techniques have provided the tools for genetically engineering plant resistance to virus diseases. One of the approaches used is the production of transgenic plants that express the coat protein gene of the target virus (coat protein mediated resistance). The aim of this study was to clone the coat protein gene of a Greek strain of CMV with the final goal to produce transgenic tomato plants resistant to the virus. After retrieving from an electronic database coat protein sequences of previously characterized CMV strains and comparing them world-wide DNA primers were designed to amplify the coat protein gene by the polymerase chain reaction (PCR). DNA amplification was based on RT-PCR. The downstream primer (complementary to the 3' end of the gene) was used for the synthesis of the first DNA strand from purified viral RNA by reverse transcription (RT). The RT product was used in a PCR reaction with both primers. To ensure directional cloning, so that the cloned gene would have the correct orientation that would allow expression in the tomato plant, cloning sites were designed at the 5' end of each primer. A *BamH* I and a *Kpn* I site was engineered at the upstream and the downstream primers respectively. A PCR product with the expected size (about 900 bp) was double-digested with the endonucleases

*BamH* I and *Kpn* I, electrophoresed on agarose gel, and the DNA recovered using a glass matrix (Gene Clean, Bio 101). Then the DNA fragment was cloned in the pBluescriptII KS+ plasmid vector of *Escherichia coli*. At the moment, subcloning of the gene to a binary vector is underway, to be transferred to *Agrobacterium tumefaciens* and used for tomato plant transformation.

**Studies on the genetics and pathogenicity of the fungus *Rhizoctonia solani*.** E.J. PAPLOMATAS and N. PERATIS. *Benaki Phytopathological Institute, 8 S. Delta Str., 145 61 Kifissia, Athens, Greece.*

Inoculation of cotton hypocotyls using the glass plate method is a quick and reliable way to test the pathogenicity and virulence of the fungus *Rhizoctonia solani*. This method was used to evaluate the suitability of bean seedlings as alternative hosts of the fungus. Moreover, to study the relation between genetics and pathogenicity of the fungus, single-spore isolates of the pathogen and heterokaryons from crosses between single-spore isolates or between single-spore isolates and wild heterokaryons (field isolates) were produced. It was found that cotton seedlings were better indicator plants than beans to differentiate isolates of *R. solani* as to their pathogenicity, since cotton hypocotyls were infected earlier and were more susceptible than bean seedlings. Furthermore, it was found that single-spore isolates derived from highly pathogenic wild heterokaryons showed a wide range of virulence from avirulent to highly virulent. Heterokaryon formed from two single-spore isolates with medium and high virulence respectively, showed intermediate virulence on cotton seedlings. The same reaction was evident for another heterokaryon formed from a single-spore isolate with medium virulence and a highly virulent wild heterokaryon. Apart from the morphological and cultural characteristics of the crosses and their pathogenicity, heterokaryon formation was further shown using molecular markers. Random DNA fragments from a wild strain of *R. solani* were cloned and evaluated as probes to differentiate between single-spore isolates and heterokaryons. Up to now, one clone able to differentiate between the above isolates has been obtained; however, screening to find a clone that will give a DNA profile characteristic of a heterokaryon formed from single-spore isolates is being continued.

**Studies on biotic and abiotic factors active against cotton seedling damping-off caused by the fungus *Rhizoctonia solani*.** E.J. PAPLOMATAS and S. TZALAVARAS. *Benaki Phytopathological Institute, 8 S. Delta Str., 145 61 Kifissia, Athens, Greece.*

In an earlier study under greenhouse and field conditions we demonstrated the biocontrol activity of the fungus *Verticillium tricorpus* against damping-off of cotton seedlings caused by *Rhizoctonia solani*. Experimental evidence suggests that this biocontrol activity is achieved through the inhibition of catalase in the pathogen, since inhibitors of catalase activity, i.e. ascorbate, decreased the virulence of pathogenic *R. solani* isolates. The goal of this study was to evaluate several formulations of biotic (*V. tricorpus*) and

