

MEETING

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OPENING LECTURE

G. LOEBENSTEIN
Dept. of Virology, ARO, The Volcani Center, Bet Dagan, Israel

A: *BIOLOGICAL CONTROL OF DISEASES; BIOLOGICAL CONTROL OF WEEDS WITH FUNGI; MYCOHERBICIDES*

TRICHODEX, A GROWTH STIMULATOR AND A BIOLOGICAL CONTROL AGENT FOR SOILBORNE DISEASES

E. BARAK, A. COHEN, H. ABIR and M. HOLIN
Makhteshim Chemical Works Ltd., Be'er Sheva, Israel

The fungus *Trichoderma harzianum* Rifai effectively controls diseases caused by soilborne plant pathogenic fungi, e.g. *Rhizoctonia solani*, *Sclerotium rolfsii*, *Fusarium* spp., *Pythium* spp. and some *Aspergillus* spp. The biofungicide Trichodex is based on *T. harzianum* isolate MTR-35. This isolate was identified as a potential control agent at the Faculty of Agriculture, Hebrew University of Jerusalem, Rehovot, Israel. The naturally occurring isolate, MTR-35, either parasitizes the phytopathogenic fungi or reduces their pathogenic activity by competition in the rhizosphere.

Greenhouse and field trials proved Trichodex to be an effective soil biofungicide when applied as a seed coating, during seedbed loading, during growth medium loading, or in the furrow.

Trichodex may be mixed with most of the commercial fungicides and pesticides used in agriculture. Its efficacy increases when applied after soil sterilization with soil fumigants, or after soil solarization. Soil application of Trichodex also enhances seed germination, root development, plant growth and flowering of various vegetables and ornamentals.

BIOLOGICAL CONTROL OF GRAY MOLD DISEASE ON VARIOUS CROPS

GILLY SHIMSHONI¹, Y. ELAD¹, A. COHEN² and I. CHÉT³

¹Dept. of Plant Pathology, ARO, The Volcani Center, Bet Dagan; ²Makhteshim Chemical Works Ltd., Be'er Sheva; and ³Dept. of Plant Pathology and Microbiology, The Hebrew University of Jerusalem, Faculty of Agriculture, Rehovot, Israel

Two hundred isolates of non-pathogenic fungi were isolated from different aerial parts of host plants naturally infected by *Botrytis cinerea*. These isolates, together with some from a collection, were tested for their ability to reduce the development of gray mold on leaves of different host plants: beans, pelargonium, cucumber, pepper, tomatoes, and rose petals. The detached leaves were inoculated with conidia or mycelium plugs of *B. cinerea*. From this screening five isolates of *Trichoderma* spp. were found to be more efficient in reducing the development of the disease. Spraying conidia of these isolates on rose flowers resulted in a 10-50% reduction of the disease severity as compared with control flowers. A concentration of at least 10^7 conidia/ml was required in order to achieve significant control. Spraying conidia of *Trichoderma* spp. on tomato fruits locally injured and inoculated with conidia of *B. cinerea* resulted in a 54-93% reduction of the disease incidence; the respective figure in bean plants was 67%. Two of the isolates of the antagonistic fungus were tested further, in a vineyard. Each of the isolates, after being sprayed separately on bunches of grapes, led to a significant 58-69% reduction in disease incidence. A combined folpet (0.25%) plus *Trichoderma* sp. treatment resulted in a reduction of 76%; this figure did not differ significantly from treatments with the antagonists alone. Treatment with vinclozolin (0.1%) resulted in a 57% decrease in disease incidence.

IMPROVEMENT OF TRICHODERMA SPP. FOR BIOLOGICAL CONTROL USING PROTOPLAST FUSION

SARA PE'ÈR and I. CHÉT

Dept. of Plant Pathology and Microbiology, The Hebrew University of Jerusalem, Faculty of Agriculture, Rehovot, Israel

Trichoderma harzianum serves as a biocontrol agent against soilborne plant pathogenic fungi. Individual strains of *Trichoderma* obtained through selection or mutation possess one or a few desirable traits, but none has all the attributes required to realize the full potential of these beneficial fungi. Since *Trichoderma* has no sexual stages, protoplast fusion techniques were used. Protoplasts from an auxotrophic mutant of *Trichoderma harzianum* Rifai (ATCC) 32173 were obtained from young thalli by digestion of cell walls with Novozym 234. Protoplasts were fused with 33% polyethylene glycol (PEG), 0.02M CaCl₂ and 0.01M tris-HCl, pH 7.5. Fusion between protoplasts of nutritionally complementary strains was detected by formation of heterokaryons developing on solid minimal medium. After 4-5 days fast-growing prototrophic colonies appeared, and after 3-4 weeks slow-growing colonies appeared. The prototrophic strains were classified into phenotype groups differing in conidia color (white and brown as the parental type and green, white-green, yellow-green and cream as the nonparental type), growth rate, and tolerance to benomyl and cycloheximide. The antagonistic ability was determined by growing *T. harzianum*

strains towards *Rhizoctonia solani* or *Sclerotium rolfsii*. The prototrophic strains were classified into three groups: (i) grew on the pathogenic fungus faster than the parents and the wild type; (ii) grew as well as the parents; and (iii) grew slower than the parents. The use of the protoplast fusion technique resulted in better antagonistic strains for biological control which are more tolerant to benomyl than the parents.

BIOLOGICAL CONTROL OF PHYTOPHTHORA ROOT ROT IN *PERSEA INDICA* SEEDLINGS

I. YONA¹, Y. PINKAS¹, Y. ELAD¹ and I. CHET²

¹Dept. of Plant Pathology, ARO, The Volcani Center, Bet Dagan; and ²Dept. of Plant Pathology and Microbiology, The Hebrew University of Jerusalem, Faculty of Agriculture, Rehovot, Israel

Biological control of root-rot disease caused by *Phytophthora cinnamomi* was demonstrated on *Persea indica* seedlings, a wild, Phytophthora-susceptible relative of avocado (*Persea americana*). Six rhizosphere bacteria and *Trichoderma harzianum* – all antagonistic to *Pythium aphanidermatum*, and *Serratia marcescens* were used as biocontrol agents. In greenhouse experiments, complete prevention of disease incidence was achieved by the introduction of *S. marcescens*, *Pseudomonas putida*, *Alcaligenes* sp. and *T. harzianum* into soil artificially infested with the pathogen. Another isolate of *P. putida*, and three isolates of *P. cepacia*, reduced the disease severity by 20-80%. *In vitro*, each of the tested organisms inhibited linear growth of the pathogen mycelium. Maximal inhibition was obtained using *S. marcescens*. Increased *P. indica* growth was observed in non-infested soil treated with *Trichoderma*, while the bacterial isolates had no effect on plant growth. The number of pathogen propagules that survived in soil was significantly reduced as compared with an untreated control when bacterial suspensions were added (separately) to the infested soil. Introduction of *S. marcescens* into infested soil with 10⁴ propagules/g soil resulted in rapid reduction of the pathogen population to a non-detectable level, whereas the untreated control showed a reduction to 10² propagules/g soil. To determine the effect of different soil types on disease development, seven soils from various locations in Israel were artificially infested with the pathogen before planting with *P. indica* seedlings. Incidence and severity of disease were less in Gilat and Bet Dagan soils than in soils from Givat Hayyim, Zemakh, Alumot, Eliad and Sha'ar haGolan. Disease severity in infested Gilat soil, previously steamed (100°C for 4 h), was significantly higher than in infested unsteamed soil, which indicates that biotic factors might be responsible for disease suppression.

BIOLOGICAL CONTROL OF VERTICILLIUM WILT OF EGGPLANT BY *TALAROMYCES FLAVUS*

T. FAHIMA, J. KATAN and Y. HENIS

Dept. of Plant Pathology and Microbiology, The Hebrew University of Jerusalem, Faculty of Agriculture, Rehovot, Israel

Eggplant (*Solanum melongena*) seedlings were grown in a greenhouse in "Speedling" trays for several weeks in the presence of *Talaromyces flavus* and then transplanted to soil infested with *Verticillium dahliae*. The antagonist was applied as an ascospore suspension to the eggplant rooting mixture. To induce germination, ascospores were activated by heat-shock.

In one experiment soil was artificially infested with *V. dahliae* microsclerotia at two levels (10 and 100 microsclerotia/g soil). *T. flavus*-treated plants showed less disease than untreated ones throughout the experimental period, and at the end of the experiment (17 wk after planting) there was a 60% and 57% (significant, $P = 0.05$) reduction in disease index on plants grown in soil containing 10 and 100 microsclerotia/g, respectively, as compared with untreated plants.

In a second experiment using soil naturally infested with *V. dahliae*, disease incidence 6, 8 and 10 wk after planting was less in *T. flavus*-treated plants than in untreated plants. At the end of the experiment (10 wk after planting), disease incidence in the treated plants was 27% as compared with 80% in the untreated ones, *i.e.*, a significant disease reduction ($P = 0.05$) of 66%.

In an attempt to evaluate the mechanism(s) involved in its ability to reduce Verticillium wilt of eggplant, the capacity of *T. flavus* to parasitize live microsclerotia of *V. dahliae* was examined. Germinability of microsclerotia incubated for 2 wk in soil amended with a wheat-bran preparation of *T. flavus* was reduced by 85% as compared with microsclerotia incubated in untreated soil. Scanning electron microscope observations revealed hyphae of *T. flavus* growing and sporulating over the microsclerotia. Examinations of attacked microsclerotia under a transmission electron microscope revealed different stages of penetration of *T. flavus* hyphae into microsclerotial cells of *V. dahliae*. Microsclerotia added to eggplant roots colonized by *T. flavus* were attacked by the antagonist's hyphae. Observation under a light microscope revealed aerial sporulating hyphae of *T. flavus* emerging directly from microsclerotia.

SUPPRESSION OF SEPTORIA BLOTCH AND LEAF RUST OF WHEAT BY 1-HYDROXYPHENAZINE PRODUCED BY FLUORESCING PSEUDOMONAD CELLS

EDNA LEVY¹, Z. EYAL¹ and I. CHET²

¹Dept. of Botany, The George S. Wise Faculty of Life Sciences, Tel-Aviv University, Ramat Aviv;
and ²Dept. of Plant Pathology and Microbiology, The Hebrew University of Jerusalem,
Faculty of Agriculture, Rehovot, Israel

Application of fluorescing pseudomonad cells of isolate LEC1 onto wheat seedlings, prior to inoculation with *Mycosphaerella graminicola* (= *Septoria tritici*) or *Puccinia recondita* f. sp. *tritici*, markedly reduced symptom expression.

Fractions active biologically against *S. tritici* *in vitro* were extracted with organic solvents. The chemical structure of one of the compounds was determined as 1-hydroxyphenazine (phOH). This compound suppressed *in vitro* growth of the following pathogens: *Fusarium oxysporum* f. *vasinfectum*, *Pythium aphanidermatum*, *Sclerotium rolfsii* and *Sclerotium sclerotiorum*. In addition, phOH suppressed the growth of Gram-negative and -positive bacteria: *Aerobacter aerogenes*, *Bacillus subtilis*, *Escherichia coli*, *Micrococcus* spp., and *Proteus vulgaris*, but did not suppress the growth of *Serratia marcescens*.

phOH at a concentration of 60 ppm, caused death of conidia of *S. tritici* grown in liquid medium. A concentration of 160 ppm was needed to cause a 60% reduction in pycnidia coverage on seedlings of the susceptible cultivar 'Shafir'. This phOH concentration caused a 75% reduction in the number of leaf rust pustules/cm². Application of LEC1 cells onto seedlings caused a greater reduction in symptom development of the two pathogens than 160 ppm of phOH. LEC1 produced additional biologically active compounds.

