

ABSTRACTS OF PRESENTATIONS AT
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A: CHARACTERIZATION OF PATHOGENS AND PATHOGENESIS

***pthG* from *Pantoea agglomerans* pv. *gypsophila* Encodes an Avirulence Effector that Determines Incompatibility in Multiple Beet Species**

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Pantoea agglomerans pv. *gypsophila* (*Pag*) causes root and crown gall disease on gypsophila, whereas *P. agglomerans* pv. *betae* (*Pab*) induces the disease on beet as well as gypsophila. Both pathogens harbor a pathogenicity plasmid (pPATH_{Pag} or pPATH_{Pab}) that determines disease development. We have previously isolated and partially characterized a pleiotropic gene from the pPATH_{Pag} designated as *pthG* that encodes a virulence factor in gypsophila and an elicitor of a hypersensitive-like response in beet roots. The present study was undertaken to characterize *pthG* further as an *avr* gene. Infiltration of beet leaves with strains expressing PthG (*i.e.*, *Pag* or *Pab* containing *pthG* in *trans*) caused an HR response within 48 h, whereas strains lacking intact *pthG* (*i.e.*, *Pab* or *Pag* mutated in *pthG*) resulted in gall formation after 5 days. HR was elicited by *pthG* on multiple beet species, whereas a marker exchange mutant of *Pag* in *pthG* extended its host range on these beet species. A marker exchange mutant of *Pag* in *hrpJ*, encoding a component of the Type III secretion system, prevented HR elicitation. Mutations in each of the *hrp* regulatory genes (*hrpY*, *hrpS* and *hrpL*) substantially reduced the transcriptional activity of *pthG* in gypsophila cuttings. Particle bombardment of GFP-PthG fusion caused cell death in beet but not in non-host (melon) leaves. Present and previous results have established *pthG* as a broad-host-range *avr* gene that functions in multiple host plant species and the first functional *avr* gene in *Pantoea* spp. These characteristics of *pthG* may be utilized for creating resistant beet plants against several pathogens. [L]

Anatomical Changes Involved in Gall Formation Caused by *Pantoea agglomerans* pv. *gypsophila* on *Gypsophila paniculata*

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L = lecture sessions; P = poster (market place) sessions.

Pantoea agglomerans pv. *gypsophila* (*Pag*) is the causal agent of gall formation in *Gypsophila paniculata*. Its pathogenicity is associated with the presence of an indigenous plasmid (pPATH_{Pag}) that harbors the *hrp* gene cluster, genes encoding Hop virulence proteins and biosynthetic genes for auxin and cytokinins. Although Type III effectors are crucial for gall initiation by *Pag*, the hyperplasia and hypertrophy involved in gall formation are triggered by IAA, cytokinin and possibly additional phytohormones. The objectives of the present study were to characterize the patho-anatomy of galls produced by *Pag* in comparison with tumors induced by *Agrobacterium tumefaciens*, and in relation to phytohormones synthesis. Microscopic observation of longitudinal section along a *Gypsophila* gall infected with *Pag* revealed the following patterns: (a) the presence of giant cells surrounded by well developed vascular bundles; (b) formation of circular vessels in parenchyma; (c) suberin deposition on the external surface of the gall cells; and (d) increase in aerenchyma tissue apparently caused by ethylene. Ethylene emission by the wild type, recorded 6 days after inoculation, was eight times higher than by non-infected controls. In contrast, *Gypsophila* cuttings infected with mutants in the two pathways of IAA biosynthesis and cytokinin showed a significant decline in ethylene production. These mutants also caused limited xylem differentiation and substantial reduction in gall size. Furthermore, giant cells and well developed aerenchyma, which characterize the anatomy patterns of galls induced by the wild type, were absent. The galls produced by *Pag* were structurally different from tumors caused by *A. tumefaciens* on *Gypsophila*: they had a rough appearance, whereas those produced by *Agrobacterium* were smooth and without suberin. In addition, galls induced by *Pag*, in contrast to *Agrobacterium*, had cells with enlarged nuclei containing several nucleoli. Confocal microscopy studies with GFP-labeled *Pag* showed that the bacteria were located at the edge of the cutting and colonized in small aggregates within the intercellular parenchymal spaces and vessels. Moreover, the presence of the pathogen *in planta* was essentially limited to the gall area. The bacterial population recorded at 5 cm above the gall was sixfold lower than in the gall tissue. [P]

The Role of Phytohormone in the Interaction between *Botrytis cinerea* and *Arabidopsis thaliana*

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The main objectives of the research were to study (a) the impact of phytohormones produced by the plant on the host-parasite interaction by using *Arabidopsis thaliana* mutants with altered hormone susceptibility or production; and (b) the effect of externally applied phytohormones, inhibitors and precursors on the host-parasite interaction using the plant mutants. Abscisic acid (ABA)-related mutants, both deficient and insensitive, were significantly more susceptible to *Botrytis cinerea* than the corresponding background lines. External applications of ABA and MVA (mevalonic acid lactone, an ABA precursor) did not change the level of disease on wild type (WT) and the ABA-insensitive mutant *abi2-1*, but significantly reduced disease on the ABA-deficient mutant *aba1-3*. Susceptibility of most of the auxin-resistant mutants was similar to that of their WT background; mutants *axr1-3* and *aux1-7* were more susceptible than WT. There was no influence of NAA (naphthaleneacetic acid) or TIBA (triiodobenzoic acid, an auxin transport inhibitor) on WT, whereas NAA but not TIBA stimulated disease on *axr1-3*. Ethylene (E)-related mutants included E-insensitive, E-overproducers and E-reduced-production mutants. E-insensitive mutants *ein2-1*, *ein-6*, *etr1-1* and *etr1-3*, E-overproducers *eto1-1* and *eto2*, and E-reduced-production mutant *hls1-1* were more susceptible than WT, whereas other mutants did not differ in susceptibility from their background. Ethephon or AVG (aminoethoxyvinylglycine, an ethylene biosynthesis inhibitor) did not affect the level of disease on WT. AVG significantly inhibited disease on both *ein2-1* and *hls1-1* mutants, whereas ethephon did not affect the level of disease on *ein2-1* and slightly stimulated disease

on *hls1-1*. All the gibberellin (GA)-related mutants were strongly affected by *B. cinerea*. GA-deficient mutants were more severely infected than GA-resistant or GA-insensitive ones. External application of GA3 or AMO1618 (gibberellin biosynthesis inhibitor) did not affect the level of disease on WT and two GA-deficient mutants tested (*ga1-4* and *ga2-1*). [P]

Development of an Effective Pathogenicity Test for *Rhizoctonia* spp. Isolates on Strawberry and Their Morphological and Molecular Characterization

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Rhizoctonia spp. are known to be involved in the black root rot complex (infecting also buds and fruit) of strawberries. The currently used pathogenicity test for *Rhizoctonia* spp. isolates is based on the reduction of strawberry plant biomass (dry weight) recorded one month after inoculation of seedlings from runners with the *Rhizoctonia* isolates. The disadvantages of this method are: (a) it is not based on defined disease symptoms; (b) it requires a relatively long incubation period; and (c) the data of the plant biomass have a relatively high variability, due to additional environmental factors. To develop a more reliable pathogenicity test for *Rhizoctonia* isolates on strawberry plants based on defined disease symptoms, three additional methods were evaluated: (i) Inoculation of detached petioles, with pathogenicity evaluated 11 days after inoculation. The sizes of the necrotic spots caused by the pathogenic isolates were significantly larger than those caused by the non-pathogenic ones, but the variability was still higher than that of the other new methods. (ii) Inoculation of detached medium-size green fruit, with pathogenicity evaluated 6 days after inoculation. Typical symptoms developed on the fruit due to the pathogenic isolates. However, latent infections by *Botrytis cinerea* interfered sometimes with reliable evaluation. (iii) Inoculation of young strawberry seedlings (at the two-leaf stage) obtained from strawberry seeds. This method proved to be the best for pathogenicity tests, having the following advantages: the pathogenic isolates incited typical disease symptoms, incubation time was only 6 days, and the data recorded had relatively low variability. Characterization of the isolates by hyphal fusion with representatives of known anastomosis groups was compared to a phylogenetic tree obtained according to a molecular method based on comparing the sequences of ITS regions of the rDNA of the isolates. The pathogenic binucleate *Rhizoctonia* isolates belonged to AG-A and AG-G of *R. fragariae* (teleomorph: *Ceratobasidium cornigerum*), whereas the multinucleate ones belonged to AG 4 of *R. solani* (teleomorph: *Thanatephorus praticola*). The pathogenic isolates of the three groups appeared in the phylogenetic tree as three defined clusters. Representative isolates of the binucleate AG-I, which are known to infect strawberry plants at relatively cold temperatures, appeared in a separate cluster, but no isolate belonging to AG-I was obtained from infected strawberry plants in Israel in the present study. [L]

The Role of Organic Acid Secretions during the Pathogenesis of *Penicillium* spp.