abiotic (ascorbate, salicylic acid) factors active against the fungus *R. solani*. For this purpose, two inert materials, diatomaceous earth and bentonite were used: a) for coating cotton seed and as carriers of *V. tricorpus* and b) for the production of pellets that encapsulated mycelia and spores of *V. tricorpus*. The effectiveness of these two treatments was compared with direct incorporation of the biological agent into the soil mix. Since effective biological control usually requires the use of large amounts of the biocontrol agent, something that is now considered not environmentally friendly even with a non-pathogenic fungus, we studied the possibility to control *R. solani* by mimicking the mode of action of *V. tricorpus*. To accomplish that, cotton seed was coated with ascorbate using either diatomaceous earth or bentonite as carriers. Moreover, salicylic acid, a known elicitor of induced resistance to other pathogens, was also evaluated for the biocontrol of *R. solani*. Greenhouse experiments showed that the survival of cotton seedlings from seed coated with mycelia and spores of *V. tricorpus* did not differ from that of untreated seeds planted in soil where *V. tricorpus* had been directly incorporated. Furthermore, both inert carriers (diatomaceous earth or bentonite) performed equally well. Ascorbate, when coated on cotton seeds, led to seedling survival equal to that of *V. tricorpus* and significantly different from the untreated control. Salicylic acid was found to perform against the pathogen similarly to ascorbate. However, preliminary pathogenicity tests using the glass plate method suggested that this effect of salicylic acid was not due to induced resistance. Finally, ascorbate was found to promote the growth of cotton seedlings, a very important point in disease development, since faster emergence of the seedlings shortens the period of their exposure to damping-off pathogens.

**Control of root and stem rot of cucumber (*Fusarium oxysporum* f. sp. *radicis-cucumerinum*) by grafting on resistant rootstocks.** G.CH. PAVLOU<sup>1</sup> and D.J. VAKALOUNAKIS<sup>2</sup>. <sup>1</sup>National Agricultural Research Foundation, Agricultural Research Station, 722 00 Ierapetra, Crete, Greece. <sup>2</sup>National Agricultural Research Foundation, Plant Protection Institute, 711 00 Heraklion, Crete, Greece.

To investigate whether grafting of cucumber on resistant rootstocks can be used to control root and stem rot caused by *Fusarium oxysporum* Schlechtend.: Fr. f. sp. *radicis-cucumerinum* D.J. Vakalounakis, an experiment was carried out during the 1995-96 crop season at the Agricultural Research Station of Ierapetra. Cucumber plants (Brunex F<sub>1</sub>) grafted on three rootstocks: PETO 42.91 F<sub>1</sub> (*Curcubita maxima* × *C. moschata*, Petoseed, France), TS-1358 F<sub>1</sub> (*C. maxima* × *C. moschata*, Hellas Hybrids, Greece) and TZ-148 F<sub>1</sub> (Tezier, France) were cultivated from October 31, 1995 to May 30, 1996 in a non-heated plastic greenhouse on soil artificially inoculated with the pathogen as well as on soil disinfected with methyl bromide (control). The following results were obtained:

1. None of the rootstocks were infected by *Fusarium oxysporum* f. sp. *radicis-cucumerinum*.
2. In midseason, a low percentage of grafted plants (approximately 8%) showed symptoms of infection by *Fusari-*

*um oxysporum* f. sp. *radicis-cucumerinum*, probably due to the contact of the cucumber stem with contaminated soil.

3. No significant difference ( $P=0.05$ ) in early and total yield of cucumber was obtained from grafted plants on any of the rootstocks.

4. No significant difference ( $P=0.05$ ) in early and total yield of cucumber was observed between grafted plants cultivated either on disinfected soil or on soil artificially inoculated with *Fusarium oxysporum* f. sp. *radicis-cucumerinum*.

5. The early (January) and total yield of grafted plants was significantly higher ( $P=0.05$ ) than that of self-rooted (non-grafted) cucumber plants, when both were cultivated on disinfected soil.

6. All self-rooted cucumber plants cultivated on soil artificially inoculated with *Fusarium oxysporum* f. sp. *radicis-cucumerinum* died gradually within four months after transplanting, giving no yield. By contrast, none of the self-rooted cucumber plants cultivated on disinfected soil was infected by the pathogen.

In conclusion, grafting cucumber on resistant rootstocks can be used as a control method against root and stem rot, and, in addition, gives higher yields of cucumber (in comparison with self-rooted plants), probably due to the larger and more vigorous root system of rootstocks.

**Morphogenetic studies of five tomato cultivars to develop transgenic plants resistant to CMV.** V. PLASTIRA. Benaki Phytopathological Institute, 8 S. Delta Str., 145 61 Kifissia, Athens, Greece.