AMPELOMYCES QUISQUALIS FOR BIOLOGICAL AND INTEGRATED CONTROL OF POWDERY MILDEW

A. SZTEJNBERG¹, S. GALPER¹, O. FRUMKIN², S. ABO-FØUL¹ and N. LISKER³

¹Dept. of Plant Pathology and Microbiology, The Hebrew University of Jerusalem, Faculty of Agriculture, Rehovot; ²Kibbutz Be'eri; and ³Dept. of Seed Research, ARO, The Volcani Center, Bet Dagan, Israel

Field and greenhouse trials were carried out to control powdery mildew (PM) of cucumber, zucchini, apple and mango by using the hyperparasite fungus *Ampelomyces quisqualis* (AQ). Disease control was assessed by measuring PM severity and the yield. High AQ hyperparasitism on PM of greenhouse-grown cucumbers (cv. Hazera 205) was achieved. Thus, PM severity was reduced, leading to the following yields: untreated cucumbers, 0.76 kg/plant; treatment with pyrazophos 0.05% and 0.1%, 1.61 and 1.43 kg/plant, respectively; AQ, 1.14 kg/plant; and two AQ treatments alternated with pyrazophos 0.05%, 1.46 kg/plant. In the field trial, PM of zucchini (cv. 'Maayan') was highly hyperparasitized by AQ, leading to a reduction of PM leaf coverage. The total yields obtained with the various treatments were: untreated, 120 kg; AQ, 167 kg; penconazole, 158 kg; pyrazophos, 183 kg; triflumizole, 184 kg; cyproconazole, 189 kg; and propiconazole, 222 kg. In apple and mango orchards, similar results were obtained using AQ for the biocontrol of PM.

YEASTS AS BIOCONTROL AGENTS OF POSTHARVEST DISEASES OF FRUITS

E. CHALUTZ¹, RUTH BEN-ARIE¹, S. DROBY¹, LEA COHEN¹, BATYA WEISS¹ and C.L. WILSON²

¹Dept. of Fruit and Vegetable Storage, ARO, The Volcani Center, Bet Dagan, Israel; and ²Fruit Disease Unit, Appalachian Fruit Research Station, USDA, Kearneysville, WV, U.S.A.

Extensive screening of naturally occurring microorganisms present on the surface of grape and citrus fruit resulted in the isolation of several bacterial and yeast organisms that exhibited antagonistic activity against postharvest diseases of fruits. Among them, one bacterial and one yeast isolate effectively inhibited *Penicillium digitatum*, *P. italicum* and *Geotrichum candidum* decay of citrus as well as *Rhizopus stolonifer* decay of grapes. In preliminary tests, the yeast antagonist exhibited activity also against *Diplodia natalensis* decay of citrus, *Penicillium expansum*, and *Alternaria alternata* decay of apple, *A. alternata* and *R. stolonifer* decay of tomato, and *A. alternata* decay of persimmon and pepper fruit.

While the bacterial and yeast antagonists were equally effective in disease inhibition, they differed in their effect on the pathogen in culture: the bacterial antagonist inhibited fungal growth while the yeast antagonist did not. Further studies with the yeast antagonist confirmed that its mode of action is not by inhibiting the pathogen through antibiotic production. Other possible modes of action under investigation are induction of host resistance processes, competition with the pathogen for nutrients or space, and direct interaction between the antagonist and the pathogen cells.

SUPPRESSION OF SOILBORNE PLANT DISEASES IN COMPOST-AMENDED MEDIA

R. MANDELBAUM, BARBARA GORODETZKI and Y. HADAR

Dept. of Plant Pathology and Microbiology, The Hebrew University of Jerusalem, Faculty of Agriculture, Rehovot, Israel

Suppression of several plant diseases was observed in compost-amended media in greenhouse experiments. In order to evaluate the role of the compost microflora in the suppression of the pathogenic fungi, we examined the propagules after incubation in the medium using light and scanning electron microscopes. Sclerotia of *Sclerotium rolfsii* were incubated in composted grape marc (CGM), raw grape marc (GM), and peat media. The sclerotia rapidly lost viability in the CGM medium but not in the peat or in the GM. In the CGM it was observed that the sclerotia were shrunken and invaded by antagonists.

Pythium aphanidermatum was grown on nylon monofilament fabric in petri dishes containing liquid growth medium. After 24 h of incubation the nylon fabric containing the fungal mycelium was transferred to composted separated cow manure (CSM), and peat media. The media had been previously adjusted to a water tension of 10 mbar and this was maintained during the incubation period. In the CSM medium the *Pythium* mycelium lysed within 24-48 h but not in the peat medium, in which the mycelium was viable even after 14 days. With neither a light nor a scanning electron microscope could we observe direct parasitism of *Pythium* by other fungi or bacteria. Addition of glucose to the compost or peat medium resulted in an increase of microbial respiration: 24 h after the glucose amendment, the respiration rate in the CSM medium was five times higher than in the peat medium, indicating that the compost microflora react more vigorously to exogenous amendment than the peat microflora, thus probably keeping available nutrients from the plant pathogens. These results emphasize the importance of the medium's microflora in the suppression of plant pathogens in compost-amended media.

FUNGAL DISEASES OF THE NOXIOUS WEED *CYPERUS ROTUNDUS* IN ISRAEL

ELINOAR BONNE-NITZANI¹, R.G. KENNETH¹, Y. KLEIFELD² and R. REUVENI³

¹Dept. of Plant Pathology and Microbiology, The Hebrew University of Jerusalem, Faculty of Agriculture, Rehovot; ²Dept. of Plant Pathology and ³Dept. of Weed Science, ARO, Newe Ya'ar Experiment Station, Haifa Post, Israel

The perennial weed purple nutsedge (*Cyperus rotundus*) is very problematic in Israel, mainly on summer crops and in orchards, and few herbicides are capable of even partly controlling it. Indigenous pathogenic fungi are therefore being sought for its biocontrol. At least three have been found here that are widespread, and have now been characterized: (i) *Pyricularia higginsii* Luttrell (= *Dactylaria higginsii*), first found in Alabama, is known in South America, Sudan and Australia. This is the first record from the Mediterranean Basin and Middle East; we identified it also on *Cyperus longus* from Anatolia. Grown in pure culture here, it incites small lesions on *C. rotundus* leaves, mainly in summer under damp conditions. (ii) A rust, comprising uredial and telial stages, corresponds morphologically to *Puccinia romagnoliana* Maire & Sacc., which differs from *P. conclusa* Thuem. by the presence of many narrow paraphyses in the telia. Telia are hypophyllous, long and narrow, opening tardily, then pulverulent. (iii) *Cercospora* sp., which does not fully match any of the many named species on Cyperaceae in morphology and/or symptoms induced on hosts. Symptoms: Large, long-elliptical chestnut-brown lesions, sometimes with narrow yellow halo, on leaves, stems, and inflorescence peduncles and leaves, often coalescing; girdled inflorescence stems collapse. Morphology: Sporulation hypophyllous, in rows within lesions, with sparse stromal tissue

in substomatal chamber. Conidiophores dark, often closely geniculate near apex, 0-3 septate, as many as 20 in each fascicle, 37-50 x 2.5-5 μm . Conidia hyaline, obclavate, subtruncate at base with dark scar, 60-200 x 2.5-4.5 μm , 4-25 distinct septa under lactophenol. Grown in pure culture, it sporulates poorly, but best on V-8 agar of *Urtica*-decoction agar under continuous or intermittent near-UV illumination. Vegetative growth: minimum ca 10°C, optimum ca 25°C; sporulation on leaf: minimum ca 10°C, maximum < 34°C; germination: minimum 5°C, maximum >32°C.

Inoculation by comminuted mycelium without any conidia induced typical symptoms, with sporulation in the lesions. Young and mature leaves were equally susceptible, with symptoms appearing from 10 to 22 d after inoculation, depending on temperature and on length of leaf wetness.

SOME FACTORS AFFECTING TELIOSPORE AND BASIDIOSPORE GERMINATION OF *Puccinia Xanthii* – A POTENTIAL MYCOHERBICIDE OF *XANTHIUM* SP. – AND CONDITIONS NECESSARY FOR SUCCESSFUL INFECTION OF THE WEED

R. REUVENI¹, Y. KLEIFELD², D. BORG² and R.G. KENNETH³

¹Dept. of Plant Pathology and ²Dept. of Weed Research, ARO, Newe Ya'ar Experiment Station, Haifa Post; and ³Dept. of Plant Pathology and Microbiology, The Hebrew University of Jerusalem, Faculty of Agriculture, Rehovot, Israel

Xanthium sp. is an annual weed which presents a problem in cotton fields in Israel in summer. *Puccinia xanthii* (Schw.) Schr., a microcyclic, monoecious fungus, was found to occur naturally on *Xanthium* sp. growing in wet areas. When alternating hot/cold treatments were given, they appeared to stimulate both teliospore and basidiospore germination. Liquid additives derived from uninfected freshly dried green leaves of *Xanthium* sp. plants, added to cultures of *P. xanthii*, generally appeared to inhibit teliospore and basidiospore germination; some increase, however, was found when additives produced from uninfected, naturally dried brown leaves, were used. Temperatures within the range of 15-30°C had little effect on teliospore germination; however, basidiospore germination decreased as the temperature increased. Light had little effect on teliospore germination but completely inhibited basidiospore germination at higher temperatures. The conditions necessary for successful infection of *Xanthium* sp. plants were found to be a pre-soaking period (by floating) of the inoculum material in 0.5% sucrose, and a very high level of humidity to ensure that host leaves remained wet during the infection period. A temperature between 28 and 35°C is needed to encourage good production of teliospore-bearing sori after the infection period.

B: HEAT TREATMENT AND SOLARIZATION, COVERING AGENTS (ANTI-TRANSPIRANTS) AND OTHER PHYSICAL METHODS

WEAKENING OF *FUSARIUM* STRUCTURES BY SUBLETHAL HEATING

S. FREEMAN and J. KATAN

Dept. of Plant Pathology and Microbiology, The Hebrew University of Jerusalem, Faculty of Agriculture, Rehovot, Israel

Sublethal heating at 38-42°C of conidia and chlamydospores of *Fusarium oxysporum* f.sp. *niveum* resulted in a weakening effect beyond the immediate heating effect. This was expressed in

a delay in germination and in mycelial growth, and in an enhanced decline of the population of conidia in soil in spite of the fact that heating reduced the population by only 0-30%. Conidia that were heat-treated or exposed to solarized soil were lysed faster than the nonheated control. Scanning electron microscope observations revealed deformations and apertures in the treated conidia and mycelium. A fluorescent staining method based on fluorescein diacetate showed that heat-treated conidia were less brightly stained than nonheated ones although initial inoculum density, as measure by dilution plating, remained 100%. Disease incidence in watermelon seedlings inoculated with sublethally heat-treated conidia was reduced beyond the immediate mortality due to the heat. Similar results were obtained with *F. oxysporum* f.sp. *melonis* in melon seedlings.

It was determined in this study that heating at sublethal ranges may weaken and adversely affect *Fusarium* viability. These findings may be applied also with other disinfection methods and possibly contribute to developing an integrated system of control.

EFFECT OF SOIL DISINFESTATION ON GROWTH AND YIELD OF GYPSOPHILA IN A MONOCULTURE SYSTEM

ESTHER HADAR¹, A. GAMLIEL² and J. KATAN²

¹Extension Service, Ministry of Agriculture, Rehovot; and ²Dept. of Plant Pathology and Microbiology, The Hebrew University of Jerusalem, Faculty of Agriculture, Rehovot, Israel

The phenomenon of "soil sickness" is typical to gypsophila growing in a monoculture system, and is characterized by nonuniform growth, delay in flowering, and yield decline. This phenomenon is not restricted to certain soil types and occurs also in soilless substrates (e.g. tuff medium). The plants growing in such soils do not evince typical disease symptoms and no known pathogen can be isolated from them. Possible ways to control soil sickness are by crop rotation, soil disinfection, and replacement of the growth medium.

Soil solarization was performed in a greenhouse which had been under gypsophila monoculture for 8 years. A 20% increase in flower yield was obtained in the solarized plots of the first crop. Then the plants were trimmed, and in the second crop there was a 46% yield increase and in the third crop a 50% increase. Improved plant growth was also detected in methyl-bromide-treated soil.

An increase in rhizosphere and root colonization by fluorescent pseudomonads, which are known plant growth-promoting bacteria, was recorded in plants from solarized plots. Populations of fungi in the rhizosphere and in plant roots were significantly reduced. In inoculation experiments in the greenhouse, some of the fungi isolated were deleterious to gypsophila growth. It is concluded that soil disinfection has a potential for plant growth improvement in monoculture systems and in controlling soil sickness.

INCREASED PLANT GROWTH RESPONSE IN SOLARIZED SOILS

A. GAMLIEL and J. KATAN

Dept. of Plant Pathology and Microbiology, The Hebrew University of Jerusalem, Faculty of Agriculture, Rehovot, Israel

Plant growth is frequently enhanced in disinfested soils, even in the absence of known pathogens. This phenomenon is referred to as increased growth response, and is related to abiotic and biological mechanisms. The biological mechanisms include stimulation of beneficial microorganisms and plant growth-promoting rhizobacteria, such as fluorescent pseudomonads (FP), and the control of deleterious microorganisms.