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The phytopathogenic fungus *Penicillium expansum* acidifies the host tissue during the *Penicillium* attack. The acidification is achieved by secretion of organic acids combined with the uptake of ammonia. The tissue acidification induces the expression of the gene *pepg1*, which encodes for the pectolytic enzyme polygalacturonase involved in the maceration of the host tissue. *P. expansum* isolates with increased pathogenicity, accumulated higher amounts of gluconic acid and reduced the

apple tissue pH to lower values than isolates with reduced pathogenicity. Glucose oxidase (GOX) activity, involved in gluconic acid production, was detected in *P. expansum* decayed tissue but not in the healthy tissue of the same fruit. Growth of the fungi at reduced oxygen levels reduced the activity of GOX, decreased the accumulation of gluconic acid in the medium and delayed development of decay. Our present results suggest that the secretion of gluconic acid and acidification of the host tissue is a first stage leading to the secretion of pectolytic enzyme and decay development. [L]

The Structure of the Uredinospore Substomatal Vesicle as a Tool for Identification of Rust Taxa

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Identification of cereal leaf rust taxa is difficult due to the great similarity in uredinospore characteristics and the overlapping of the range of the main host. It is of major importance to find a rapid, reliable method of identifying a rust taxon for scientific as well as practical ends. A relatively simple and fast (4 days) method for identification is to study the host penetration structures of the rust, mainly the substomatal vesicle (SSV) of uredinospores (or aeciospores) within the cereal leaf. Two groups of rust taxa have been demonstrated: (i) crown rusts of oats, barley, and brome grass; and (ii) leaf rusts of wheat and relatives: wheat leaf rusts, leaf rust of *Aegilops speltoides*, and leaf rust of *Aegilops longissima*. The first to use the SSV shape for rust fungi identification was R. Niks. In addition to SSV study, other methods for the study of systematics were developed at the Institute for Cereal Crops Improvement, Tel-Aviv University. These include main host range study; alternate host range and inter-rust-taxa crosses; dimensions of different spore forms throughout the life cycle; and DNA content in pycniospore nuclei. Used together, these methods enable establishment of species and *formae speciales* borders. [L]

Characterization, Survival and Effect of Heat Treatments on *Colletotrichum gloeosporioides*, Causal Agent of Limonium Decline

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Colletotrichum gloeosporioides is the causal agent of Limonium decline. In this research we characterized vegetative compatibility groupings (VCGs) in *C. gloeosporioides* isolates affecting *Limonium* and identified three major VCGs. The three VCGs were also characterized according to arbitrarily primed (ap)-PCR and isolates from each of the molecular genotypes were grouped according to their corresponding VCG. The sexual cycle in *C. gloeosporioides* was also examined. Perithecia were formed on infected plant tissue that generated viable ascospores that subsequently produced appressoria on plant tissue. Survival of isolates of the fungus *Colletotrichum* to heat treatments was examined under laboratory conditions. At 48°C, conidia of all isolates of *Colletotrichum* that were produced from artificially inoculated *Limonium* stems were more resistant to heating than conidia originating from culture. The evidence of survival ability suggests that *C. gloeosporioides* affecting *Limonium* is not a typical soil-inhabiting pathogen, since populations of the fungus decline rapidly in soil under laboratory and field conditions. Results of this investigation show that *C. gloeosporioides* in soil is not likely to be a major source of primary inoculum causing anthracnose epidemics in *Limonium* in Israel. [L]

Environmental Effect on Gene Regulation of *Colletotrichum gloeosporioides* during Pathogenicity

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During the development of *Colletotrichum gloeosporioides* on ripening avocado fruits, the pathogen modulates accumulation of ammonia, and the pH increases from 4.0 to 6.0. Alkalinization of the fruit tissue induces secretions of pectate lyase (PL), a pathogenicity factor involved in decay development. In addition to pH, it was found that also nitrogen (organic and inorganic) is essential for PL secretion, even when the pH is optimal (>5.5). Mutants which are defective in their ability to take up nitrate did not cause alkalinization and we could not detect any secreted ammonia in the media. Those mutants were also less virulent. Growth of the mutants in liquid media, in the presence of glutamine or glutamate as nitrogen source, restored their ability to secrete PL. Analysis of the 5' prime of the *pelB* promoter (the *pelB* gene encodes to PL) revealed a putative consensus-binding site for the transcription factor AREA. We suggest that the pH and the nitrogen source are two different, independent factors, which take part in the regulation process of PL secretion and virulence of the pathogenic fungus *C. gloeosporioides*. [L]

Molecular Characterization of Benomyl-Resistant and -Sensitive Populations of *Colletotrichum gloeosporioides* from *Limonium*

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The fungus *Colletotrichum gloeosporioides* (*C.g*) causes *Limonium* decline and plant mortality in the Arava desert of Israel. The species *C.g* is sensitive to benomyl but may rapidly develop resistance to the fungicide. Previous reports have shown that with various fungi, a point mutation of the β -tubulin gene of sensitive isolates may change the phenotype and thus confer resistance of an isolate to the fungicide. Sixty-five isolates of *C.g* from *Limonium* were screened for resistance to benomyl, because fungicide application did not reduce field infections. At a concentration of 10 ppm benomyl, 35 isolates had acquired resistance to the fungicide and grew at a rate similar to control. Twelve isolates of *C.g* from *Limonium* and five *C.g* isolates from avocado (sensitive, resistant and induced resistance to benomyl) were used to amplify the specific fragment of the β -tubulin genes, *TUB1* and *TUB2* [from *Colletotrichum: C.g. f.sp. aeshynomene* (*C.g.a*) and *C. graminicola* (*C.gr*)], known to confer resistance. The fragments were sequenced and comparisons made between the resistant and sensitive isolates at the DNA and protein levels. Changes at the DNA level of *TUB1* of all the isolates were not reflected at the protein level and were identical to the fragment of *TUB1* of *C.g.a*. Translation of the nucleotide sequence of the *TUB2* fragment revealed complete identity among the sensitive *Limonium* and avocado isolates and that of *C.g.a* and *C.gr*. However, a single codon mutation at position 198 encoding alanine was found in all resistant *Limonium* isolates, similar to numerous studies conferring benomyl resistance in other fungi. In induced resistant *Limonium* isolates a base change at position 167 of *TUB2* was found, similar to that reported in other fungi. Representative isolates were compared by arbitrarily primed PCR and rDNA sequence analyses, indicating that the resistant and sensitive populations of *C.g* from *Limonium* belong to two separate genotypes. [P]

Identification, Characterization and Survival of *Phytophthora cactorum*, Causal Agent of Crown and Leather Rot of Strawberry in Israel

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Phytophthora is known as one of the most destructive pathogens of strawberry worldwide. The two major species affecting strawberry, *P. cactorum* and *P. fragariae*, are considered quarantine organisms in Israel. Field-cultivated strawberry plants exhibiting wilt symptoms and leather rot were diagnosed for *Phytophthora* by morphological and molecular methods. Koch's postulates were performed with the isolated pathogen and disease symptoms typical for *Phytophthora* were reproduced on inoculated strawberry plants. Coenocytic mycelia bearing typical sporangia for *Phytophthora* were isolated from infected crowns and fruit. Nested polymerase chain reaction on DNA from 30 local and worldwide representatives resulted in amplification of a species-specific band of 520 bp for *P. cactorum*, whereas primers specific for *P. fragariae* failed to produce amplification results. Survival of the pathogen in inoculated fruits was assessed. No significant differences were determined in survival of the pathogen in fruits when placed in autoclaved as opposed to field soil. Percentage infection of fruits declined after 4 days' incubation to approximately 80% while survival of *P. cactorum* in autoclaved and untreated soil decreased further to 50% and 20%, respectively, 28 days after incubation. Survival of pathogen inoculum in fruits not exposed to soil and maintained under controlled conditions remained stable at 100%. Preliminary experiments indicated that safflower can be used as a reliable indicator, since wilt symptoms were observed 4 days after planting in infested soil and typical sporangia of *P. cactorum* were observed on infected roots. Further survival experiments should be conducted to assess the survival capacity of pathogen inoculum in soil from season to season. [P]