To develop transgenic tomato plants resistant to CMV with *Agrobacterium*-mediated transformation, the regeneration frequency of five tomato cultivars was investigated in *in vitro* cultures. Important genotypic differences were observed among cultivars in regeneration frequency, the time the explants began to regenerate shoots and the average number of shoots per explant. The rate of explants expressing shoot regeneration after four weeks of culture was 70, 20 and 40% for the cultivars San Marzano nano, Principe Borghese and San Marzano respectively but 95, 100 and 95% for the same cultivars after eight weeks of culture, the corresponding average number of shoots per explant was 7.95, 5.35 and 7.05 respectively. By contrast, the regeneration frequency of the ACE 55VF and Pakmor cultivars did not exceed 80%, and the average number of shoots per explant was 2.62 and 2.75 respectively.

**Morphogenetic studies of tomato leaf, cotyledon and hypocotyl explants, to develop transgenic plants resistant to CMV.** V. PLASTIRA and A. PERDIKARIS. Benaki Phytopathological Institute, 8 S. Delta Str., 145 61 Kifissia, Athens, Greece.

To develop transgenic tomato plants expressing the CMV coat protein gene with *Agrobacterium*-mediated transformation, the regeneration ability of leaf, cotyledon and hypocotyl explants was investigated. The regeneration responses of explants depended on the presence of plant growth regulators. In their absence the explants were en-

larged and almost doubled in size, but did not produce callus or shoots, although frequently they produced roots. Benzyladenine (BA) and Zeatin at concentrations ranging from 0.1-10 mg l<sup>-1</sup> induced multiple shoot regeneration. Kinetin in most cases failed to cause shoot differentiation. All used levels of BA, alone or in combination with indol-acetic acid (IAA) induced adventitious shoots, but in the second case the regeneration frequency was higher. The best results were observed when BA was used in combination with IAA at concentrations 1-2 mg l<sup>-1</sup> and 0.01-0.02 mg l<sup>-1</sup>, respectively. Hypocotyl explants expressed a high regeneration ability. Depending on the BA concentration, 50-94% of hypocotyl explants regenerated shoots *in vitro*. Under the same conditions, 12-75% of cotyledon explants and 0-45% of leaf explants expressed shoot regeneration. Furthermore, the average number of shoots per explant was 4.73 for hypocotyls and 1.58 for cotyledons.

**Infection of gypsophila (*Gypsophila paniculata* L.) by *Rhizoctonia solani* Kühn in Greece.** P.N. ROZA and K. TZAVELLA-KLONARI. *Aristotelian University, Faculty of Agriculture, Plant Pathology Laboratory, P.O.Box 269, 540 06 Thessaloniki, Greece.*

Severe necrosis of young plants of *Gypsophila paniculata* L. was observed in open cultivation in the area of Plati Imathias during the spring of 1994 and 1995. Symptoms of brown spots leading to collar-rot, root-rot and damping-off were detected on young plants. The fungus *Rhizoctonia solani* Kühn was isolated from all the infected parts in isolations made in the Plant Pathology Laboratory of our University. Pathogenicity tests carried out by simple contact of inoculum on stems of young cuttings, produced the same symptoms after three days at room temperature (26-29°C), while wilt and damping-off appeared after six days. Following anastomosis tests the fungal isolates were shown to belong to anastomosis group 4 (AG-4). The colour of the culture on PDA after 21 days of incubation at 25°C in the dark was whitish brown with a membranous surface. There were neither concentric rings nor sclerotia. The hyphal width was 7.5 µm as found by measuring 30 cells at equal distance from the hyphal tip on the running hyphae. The above morphological characteristics were typical of that anastomosis group. The optimum temperature, measured by growth rate of the colony at 10, 15, 20, 25 and 30°C, was 25°C. The disease is known to occur in America since 1947 and also in Israel and Europe but as far as we know this is the first report for Greece.