Dry weight of tomato plants, bioassayed in solarized soils brought from different sites in Israel, was increased by 20-180% as compared with plants in the respective untreated soils. Emergence of pepper and eggplant seedlings was enhanced. A significant increase (20- to 10,000-fold) in root and rhizosphere FP populations was recorded in solarized soils, in spite of the fact that these organisms are heat sensitive. It appears that these bacteria recover after solarization and rapidly colonize the roots. Fungal populations in the rhizosphere and roots of plants in solarized soils were reduced considerably. Some of the fungi isolated from nonsolarized soils were tested in a greenhouse and found to be deleterious to tomato plants. Actinomycete populations were not significantly changed.

Similar microbial trends were observed in monoculture experiments. FP populations decreased, and populations of deleterious fungi increased. Soil disinfestation has the potential to improve plant growth and yield in monoculture soils.

INHIBITION OF WHEAT LEAF RUST DEVELOPMENT ON WHEAT SEEDLINGS WITH ARTIFICIAL FILMS

JEHUDITH ZEHA¹, O. 7W² and Z. EYAL¹

¹*Dept. of Botany, Tel-Aviv University, Ramat Aviv; and* ²*Dept. of Field Crops, ARO, The Volcani Center, Bet Dagan, Israel*

Control of foliar diseases with artificial films is considered an important part of integrated pest management (IPM) strategy, the main aim of which is to reduce the use of chemical fungicides and to lessen environmental pollution. In this research we tested the efficiency of various coating polymers as protective agents against wheat leaf rust. Several antitranspirants, such as Vapor Gard, Bio Film, Folicote and Wilt Pruf, were found to control effectively leaf rust development on wheat seedlings, if treated before infection. Bio Film, Folicote, and Vapor Gard significantly reduced the number of pustules/cm² when applied prior to inoculation with uredospores (by 99%, 80% and 85%, respectively). Application of these compounds 24 h after inoculation had a much smaller effect on suppression of rust development (34%, 19% and 14%, respectively). Application of the three compounds at a concentration of 0.5% (v/v) resulted in a reduction in pustule number/cm² of 95% with Bio Film, as compared with 38% and 44% with Folicote and Vapor Gard, respectively. A 2% concentration resulted in suppression of more than 80% with the two last compounds. The concentration of the film-forming compounds did not affect pustule development when the material was applied post-inoculation. The compounds did not affect germination of uredospores, but the number of appressoria and their association with stomata were greatly reduced.

The mechanism of inhibition seems to act by changing the microstructure of the leaf surface and by reducing significantly appressoria production and location of appressoria on stomates.

Scanning electron microscopy showed different stability and uniformity of the various coating films on wheat leaves.

DEVELOPMENT OF A SOLAR-RADIATION-SELECTIVE GREENHOUSE COVER FOR FOLIAR PLANT PATHOGEN CONTROL

R. REUVENI¹, M. RAVIV², YAEL ALLINGHAM³ and R. BAR³

¹Dept. of Plant Pathology and ²Dept. of Ornamental Horticulture, ARO, Newe Ya'ar Experiment Station, Haifa Post; and ³Ginegar Plastic Co., Ginegar, Israel

The use of plastic sheets in greenhouses to control microclimatic conditions and reduce fuel consumption, is expanding in many parts of the world. On the other hand, such an ecosystem creates favorable conditions for the development of plant diseases caused by *Botrytis cinerea*, downy mildew, etc. Modern production of plastic sheets based on three-layer films enables the distribution of various active ingredients according to their optimal location in the film. It provides better use of materials as to their light transmittance, light diffusion and sheet strength. For instance, antifog material to prevent water droplets on the sheet is incorporated in the middle layer, while an ultraviolet absorber is located mainly in the external layer. A light-diffuser, infrared-absorber and pigments may be incorporated in the middle layer. The use of a UV-absorber in order to remove the UV spectrum of sunlight radiation and to improve sheet strength has been accompanied by a reduction of several fungal greenhouse pathogens. Modification of the spectrum passing through the greenhouse cover can be achieved by incorporating various additives to the plastic. Raising the blue/UV-B (280-320 nm) ratio has a significant inhibitory effect on the sporulation of *B. cinerea* in growth chambers as well as in sunlight. Such a correlation was found also when using the same sheets in a greenhouse experiment, in which a reduction in severity of *B. cinerea* epidemics on tomato was demonstrated.

RELATIONSHIPS BETWEEN CLIMATIC CONDITIONS UNDER PLASTIC AND EPIDEMICS OF GRAY MOLD DISEASE OF CUCUMBERS GROWN IN GREENHOUSES

H. YUMIS¹, Y. ELAD¹ and Y. MAHRER²

¹Dept. of Plant Pathology, ARO, The Volcani Center, Bet Dagan; and ²Dept. of Soil and Water Sciences, The Hebrew University of Jerusalem, Faculty of Agriculture, Rehovot, Israel

Epidemics of gray mold are very common in Israel in cucumbers grown in greenhouses during winter. The pathogen, *Botrytis cinerea*, attacks flowers, female fruits, stems and leaves of this host from January until April. Infection of fruits starts usually on the senescing flowers; that on stems is *via* wounds. The development of epidemics and several meteorological parameters were studied in walk-through polyethylene tunnels in a 2-year experiment in the central coastal plain of Israel. Five brands of infrared-absorbing (IR) polyethylene and ultraviolet-absorbing polyethylene (UVA) were tested. During the relatively dry winter of 1985/86, non-persistence of dew on the canopy of plants was the factor limiting development of epidemics under IR covers. More hours of dew persistence and lower temperatures were recorded in UVA greenhouses as compared with IR tunnels. During the rainy season of 1986/87, dew persistence was greater. Disease counts were correlated with accumulated degree hours in the range of 15-20°C. It is suggested that compensation exists between air temperatures and water duration on the host, allowing development of severe epidemics. There are fewer hours of dew and higher temperatures under IR polyethylene as compared with the traditional UVA covers. Counts of *B. cinerea* conidia in greenhouses of cucumber showed a high population around noon. Counts were very low both outside and inside greenhouses during periods of dew persistence, *i.e.*, in the morning and evening.

C: DISEASE-RESISTANT PLANTS AND BREEDING FOR PLANT RESISTANCE TO FUNGI

MECHANISMS OF RESISTANCES AGAINST DOWNY AND POWDERY MILDEWS IN MUSKMELON

Y. COHEN and HELENA EYAL

Dept. of Life Sciences, Bar-Ilan University, Ramat Gan, Israel

Cucumis melo L. PI 124111F is highly resistant to downy mildew (*Pseudoperonospora cubensis* pathotype 3), powdery mildew (*Sphaerotheca fuliginea* races 1 and 2) and Fusarium wilt (*Fusarium oxysporum* f.sp. *melonis* races 0, 1, and 2). Resistance against *P. cubensis* is conferred by two incompletely resistant genes, *Pc-1* and *Pc-2*. Resistance against race 1 of *S. fuliginea* is conferred by a single dominant gene, *Pm-3*, and resistance against race 2 of this pathogen is conferred by a single incompletely dominant gene, *Pm-6*. No linkages were found between either of these genes. Preliminary observations revealed that resistances against Fusarium wilt are dominant. Resistances against downy and powdery mildew were associated with enhanced formation of callose, which accumulated in host cell walls and around haustoria. Enhanced lignin formation by host cells was also noticed in mildew-resistant plants.

Breeding line '29' was developed from *C. melo* PI 124111F. F1 hybrids of '29' with Charantais or a Perlita-type cantaloupe were resistant to mildews in the field and gave high-quality fruits.

INHERITANCE OF RESISTANCE IN WINTER AND SPRING BREAD WHEATS TO TWO SEPTORIA TRITICI ISOLATES

TAMAR DANON¹, ILANA GELERENTER² and Z. EYAL¹

¹Dept. of Botany, The George S. Wise Faculty of Life Sciences and ²Dept. of Statistics, Faculty of Exact Sciences, Tel-Aviv University, Ramat Aviv, Israel

The winter wheats 'Aurora' (PI 167407) and 'Kavkaz', sib selections of the same cross and which possess 1B/1R rye translocation, 'Bezostaya 1' x 'Trakia', all having Bezostaya 1 germplasm, were crossed in a diallel fashion (with reciprocals) with the susceptible spring wheat 'Hazera 2230' and 'Lakhish'. A 4 x 4 diallel cross was prepared with the resistant South American cultivars 'Colotana' (CI 13556) and 'Klein Titan' (CI 12615) with the same susceptible spring parents.

Parents, F₁, F₂ and backcross populations were inoculated in the field and at the seedling stage in the greenhouse, with *Septoria tritici* isolates ISR398 (not virulent to resistant cultivars) and ISR8036 (virulent to winter wheats). Populations were sown in the field in replicates in two separate blocks, each inoculated with one isolate. Plants were assessed for heading date, plant height and pycnidial coverage. Significant cultivars x isolates interactions were recorded both for seedlings in the greenhouse and for adult plants in the field.

Resistance in winter wheats to ISR398 is explained by a combination of additive and partially dominant gene action. The specific resistance is controlled by a small number of genes. Resistance in these winter cultivars is not associated with the 1B/1R translocation. Resistance in the South American wheats is based mainly on additive gene action. A high correlation was found between the response of seedlings and adult plants inoculated with ISR398.

SUSCEPTIBILITY OF WILD EMMER WHEAT FROM EASTERN GALILEE TO TAKE-ALL DISEASE

Z. SOŁEL¹ and Y. ANIKSTER²

¹Dept. of Plant Pathology, ARO, The Volcani Center, Bet Dagan; and ²Dept. of Botany, Tel-Aviv University, Ramat Aviv, Israel

Within the framework of a study of the native population of wild emmer wheat (*Triticum turgidum* var. *dicoccoides*) near Kibbutz Ammiad (eastern Galilee), natural infection with take-all disease was detected. The causal organism, *Gaeumannomyces graminis* var. *tritici*, was first recorded in Israel in 1981 on bread wheat. However, the pathogen may have existed in uncultivated soil for many years without being noticed, and thus could have induced selection for resistance to take-all within the population of wild emmer wheat.

A number of accessions, established from seed collected at various locations at the site, were evaluated for susceptibility to take-all. Single seeds were planted in vermiculite in conical vials and inoculated with isolates of *G. graminis* var. *tritici*. After a 4-week incubation at 16°C the seminal root system was rated for severity of symptoms, based on the proportion of discoloration. Fifty accessions were ranked as moderately to very susceptible, but two accessions demonstrated moderate resistance. If this resistance will be verified under natural field conditions, transfer of the trait into *T. aestivum* will be attempted.

PHYSIOLOGICAL RACES OF *EXSEROHILUM TURCICUM* IN ISRAEL

RACHEL ABADI¹, A. BAR-TSUR² and Y. LEVY¹

¹Dept. of Life Sciences, Bar-Ilan University, Ramat Gan; and ²Dept. of Field Crops, ARO, Neve Ya'ar Experiment Station Haifa Post, Israel

Host plant resistance is one of the most efficient methods of controlling Northern leaf blight of corn, caused by *Exserohilum turcicum*. Single dominant genes which confer resistance against this species have been identified by many workers and backcrossed into corn inbred lines. Races of *E. turcicum* which are virulent on corn possessing resistant genes exist in many parts of the world. Races 1, 2 and 3 have the virulence formulae (effective/ineffective host genes) Ht1, Ht2, Ht3, Htn/0, Ht2, Ht3, Htn/Ht1, and Ht1/Ht2, Ht3, respectively. The genes Ht1, Ht2 and Ht3 were backcrossed, individually, into two sensitive dent corn inbred lines (A619 and WI53R) at the University of Illinois and used to determine the physiological races of *E. turcicum* in Israel. Differentials containing Ht1, Ht2, Ht3 or Htn were highly resistant to isolates of *E. turcicum* collected from northern and southern parts of Israel, whereas the inbred lines without Ht genes were highly sensitive.

Resistance was characterized by increased latent period and reduced lesion area and sporulation capacity. The level of resistance decreased when plants were inoculated with a relatively high inoculation concentration (10,000 spores/ml). A sensitivity index (SI) was developed according to the following formula:

$$SI = \frac{\text{number of spores/cm}^2 \times \text{average lesion area} \times \text{infection efficiency}}{\text{latent period}}$$

The sensitivity index expressed in percent from the sensitive inbreed, in differentials containing Ht1, Ht2, Ht3 and Htn genes was, respectively, 0.2%, 3%, 11% and 0%, in plants

inoculated with isolates collected in the north of Israel. The same trends were observed in plants inoculated with isolates collected in the south of the country, even though isolates collected in the north were significantly more virulent than those collected in the south.