Suppression of One Mating Type Does not Prevent Oospore Formation by the Other Mating Type of *Phytophthora infestans* in planta

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The heterothallic oomycete *Phytophthora infestans* reproduces both sexually and asexually. Sexual sporulation (oospore formation) requires the simultaneous presence of A₁ and A₂ mating types. The A₁:A₂ ratio which enables oospore production ranges between 5:95 and 95:5. In the present study we demonstrated that differential suppression of one mating type in a mixture of A₁+A₂ isolates does not prevent oospore production by the other mating type. The effect was observed in potato and tomato leaf tissues and potato tuber discs. Oospore formation was observed when either the A₁ or the A₂ isolate was suppressed. Differential suppression was achieved in three ways: (i) By metalaxyl: potato and tomato tissues, inoculated by various A₁+A₂ mixtures, of which one isolate was sensitive and one resistant to metalaxyl, were maintained on 1 or 10 ppm metalaxyl; (ii) By resistant host genotypes of potato or tomato: leaves of various cultivars were inoculated with A₁+A₂ mixtures, of which one isolate was non-pathogenic to that specific cultivar; and (iii) By killing one of the isolates in the mixture by liquid nitrogen, prior to inoculation. All the above-mentioned treatments significantly diminished, but did not prevent, oospore formation in potato or tomato tissues. We assume that a trace amount of one isolate may induce oosporogenesis in the other isolate by selfing. It is known that oospore production may be stimulated by pheromone secretion by isolates of the opposite mating type. Our data suggested that such pheromones are produced by killed sporangia or by mycelia which were depressed at initial stages of colonization. [P]

Phenotypic Characterization of Oosporic Isolates of *Phytophthora infestans* Derived from Tomato Leaves

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A₁, A₂ and A₁+A₂ sporangial suspensions of *Phytophthora infestans* (late blight) were inoculated onto detached tomato leaflets in growth chambers. At ~10 days post-inoculation the infected leaflets were homogenized in distilled water and exposed to two cycles of drying and wetting at 30°C to kill sporangia and mycelia of the pathogen. Homogenates were mixed with perlite on which healthy tomato leaflets were floated, to allow for oospore infection. In various experiments 0–50% of the floated leaflets became infected with late blight, depending on the A₁+A₂ combination used. None of the leaflets floating on A₁ or A₂ homogenates became infected, indicating effective kill of sporangia and mycelia. One isolate pair, consisting of isolates AD (A₁, sensitive to metalaxyl) and 367 (A₂, resistant to metalaxyl), was most infectious. Sporangia produced on each infected leaflet were collected separately and single-sporangium isolates (SSI) were produced with the aid of potato tuber discs. Approximately 100 SSI were tested for metalaxyl sensitivity, mating type and virulence profile, and some also for aggressiveness with tomato leaves. Whereas the MIC values of metalaxyl for isolates AD and 367 were 0.1 and >100 µg ml⁻¹, respectively, those for the SSI's, derived from the cross AD×367, ranged between 1 and 10 µg ml⁻¹, indicating a possible oospore origin. SSI's derived from a single infected leaflet belonged to A₁ and/or A₂, suggesting a multiple oospore infection of a single leaflet. Virulence profiles and aggressiveness of the SSI's generally resembled those of the parents. All SSI's were incompatible with *Lycopersicon pimpinellifolium* LO-3707, as were their parents. [P]

A Method for Infecting Tomato Leaves with Oospores of *Phytophthora infestans*

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Phytophthora infestans, the causal agent of late blight in potato and tomato, reproduces asexually by producing sporangia, or sexually by mating of A₁ and A₂ isolates to produce oospores. Oospore formation occurs in leaves subjected to wet conditions, and in tomato fruits. Only rarely are oospores formed in potato tubers. Oospores may survive in soil, in tomato fruits and in tomato seeds. They were reported not only to initiate late blight epidemics in the field but also to establish new recombinant isolates of the pathogen. Oospores germinate at a very low frequency in the laboratory. It is also very difficult to isolate them from the host tissue. Therefore, oospore-derived infections are seldom obtained. Oospores produced *in planta* are always mixed with sporangia and mycelia of their two parents, thereby enabling both oospores and parents to cause new infections. The technique described here makes it possible to kill sporangia and mycelia differentially in leaves carrying oospores but to maintain oospores infective. Detached tomato leaflets (cv. 'ZH') were placed on wet filter paper in 20×20 cm Nunk dishes and inoculated with mixed A₁+A₂ sporangia of *P. infestans*. Control leaves were inoculated similarly with only A₁ or A₂ sporangia. Dishes were incubated at 18–20°C (12 h light per day) for 8–10 days to allow for oospore formation. Thereafter, leaves were homogenized in cold distilled water (20 leaflets in 5 ml water) and the homogenate was pipetted into 9 cm petri dishes (5 ml per dish). Oospore counts in leaf homogenates ranged between 1000 and 5000 per ml for different A₁+A₂ combinations. No oospores were seen in control inoculated leaf tissues. Our earlier data showed (*Can. J. Bot.* 52:447-450, 1974) that sporangia of *P. infestans* fail to survive wetting, drying and rewetting. We therefore exposed the leaf homogenates to two cycles of drying and wetting at 30°C. Homogenates were thereafter suspended in 25 ml of water and mixed with 1.5 g of perlite (No. 4) in 9 cm petri dishes (3 cm height). Two leaflets of tomato (cv. ZH) were floated on the mixture. Plates were incubated at 18–20°C (12 h light per day)

for 3 weeks to allow for oospore infection. None of the leaflets floating on A₁- or A₂-inoculated leaf homogenates became infected, whereas 0–50% of the leaflets floating on A₁+A₂-inoculated leaf homogenates became infected, producing sporangia of *P. infestans*. Phenotypic characterization of the recovered isolates revealed that all were of oosporic origin. [P]

B: SOILBORNE PATHOGENS – ENVIRONMENTAL INFLUENCE AND CONTROL

Pathogen Effects on the Microbial Ecology of the Root and Rhizosphere

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The interactions of pathogen and host plant have been widely studied. Very little is known, however, about the effect of the presence of pathogens on the microbial ecology in the plant's environment. Plant-associated microbial communities can play a critical role in plant nutrition and health. The present study examined such microbial communities in soil, rhizosphere and root samples from pathogen-amended experimental systems. These communities were compared in control soil treatments and in treatments amended with disease-conducive and disease-suppressive composts. *Pythium aphanidermatum* was used as the model pathogenic organism, and caused damping off in cucumber. Cultivation and molecular methods were employed to follow bacteria and fungi in the systems, and focused in greater detail on several narrower taxa, including the phylum Bacteroidetes, the class Actinobacteria, the families Streptomycetaceae and Oxalobacteraceae, and the genera *Pseudomonas* and *Chryseobacterium*. Root-, rhizosphere- and seed-dominant microorganisms were detected. Those organisms with higher affinity to plant surfaces (root and seed) or that reacted to the presence of the pathogen were discussed. The research strategy described has great potential for facilitating the resolution of complex plant-oriented microbial communities and for identifying reactions to pathogens that will assist in the identification and isolation of disease-suppressive organisms. [L]

Characterization of *Streptomyces* Structure in *Pythium*-Suppressive and -Non-Suppressive Compost-Amended Plant Systems