**Viruses of garlic (*Allium sativum*) in Greece.** R. SALOMON<sup>1</sup> and N.I. KATIS<sup>2</sup>. <sup>1</sup>ARO, *The Volcani Center, Department of Virology, P.O.Box 6, Bet Dagan, 50-250 Israel.* <sup>2</sup>Aristotelian University, *Faculty of Agriculture, Plant Pathology Laboratory, 540 06 Thessaloniki, Greece.*

Garlic (*Allium sativum*) is propagated exclusively by vegetative means (cloves) and there is ample opportunity for multiple virus infections to accumulate in old garlic clones. The use of virus-free material is the only way to combat virus diseases. The first stage in the development of an im-

proved garlic propagation scheme through meristem culture is the identification of the viruses involved. The purpose of our work was to identify garlic viruses. About eighty symptomatic garlic leaves, were collected from two areas and tested for the presence of specific viruses by ACP-ELISA. Seventeen bulb cloves were also tested by the same method. The viruses looked for were onion yellow dwarf virus (OYDV), leek yellow stripe virus (LYSV), a carlavirus (193) not yet classified, turnip mosaic virus (TuMV) and garlic virus 2 potyvirus (GV-2). In leaf samples from Larissa 69.7% (46 out of 66), 90.9% (60 out of 66), 89.4% (59 out of 66) and 3% (2 out of 66) were infected by OYDV, LYSV, TuMV and 193 (carlavirus) respectively. In samples from Thessaloniki 58.3% (7 out of 12), 16.6% (2 out of 12) and 75% (9 out of 12) were infected by OYDV, LYSV and 193 respectively. OYDV and the carlavirus were found only in two clove samples. LYSV and GV-2 were not detected, but TuMV was found in all samples tested. However, the extent of damage caused by TuMV infection in garlic crops is unknown.

**ACROBAT (dimethomorph). A new selective fungicide for the control of Oomycete diseases in vines, potatoes and vegetables.** D. SERVIS and P. SILELOGLOU. *Technical Department of Cyanamid Hellas SA, 48 Egialias Str., 15125 Maroussi, Athens.*

Dimethomorph is a new active compound with fungicidal activity against oomycetes of the Pythiaceae (*Phytophthora* spp.) and Peronosporaceae (*Plasmopara viticola*, *Peronospora tabacina*, *P. destructor*, *Pseudoperonospora cubensis*, *Bremia lactuca*) families. It belongs to the chemical group of cinnamic acid derivatives. It is active at very low concentrations (0.2 - 1.6 ppm) causing the lysis of cell wall in all stages involving active cell wall growth and inhibition of sporangiophore formation and sporangial or spore germination. It has extremely low toxicity to non target organisms. Dimethomorph exhibits local systemic (penetrant) action when applied on the leaves, and systemic acropetal movement when applied on the root system. In the field it is used at concentration ranges of 0.015 - 0.019% in formulations containing mancozeb or copper oxychloride. It is active against strains resistant to other fungicides groups. In Greece ACROBAT was tested in field trials during 1992-1996. It showed high efficacy and long residual activity in protecting vegetation and fruits of vine, potato and cucumber.

**Temporal and spatial aspects of aphid movement between plants and spread of BYDV.** I. SMYRNIODIS<sup>1</sup>, J. MANN<sup>2</sup> and R. HARRINGTON<sup>2</sup>. <sup>1</sup>Aristotelian University of Thessaloniki, *Faculty of Agriculture, Laboratory of Plant Pathology, 540 06 Thessaloniki, Greece.* <sup>2</sup>Department of Entomology and Nematology, *AFRC Institute of Arable Crops Research, Rothamsted Experimental Station, Harpenden, Herts AL5 2JQ, UK.*

In an experiment carried out in a greenhouse with fluctuating temperature at Rothamsted Exp. Station, dispersal, time spent on a plant and ability of aphid species *Sitobion*

*avenae* and *Rhopalosiphum padi* to transmit Barley Yellow Dwarf Leutovirus (BYDV) were studied. Adult apterae, previously reared on plants infected with BYDV (isolates PAV and MAV), were placed on a tray with barley plants (*Hordeum vulgare*) at two differed growth stages (GS 12 and GS 22). More than 40% of apterous aphids were found to move from the release plant within 72 h. *S. avenae* seemed to move further than *R. padi* but there were no significant differences between the two species. The inoculation access period for BYDV varied. Apterous adult of *S. avenae* released on individual barley plants of different growth stages were found to move more from 5 weeks old plants than from one and two weeks old plants.