Our results indicate that the physiological race of *E. turcicum* in Israel is race 1.

COMPARISON OF THE SUSCEPTIBILITY OF CARNATION VARIETIES TO INOCULATION WITH *FUSARIUM OXYSPORUM* F.SP. *DIANTHI*

HILLELA VIGODSKY-HAAS¹ [deceased], A. BAR-YOSEF², MICHAL REUVEN¹ and Y. MOR³

¹Dept. of Plant Pathology, ARO, The Volcani Center, Bet Dagan; ²Extension Service, Ministry of Agriculture, Rehovot; and ³Extension Service, Ministry of Agriculture, HaQiryia, Tel Aviv, Israel

Resistance of carnation varieties to Fusarium wilt was tested in a field trial which was repeated for 3 years. The trial was carried out in a field which was infested with 50 isolates collected from various locations in Israel (and proved pathogenic in preliminary experiments). Randomized-block plots were used for the trial, and each variety was planted in eight replicates. The progress of the disease was measured by recording the number of plants with symptoms during the season. Susceptible varieties started to show disease symptoms ~2 months after planting and reached a maximal level 3 months later. Moderately resistant varieties evinced the first symptoms ~3 months after planting. Disease progress in the plants was gradual in all the varieties, although it was faster in the more susceptible varieties than in those which showed higher resistance to the disease. Some varieties, which seemed to be resistant at first, developed symptoms only ~5 months after planting and by the end of the trial they had attained the same disease level as the most susceptible group. At the end of the trial, the most resistant variety had no diseased plants, whereas in the most susceptible varieties 97% of the plants were diseased. The other varieties evinced all intermediate resistance levels between these two extremes. Among 20 varieties tested in this trial, only three varied in their reaction from one year to another; the others reacted consistently in the same way during the 3 years. All the varieties exhibited the same level of resistance in this trial as they are known to have in commercial fields.

The availability of resistant carnation varieties will enable flower production in fields which suffer from Fusarium wilt. The varieties can also be used as a source of disease resistance in carnation breeding.

REACTION OF *TRITICUM DICOCOIDES* POPULATIONS AND SEVERAL *AEGILOPS* SPECIES TO ISRAELI ISOLATES OF *Puccinia recondita* *TRITICI*

HANNA ROTMCHILD, J. MANISTERSKI and Y. ANIKSTER

Institute for Cereal Crops Improvement, The George S. Wise Faculty of Life Sciences, Tel-Aviv University, Ramat Aviv, Israel

In order to study specific resistance in populations of *Triticum dicoccoides* and of *Aegilops* spp., a survey was carried out of isolates of *Puccinia recondita*. A group of 50 isolates that were collected in different locations in Israel was tested. Two sets of nearly isogenic varieties containing each a single Lr gene were used: set 1 included 15 varieties received from Dr. J.G. Moseman, USDA, Beltsville, MD; and set 2 consisted of 33 varieties supplied by Mr. D. Long, Cereal Rust Laboratory, St. Paul, MN. Five isolates of diverse virulence were selected and inoculated on ten populations of *T. dicoccoides* and ten species of *Aegilops* spp. The plants were inoculated with the five cultures on the first leaf of the seedling, four seedlings for each line. All *T. dicoccoides*

accessions were susceptible to the five isolates, except one accession from Ammiad, which was resistant to the least virulent isolate. Much specific resistance was located in some *Aegilops* species; almost all *A. speltoides* were highly resistant, and *A. searsii* and *A. bicornis* were susceptible. A significant correlation was found between susceptibility of *Aegilops* spp. and ecogeographic parameters: latitude, altitude and annual rainfall. The reaction of several *Aegilops* spp. was influenced by the soil type. On heavy soils there is a higher level of resistance. Correlation of resistance with ecologic parameters will render future search for resistance more economic. The concentration of specific resistance in *Aegilops* spp. may serve as a basis for breeding programs.

STUDIES OF THE SEXUAL STAGES OF *PUCCINIA RECONDITA TRITICI*

J. MANISTÉRSKI and Y. ANIKSTER

Institute for Cereal Crops Improvement, The George S. Wise Faculty of Life Sciences, Tel-Aviv University, Ramat Aviv, Israel

Puccinia recondita tritici is the causal agent of leaf rust in *Triticum aestivum*, *T. durum*, *T. dicoccoides*, and *Aegilops* spp. in Israel. Three types of this parasite can be distinguished on the basis of teliospore source and by the alternate host that is infected by them. Cultures originating from *T. aestivum*, *T. durum* and *T. dicoccoides* infect *Thalictrum speciosissimum*, while those originating from *Aegilops longissima*, *A. variabilis* and *A. sharonensis* infect *Anchusa aggregata*. A third group, originating from *A. ovata*, infects *Echium glomeratum*. Infection of the alternate host caused the formation of pycnia. Self-fertilization was done and aecia were formed. Aeciospores from the alternate host infected the following main host. Aeciospores from *Thalictrum* infect *T. aestivum*, *T. durum* and *T. dicoccoides*; those from *Anchusa* infect *Aegilops* spp.; and those from *Echium* infect *A. ovata*. *Hordeum marinum* was found to be infected by leaf rust, and teliospores from it then infected *Thalictrum*. Aeciospores from this *Thalictrum* infected *T. aestivum* as well as *H. marinum*. *H. marinum* was found to be resistant to inoculation by aeciospores from *Anchusa* or *Echium* and by urediospores from *Aegilops* spp. In tests on a set of differential varieties, the virulence spectrum of cultures from *Thalictrum* was identical to that found in a countrywide survey of urediospores of *P. recondita tritici*.

The population of *P. recondita tritici* harbors three types, two of which have a complete life cycle, including an alternate host, in Israel: *Aegilops* spp. – *Anchusa*, and *A. ovata* – *Echium*; the third type, *T. aestivum* – *Thalictrum*, which is of the greatest economic importance, has no sexual stage in Israel. Its spores arrive from an outside source each year. This type is similar to the European population in the infection of *T. speciosissimum*, which is a European species. There seems to be no connection between the *Aegilops* leaf rusts and the *Triticum* leaf rust. No dangerous recessive genes were found in aeciospores originating from *Thalictrum*. We can assume that the potential danger in the population is not large. *H. marinum* may serve for differentiation among *P. recondita* types.

INFLUENCE OF CALCIUM NUTRITION ON HOST SUSCEPTIBILITY TO *BOTRYTIS CINÉREA*

HANNE VOLPIN and Y. ELAD

Dept. of Plant Pathology, ARO, The Volcani Center, Bet Dagan, Israel

Botrytis cinerea, the causal agent of the gray mold disease, is a major pathogen in Israel. The disease is most severe in crops grown in greenhouses or plastic tunnels during the winter. Over the

years *B. cinerea* had developed strains resistant to fungicides used for disease control and therefore alternative control methods were tested. In this work the influence of calcium nutrition on the gray mold disease was examined. Stems of cut rose flowers were dipped in solutions of CaSO_4 (3 mM) and/or solutions of the chelate EGTA; 5 days later, disease severity was evaluated. Disease severity was found to be reduced significantly ($\sim 30\%$) on the flowers previously treated with CaSO_4 , as compared with untreated flowers. On the flowers treated with EGTA, disease was increased by 45%, but the disease incidence on flowers treated concomitantly with CaSO_4 and EGTA was not significantly different from the control. Similar results were obtained on bean.

Rose plants in a commercial greenhouse were given a fertilizer containing calcium and compared with those receiving the same fertilizer but without calcium. Their flowers were picked, transferred to a humidity chamber, and inoculated with *B. cinerea*. Disease incidence was reduced about 45% on flowers treated with the calcium-containing fertilizer when compared with flowers from the control treatment in the greenhouses. Ethylene production was measured on roses treated with CaSO_4 (3 mM) and EGTA in the dip-water. The flowers treated with calcium produced 0.003 nl ethylene/g/h; those treated with EGTA and untreated flowers produced 0.66 and 0.20 nl/g/h, respectively. Ethylene was found to induce susceptibility of roses to development of the gray mold disease. This suggests that the influence of calcium on host susceptibility to gray mold is connected with the influence of calcium on ethylene production. However, other possible mechanisms are not excluded.

INDUCED RESISTANCE AGAINST PHYTOPHTHORA ROOT ROT IN A SUSCEPTIBLE AVOCADO ROOTSTOCK

MIRYAM ZILBERSTEIN and Y. PINKAS

Dept. of Plant Pathology, ARO, The Volcani Center, Bet Dagan, Israel

In avocado cv. 'Topa-Topa' seedlings (susceptible) inoculated with *Phytophthora cinnamomi*, weakly virulent isolates (WVI) develop confined necrotic lesions on the infected root. With highly virulent isolates (HVI) the entire infected root dies. Isolates of both groups were recovered from diseased avocado trees and nursery plants in Israel and were identified as the A^2 mating type. Within the first 48 h after inoculation the necrotic lesion as well as the pathogen mycelia developed more rapidly in the vascular cylinder than in the cortex. Based on this observation three distinct zones in the infected root were distinguished: Zone A – the necrotic portion at the root tip, Zone B – the root portion without external disease symptoms, reaching up to the point where discoloration of the vascular cylinder ends, and Zone C – the symptomless and pathogen-free root portion. In Zone C, even in a susceptible rootstock, a resistant reaction could be invoked. Initial inoculation with WVI at the root tip induced resistance in Zone C against challenge inoculation with HVI (after removal of both the A and B zones). Resistance intensity increased when the incubation period (after the initial inoculation) was extended to 5 days. Similarly, resistance induction against HVI was invoked by pulse treatment with HgCl_2 or fosetyl-Al as a substitute for the initial inoculation by WVI. The resistance build-up was positively correlated with phenylalanine ammonia-lyase (PAL) concentration. Resistance induction by WVI was prevented when, prior to inoculation, the roots were treated with aminooxyacetic acid, an anti-metabolite of PAL.

MODELING GENETIC AND BIOLOGICAL INFLUENCES ON THE DEVELOPMENT OF FUNGICIDE RESISTANCE

MICHAL BENDERLY and Y. LEVY

Dept. of Life Sciences, Bar-Ilan University, Ramat Gan, Israel

Resistance to systemic fungicides became a major problem soon after such materials came into commercial use. It has been suggested that the use of a systemic fungicide in combination with a low-risk fungicide would prevent or at least delay the buildup of resistant subpopulations. A number of theoretical models have been published which aim at assessing the development of fungicide resistance in a pathogen population consisting of two subpopulations. These models did not consider the occurrence of mutation in more than one locus. If such a case did occur, the fungal population would be composed of several subpopulations exhibiting different levels of resistance.

A mathematical model was constructed in which polygenic resistance was assumed, in order to assess the effect of various strategies on both control efficacy and the buildup of resistant subpopulations. The parameters considered in the model were: number of mutations for resistance, relative fitness of resistant phenotypes, and degree of selection pressure due to systemic fungicides. Buildup of resistance in a fungal population was simulated when controlled by a systemic fungicide, protectant fungicide or a mixture, in a preventive or responsive treatment. The fungus was assumed to be haploid or diploid with dominant, semidominant or recessive mutations.

Increasing the concentration of the systemic fungicide, when applied singly or in a mixture, caused an increase in selection pressure toward resistance. The use of systemic fungicides in combination with protectant fungicides enables us to reduce the selection pressure by decreasing the amount of the systemic component per treatment. Another factor found to be of importance was the number of treatments given per season. Therefore, responsive treatment delayed the buildup of resistant populations as compared with preventive treatment. Simulation of the model also revealed that when mutation is not accompanied by reduced fitness, the development of resistance is faster. In most of the treatments that were simulated, an increase in the frequency of partially resistant populations occurred when the selection pressure was high. The very resistant populations increased in frequency only when selection pressure was very high and the disease severity at the end of the season was low. In haploid fungi or diploid fungi with a dominant allele for resistance, the increase in resistant forms was faster than in diploid fungi with a semidominant or recessive allele for resistance. Resistance level at the end of one growing season (as simulated) depends on the combination of the various factors that were studied in this work.