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A study was conducted of the disease-suppressive capability of three different composts in a compost–pathogen–plant experimental system, and associated bacterial populations were characterized. Cucumber plants were grown in compost-amended potting mixes inoculated with *Pythium* mycelium, with a peat-only treatment serving as a control. Disease rates were determined 21 days after planting. Two of three composts were effective in reducing damping off, a disease caused by the pathogenic Oomycete *Pythium*. Our microbial analyses were focused on the antibiotic-producing genus *Streptomyces* (Class Actinobacteria) using PCR primers and culturing media selective for *Streptomyces* and Actinobacteria, respectively. Concurrent to isolation of specific bacterial populations, DNA was extracted from potting mix (0, 1, 4, and 21 days), seed (1 day), seedling (4 days), rhizosphere (21 days) and root (21 days) samples. Using denaturing gradient gel electrophoresis (DGGE) of PCR-generated rDNA fragments, *Streptomyces* populations from these samples were profiled. Prominent DGGE bands were excised and sequenced, and the phylogeny of the respective organisms was inferred. Compost application yielded significantly different population

profiles at both levels of taxonomic analysis (*i.e.*, Actinobacteria and *Streptomyces*). Variations between composts were significantly greater than variations between *Pythium*-amended and control treatments. The physiology and distribution of relevant organisms, including those detected only in *Pythium*-amended treatments, were discussed. [L]

Microbial Changes on Disinfested Soils

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Microbial changes were studied in infested and disinfested soils and growth substrates, either solarized for 1–15 years or treated with methyl bromide. In solarized soils or substrates, the population density of thermotolerant microorganisms, *viz.* those surviving temperatures of 42–44°C, was higher than in the untreated soils. In solarized and untreated tuff medium, 58% and 1% of the fungi and 98% and 0% of the bacteria, respectively, were able to grow at 38°C. A similar trend was observed with soils solarized from up to 12 years. Populations of fungi (especially *Aspergillus* spp.) and *Bacillus* sp. which have the capacity to produce antibiotics against *Fusarium oxysporum* f.sp. *radicis-lycopersici* (FORL) and *Rhizoctonia solani*, were higher in solarized soils and growth substrates than in nonsolarized ones, whereas in methyl bromide-treated soils the results varied. Some of the antibiotic-producing fungi from solarized soils suppressed as many as seven tested pathogenic fungi. Populations of *Fusarium* conidia added to solarized soils declined faster than in untreated soils, indicating induced suppressiveness in the solarized soils. FORL isolates from the desert region were more tolerant to high temperatures than were isolates from another region. [P]

The Root-Knot Nematode *Meloidogyne marylandi* Isolated from Turf Grass in Israel

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A population of a root-knot nematode species was isolated from *Zoysia japonica* in a turf nursery in Israel. Measurements and morphology of the second-stage juveniles and adult females, including perineal pattern, indicated that this nematode was *Meloidogyne marylandi*. In addition, esterase (Est) and malate dehydrogenase (Mdh) isozymes were studied for the first time for this species which is shown to be characterized by VS1 Est band and a N1c Mdh pattern. Host range tests showed that the turf grasses *Stenotaphrum secundatum*, *Dactyloctenium austale* and *Paspalum vaginatum*, corn (*Zea mays*) and oat (*Avena sativa*) were non-hosts or resistant. Kikuyu grass (*Pennisetum clandestinum*), wheat (*Triticum aestivum*), barley (*Hordeum vulgare*), bristle oat (*Avena strigosa*), Siberian millet (*Echinochloa frumentacea*) and pearl millet (*Pennisetum glaucum*) were susceptible to the nematode. The second-stage juveniles of *M. marylandi* penetrated the elongation zone of wheat roots but did not reach the meristematic zone and settled with their anterior end toward the root tip. This contrasted with *M. javanica* or *M. incognita*, which migrated to the meristematic zone, and turned around with their anterior toward the root base. *M. marylandi* juveniles induced giant cells from vascular parenchyma cells and caused no or only small galls on the roots. [P]

Soil Solarization and Organic Amendments: A Laboratory System to Study Physical, Chemical and Microbial Aspects of the Process

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Solarization of organic amended soil involves several aspects of activity in soil. Pathogen control is attributed to lethal temperatures, weakening effects, generation of toxic volatile compounds, and shift toward populations of antagonistic microorganisms. In small-plot field experiments, solarization of soil amended with wild rocket (*Eruca sativa* Mill.) effectively controlled populations of pathogenic fungi and of root-knot nematodes down to a depth of 40 cm. In contrast, solarization alone was only partially effective in controlling these pathogens. Solarization of soil amended with wild rocket results in a sharp decrease in oxygen concentration in the soil atmosphere during the first few days, followed by a gradual increase back to the original concentration. The microbial and enzymatic activity in the soil was accelerated simultaneously; the bacterial population increased, while the fungal population declined. The objectives of the study were to develop a laboratory system that combines heating and organic amendments, and enables us to study separately the factors that are involved in the process. We developed a laboratory system which consists of heating soil containers similar to the situation occurring in the upper soil layer during solarization. The simulation system enables us to assess simultaneously many physical and chemical variables such as the soil atmosphere (pressure, oxygen concentration, volatile compounds). It also makes it possible to test various biological changes such as pathogen control, enzymatic activities and shifts in microbial populations. With this system it was found that heating tarragon (*Aremisia dracunculus*)- or wild-rocket-amended soil, completely controlled chlamydospores of *Fusarium oxysporum* f.sp. *radicis lycopersici* when they were buried in soil, or exposed to heat and soil atmosphere only. On the other hand, volatile compounds that were generated in this process controlled *Rhizoctonia solani* at room temperature. Changes in oxygen concentration, pH, soil enzymatic activity, and microbial populations during this process were similar to those observed in the field during solarization of organic amended soil. The simulation system which was developed is an important tool for rapid screening of effective organic amendments. It allows adoption of alternative methods to chemical fumigants for soil disinfection. Further studies with this system will enable better understanding of the processes which are involved in solarization of organic amended soil. [L]

Enhanced Solarization Efficacy to Control Soilborne Pathogens

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Soil solarization is a non-chemical method to control a wide range of soilborne pathogens. One of its limitations is the inability to control heat-tolerant pathogens such as *Fusarium oxysporum* f.sp. *radicis-lycopersici* (FORL), the causal agent of crown and root rot of tomatoes. The objective of this study was to enhance solarization intensity and performance by elevating soil temperatures. At the initial stage we assayed the relationships between heat and survival of resting structures of FORL and *Sclerotium rolfsii* in soil. We found that exposure of the fungal propagules to a temperature above 50°C was very effective in reducing their viability (90% reduction for FORL after 2 h, and 90% reduction for *S. rolfsii* after 20 min). In small plot experiments a wide range of plastic films was screened in an attempt to achieve increasing soil solarization intensities and a better film for

solarization purposes. Additionally, we tested a combination of two layers of mulch, including sprayable black mulch under a clear plastic film tarp. The double layer mulch yielded higher soil temperature and the shortest time required to control the propagules of soilborne pathogens. Another film that was successful in the tests was a polyethylene film which was formulated with the addition of anti-drip components (AD). This formulation prevents condensation of water droplets on the film surface, leading to 30% greater irradiation transmittance compared with the regular film. Soil temperatures under AD film were 2–7°C higher than under regular film. The efficacy of the improved films in increasing soil solarization in tomato and melon crops infested with *FORL* and *Monosporascus cannonballus* was tested in a field experiment in the Arava region of Israel. Soil solarization using a double layer mulch was effective in controlling crown and root rot disease in tomatoes and sudden wilt of melons, whereas solarization with regular films was not effective. Preliminary tests with the AD films gave similar results. The use of the new films can provide a solution for improved control of soilborne pathogens by solarization. Further studies to improve film performance are under way. [L]

Cultivit - A New Machine for Soil Thermal Disinfestation

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Thermal disinfestation of soil by Cultivit has been employed commercially since 1999. In 2002/03, 50 ha of vegetables, flowers and spices were disinfested by this method. The action consists of blowing very hot air into granulated soil for a few seconds. The soil is lifted to the blower by a rotavator. Adjustment of the machine's speed to soil conditions ensures that every grain of soil will be heated and disinfested. The very short heating time avoids damages to organic material and does not create phytotoxic volatile compounds. Sowing or planting is permitted immediately after disinfesting, and there is no need to leach or aerate the soil. Some phenomena after disinfestation hint that there is a positive shift in the soil biological equilibrium. The machine is built on a tractor and operated by remote control, thus enabling the operator to avoid being close to the very high temperatures.