**The significance of meteorological parameters in forecasting, monitoring and suppressing fungal diseases in vineyards.** S.I. SPANOS and C.D. ZARPAS. *University of Thessaly, Laboratory of Agrometeorology, Department of Agriculture, Pedion Areos, 383 34 Volos, Greece.*

An overview of the significance of meteorological parameters in forecasting, monitoring and suppressing fungal diseases in vineyards was conducted. The impact of meteorological conditions on plants, on fungi and on their interaction leading to infection was examined separately. The impact on plants was analyzed by using remote sensing techniques of phenological stages through NDVI (Normalized Difference Vegetation Index) and by using the thermal unit accumulation based on ground measurements. Examples are given from the database of satellite and ground stations of University of Thessaly. The impact on fungi is examined through direct link to biological circle or through the modification of micrometeorological conditions by the weather. Calculation and measurement techniques of the most significant micrometeorological parameters (leaf temperature and wetness) are described and examples are given from automatic ground stations. A comparison of these parameters with typical meteorological parameters such as air temperature, relative humidity and rainfall is also discussed. Finally computational techniques of conidia dispersion by wind are presented and discussed.

**Preliminary studies of a seed-transmitted virus of vetch.** M.G. TERZAKIS<sup>1</sup> and N.I. KATIS<sup>2</sup>. <sup>1</sup>*National Agricultural Research Foundation, Plant Protection Institute, 711 10 Heraklion, Crete, Greece.* <sup>2</sup>*Aristotelian University, Faculty of Agriculture, Plant Pathology Laboratory, P.O.Box 269, 540 06 Thessaloniki, Greece.*

A seed-transmitted virus was isolated from a seedlot of vetch (*Vicia sativa* L.) originating from the Union of Agricultural Cooperatives of Ioannina. Infected seedlings showed severe stunting, reduced lamina and leaf mottling. The host range of the virus was restricted to a few plant species, mainly belonging to the families Leguminosae and Solanaceae, and exhibiting ringspots, severe malformations and leaf necrosis. The dilution end point (DEP) of cucumber sap was 10<sup>-5</sup>, the thermal inactivation point was 50°C, and its longevity *in vitro* was 2-5 days. Transmission tests in the non-persistent manner by using the aphids *Myzus persicae* Sulzer

and *Aphis fabae* Scop. were negative. The virus was transmitted through the seed of *Chenopodium quinoa* Willd. (4.2%), *Nicotiana rustica* L. (5.5-11%) and *C. amaranticolor* Coste et Reyn (up to 93%). Eradication treatments of *Ch. amaranticolor* seeds using either 10% Na<sub>3</sub>PO<sub>4</sub> or 10 min thermotherapy at 60°C for 2 days were ineffective, while 3 days at 70°C resulted in total loss of seed germination. The sedimentation profile of partially purified preparations revealed particles of three classes, while UV analysis of purified preparations indicated that nucleic acid content ranged from 12.5 to 40%.

**Infection of gypsophila (*Gypsophila paniculata* L.) by *Rhizoctonia solani* Kühn in Greece.** P. ROZA and K. TZAVELLA-KLONARI. *Aristotelian University, Faculty of Agriculture, Plant Pathology Laboratory, P.O.Box 269, 540 06 Thessaloniki, Greece.*

Severe necrosis of young plants of *Gypsophila paniculata* L. was observed in open culture in the area of Plati Imathias during the spring of 1994 and 1995. Symptoms of brown spots leading to collar-rot, root-rot and damping-off were detected on young plants. The fungus *Rhizoctonia solani* Kühn was isolated from all the infected parts, in isolations made in the Plant Pathology Laboratory of our University. Pathogenicity tests, carried out with simple contact of inoculum on stems of young cuttings, showed the same symptoms after three (3) days at room conditions (26-29°C), while wilt and damping-off appeared after six (6) days. The isolations of the fungi were proved to belong at the anastomosis group 4 (AG-4) after anastomosis tests. The colour of the culture on PDA at 25°C after 21 days of incubation in dark was whitish brown with membranous surface. There were neither concentric rings nor sclerotia. The hyphal width was 7.5 µm as found after measuring 30 cells of the same distance from the hyphal tip in the running hyphae. The above morphological characteristics are typical of the particular anastomosis group. The optimum temperature, measured by growth rate of the colony at 10, 15, 20, 25, and 30°C, was that of 25°C. The disease is known in America since 1947 and also in Israel and Europe but as far as we know it is for first time mentioned in Greece.