NON-COMPETITIVE FITNESS OF METALAXYL-SENSITIVE AND -RESISTANT POPULATIONS OF *PHYTOPHTHORA INFESTANS*

D. KADISH and Y. COHEN

Dept. of Life Sciences, Bar-Ilan University, Ramat Gan, Israel

Twenty field isolates of *Phytophthora infestans* from different locations in Israel were compared in growth chamber experiments and six isolates were compared in walk-in plastic tunnels. Lesion areas induced in potato plants (cv. 'Alpha') by metalaxyl-resistant (MR) isolates were significantly ($P < 0.0001$) larger than those induced by metalaxyl-sensitive (MS) isolates. No significant differences were recorded between isolates in sporulation capacity or infection

efficiency. The infectious period of MS isolates lasted 7 days, as compared with 5 days in the MR isolates. Maximal sporulation in MS isolates was obtained at 7 days after inoculation, vs 6 days in MR isolates. Composite fitness indices in growth chambers and epidemics development in plastic tunnels were significantly higher for MR than for MS isolates, thus providing a possible explanation for the severe MR-induced late blight epidemic outbreaks in commercial potato fields in Israel.

A METHOD FOR ESTIMATING THE FREQUENCY OF METALAXYL-RESISTANT SPORANGIA IN POPULATIONS OF *PHYTOPHTHORA INFESTANS*

D. KADISH and Y. COHEN

Dept. of Life Sciences, Bar-Ilan University, Ramat Gan, Israel

A method for detecting low frequencies of resistance to metalaxyl was developed for *Phytophthora infestans*. Potato (cv. 'Alpha') tuber disks were used because they were more susceptible to the blight fungus than leaf disks or intact plants. When resistance of 5-95% was anticipated in a mixed sporangial population, 20 tuber disks (10 x 3 mm) were placed in petri dishes on either water or 100 mg/l metalaxyl and inoculated with three to six sporangia per disk. The percent resistance was computed by dividing the number of tuber disks supporting fungal sporulation on metalaxyl by the number of tuber disks supporting fungal sporulation on water and multiplying the result by 100. When 0.01-1% resistance was anticipated, 40 disks were placed on 100 mg/l metalaxyl and inoculated with 50-800 sporangia per disk. The number of disks supporting fungal sporulation on metalaxyl at 1 wk after inoculation was used to compute resistance frequency (%R), according to the formula $\%R = P (IE/S)$, where P = percent disks supporting fungal sporulation, IE = number of resistant sporangia required to produce infection (with sporulation) in 90% of the disks inoculated, and S = number of sporangia inoculated per disk. The bioassay is simple, accurate, and may be used as a tool to assess aspects of selection pressure imposed on the pathogen by phenylamide fungicides in the field.

CONTROL OF LATE BLIGHT IN FIELD-GROWN POTATOES BY SYNERGISTIC FUNGICIDAL MIXTURES

Y. SAMOUCHA and Y. COHEN

Dept. of Life Sciences, Bar-Ilan University, Ramat Gan, Israel

Five fungicides and six fungicidal mixtures were compared on field-grown potatoes (cv. 'Alpha') for the control of a phenylamide-sensitive (MS) and a phenylamide-resistant (MR) field isolate of *Phytophthora infestans*. The cymoxanil-containing fungicidal mixtures of Mancur, Pulsan, and Sandocur-M provided acceptable control of both MS and MR in the field. The fungicidal mixtures SAN-518, SAN-518P (SAN-518 + Previcur, 4:1) and Ridomil-MZ were potent in controlling the MS isolate but less effective against the MR isolate of the fungus. The effectiveness of Mancur, Pulsan and Sandocur-M was reflected not only in the relatively low dosages required to achieve 90% control of the disease (ED_{90}) incited by the MR isolate, but also in the low ratio between dosages required to achieve 90% control of the MR and the MS isolates. Dosages required for 90% control of the MR isolate were far smaller than those expected based on the efficacy of the ingredients in these mixtures. This finding indicated that synergistic interactions occurred between ingredients of Mancur (mancozeb + cymoxanil) and between those of Pulsan and Sandocur-M (mancozeb, cymoxanil and oxadixyl). It was concluded that for the control of Israeli

MR isolates of *P. infestans*, Mancur, Pulsan and Sandocur-M are preferable to the other fungicides tested.

EFFICACY OVER TIME OF CYMOXANIL MIXTURES IN CONTROLLING LATE BLIGHT IN POTATOES INCITED BY PHENYLAMIDE-RESISTANT *PHYTOPHTHORA INFESTANS*

Y. SAMOUCHA and Y. COHEN

Dept. of Life Sciences, Bar-Ilan University, Ramat Gan, Israel

Field-grown potatoes (cv. 'Alpha') were sprayed in autumn 1986 and spring 1987 with ten fungicides at recommended rates and inoculated *in situ* with 200 sporangia/ml of metalaxyl-resistant *Phytophthora infestans* at 0-15 days after spray. Two similar experiments were conducted in autumn 1986 and winter 1986 with potted potato plants grown outdoors and inoculated with 20,000 sporangia/ml in growth chambers at 20°C. In the field, the reduction in lesion number per row-treatment as compared with fungicide-non-treated rows was used to estimate the percent disease control; in growth chambers, the reduction in disease severity in treated compared with non-treated plants was used. The curve-fitting method employed for percent disease control data over time showed a high goodness-of-fit to a declining logistic equation. Results from the field showed that while the 3-way mixtures Pulsan and Sandocur-M provided 90-100% control of the disease for a period of 13-15 days, the 2-way mixtures Ridomil-MZ and SAN-518 sustained that level of control for only 4-6 days. Predicted T 0.5 (half-time) values in potted plants were 8.7-11.1 days for the 2-way mixtures vs 13.5-17.7 days for the 3-way mixtures. We predicted that phenylamide + mancozeb mixtures may have a slight advantage over mancozeb in controlling late blight incited by phenylamide-resistant *P. infestans*, whereas the 3-way mixtures of phenylamide + mancozeb + cymoxanil may be far superior to 2-way mixtures. Three-way mixtures should be applied at intervals of about 2 weeks.

SYNERGISM IN FUNGICIDE MIXTURES AGAINST *PSEUDOPERONOSPORA CUBENSIS*

Y. SAMOUCHA and Y. COHEN

Dept. of Life Sciences, Bar-Ilan University, Ramat Gan, Israel

Cymoxanil mixtures with either mancozeb (Mancur) or with mancozeb plus oxadixyl (Pulsan, Sandocur-M) were effective in controlling either metalaxyl-sensitive (MS) or -resistant (MR) isolates of *Pseudoperonospora cubensis* in cucumbers (cv. 'Elem') in growth chambers. Metalaxyl + mancozeb (Ridomil-MZ) and oxadixyl + mancozeb (SAN-518) mixtures were effective against the MS but less so against the MR fungus. Fungicide mixtures were far more effective in controlling either the sensitive or the resistant isolates than the individual components applied alone, representing significant levels of synergistic interactions. Increased efficacy of mixtures relative to their combined components (synergy factor, SF) was calculated by the Wadley method. Mixtures containing cymoxanil showed high energy factors against the resistant isolates, whereas Ridomil-MZ and SAN-518 did not evince this phenomenon. It was concluded that cymoxanil mixtures are suitable for downy mildew control in areas of Israel where metalaxyl-resistant *P. cubensis* populations prevail.

SYNERGISTIC INTERACTIONS BETWEEN RESPIRATION INHIBITORS AND PHENYLAMIDE FUNGICIDES IN CONTROLLING *PHYTOPHTHORA INFESTANS*

BILHA BASHAN, Y. LEVY and Y. COHEN

Dept. of Life Sciences, Bar-Ilan University, Ramat Gan, Israel

Recent publications have shown that synergistic interactions occur between phenylamide systemic fungicides such as metalaxyl and oxadixyl, and the protectant fungicide mancozeb, in controlling *Oomycetes* fungal diseases. Mancozeb is known to inhibit respiration in fungi, whereas phenylamides inhibit RNA synthesis.

The relationship between respiration inhibition and synergistic interaction was investigated in the present study. Sporangia of *Phytophthora infestans* were treated with sublethal doses of different respiration inhibitors, and used to inoculate potato tuber disks pretreated with phenylamide fungicides. One week later the intensity of fungal development on the inoculated disks was evaluated. Results showed that when applied singly in a sublethal dose, uncoupler agents (e.g. DNP), inhibitors of oxidative phosphorylation (e.g. oligomycin), ionophores (e.g. valinomycin), and inhibitors that block specific carriers in the electron-transport chain (e.g. rotenone, antimycin A), did not inhibit the development of the late blight fungus. However, when applied to discs pretreated with metalaxyl or oxadixyl, blight development was significantly decreased. This phenomenon was more pronounced in *P. infestans* isolates resistant to metalaxyl and oxadixyl than in isolates sensitive to metalaxyl.

The synergism between mancozeb and metalaxyl was found to be related to the inhibition of respiration of the pathogen, in spite of the fact that the inhibition did not affect the development of the pathogen itself.

Addition of ascorbic acid, a chemical which annuls the inhibitory effect of antimycin A (a molecule which inhibits electron transfer between cytochromes b and c), decreased significantly the synergistic interaction between antimycin and phenylamides.

CAN WE CONTROL PHENYLAMIDE-RESISTANT OOMYCETES?

Y. COHEN and Y. SAMOUCHEA

Dept. of Life Sciences, Bar-Ilan University, Ramat Gan, Israel

Phenylamides manufacturers market their products (metalaxyl, oxadixyl, benadaxyl) as prepacked mixtures together with a broad-spectrum surface (protectant) fungicide, usually mancozeb. This marketing policy is based on simulation models and some laboratory and field observations which showed a delayed buildup of resistance when such mixtures were used. In the present experiment, the control of late blight by metalaxyl, metalaxyl + mancozeb (Ridomil-MZ), and Ridomil-MZ + cymoxanil (Curzate) was examined in potato crops (cv. 'Alpha') grown in walk-in plastic tunnels inoculated with *Phytophthora infestans* sporangial suspensions containing 0.1%, 1%, or 10% resistant sporangia. Fungicidal sprays were applied at biweekly intervals. Results showed that metalaxyl was poorly effective compared with Ridomil-MZ in controlling the blight, especially when the original inoculum contained 1% or 10% resistant sporangia, thus proving that a systemic + protectant is more effective than a systemic alone when resistance to the systemic already exists. Moreover, Ridomil-MZ + cymoxanil was more effective than Ridomil-MZ alone, regardless of the percent resistant sporangia in the original inoculum. At 6 wk after inoculation, the portion of the foliage blighted was 100%, 80% and 10% in crops treated with metalaxyl, Ridomil-MZ and Ridomil-MZ + cymoxanil, respectively. We suggest combining cymoxanil with Ridomil-MZ for the control of phenylamide-resistant *P. infestans*.

E: ENHANCEMENT OF PLANT SUSCEPTIBILITY BY INTERACTION BETWEEN PATHOGENS OR BY CHEMICAL FACTORS

INTERACTION OF *ERWINIA CAROTOVORA* VAR. *ATROSEPTICA* WITH *VERTICILLIUM DAHLIAE* ON POTATO

LEAH TSOROR (LAHKIM)¹, A. NACHMIAS¹, M. PEROMBELON² and Z. BARAK³

¹Dept. of Plant Pathology, ARO, Gilat Experiment Station, Mobile Post Negev, Israel; ²Scottish Crop Research Institute, Dundee, Scotland; and ³Dept. of Biology, Ben-Gurion University of the Negev, Be'er Sheva, Israel

The interaction between *Verticillium dahliae* and *Erwinia carotovora* var. *atroseptica* (ECA), the causal agents of Verticillium wilt and Black leg on potato, respectively, was demonstrated under field conditions. Four potato cultivars, representatives of four combinations of Verticillium wilt/Black leg susceptibility, were examined. Potato seed tubers were inoculated with ECA in Scotland and planted in *V. dahliae*-infested and -free soil in Gilat. In the *V. dahliae*-tolerant cvs. 'Cara' and 'Désirée', a highly significant difference was observed in colonization level, chlorosis and stunting effect between plants infected with *V. dahliae* and ECA compared with *V. dahliae* alone. In the *V. dahliae*-susceptible cvs. 'Pentland-Crown' and 'Maris-bard' the levels of the above parameters were high, even without the bacterial infection.

The involvement of bacterial pectolytic enzymes in *V. dahliae* pathogenesis was demonstrated using *Escherichia coli* cloned with the pectate lyase gene from *Erwinia chrysanthemi* (PL 748). Cloned bacteria and extracellular fluid were injected together with *V. dahliae* spore suspension into potato leaflets. The symptoms, as expressed in reduction of chlorophyll content, increased when both agents were used together. The results indicate that there may be a synergistic effect between *V. dahliae* and *Erwinia* enzymes during pathogenesis.