Technical data: Air temperature in the blower, 700–750°C; air speed, 25 m/sec; speed, 250 m/h; width of the disinfested line, 180 cm; depth of rotavating, 35 cm. **Pests that are eliminated:** Nematodes, both free and cyst forming; Verticillium wilt; potato scab; seedling damping off (*Pythium*, *Rhizoctonia*, etc.); weeds (more than ten annual species). **IGR (Improved Growth Response).** Stronger and healthier plants are grown and better yields are obtained over 2 years and sometimes even in the third year after disinfestation. **Mode of action.** It seems that the hot air affects some systems in soil. There is a direct impact of weakening pest agents. The hot air acts on soil by inducing soil suppressiveness against pest agents and affects the plants by making them stronger, healthier and more resistant to pests. The combined outcome is probably synergistic. [L]

Accelerated Degradation of Metam-Sodium in the Field and Its Management

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Accelerated degradation of metam-sodium (MS) in soil was documented in Holland, Australia and Israel. The objectives of the present study were to determine the possible occurrence of

accelerated degradation of methyl isothiocyanate (MITC) under field conditions in Israel, and first assessing prevention strategies in order to delay and/or control the development of accelerated fumigant degradation. A wide survey, which included 40 soil samples from different agricultural fields, was conducted to assess the dissipation profile of MITC in each soil after MS application. The results indicate that the soils differ in their capacity to degrade MITC. Different dissipation curves were obtained for tested soils, ranging from rapid dissipation (24 h) to slow and long with a high concentration of MITC in soil. The dissipation curves in each soil were highly correlated with the efficacy of pathogen control in the tested soil. The differences of MITC dissipation among the tested soils can explain failures in pest control in certain fields following MS application for disease management. Two field experiments were established in plots where insufficient management of soilborne disease had been documented during the previous crop. One field was infested with *Verticillium* wilt of potatoes and the other with *Pythium* and pod wart pathogens in peanuts. Soil fumigation included one and two applications of MS, and a combination of MS and formalin. The dissipation curve of MITC for each soil history was tested in the lab. Disease development and crop production were determined during two seasons. MS was effective in controlling *Verticillium* wilt in potato and maintaining a commercial yield during the first year. The combination of MS and formalin further improved pest control. The loss of disease control was observed with MS after the second application, indicating rapid dissipation of MITC in the soil. In the second experiment MS was not effective in controlling *Pythium* pod rot in peanuts after repeated application. The inefficient control of pod rot was correlated with rapid dissipation of MITC in this soil. The accelerated degradation phenomenon of soil fumigants has important practical implications and further emphasizes the importance of alternating pesticides and avoiding frequent application of the same pesticide. This phenomenon should be studied also for other fumigants in order to prevent the rapid loss of their efficacy. (L)

Effect of Alternating Applications of Metam-Sodium and Formaldehyde on Control of *Pythium aphanidermatum*

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The present study was conducted to compare the efficacy of alternating applications of metam-sodium (MS) and formaldehyde, with repeated applications of each chemical alone, in controlling *Pythium aphanidermatum* and on total populations of *Fusaria* and bacteria. A sandy soil sample collected from a commercial field was used to study the effect of the following four treatments: control, MS, formaldehyde, and alternating between MS and formaldehyde. Chemicals were applied at 2-week intervals, six times successively to soil before sterilization, and four times to soil after sterilization. Five days after chemical application, viability of *P. aphanidermatum* and also the count of bacterial and *Fusaria* populations in soil samples, were determined. Effective control of *P. aphanidermatum* (100% mortality) was observed only following the first application of each chemical, at 300 ppm active ingredient (a.i.). In the second application, including alternating applications, none of the chemicals was effective in controlling the fungus. In the four following applications a gradual increase of each chemical concentration up to 1000 ppm a.i. in the sixth application did not affect the fungus. Bacterial population after chemical applications was similar to the control. *Fusarium* level, however, was higher than the control following formaldehyde but lower following MS or alternating applications. Application of each chemical at 300 ppm a.i. to sterilized soil, controlled the fungus effectively (100% mortality) in two successive applications but was less effective in two additional applications. Bacterial populations were similar in all treatments and *Fusaria* populations were very low in all chemical treatments. The results obtained in the present study confirmed that a reduction in efficacy of each chemical following repeated applications was due

to the microbial population. Alternating applications did not improve control efficacy as compared with repeated applications of each chemical alone, although it improved slightly the control efficacy of MS. Reduction in formaldehyde efficiency was not due to an increase in *Fusaria* populations but rather to other microbial populations, probably from bacterial groups. [L]

Combination of Soil Fumigants: Synergistic Performance and Improved Yield

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The currently available fumigants control a narrow range of pests compared with methyl bromide. Combination of fumigants with a different spectrum of target pests can extend the application of these fumigants. Formalin (Fordor, Dor Chemicals, Haifa) and metam-sodium (MS) are registered in Israel against bacterial and fungal diseases. A combination of the two fumigants provides relief to farmers in potato and peanut fields that are heavily infested by soilborne fungi (*Pythium* and *Verticillium*) and bacteria (*Streptomyces* spp.). The objective of this study was to evaluate the performance of MS combined with formalin in controlling soilborne pests in agricultural fields. In laboratory studies we found that application of formalin and MS resulted in a synergistic control of fungal pathogens. It was evident that formalin enhances MS toxicity at a low concentration at which MS alone was not effective. Field experiments in small plots showed that combined application of formalin and MS at reduced dosages improved the control of fungal pathogens and increased mortality in deeper soil layers. The effect of combined fumigation was tested in controlling diseases of tomatoes, melons and potatoes. In all the experiments standard dosages were compared with the combination at half and even further reduced dosages. Application of MS at 200 l ha⁻¹ combined with formalin at 1500 l ha⁻¹ (30% of the standard dosage for each fumigant alone) resulted in effective control of sudden wilt of melons, crown rot of tomatoes and *Verticillium* wilt of potatoes. In all the experiments the combination of fumigants resulted in increased yield quality and quantity. Application of formalin and MS was further validated in large commercial fields with successful results, the combination having a synergistic effect in pest control. This enables us to extend the use of these fumigants in fields with a broad spectrum of pests, as well as to reduce dosages while maintaining a high level of control and minimizing negative attributes. Further research should be conducted to explore additional possibilities of fumigant combinations. [L]

C: RESISTANCE

The Role of Sorbitol in Progression of Fire Blight Symptoms in Perennial Pear Branches in Israel

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Fire blight, caused by *Erwinia amylovora*, is a devastating disease of pear trees in Israel and elsewhere. The bacteria invade the trees through the blossoms mainly during spring bloom, but also in the autumn when autumn flowering occurs. The bacteria then progress to the perennial branches at a rate that is governed by the vigor of the trees and the season of infection. In the spring, the rate of disease progression is higher in trees with high vigor than in trees with low vigor, but in the autumn

the situation is reversed. Moreover, the disease rates are much higher and the symptoms progress for a longer period during the following autumn vs spring infections. Sorbitol is the main soluble sugar of rosaceous trees and 30–40% of the soluble sugars of pear trees are stored as sorbitol. It was hypothesized that the different rates of disease progression between trees with low and high vigor and between spring and autumn infections are related to the sorbitol content of perennial branches. Pear shoots in low and high vigor trees were artificially inoculated in the spring and autumn, and the rate of disease progress was recorded periodically. In addition, phloem tissues were sampled and their sorbitol content was determined. In the autumn, sorbitol content was higher in high vigor trees but following the breakage of dormancy the situation was reversed. A significant coincidence ($P < 0.01$, $r^2 = 0.826$) was found between the rate of symptom progression in perennial branches and the change in sorbitol content. It is not yet known whether sorbitol content directly affects *E. amylovora* or whether it is just an empirical measure for physiological host resistance. [L]

Race-non-specific Resistance against *Phytophthora infestans* in *Lycopersicon pimpinellifolium*