**Comparative study on some isolates of the fungi *Sclerotinia trifoliorum* and *Sclerotinia sclerotiorum*.** I. THEOCHARI-ATHANASIOU. *National Agricultural Research Foundation, Fodder crops and Pastures Institute, 411 10 Larissa, Greece.*

Some isolates belonging to the genus *Sclerotinia* and deriving from winter leguminous infected plants were studied with regard to *in vitro* development at different temperatures, conditions required for the sclerotia germination, subsequent apothecia formation and dimensions of asci and ascospores, in order to clarify their taxonomic position in the *S. trifoliorum* and *S. sclerotiorum* species. Isolates classified as *S. sclerotiorum* showed good development even at temperatures as high as 30°C, while those classified as *S. trifoliorum* developed poorly at 25°C. For all the isolates tested sclerotia germination and the apothecia formation

required very high soil humidity, near the saturation level, as well as a relative humidity at 95-100%. However, a distinction concerning temperature requirements at this stage was observed. The isolates considered to be *S. sclerotiorum* needed a constant temperature around 10°C for at least 48 h, while a fluctuating daily temperature between 7°C and 15°C was needed for *S. trifoliorum* isolates. Average ascus length, was about 143 µm for the *S. sclerotiorum* isolates and 225 µm for *S. trifoliorum*. In the case of *S. sclerotiorum* isolates the size of the ascospores averaged 12x6.2 µm. The ascospores of *S. trifoliorum* isolates exhibited a dimorphism in their size and all the asci contained consistently four small and four large spores, measuring about 15.5x7.5 µm and 20x10.5 µm respectively.

**Infection of *Phoenix theophrastii* by the fungus *Graphiola phoenicis* in the natural forest of Vai in Crete, Greece.** P. TSOPELAS. *National Agricultural Research Foundation, Institute of Mediterranean Forest Ecosystems, Terma Alkmanos, 115 28 Athens, Greece.*

The palm tree *Phoenix theophrastii* Gr. is an indigenous species of Crete in the natural forest of Vai, located in the Sitia region. Infections by the fungus *Graphiola phoenicis* (Moug.) Poit., mainly on the lower leaves were observed in a limited number of palm trees of the forest. The infected trees were located near running or stagnant water in the forest area, where humidity was always high. Infections by the same fungus were also observed on young trees of *P. theophrastii* in a private nursery near the forest of Vai. The trees in the nursery were watered by sprinkler irrigation, which created conditions of excessive moisture both on leaves and in the atmosphere and resulted in infection of a great number of trees. Sori with spores of *G. phoenicis* were evident on both sides of the infected leaves, but leaf tissue destruction was limited in most leaves. The fungus is a biotrophic parasite obtaining its nutrients from living cells without creating extensive damage on the host tissues. However, premature necrosis was observed on some severely infected leaves. Leaf pruning and destruction of the infected plant material was suggested for controlling the disease in the ornamental forest of Vai. In nurseries, for the production of disease-free plants the use of the fungicide carbendazimol alternate with thiophanate-methyl, zineb, and mancozeb was suggested.

**First record of *Puccinia horiana* on *Chrysanthemum x morifolium* in Greece.** D.J. VAKALOUNAKIS. *National Agricultural Research Foundation, Plant Protection Institute, 711 10 Heraklion, Crete, Greece.*

In February 1995, a severe attack of *Chrysanthemum morifolium* Ramat by *Puccinia horiana* P. Henn was observed in a glasshouse cultivation in the area of Folede (Crete). The first symptoms of the disease appeared as light green, sunken lesions on the upper surface and as blisters on the lower surface of young leaves. The blisters were initially white, but later they became gray or light brown in colour. Following the rupture of epidermis telia with numerous elongated teliospores, 39.2±5.56 (27.8-50.8) x 14.5±1.45

(12.1-18.2)µm, were observed. The wall of the teliospores was of a pale yellow colour (1-2µm in width), whereas the pedicel was hyaline up to 34.4±6.49 (24-2-41.1) x 6.0±1.09 (4.8-7.3)µm. This is the first report of *P. horiana* in Greece.