INCREASED SUSCEPTIBILITY OF POTATO PLANTS TO VERTICILLIUM WILT BY IRRIGATION WITH SALINE WATER

Z. KAUFMAN¹, A. NACHMIAS¹, L. LIVESCU¹, A. MEIRI²,
M. BEN-HUR³ and M. TIBOR³

¹Dept. of Plant Pathology, ARO, Gilat Experiment Station, Mobile Post Negev; and ^{2,3}Inst. of Soils and Water, ARO – respectively, The Volcani Center, Bet Dagan, and Gilat Experiment Station, Mobile Post Negev, Israel

Verticillium wilt of potato plants caused by *Verticillium dahliae* induces economic yield losses. Development of disease-tolerant cultivars is one of the main tasks of potato breeders. Tolerant potato cultivars such as 'Désirée' and 'Cara' are commonly used in semi-arid zones where there is a high risk of Verticillium wilt. Usually the yield losses due to Verticillium wilt are 10-20%, in comparison with 50% in susceptible cultivars (such as 'Nicola', 'Atica', 'Cloustar', etc.).

The allocation of high quality water for agriculture is decreasing, while the use of saline water is becoming prevalent. The present work was carried out to study the potato response to Verticillium wilt under a saline water irrigation regime. All experiments were conducted in the fields of the Gilat Experiment Station (arid Mediterranean climate, with mild winters and hot, rainless summers) on a loessial silt loam soil. Four potato cultivars were grown on *Verticillium*-infested soil, with *Verticillium*-free soil as the control. The double single-line irrigation technique was adopted to obtain a steady rate change in salinity with uniform watering. At the end of each irrigation the foliage was rinsed with a small amount of non-saline water, to prevent direct damage

by salt to the leaves.

Incidence and severity of the disease were greatly enhanced, and the aging process was accelerated, by the salinity of the irrigation water. *Verticillium* colonization in the potato stems occurred earlier and at a higher level with saline irrigation water than in the control. Cv. 'Desirée' lost 50% of its yield under the combined stresses of salt and disease, and only 20% by exposure to salinity stress alone.

These findings have crucial economic implications on the use of saline water to irrigate potato fields infested with *V. dahliae*.

F: LATENT INFECTIONS AND STRESS

IMPLICATIONS OF THE LATENCY PHENOMENON IN THE DEVELOPMENT OF LEAF SPOT DISEASES IN CROTON (*CODIAEUM VARIEGATUM*), AS A MODEL OF FOLIAGE PLANTS

E. PERETZ¹, NAVA ESHED¹, A. DINCOR² and D. PRUSKY²

¹Dept. of Plant Pathology and Microbiology, The Hebrew University of Jerusalem, Faculty of Agriculture, Rehovot; and ²Dept. of Fruit and Vegetable Storage, ARO, The Volcani Center, Bet Dagan, Israel

Leaf spots in foliage plants can cause problems for growers, since even small amounts of infection are likely to be significant in plants whose advantage lies in the beauty of their leaves. The fungi *Glomerella cingulata*, *Phoma* sp. and *Colletotrichum variegatum*, which were isolated from leaf spots on croton, were found to be preserved in a latent state for an extended period of time without showing any symptoms. Latent infections of these fungi and others were found throughout the year in a commercial greenhouse that was extensively surveyed, in several other greenhouses, and in a number of other foliage plants.

In an attempt to cause a change in *G. cingulata*, *Phoma* sp. and *C. variegatum* from latency to a visible state, it was found that stress in infected plants, caused by exposure to sunlight and/or heat, and possibly even by exposure to cold, as well as scorching by herbicides, results in drying of the leaves, or sections of them. Under moist conditions the latent fungus develops reproduction bodies on the dry sections.

It was found that these fungi can also behave as saprophytic fungi and develop on necrotic tissue following inoculation. *Cytospora*, *Phoma* sp., *G. cingulata* and occasionally also *Alternaria* sp. were isolated from leaf spots, termed "dry spots." Koch's test was successfully completed with *Cytospora* and the conditions for disease development were determined. In *Phoma* sp., factors conducive to the transformation from latency to disease development were found (e.g. interactions with *Cytospora*). In *G. cingulata* the development cycle of the fungus in the greenhouse was described, including the latent state in live plants and the visible state in dry parts of leaves and in dry defoliated leaves. No fungicide of those tested was very effective against all three fungi: *Cytospora* (a lesion-causing fungus), *Phoma* sp. and *G. cingulata* (latent fungi); some of the fungicides were effective against one or two of the fungi. The fungicides to be used, singly or in combination, should be chosen in accordance with the pathogen identified at the problem site.

G: EPIDEMIOLOGY

A NEW CAUSAL AGENT OF ALTERNARIA BLIGHT OF COTTON: ITS OVERWINTERING IN WILD BEET, AND TRANSFER IN AIR CURRENTS DURING THE GROWING SEASON

HANNA LEVANOY¹, Y. BASHAN¹, R. OR² and R.G. KENNETH³

¹Dept. of Plant Genetics, The Weizmann Institute of Science, Rehovot; ²Eden Regional Experiment Station, Bet She'an Valley; and ³Dept. of Plant Pathology and Microbiology, The Hebrew University of Jerusalem, Faculty of Agriculture, Rehovot, Israel

Alternaria blight of cotton is caused by the pathogenic fungus *Alternaria macrospora*. However, this species has also been implicated as a disease agent. The fungus, which is capable of infecting cotton plants (cv. 'Pima'), has not been fully characterized, but is systematically close to *A. tenuissima*. A continuous 2-year field survey was carried out in Bet She'an cotton fields, based on the detection of airborne propagules on solid growth media. In this study, airborne propagules of both fungi were trapped. Monitoring the trapping levels throughout the entire year revealed significant peaks in April-May and in August-September. During these periods *Alternaria* blight in cotton fields is most severe. Controlled experiments showed that the flying range of *Alternaria* spores in the wind velocities normally prevailing in the Bet She'an Valley was several tens of meters.

The survival of spores in soil and in dormant mycelium in cotton plant debris buried in the soil is quite poor. Although the fungus can survive in off-season cotton plants and in above-soil plant debris, such a situation is rare in Israel because of correct cultivation procedures. In addition, the percent of internal seed infection capable of initiating a disease, is very low. Thus, with the goal of detecting a possible off-season carrier of the *Alternaria* blight agent – which is capable of infecting cotton at its most susceptible seedling stage, we surveyed wild plants grown at the edges of fields prepared for cotton. Wild beet plants that were heavily infected with *Alternaria* blight were detected at 14 locations in the Bet She'an Valley during the winter season. Pathogenic isolates of *Alternaria* sp. were obtained from these plants and their pathogenicity on cotton, as well as on forage beet and sugar beet plants, was verified by Koch's postulates. Subsequently, a second survey was carried out at the same sites at the beginning of the new cotton season. Severe *Alternaria* blight infections were detected in all cotton fields adjacent to diseased wild beet plants. We propose that there is more than one causal agent for *Alternaria* blight of cotton. This new agent can apparently overwinter in wild beet plants and then, during the growing season, be transferred locally from one cotton field to another by air currents.

H: SOILBORNE DISEASES; SEEDBORNE DISEASES AND THEIR CONTROL; FOREST TREE DISEASES

SURVIVAL OF SCLEROTIA OF *SCLEROTINIA SCLEROTIUM*

Y. BEN-YÉPHET¹, E. SITI² and Y. SZMULEWICH¹

¹Dept. of Plant Pathology, ARO, The Volcani Center, Bet Dagan; and ²Extension Service, Ministry of Agriculture, Be'er Sheva, Israel

The survival of *Sclerotinia sclerotiorum* sclerotia was tested in two regions of Israel, the Negev and the Jordan Valley, with semi-arid and arid conditions, respectively, in fields where

lettuce drop was present. In the Jordan Valley survival of sclerotia was tested once a year in one field for 5 years. In the Negev, survival of sclerotia was tested only once in four fields where lettuce drop had been prevalent 1, 2, 3 and 4 years previously. Five soil samples, each of 10 kg, were taken from each field. Sclerotia were separated from soil and their viability and ability to produce apothecia were tested. The same test was also done with sclerotia stored in a laboratory at $21 \pm 4^\circ\text{C}$ for the same periods. The viability of sclerotia was tested by plating them on a growth medium and the ability to produce apothecia was determined by placing sclerotia at different soil depths in pots filled with soil. In the Jordan Valley field samples the number of sclerotia was reduced during the 4 years following lettuce drop and increased in the fifth year: 7.6, 4.8, 4.6, 2.4 and 4.8 after 1, 2, 3, 4 and 5 years, respectively. Viability and the ability of sclerotia separated from soil to produce apothecia placed in pots of soil at a depth of 2 cm were similar in all the fields, the figures being $\sim 90\%$ and 33% , respectively. The viability of sclerotia stored at 23°C was 100% , 94% , 75% and 15% after 1, 2, 3 and 4 years of storage, respectively. Apothecia production, however, was poor: 7% and 2% of the sclerotia, and only those stored 2 and 1 years, respectively, produced apothecia.

In conclusion, although the total number of viable sclerotia decreased with time, viability and the ability to produce apothecia from the remaining sclerotia were stable.

EFFECT OF *MACROPHOMINA PHASEOLINA* INOCULUM POTENTIAL ON FREQUENCY OF MELON ROOT INFECTION AND FRUIT DEVELOPMENT

R. REUVENI¹, N. OMARY¹, B.D. BRUTON² and M.J. JEGGER³

¹Dept. of Plant Pathology, ARO, Newe Ya'ar Experiment Station, Haifa Post, Israel;

²USDA-ARS-SCARL, P.O.Box 159, Lane, OK 74555, U.S.A.; and ³Fruit, Vegetable

and Root Crops Section, Tropical Development and Research Institute, London, U.K.

As few as five microsclerotia of *Macrophomina phaseolina* per gram dry soil was demonstrated to have an adverse effect on the growth and development of melon plants. The penetration of the fungus into melon roots occurred 25 days after planting and was accompanied by a decrease in chlorophyll content and a reduction in foliar fresh weight of the plants. There was a significant interaction between soil volume and microsclerotia density on infection of melon roots. Fresh foliar weight of melon decreased significantly as inoculum density increased or as soil volume decreased. There were significant effects due to soil volume, inoculum density, and the interaction between the two. Increasing soil volume resulted in a significant increase in fresh root weight, although the soil volume x inoculum density was not significant. This study demonstrated that a relatively low inoculum density of *M. phaseolina* can cause considerable damage to the melon plant.

SEEDBORNE *PENICILLIUM* IN SWEET CORN: ITS DAMAGE AND CONTROL

ALIZA MEIRI¹, Z. SOLEL², R. TAMARI³ and RONIT COHEN¹

¹Dept. of Seed Research and ²Dept. of Plant Pathology, ARO, The Volcani Center, Bet Dagan; and

³Sunfrost Ltd., Ashdod, Israel

In 1985, sweet corn seedlings of cv. 'Jubilee' suffered from substantial growth retardation and blight. Isolation from affected seedlings and from fungicide-treated seed lots, imported from the USA, yielded a high proportion of *Penicillium oxalicum* Currie and Thom.

In infected seeds the fungus is carried mainly on the seed surface but it was isolated also from the inner parts of seeds. The pathogenicity and seedborne nature of the fungus were proved

with naturally and artificially infected seeds. In laboratory experiments, the disease severity caused by the fungus was affected by temperature, inoculum concentration and planting media.

The standard commercial treatment of imported seed lots with a blend of captan, thiram and carboxin was ineffective against *Penicillium* blight. In a bioassay screening, the fungicide imazalil (Fungaflor) was highly toxic to the pathogen and therefore selected for seed treatment in a blend with captan and thiram. This combination was found very effective in laboratory tests. In field trials with corn cv. 'Jubilee' in an early date sowing, the combined seed treatment with imazalil+captan+thiram improved stand rate and seedling development. However, no significant differences in yield were found between the standard commercial treatment, the new combined treatment and the untreated control.

DISINFECTION OF POTATO SEED TUBERS IN A LOW-VOLUME SPRAY CHAMBER

A. GRINSTEIN¹, G. KRITZMAN², H. FRANKEL¹, MIRIAM AUSTERWEIL¹, YEHUDITH RIVAN¹ and BRACHA SHTAINER¹

¹Dept. of Pesticide Application, and ²Dept. of Plant Pathology, ARO, The Volcani Center, Bet Dagan, Israel

Potato seed tubers are known disease carriers. The tubers, like other kinds of propagative material, are commonly treated to control externally borne pathogens and pests by dip and drench techniques.