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Seven accessions of *Lycopersicon pimpinellifolium* (wild tomato) were obtained from Agrogene Ltd. (Kibbutz Keramim, M.P. Negev, Israel): LO3707, LO3708, 14277, 14322, 14341, 14345 and 14377. Approximately 200 plants of each accession were grown in 'Speedling' trays and inoculated at the 4-leaf stage with a sporangial mixture of *Phytophthora infestans* composed of 68 field isolates. LO3707 was highly resistant, whereas the other accessions exhibited various levels of susceptibility to the blight. Twenty-eight LO3707 plants were transplanted to a shade house and after 2 months leaves were sampled for inoculation. Leaflets were placed in moist petri dishes and inoculated with each of 73 field isolates of the pathogen as well as with each of 40 recombinant isolates produced from $A_1 \times A_2$ isolates (see Rubin and Cohen abstract). Seven plants showed resistance to all 113 isolates. Each of these plants was self-pollinated and also crossed with plants of accession 14377, which was found susceptible to the above isolates. The F1 plants and their parents were grown in the following season in a shade house to produce F2, BC_S and BC_R families. Leaves were sampled from LO3707, 14377 and their F1 plants and inoculated with a sporangial suspension of mixed isolates. The number of sporangia produced after a week on the inoculated leaflets of LO3707, 14377 and their F1 hybrid was 260, 76,000 and 850 sporangia per leaflet, respectively, suggesting that resistance was inherited in a dominant manner. Epiphytotics of late blight developed later in the shade house only on the susceptible parent plants, thus supporting the notion of dominant resistance. Current work is aimed at determining the number of loci controlling race-non-specific resistance in LO3707. [L]

Identification of Genes Involved in Dinitroaniline Herbicide-Induced Resistance of Melon to Fusarium Wilt

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Fusarium wilt of melons caused by the fungus *Fusarium oxysporum* f.sp. *melonis* (*Fom*) is one of the most destructive diseases of melons. Dinitroaniline herbicides have been demonstrated to induce resistance of melon plants to Fusarium wilt. The objective of the present project was to identify changes in gene expression following treatment with the herbicide trifluralin and inoculation with

Fom. Based on the colonization of the plant's tissues by the fungus, the 3rd and 6th days after inoculation were chosen for sampling the plant material for molecular analysis. Two techniques, SSH and cDNA-AFLP, were used to identify genes that exhibited differential expression following the trifluralin treatment and inoculation. Employing these techniques, approx. 120 clones were isolated and sequenced. Four suppression libraries were constructed using the SSH technique, two from root/stem tissues and two from cotyledon tissue.Suppressions were performed between the non-treated control plants and the treated (trifluralin, *Fusarium*) plants in direct and reciprocal procedures. The SSH libraries were spotted on array membranes. Among the SSH technique-isolated clones, three exhibited differential expression. These genes exhibited the best similarity to sequences of: (i) zinc finger osmotic/cold-induced stress protein-encoding gene; (ii) gene encoding glycolate oxidase, a key enzyme in the respiratory process; and (iii) gene encoding an DXR enzyme that plays a role in terpenoid biosynthesis. Other genes, isolated by the cDNA-AFLP technique, exhibited only minor differences between non-treated controls vs treated plants. In order to determine if the genes that exhibited differential expression are also characteristic to other abiotic stresses, their expression to salinity was tested. Similar expression patterns of these genes were found in the trifluralin-*Fusarium* and the salinity experiments, suggesting they are common denominators of biotic and abiotic stress responses in melon plants. [L]

Use of a Diagnostic Medium for *in situ* Determination of the Response of *Erwinia amylovora* Strains to Oxolinic Acid and Streptomycin

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Erwinia amylovora, the causal agent of fire blight, is managed by application of bactericides to protect the blossoms from infection. Monitoring the response of *E. amylovora* strains to bactericides is crucial for adequate disease management. The coliform agar medium produced by Merck (RD-medium) was recently reported as an effective tool for rapid diagnosis of *E. amylovora*. The objective of the present study was to examine the possibility of using the RD-medium for *in situ* determination of the response of *E. amylovora* strains to oxolinic acid and streptomycin. The phenotypic responses of 48 *E. amylovora* strains, isolated in 2002, to both bactericides were determined with the RD-medium and, for comparison, by a routine laboratory test. The results of 45 samples (93.7%) were in agreement with the findings of the routine laboratory test. A χ^2 test rejected the null hypothesis that the phenotypic characteristics as determined by the two respective methods differed significantly ($P=0.389$). In 2003, the response to oxolinic acid of *E. amylovora* strains isolated from 61 different orchards, was determined in a two-step procedure. In the first step samples were imprinted onto the RD-medium plates in the orchards. Suspected samples with oxolinic acid-resistant strains were re-examined in the ARO laboratory (second step). Results were in agreement with those obtained in 2002, which suggests that this medium can be used *in situ* for monitoring the appearance of resistance in *E. amylovora* populations. [P]

Screening Tomatoes for Race-Non-Specific Resistance against Late Blight

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Late blight caused by *Phytophthora infestans* is a devastating disease of tomato worldwide. In Israel, all commercial cultivars are susceptible to the disease. *P. infestans* has undergone major

genetic changes in Israel over the past 20 years, with newer races appearing very often. During the years 2000–2002, 25 new races were recorded. Cultivars carrying the monogenic resistance genes *Ph-1* and *Ph-2* were tested but found to be susceptible to many isolates prevailing in the country. Approximately 100 cultivars were tested and found to be susceptible to mixed inoculation with 60 field isolates of the pathogen. We therefore examined wild tomato accessions to discover the possible occurrence of race-non-specific resistance against late blight. Among accessions of *Lycopersicon pimpinellifolium*, one accession, LO3707, was highly resistant to *P. infestans*. Highly resistant individuals were selfed and progenies were tested for race-non-specific resistance in growth chambers and against natural infection in shade houses at two locations, Keramin and the Bar-Ilan Farm. Resistant, stable lines were obtained after eight generations of selfing and selection. LO3707 (having small red fruits of ~0.7 g) was crossed with high-quality tomato inbred lines, and the F2 families were screened for resistance at the 4-leaf stage with mixed sporangial inoculum made of 60 isolates of *P. infestans*. Resistant F3 individuals were selfed and selected for resistance for three more self-generations. These S8BC3 inbred lines possess race-non-specific resistance to the blight as well as nearly adequate fruit characteristics. The research was supported by a grant from Hazera Genetics to Agrogen Ltd. [P]

D: VIROLOGY

Development of an IPM System to Reduce the Damage of Squash Leaf Curl Virus in Zucchini Crops

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Squash leaf curl virus (SLCV) is a 'New World' *Begomovirus*; however, it was recently found in Israel in zucchini crops grown in the open field during summer and autumn. This whitefly-borne virus has a circulative relationship with *Bemisia tabaci* that is responsible for its spread in nature. The virus has a relatively wide host range in the Cucurbitaceae and other plant families. During the last 2 years zucchini crops grown in the open field were devastated by the virus despite aggressive treatments with insecticides. The goal of this study was to develop an IPM system to protect zucchini crops from the spread of the disease. In field experiments carried out in the Besor area, zucchini plants var. 'Erlika' that were grown over yellow, silver or metallic soil mulches and sprayed twice a week with insecticides against whiteflies, were efficiently protected from the effects of SLCV disease compared with plants that were under the same spray regime but were planted on bare soil. Zucchini plants that were covered with Agryl or a 50-mesh screen for 3 weeks from planting, produced a lower yield than plants that were grown on soil mulches. A positive correlation was found between the growth vigor of zucchini varieties and their tolerance to SLCV disease. The zucchini varieties 'Zuk' and '1336' were significantly more tolerant to the disease than var. Erlika, which was found to be the most susceptible. Our results suggest that an IPM system incorporating yellow soil mulches, limited sprays with anti-whiteflies insecticides, and tolerant varieties would be efficient in reducing the damage caused by SLVC to zucchini crops.