**Preliminary study on the vegetative compatibility of *Fusarium oxysporum* f. sp. *radicis-cucumerinum* causing root and stem rot of cucumber.** D.J. VAKALOUNAKIS. *National Agricultural Research Foundation, Plant Protection Institute, 711 10 Heraklion, Crete, Greece.*

Root and stem rot of cucumber (*Cucumis sativus* L.), caused by the new worldwide fungal parasite *Fusarium oxysporum* Schlechtend.: Fr. f. sp. *radicis-cucumerinum* D.J. Vakalounakis, was recently described in Crete where it is causing severe losses in greenhouse crops. To investigate the genetic relatedness among various *F. oxysporum* f. sp. *radicis-cucumerinum* isolates and develop a reliable and quick technique for pathogen identification we carried out a preliminary study on the vegetative, or heterokaryon, compatibility of a collection of 28 monoklonal isolates obtained from greenhouse crops in Crete during 1990-96. Nitrate non-utilizing (*nit*) mutants were generated from all isolates on *Fusarium* minimal medium (FMM) amended with 1.5% potassium chlorate (FMKC) and used for complementation tests. Our data demonstrated the homogeneity of the *F. oxysporum* f. sp. *radicis-cucumerinum* population; all 28 isolates were classified into one vegetative compatibility group (VCG). Heterokaryon formation was never observed among the testers of *F. oxysporum* f. sp. *radicis-cucumerinum* and complementary *nit* mutants of the three known races, 1, 2 and 3, of *F. oxysporum* f. sp. *cucumerinum* J.H. Owen, which causes fusarium wilt of cucumber. According to the Puhala numbering system we suggest that the VCG of *F. oxysporum* f. sp. *radicis-cucumerinum* be numbered 0260. Complementation tests will continue with more isolates of both *F. oxysporum* f. sp. *radicis-cucumerinum* and *F. oxysporum* f. sp. *cucumerinum*.

**Virus assessment of apricot mother-trees in the state farm of Evinochori with ELISA.** C. VARVERI and A. KAFCHITSAS. *Benaki Phytopathological Institute, Laboratory of Virology, 8 S. Delta Str., 156 61 Kifissia, Athens, Greece.*

During spring of 1995 a survey was undertaken in an apricot mother-tree block situated at the state farm of Evinochori, Etoloakarnania, aiming at the characterization of its sanitary condition regarding virus-diseases and, further, at the production of propagation material free of them. The 178 trees tested were seven years old and belonged to five different cultivars. Five samples of three leaves each were collected from every tree and tested separately for the presence of plum pox potyvirus (PPV), prunus necrotic ring-spot (PNRSV) and prune drarf (PDV) ilaviruses, apple chlorotic leaf spot trichovirus (ACLSV) and tomato ringspot nepovirus (TomRSV). The DAS-ELISA immunochemical method was used with reagents either produced in the laboratory (PDV, PNRSV) or purchased from the market (ACLSV, PDV, TomRSV). The tests revealed that 14 trees (7.9%) were infected with PPV (only two of which showed

sharka symptoms), 14 with PNRSV (7.9%) and four with ACLSV (7.9%). The eradication of all these trees was suggested.

**Establishment of the critical levels of ozone in Europe and its effects on crop plants, forests and natural vegetation.** D. VELISSARIOU. *Benaki Phytopathological Institute, 8 S. Delta Str., 145 61 Kifissia, Athens, Greece.*

Ozone is formed in the biosphere as a result of photochemical air pollution and has proved to be the most toxic air pollutant for plants because of its biological effects and its wide spatial dispersion. Air pollution problems in general, apart from their local or national importance, also have a global dimension (transboundary air pollution, forest decline, global heating, etc.). The last decade four Workshops were held in Europe under the auspices of the UN-ECE Convention on Long-Range Transboundary air pollution (Bad Harzburg, Germany 1988; Egham UK 1992; Bern, Switzerland 1993 and Kuopio, Finland 1996), to establish the critical level in Europe for the most important phytotoxic air pollutants. The two last Workshops were held specifically for critical level of ozone. Critical levels are defined as those concentrations of pollutants in the atmosphere above which direct adverse effects may occur on crops, forests and natural vegetation. The Critical level for ozone in Europe was expressed as the cumulative exposure over the threshold concentration of 40 ppb (parts per billion) for a specific time period (AOT40 = Accumulated exposure Over a Threshold of 40 ppb). For agricultural crops and natural vegetation the critical level was defined as 3.000 ppb/hours for the 3 month period May-July. For forests the value was 10.000 ppb/hours for the 6 month period April-September. With the definition of those values, the climatic character of the Mediterranean area and particularly Greece, mainly due to the strong and prolonged solar irradiation favouring ozone formation, becomes apparent. As an example we can report that using the database of the State air pollution monitoring network at Athens, the AOT40 mean value for the 3 month period May-July for the station of Lioussa, 14 km N of the centre of Athens for the years 1991-1994 was calculated as 19.000 ppb/hours.