Reduced-volume application may be defined as the deposition of drops which remain discrete on the target. An aerosol spray chamber, utilizing a very low volume application technique, was used for disinfection trials. The tubers are carried through the exposure chamber on a roller belt while rotating on their own axis. A spray cloud consisting of drops with a 90 μm VMD (volume median diameter) is produced by a kinetic energy nozzle and the spray deposit is due to gravitational forces. Two chemicals are applied simultaneously by two atomizer heads. An exposure of 8 sec with tubers rotating no fewer than 10-13 times, with feed rates of 30 ml/min, resulted in a cover density of ~ 350 -500 droplets/cm². Presuming a spread factor of 2, the corresponding contact area is 4-7%; treatment capacity is 10-12 t/h (1500 m²). No subsequent drying is required.

Laboratory and field tests conducted over a 3-year period with C-103 (2,2-dibromo-3-nitropropionamide) and TOG (15% thiabendazole + 15% 8-hydroxyquinoline) resulted in complete control of tuber-borne *Rhizoctonia solani* and externally borne *Verticillium dahliae* fungi and of the potato common and deep scab *Streptomyces* spp. Control of the soft rot bacterium *Erwinia* spp. by this method was equal to or better than that obtained with the recommended organic mercury dipping procedure. It is postulated that the mode of action is cumulative due to multiple controlling effects which are caused by direct contact and also by secondary distribution by diffusion or by vapor action.

C-103 has not yet received official clearance. Research efforts were therefore concentrated on finding alternative compounds with no or low mammalian toxicity, permitting pre-storage treatment of the entire crop, with obvious benefits.

**CYPRESS DISEASES CAUSED BY *SEIRIDIUM CARDINALE* AND *DIPLODIA PINEA*
F.SP. *CUPRESSI***

Z. MADAR¹, Z. SOLEL² and M. KIMCHI²

¹Dept. of Forestry, Land Development Authority, Qiryat Hayyim; and ²Dept. of Plant Pathology,
ARO, The Volcani Center, Bet Dagan, Israel

In recent years two important cypress diseases were observed in Israel, namely, *Seiridium* canker caused by *Seiridium (Coryneum) cardinale*, and bark fissuring disease caused by *Diplodia pinea* f.sp. *cupressi*. The following hosts were attacked by *S. cardinale* under natural conditions: *Cupressus sempervirens* (varietas *horizontalis* and *pyramidalis*); *C. macrocarpa*, *C. glabra* and *C. goveniana*. Hosts infected by *D. pinea* f.sp. *cupressi* under natural conditions were *C. sempervirens* (varietas *horizontalis* and *pyramidalis*) and *Callitris vericosa*. Variability in pathogenicity among *Seiridium* isolates was not significant, whereas the differences among *Diplodia* isolates were considerable. When inoculated at terminal parts of the stem or branches, development of *Diplodia* canker was faster than following basal inoculations, whereas *Seiridium* cankers developed similarly, irrespective of the location of the inoculation. Infection with these pathogens occurred through wounds only. Wounds existed in the bark or in the external wood and enabled pathogen establishment as long as 20 days after wounding.

Seiridium cankers developed faster during autumn, winter and spring than in summer, whereas the rate of enlargement of cankers caused by *D. pinea* f.sp. *cupressi* was rapid during the warm months but slow in winter. Under temperature-controlled greenhouse conditions and in a field experiment, a long period of water stress induced expansion of *Diplodia* cankers. The size of *Seiridium* cankers was not influenced by water stress under natural conditions.

I: DIAGNOSIS – IDENTIFICATION OF ISOLATES OF DIFFERENT PATHOGENIC SPECIFICITY OR DIFFERENT VIRULENCE

A LABORATORY METHOD FOR IDENTIFICATION OF *FORMAE SPECIALES* OF *FUSARIUM OXYSPORUM* BY THEIR VEGETATIVE COMPATIBILITY

TALMA KATAN¹ and J. KATAN²

¹Dept. of Plant Pathology, ARO, The Volcani Center, Bet Dagan; and ²Dept. of Plant Pathology
and Microbiology, The Hebrew University of Jerusalem, Faculty of Agriculture, Rehovot, Israel

The *Fusarium oxysporum* complex includes pathogenic and non-pathogenic fungi which cannot be distinguished morphologically. *Formae speciales* and physiologic races are recognized by means of pathogenicity tests on host plants, but no other methods are available to subdivide the complex. *F. oxysporum* lacks a sexual cycle. Recently, the vegetative-compatibility grouping (VCG) method was introduced to dissect the *Fusarium* complex, based on the ability of isolates to form heterokaryons *in vitro*. The VCG theory suggests a systematic and evolutionary relationship between vegetative compatibility and *forma specialis*.

Isolates of the cotton wilt pathogen *F. oxysporum* f.sp. *vasinfectum*, and of non-pathogenic *F. oxysporum*, were obtained from roots and the rhizosphere of diseased 'Pima S-5' plants, growing in naturally infested soils. Each isolate was tested for pathogenicity to cotton plants and for heterokaryon formation with other isolates. All the pathogenic isolates belonged to a single VCG and could be distinguished from the non-pathogenic isolates, none of which belonged to this VCG. Similarly, isolates of the cotton pathogen could be distinguished from isolates pathogenic to other crops, such as tomato and watermelon.

This approach may help us to develop a laboratory test to identify *formae speciales* of *F. oxysporum* without the need for pathogenicity tests.

VARIABILITY IN VIRULENCE AMONG ISOLATES OF *PHYTOPHTHORA CINNAMOMI* FROM AVOCADO TREES IN ISRAEL

ARNA KARIV, MIRYAM ZILBERSTEIN and Y. PINKAS
Dept. of Plant Pathology, ARO, The Volcani Center, Bet Dagan, Israel

Isolates of *Phytophthora cinnamomi* recovered from infected avocado trees and nursery plants in Israel differed in their aggressiveness toward young avocado seedlings. All isolates were identified as the A² mating type. Based on their aggressiveness, the different isolates can be classified into two distinct groups: highly virulent isolates (HVI) and weakly virulent ones (WVI). The isolates of both groups are attracted to the elongation zone at the root tip, but while the HVI proceed upward until the entire root is killed, the WVI produce a confined necrotic lesion that develops to a maximum of 2 cm long while the upper part of the root remains pathogen- and symptom-free. In cv. 'Topa-Topa' seedlings (susceptible) inoculated with HVI, 80% of the roots evinced disease symptoms. Due to the infection, 85% of the increase in root dry weight which occurred with time in the non-infected control, was prevented. After inoculation with WVI, only 50% of the roots showed disease symptoms. The increase in root dry weight did not differ significantly from that of the uninoculated control. Both groups could be distinguished from each other morphologically by the smaller chlamydospores which characterize the WVI isolates. Most infected groves discovered in Israel yielded HVI. However, under excessive moisture, in undrained soils, pronounced disease symptoms were apparent also due to infections by WVI.

J: *CHEMICAL CONTROL*

THE USE OF PACLOBUTRAZOL TO REDUCE THE INCIDENCE OF SUDDEN WILT AND COLLAPSE OF CUCURBITS

R. COHEN¹, A. GREENBERGER² and J. KATAN³

^{1,2}*Dept. of Vegetable Crops, ARO – respectively, Newe Ya'ar Experiment Station, P.O. Haifa, and The Volcani Center, Bet Dagan; and* ³*Dept. of Plant Pathology and Microbiology, The Hebrew University of Jerusalem, Faculty of Agriculture, Rehovot, Israel*

Sudden wilt (also known as vine decline) is a disease causing collapse of melon and watermelon plants toward the end of the growing season, which may cause a total yield loss. Sudden wilt became widespread in the last few years and constitutes a significant threat to cucurbit production in Israel. Dinitroaniline herbicides are known to induce resistance to Fusarium wilt of melon and watermelon. The resistance induced in the plant by the herbicide is accompanied by plant growth retardation. Similarly, application of the growth retardant paclobutrazol reduced incidence of Fusarium wilt by approximately 50%, significantly reduced incidence of sudden wilt and increased the crop yield. The effectiveness of paclobutrazol in reducing the incidence of sudden wilt and in increasing yields depends on a variety of factors, such as: date of planting, cultivar, mode of application, and concentration of the chemical. Paclobutrazol application had additional effects. It altered plant habit toward compactness, increased fruit size, and resulted in earlier and more concentrated yields. However, these effects were not observed in all cases. The

effectiveness of paclobutrazol in reducing sudden wilt can be influenced sometimes by environmental factors.

CONTROL OF PHYTOPHTHORA ROOT ROT OF AVOCADO BY INJECTION OF FOSETYL-AL OR PHOSPHOROUS ACID INTO INFECTED TREES

Y. PINKAS¹ and D. BAUM²

¹*Dept. of Plant Pathology, ARO, The Volcani Center, Bet Dagan; and* ²*Extension Service, Ministry of Agriculture, Hadera, Israel*

In the spring of 1984, experiments were initiated to control *Phytophthora* root rot of avocado. Moderately to severely root-rot affected avocado trees were pressure-injected twice a year with fosetyl-A1 or neutralized phosphorous acid (0.6 and 0.4 g/m² of the canopy image, respectively). Differences between control and injected trees were noticeable only after 1.5 years. The two chemicals were equally effective but improvement due to the injections was minimal. The chemicals primarily prevented the deterioration occurring in the control trees. The injection method, as developed in South Africa, was found to be awkward and slow. Introduction without pressure of concentrated phosphorous acid into 9-mm-diam holes in the trunk was tried and proved to be much faster and no less effective. Curing of heavily diseased trees could be demonstrated using an integrated control scheme. The infected trees were pruned to the main branches (ca 1 m high) to replace the damaged canopy with a healthy one. After 9 months, when long healthy branches were produced, the trees were injected (twice a year) with phosphorous acid. When the disease was very severe, prior to the pruning the soil around the trees was treated with metalaxyl (every 3 months until the trees were injected) or solarized. This resulted in the development of new healthy and abundant growth.

POWDERY SCAB AND ITS CONTROL IN ISRAEL

L. LIVESCU, A. NACHMIAS and J. KRIKUN

*Dept. of Plant Pathology, ARO, Gilat Experiment Station,
Mobile Post Negev, Israel*

Powdery scab of potato, incited by *Spongospora subterranea* var. *subterranea*, was identified recently in Israel. The occurrence of this disease was unexpected, due to the particular moisture and temperature requirements for infection described in the literature. Studies were undertaken to determine how the pathogen was introduced, whether tuber seed treatments could eliminate seedborne inoculum, and if soil inoculum could be eradicated. Our results suggest that the organism was introduced on imported seed potatoes and that the recommended seed treatment, organic mercury + PCNB, eliminates the inoculum. Fumigation with methyl bromide or with metham-sodium greatly reduced soilborne inoculum. The importance of proper seed treatments to prevent the introduction of a potentially highly damaging pathogen was demonstrated. This is of special importance with regard to soilborne pathogens which, due to conducive edaphic factors, could readily establish themselves and pose a severe threat to the potato industry.

PURIFICATION AND CHARACTERIZATION OF *PENICILLIUM ITALICUM*
POLYGALACTURONASES FROM CULTURE AND FROM INFECTED VALENCIA ORANGES

J. HERSHENHORN and I. BARASH

*Dept. of Botany, The George S. Wise Faculty of Life Sciences,
Tel-Aviv University, Ramat Aviv, Israel*

Penicillium italicum secretes polygalacturonases in a growth medium containing sodium polypectate and citrus pectin. Three isozymes of polygalacturonase were revealed by means of Iso-Electric-Focusing (IEF) column. The isozymes were purified to homogeneity with IEF and Sephadex G-100 columns. The molecular weights, as determined by SDS electrophoresis and Sephadex G-100 column, were 50,000, 36,000 and 38,000 daltons and the iso-electric points were pIs 3.6, 7.5 and 8.0 for PG1, PG2 and PG3, respectively.

PG1 was characterized as exopolygalacturonase and PG2 and PG3 as endopolygalacturonase. The pH optima were 4.0, 4.5 and 4.5, the temperature optima were 40°, 50° and 50°C, and the Km for sodium polypectate were 0.056, 0.141 and 0.09 g/100 ml for PG1, PG2 and PG3, respectively.

Three isozymes of polygalacturonase were also isolated from infected orange tissue and found to be identical to the isozymes secreted *in vitro*, and to all other properties, as determined by purification profile.