Role of the Potyviral Helper Component in Binding to Cuticular Proteins of Aphids

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Potyvirus are non-persistent viruses that require a non-structural viral protein, named helper component (HC), for transmission by aphids. The mode of action of the HC is believed to be by bridging between the virion and the aphid stylet. The HC shows specificity for certain viruses and/or aphid species. Potyviruses were shown to be attached within the lumen of the food canal of the aphid's stylets. The stylet is made of epicuticle [composed of cuticular protein (CuPs) and waxes]. The CuPs together with chitin comprise the insect skeleton. A first CuP gene from aphids was reported recently by us (Dombrovsky et al., 2003, *J. Insect Biochem. Mol. Biol.* 33:709). The working hypothesis herein is that the CuPs are involved in binding the HC, thus indirectly binding virus particles. After hardening, the CuPs are insoluble. Several extraction procedures using the compound 8M urea, 1% calcofluor, 1% congo red or 5% sodium dodecyl sulfate were reported. The extracted proteins were separated by electrophoresis, electroblotted onto nitrocellulose paper, and exposed to a recombinant HC that includes seven histidine residues. Bound HC was visualized using a commercial monoclonal antibody to the histidine residues. This procedure enabled us to determine that CuPs bands of 31 and 33 kDa that were extracted with either 1% congo red or 1% calcofluor, reacted with the HC. This is the first report of an affinity between the potyviral HC and the aphid CuPs. The significance of this finding and future work were discussed. [L]

E: EPIDEMIOLOGY

Epidemiological Aspects and Survival of *Fusarium mangiferae*, Causal Agent of Mango Malformation Disease

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Mango malformation caused by the fungus *Fusarium mangiferae* is a major disease of this crop worldwide. Conidia declined rapidly in soil under controlled (laboratory) and outdoor (winter and summer) conditions and could not be recovered after 102, 72 and 10 days, respectively. Complete recovery (100%) was determined from natural infections of flowers and fruitlets from diseased tissues. The pathogen could not be recovered from seed and seed coats of fruit harvested from diseased trees. The pathogen was quantified from the surface of 2- and 3-month-old fruits from the same diseased trees, with respective concentrations of ca 3,500 and 2,000 colony forming units (cfu) g⁻¹ tissue. Natural infections in vegetative shoots from infected trees were quantified in 2001 and 2002 with respective concentrations of ca 3 × 10⁶ and 10⁶ cfu g⁻¹ tissue. All malformed vegetative shoots from infected trees were 100% infected by the pathogen. Sensitivity tests of conidia to U.V. showed a rapid decline in viability resulting in 100% mortality when exposed to sunlight for 3 min. *Nit* mutants of *F. mangiferae* as 'tagged' inoculum in inoculated soil declined rapidly after 30 days and could not be recovered after 120 days but were detected 30 days after inoculation in plant roots. Fifteen months later, colonies of the mutant isolate were recovered from the roots and within the stems, 40 cm from the crowns of the inoculated plants. Characterization of vegetative compatibility groups (VCGs) within the population of *F. mangiferae* used in this study resulted in 96% of total isolates belonging to a single VCG group, including all the Israeli isolates and some from Florida and South Africa. [L]

Fusarium Stem and Root Rot of Cucumber in Israel

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A new disease in cucumber, brought about by a distinct *forma specialis*, *Fusarium oxysporum* f.sp. *radicis-cucumerinum* that causes a stem and root rot, was evident in the Ahituv region of Israel in the winter and spring of 2002 and 2003. The identity of the pathogen was verified by pathogenicity and VCG tests. The first record of this disease in Israel was in 1998 at Hatzav, with subsequent appearance at additional sites. This pathogen produces masses of aerial macroconidia on the plant stem, which have the potential of spreading the pathogen. Means to reduce the inoculum and disease incidence were examined. Application of 0.1 g per plant of the fungicide Bavistin at 2-week intervals, starting 7 days after planting, reduced disease incidence. In three greenhouse experiments applying structure solarization and reaching maximal temperatures of 50–70°C, inoculum reduction was 46–97% after 22 to 27 days of solarization. Dipping contaminated support strings in 1% sodium hypochlorite eradicated the pathogen. The disease was totally controlled when the resistant rootstock 148-TZ was used. It is concluded that a holistic approach to control that integrates sanitation, grafting, fungicides and crop rotation, is necessary in order to achieve satisfactory reduction of inoculum and disease. [L]

UV-Absorbing Greenhouse Covers and Nutritional Regime as Measures to Suppress Gray Mold of Basil

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Basil (*Ocimum basilicum*), used for medicinal purposes and as a spice in the food industry, is an economically important crop in Israel, cultivated during the winter. The gray mold disease, caused by the fungus *Botrytis cinerea* Pers.:Fr, threatens greenhouse-grown basil plants. The disease may result in the plant's death and collapse of the entire crop. To suppress the fungus, growers frequently apply fungicides. The objective of this study was to examine the efficacy of non-chemical measures in disease suppression. The first measure tested was UV-absorbing polyethylene sheets. The effects on gray mold of polyethylene covers with varying UV-filtering properties were tested in petri dishes, detached stems and intact plants. It was found that under controlled conditions sporulation was reduced by films with a radiation cutoff of 360 to 380 nm. However, no such inhibition of sporulation was found on greenhouse-grown intact plants. There were variable responses of different *B. cinerea* isolates to the various tested films. The second measure tested was different fertilization regimes, as it is known that plant diseases are affected by the nutritional status of the host. A series of experiments examined the effects of different concentrations of nitrogen, phosphorus and calcium on infected detached stems and on intact plants, grown in pots and in beds in a greenhouse. Gray mold was inhibited by low levels of nitrogen and phosphorus and by high levels of calcium as compared with the standard commercial levels. [L]

Effect of Temperature on Development of Scytalidium Wilt of Star Ruby Grapefruit

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Outbreaks of *Scytalidium* wilt of Star Ruby grapefruit, caused by *Scytalidium lignicola*, in the Jordan Valley in northeastern Israel during spring to mid-summer, were preceded by a spell of a few days of extremely hot and dry weather. In this work we studied the effect of nine temperature regimes, 3 pre-inoculation × 3 post-inoculation, on the disease development in young Star Ruby trees in a glasshouse. The temperature regimes (day/night, in °C) were: cold, 33/21; medium, 37/28; and hot, 48/34. After pre-treatment for one week, the plants were inoculated with a mycelium disk of *S. lignicola* placed over a wound in the stem, and transferred to the post-inoculation conditions. At various time intervals after inoculation, disease intensity was assessed by two parameters: canker length, and percentage of dead trees. In the cold or medium pre-treatments × cold or medium post-treatments no disease developed. In the hot × hot regime, the average canker length was 120 mm and 100% of the trees died within 14 days after inoculation. In the hot × medium regime, the average canker length was 35 mm and 50% of the plants were dead after 14 days. In the hot × cold regime, the average canker length was 20 mm and only one tree (20% of plants) died. In the medium × hot regime, the average canker length was 35 mm, and mortality rate within 14 days was 75%. In cold × hot regime the cankers expanded to an average of 20 mm and then their development ceased; no tree died. It can be concluded that hot conditions enhance disease development. Pre-inoculation exposure of plants to high temperatures induced disease development in all post-inoculation regimes. This indicates a predisposing effect of the heat, reducing the resistance of the trees to fungal invasion and subsequent disease development. The predisposing effect of the heat may account for the observation that an outburst of *Scytalidium* wilt occurred only after extremely hot and dry weather, characteristic of the Jordan Valley. [L]

Management of Late Blight in Greenhouse Tomatoes: A Two-Step Procedure

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Late blight, caused by *Phytophthora infestans*, is one of the most devastating diseases of greenhouse-grown tomatoes in Israel and elsewhere. Growers frequently apply fungicides to suppress late blight, but it is not uncommon that severe epidemics develop even in fungicide-treated crops. Determining the quantitative effects of the relevant factors on the pathogen may lead not only to improved disease suppression, but also to reduction in fungicide use. The effects of environmental factors (temperature and relative humidity) and management actions (application of fungicides and sanitation) were studied in a series of experiments conducted in growth chambers and commercial-like greenhouses. It was found that disease development may be divided into two steps. In the first step foliar infection occurs at low temperatures (<20°C) accompanied by wet conditions. The resultant late blight severity is governed by the suitability of the weather conditions to the pathogen and by the quantity of prevailing inoculum. At this stage the disease may be suppressed by manipulating the environmental conditions (for example, by reducing leaf wetness) and by application of fungicides. The second step follows when temperatures are high (>20°C) and the foliage is dry. Under these conditions the pathogen progresses from infected leaf-blades via the petioles, to the stems, where it causes stem lesions that eventually lead to plant death. Observations made in the greenhouses suggested that the damage resulting from these types of symptoms is more significant than that which results from foliar infection. It is possible to prevent stem infections by

sanitation (removal of infected leaves). Moreover, it was observed that the rate of disease progression in infected leaves was reduced, and fewer plants died from stem infections, when temperatures exceeded 30°C. In conclusion, late blight management should incorporate this two-step procedure. [L]