**New fungal diseases on ornamental plants in Greece.** I. VLOUTOGLOU. *Benaki Phytopathological Institute, 8 S. Delta Str., 145 61 Kifissia, Athens, Greece.*

During examination of diseased plant specimens in the Benaki Phytopathological Institute, the following fungal diseases, reported for the first time in Greece, were identified: 1. *Gibberella fujikuroi* (Saw.) Wollenw. on *Dracaena fragrans* L.

In May 1995, presence of numerous small (1-2 mm), circular, chlorotic lesions was observed on glasshouse-grown *Dracaena fragrans* plants in Kifissia. In some cases, the lesions coalesced forming larger lesions of irregular shape with

a necrotic centre and chlorotic halos. The fungus *Fusarium moniliforme* (perfect stage *Gibberella fujikuroi*) was isolated from infected tissues.

2. *Botrytis cinerea* Pers. ex Fr. on *Dracaena marginata* L. In January 1995, a serious leaf spot was observed on glasshouse-grown *Dracaena marginata* plants in Markopoulo, Attika. Lesions were circular (0.5 - 3 mm) or slightly elongated with light brown or orange-brown centre, dark brown margin frequently surrounded by chlorotic halos. Gradually the lesions enlarged and increased in number resulting in necrosis and fall of infected leaves. The fungus *Botrytis cinerea* was consistently isolated from the lesions.

3. *Botrytis cinerea* Pers. ex Fr. on *Yucca* sp. In October 1995, a serious leaf spot was observed on glasshouse-grown *Yucca* plants in Drama. The lesions, initially confined to the periphery of the leaf blade, were elongated, (1-2.5)×0.5 cm, with a light brown or orange-brown centre, dark brown margin surrounded by chlorotic halos. In severe infection, the lesions coalesced forming large necrotic areas resulting in the death of the leaves. The fungus *Botrytis cinerea* was isolated from the infected tissues.

**New pathogenic fungi of the genus *Alternaria* in Greece.** I. VLOUTOGLOU and D. LASCARIS. *Benaki Phytopathological Institute, 8 S. Delta Str., 145 61 Kifissia, Athens, Greece.*

In 1995 and 1996, serious leaf spotting was observed on *Dichondra repens* L. (Convolvulaceae) plants caused by the fungus *Alternaria dichondrae* Gambogi, Vannacci & Triolo. The symptoms appeared on the leaf blades as small (1-2 mm), brown, lesions of irregular shape, which often showed a dark brown margin surrounded by chlorotic halos. In severe infection, the lesions appeared necrotic and covered most or the whole leaf surface. The fungus was isolated on PDA medium, on which it produced a few conidia. Typical disease symptoms were reproduced on *D. repens* plants artificially inoculated with a conidial suspension of  $5 \times 10^4$  conidia/ml. The pathogen was re-isolated from artificially inoculated plants. This is the first report of the occurrence of *A. dichondrae* on *D. repens* plants in Greece, and the third record world-wide. In October 1995, a serious disease caused by the fungus *Alternaria alternata* (Fr.) Keissler was observed on a commercial, outdoor cultivation of *Jasminum grandiflorum* plants in Kato Souli in Attica. The symptoms appeared as necrotic areas, mainly at the tip of the leaflets. In many cases the necrotic areas covered most or the whole leaflet blade and spread to the petiole of the composed leaf and through it to the stem. Similar symptoms were also observed on the flower petals. In severe infection, necrosis of stems or of the whole plant was observed. The fungus was isolated on PDA medium and Koch's postulates were applied to healthy *J. grandiflorum* plants at the flowering stage. This is the first report of the occurrence of *A. alternata* on *J. grandiflorum* plants in Greece and the second world-wide.