INVOLVEMENT OF EPICATECHIN IN THE REGULATION OF THE ANTIFUNGAL DIENE
DURING REACTIVATION OF LATENT *COLLETOTRICHUM GLOEOSPORIOIDES* INFECTION
OF AVOCADO FRUIT

LEAH KARNI¹, D. PRUSKY¹, ILANA KOBILERA, EINAV BAR-SHIRA¹,
D. KOBILER² and B. JACOBY³

¹*Dept. of Fruit and Vegetable Storage, ARO, The Volcani Center, Bet Dagan; Israel Biological Institute, Nes Ziyiyona; and* ²*Dept. of Agricultural Botany, The Hebrew University of Jerusalem, Faculty of Agriculture, Rehovot, Israel*

Lipoxygenase is involved in the regulation of the antifungal diene during reactivation of latent *Colletotrichum gloeosporioides* infections of avocado fruits. Increased avocado lipoxygenase activity from 20 to 40 $\mu\text{mole O}_2/\text{h/g f.wt}$ was detectable at the end of the first hour, and at 24 h and at 4-5 days after the fruit harvest.

The concentration of the antifungal compound 1-acetoxy-2-hydroxy-4-oxo-heneicosa-12,15-diene declined from about 1700 to 200 $\mu\text{g/g f.wt}$ during periods of increased lipoxygenase activity.

The concentration in the fruit of the naturally occurring antioxidant, epicatechin, was inversely correlated with lipoxygenase activity during ripening.

The amount of lipoxygenase in the crude extract of the fruit peel was determined by the ELISA technique. It was found to fluctuate between 10 and 30 $\mu\text{g/g f.wt}$ during ripening, but there was no linear relationship with the fluctuations in the enzyme activity.

The results suggest that the differences in lipoxygenase activity during fruit ripening could not be correlated with changes in the amount of this enzyme, but rather result from changes in concentrations of one of its activity inhibitors – epicatechin.

ZEARALENONE PRODUCTION BY *FUSARIUM EQUISETI*, AS AFFECTED BY CULTURAL CONDITIONS

JILL BLUMENTAL-YONASSI¹, N. PASTER¹ and RIVKA BARKAI-GOLAN²

¹Dept. of Stored Products and ²Dept. of Fruit and Vegetable Storage, ARO, The Volcani Center, Bet Dagan, Israel

Zearalenone is a mycotoxin produced by several *Fusarium* species, causing hyperestrogenism and other disorders in swine and dairy cattle. The effect of cultural conditions on zearalenone production by *Fusarium equiseti* (Corde) Sacc. was studied. Three of the eight *F. equiseti* strains tested were found to be zearalenone producers (ca 2000 µg/60 ml of Czapek's medium), three produced trace amounts, and two gave negative results. Additional tests were carried out using one of the high-producing strains (NRRC 6470). When xylose at $3 \times 10^{-4}M$ was the sugar used, no zearalenone was produced. Of the sulfur-containing amino acids tested at $10^{-3}M$, only methionine inhibited toxin production; fungal growth (as measured by radial growth and mycelial dry weight) was not affected. At $10^{-2}M$, cystine inhibited zearalenone production without affecting fungal growth. At this concentration methionine both inhibited toxin production and caused a 20% reduction in fungal growth. Alanine and tryptophan ($3.5 \times 10^{-2}M$) also inhibited toxin production, while glycine enhanced zearalenone formation by 200% as compared with the control. Of the inorganic nitrogen sources, NH_4Cl and NH_4NO_3 significantly reduced fungal growth and toxin production.

Avocado, tomato, banana and melon were found to be good substrates for zearalenone production, yielding 3,500, 50, 50 and 200 µg of toxin, respectively, per 40 g fresh weight.

BIOLOGICAL ACTIVITY OF *VERTICILLIUM DAHLIAE* TOXIN ON POTATO PLANTLETS IN TISSUE CULTURE

JANETTE ORENSTEIN and A. NACHMIAS

Dept. of Plant Pathology, ARO, Gilat Experiment Station,
Mobile Post Negev, Israel

Verticillium dahliae, a soilborne pathogen, is the causal agent of the early dying syndrome of potato. During the pathogen's growth in a synthetic liquid medium it secretes a toxic protein, lipopolysaccharide. This toxin induces wilt symptoms in injected leaves and causes root growth inhibition in culture.

The biological activity of the partially purified toxin on potato plantlets' growth in tissue culture, was studied in order to evaluate the possibility of using this technique for *Verticillium*-tolerance screening *in vitro*. The potato cultivars used were 'Alpha' and 'Désirée' as tolerant cultivars, and 'Russet-Burbank' (RB) and 'Blanka' as susceptible ones. *V. dahliae* toxin was purified as described previously by us, and the partially purified fraction was used. The plantlets were grown on Murashige and Skoog medium under sterile conditions. The biological activity of the toxin was measured as reduction in plantlet height, number of leaves, root development and fresh weight. The development of susceptible plants was inhibited significantly but little damage was observed in the tolerant cultivars.

The biological activity of the toxin was studied also in the presence of benzyladenine; in susceptible cultivars, benzyladenine inhibited the phytotoxic activity of the toxin.

The stunting effect on plantlets *in vitro* confirms the observation that plant stunting is a major symptom of *V. dahliae* on potato.

DETECTION AND CHARACTERIZATION OF HYDROXAMATE SIDEROPHORES PRODUCED BY *GAEUMANNOMYCES GRAMINIS* VAR. *TRITICI*

S. DORI, Z. SOLEL and I. BARASH

Dept. of Plant Pathology, ARO, The Volcani Center, Bet Dagan, Israel

Isolates of *Gaeumannomyces graminis* var. *tritici*, collected in Israel, were cultured on low-iron-content liquid media, and the filtrates were analyzed for siderophore production. Accumulation of siderophores per unit of mycelium weight was maximal after 3 weeks of active growth. The amount of siderophore synthesis varied among isolates. Siderophore production was maximal when iron concentration in the growth medium was 0.01 mg/l, decreased with increasing iron concentration in the medium, and became negligible when the iron concentration was 1.0 mg/l. Monosodium glutamate as a nitrogen source was superior to asparagine, nitrate or ammonium for both mycelium growth and siderophore production. The siderophores were removed from the culture filtrates and separated, according to their molecular weight and color, into three distinct substances that were designated A, B and C. Synthesis of the various types was affected by the pH of the medium: at acidic conditions mainly siderophore C was produced, while at neutral pH types A and B were dominant. The siderophores were initially separated on a Bio-gel column, and brought to homogeneity by means of carboxymethyl cellulose and Sephadex LH-20 columns. Siderophores B and C were identified as dimeric acid and Coprogen b, respectively, by ¹HNMR and ¹³CNMR spectra; the structure of siderophore A appears to be similar to dimerum acid, but its exact structure has not yet been determined.

L: VIRUSES, VIROIDS AND DISEASES OF UNKNOWN ETIOLOGY

UTILIZATION OF AN ENZYME-AMPLIFIED ELISA TECHNIQUE FOR IMPROVED DETECTION OF PLANT VIRUSES

I.S. BEN-ZE'EV, A. FRANK and M. BAR-JOSEPH

The S. Tolkowski Laboratory, Dept. of Virology, ARO, The Volcani Center, Bet Dagan, Israel

Enzyme-linked immunosorbent assays (ELISA) are commonly used for the detection of plant viruses. Concentrations of most plant viruses are high enough to be detected directly in sap by routine ELISA methods. However, several cases where the virus titer did not reach detectable concentrations have been encountered, e.g. citrus tristeza virus (CTV) in certain hosts and/or during hot seasons, and papaya ringspot virus (PRV) during summer in the Jordan Valley. A recent development in the ELISA is the use of improved detection systems, resulting in the amplification of substrate conversion (colorimetrically detected), comparable to the amplification effect in electronics. The resulting assay was termed by its developers 'enzyme-amplified ELISA'.

We routinely use the double antibody sandwich, and the heterologous antiglobulin double antibody methods of ELISA, having alkaline phosphatase as the enzyme tag, for the detection of CTV and PRV. Both ELISA systems were modified to enzyme-amplified ELISA by replacing the usual substrate, para-nitrophenyl phosphate (pNPP), with beta-NADP, which is dephosphorylated by the enzyme to NAD. The latter served as a catalytic trigger for a redox cycle involving the secondary enzyme/substrate reactions (amplifier): alcohol dehydrogenase/ethanol, and diaphorase/p-iodonitratetrazolium violet (INT). During each turn of the cycle, a molecule of NAD is reduced to NADH and reoxidized to NAD, transferring H⁺ from ethanol to INT and reducing the latter to an intensely red-colored formazan.

Our results show increased sensitivity of detection of CTV and PRV in plant sap by 25-fold and 125-fold, respectively, by enzyme-amplified ELISA as compared with ordinary ELISA.

REDUCING PAPAYA RINGSPOT VIRUS DAMAGES IN THE JORDAN VALLEY BY SANITATION PROCEDURES

A. FRANČEK and M. BAR-JOSEPH

Dept. of Virology, ARO, The Volcani Center, Bet Dagan, Israel

In 1982 a few cases of infection by papaya ringspot virus (PRV) were first observed in a small papaya (*Carica papaya*) variety collection block at Gilgal, the Jordan Valley experiment station. During the years 1984-1985 the disease became widespread in the experimental plots at Gilgal and in a few other commercial papaya plots both in the Jordan Valley and in other parts of the country.

In order to prevent PRV spread to new plantings and to other sensitive crops, sanitation and cross protection procedures were tested as measures to reduce disease incidence and severity. Preliminary results after 2 years of sanitation in the Jordan Valley and in the coastal plain indicate that eradication of all PRV-infected papaya plants and a 6-month rest period (the minimum period tested) during which no papaya plants were grown, completely prevented new cases of PRV infection in the treated plots. This indicates that at this stage PRV has not spread to either the natural vegetation or to other crops growing in the vicinity of the tested plots, and apparently most previous PRV infections in these areas were acquired from infected papaya plants. The importance of continuous surveys for early elimination of any new incidence of PRV infection, and diagnosis of all nursery sources is stressed.

CONTROL OF PAPAYA DECLINE IN THE JORDAN VALLEY

A. FRANČEK and M. BAR-JOSEPH

Dept. of Virology, ARO, The Volcani Center, Bet Dagan, Israel

A disease causing the decline of papaya (*Carica papaya*) plants was noticed for the first time in the Jordan Valley, in 1982. The disease caused severe yellowing of the upper leaves and dieback of the apex. Petioles on the infected plants were water-soaked and stopped exuding latex. The primary symptom of the disease was a curvature of the apex followed by shortened and declined growth, resulting within 4-6 weeks in total necrosis of the tops of infected plants. Fruits on infected plants showed only a slight discoloration but were unsuitable for marketing. Observations in the Jordan Valley over the last 5 years indicated that the disease commenced regularly during the second half of October, but the annual incidence of infection fluctuated considerably, between 1% and 60%. A similar dieback disease with variable annual appearance has been reported in Australia.

The disease was not transmissible mechanically and continued efforts to associate mycoplasmas, viruses and viroids have so far been unsuccessful. Epidemiological observations suggested that the disease is air-borne and probably caused by a mollicute. In order to test this hypothesis, plots were covered with white nets providing 15%, 30% and 50% shade. The 30% and the 50% netting provided complete protection, while the 15% netting reduced disease incidence to less than 1%, compared with >30% infection in the non-covered control plot. Spraying plants at weekly intervals with a commercial whitewash solution was also found to be effective in reducing disease incidence.

ISOLATION AND CHARACTERIZATION OF MYCOVIRUSES FROM CONIDIA
OF *SEPTORIA TRITICI*

NOGA ZELIKOVITCH¹, Z. EYAL¹ and Y. KÖLTIN²

¹Dept. of Botany and ²Dept. of Microbiology, The George S. Wise Faculty of Life Sciences,
Tel-Aviv University, Ramat Aviv, Israel

Thirteen conidial isolates of the fungus *Septoria tritici* (= *Mycosphaerella graminicola*) from different geographical locations, with different virulence spectra (determined on ten differential wheat cultivars), were examined for the presence of mycoviruses.

Isolates ISR398 and ETH8205 of *S. tritici*, which differ in their virulence, contain double-stranded RNA (dsRNA) mycoviruses. The two viruses contain one dsRNA segment with the same molecular weight (1.3×10^6) and the segments are homologous. The two mycoviruses are isometric and ~ 35 nm in diameter. The capsid consists of one major protein with a molecular weight of 60 kD. Thus, the two *S. tritici* isolates, which differ in their virulence, contain mycoviruses with the same size capsid and dsRNA segments. The relation between virulence and the presence of mycoviruses in *Septoria tritici* is still not clear.