Plant Pathogenic Nematodes in Recycled Water

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The levels of plant pathogenic nematodes were tested in samples of recycled water obtained from glasshouses in two geographical locations in Israel (Lakhish and the Carmel coast), cultivated with roses and vegetables, respectively. Weekly assays of water samples from different sources of recycled water, including the main containers, irrigation pipes and drippers, revealed large populations (8–45 larvae) of *Meloidogyne hapla* and *M. javanica* from Lakhish and Carmel glasshouse operations, respectively. The observed distribution of plant parasitic nematodes among plants cultivated within glasshouses in both areas revealed nematode infestation at a rate and distribution far higher and more uniform than expected for natural infestations under normal field conditions. Since soil-less glasshouse cultivation of roses and vegetables is highly conducive to the build-up of large populations of plant parasitic nematodes and because of regulations prohibiting the use of methyl-bromide for soil disinfection, it is possible that future soil-less cultivation in glasshouses will be faced with massive damage from nematode spread *via* recycled water. The urgent need to develop new and effective ways of nematode elimination from recycled water was discussed. [L]

F: CHEMICAL AND BIOLOGICAL CONTROL

Postharvest and Soilborne Disease Control with the Fungal Biofumigant *Muscodor albus*

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The biofumigant fungus *Muscodor albus* produces a mixture of antimicrobial volatiles which is lethal to storage pathogens such as *Botrytis cinerea*, *Monilinia fructicola*, *Penicillium* spp., *Colletotrichum* spp. and *Erwinia carotovora*, as well as the soilborne pathogens *Rhizoctonia solani*, *Phytophthora capsici* and *Sclerotinia* spp. The fungus, which was isolated from a cinnamon tree, is a sterile mycelium belonging to the family Xylariaceae based on molecular evidence. The main volatiles detected were alcohol, ester and acid derivatives such as ethyl propionate, isobutyl alcohol, isobutyric acid, 2-methyl-1-butanol and phenethyl alcohol. Biofumigation of wound-inoculated apples, peaches and lemons with grain culture of *M. albus* for 24 to 72 h provided excellent control of diseases such as blue mold (*Penicillium expansum*), gray mold (*B. cinerea*), brown rot (*M. fructicola*), sour rot (*Geotrichum citri-aurantii*) and green mold (*Penicillium digitatum*). In the case of gray mold and blue mold of apple and green mold of lemon, biofumigation was effective when applied at ambient room temperature 24 h after inoculation. Gray mold was also controlled on cut flowers. Biofumigation was effective in reducing populations of bacterial human pathogens on fresh produce, such as *Salmonella* spp. on melon. Adding grain culture of *M. albus* to an infested potting mix or soil effectively controlled seedling and root diseases such as damping-off and *Phytophthora* root rot, resulting in enhanced plant growth. Dried grain culture of *M. albus* can be readily reactivated by re-hydration and used immediately. [L]

Control of Plant Diseases by Tea Tree Oil

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Tea tree oil is an essential oil steam-distilled from the Australian plant *Melaleuca alternifolia*. Tea tree oil contains over 100 components, mostly monoterpenes, sesquiterpenes and their alcohols. This natural oil is an effective antiseptic, fungicide and bactericide, and has many safe and effective uses in the health and cosmetics industries. Its use against plant pathogens has not been investigated. Recently, together with the Biomor company, we have developed two new formulations, Timor and Timorex, containing 50% and 66% tea tree oil, respectively, effective against a broad spectrum of plant diseases in vegetables, herbs, grapevines and fruit trees, with no phytotoxicity to plant foliage. *In vitro* tests showed that Timor and Timorex at 0.001–0.01% inhibited spore germination and at 0.01–0.1% inhibited mycelial growth of various fungal pathogens of the powdery and downy mildews, and species of *Alternaria*, *Aspergillus*, *Stemphylium* and *Penicillium*. Growth chamber tests revealed that both compounds inhibited powdery mildews in grapevine (*Uncinula necator*) and cucurbits (*Sphaerotheca fuliginea*). In greenhouses and open fields Timor at 1% inhibited mainly powdery mildews in cucurbits, pepper and grapevines. Pre-plant dipping of potato tubers in a 0.5% solution of Timor inhibited *Rhizoctonia solani* infection by 71% and 99% in tubers of organic and conventional growth, respectively, compared with controls. Timorex at 1% controlled powdery mildews in herbs, carrot, mango, apple and nectarine, and was also effective in controlling cucumber downy mildew and potato early blight. Tea tree compounds do not harm natural enemies and can be used to replace sulfur and/or copper treatments. Their mode of action is not clearly understood, but they act as protectants against a wide range of fungi by inhibiting spore germination, mycelial growth and sporulation and by suppression of mildewed tissue. [L]

Antagonist Activity of the Fungi *Meira argovae*, *Meira geulakonigii* and *Acaromyces ingoldii* on Plant Pathogens

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The fungi *Meira argovae* (*Ma*), *M. geulakonigii* (*Mg*) and *Acaromyces ingoldii* (*Ai*) caused mite mortality and showed antagonism to plant pathogens *in vitro*. The fungi inhibited the sporulation of *Fusarium* wilt diseases in garlic, cucumber, tomato, cotton, basil and melon. Colonies of *Fusarium oxysporum* f.sp. *melonis* that were exposed to *Ma*, *Mg* and *Ai* produced only $\sim 5 \times 10^4$ spores ml⁻¹ as compared with 37×10^4 spores ml⁻¹ in the controls. *Fusarium mangiferae* inhibition of sporulation was approximately 60%. The fungi *Ma*, *Mg* and *Ai* decreased the leaf coverage of powdery mildew disease (*Sphaerotheca fusca*) on cucumber cotyledons by ca 83–97%. The secretion of *Ai* inhibited 98–100% of the spore germination of rust fungi in oat, faba bean, poplar, almond and snapdragon. Secretion of the fungi limited spore germination of *Botrytis cinerea*: *Ai* (98%), *Ma* (95%) and *Mg* (50%). Substances that were extracted from the fungi showed antagonism to bacterial species such as *Agrobacterium tumefaciens*, *Clavibacter michiganensis*, *Erwinia amylovora* and *Xanthomonas campestris*. These fungi have great potential for biological control of a wide range of plant pathogens, and for the production of substances antagonist to these pathogens. [L]

Moderation of Virulence of *Alternaria alternata* by Environmental pH

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Alternaria alternata (Fr.) Keissler is the causal agent of black spot disease in persimmon and mango fruits, and of moldy-core disease in apple fruit cv. 'Red Delicious'. *Alternaria* secretes extracellular enzymes as a mechanism for tissue colonization. Cell-wall digestion was used to obtain nutrients for further growth, and colonization of the host. On media containing persimmon cell wall, *A. alternata* secretes mainly endo-1-4- β -glucanase as a function of alkali environmental pH. Based on this principle, acidic solutions were used to moderate black spot development in fruits. When mango fruits cv. 'Tommy Atkins' were treated with acidic solutions the fruits showed reduced disease incidence similar to the situation following commercial chlorine treatment. The present results suggest that treatment with acidic solutions represents a new approach for disease control of *A. alternata*. [L]

Interaction between Fungi Pathogenic to Egyptian and Sunflower Broomrape

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Broomrapes (*Orobancha* spp.) are the most troublesome weeds in Israel, causing severe yield and quality losses. The most important species are Egyptian (*O. aegyptiaca*) and sunflower (*O. cumana*) broomrape. Recently *Fusarium solani* (*Fs*) was isolated in Israel from diseased Egyptian broomrape inflorescences. In spite of its high pathogenicity to Egyptian broomrape, the fungus did not prevent completely the damage caused to the tomato plants by the parasite. The fungus *F. oxysporum* f.sp. *orthoceras* (*Foo*) was isolated from *O. cumana*-diseased inflorescences in Bulgaria and attacks only *O. cumana*. In a field experiment conducted at Neve Ya'ar Research Center the fungus did not prevent the damage caused to sunflower by the parasite. In the present project the efficacy of each of the isolates and their mixtures was tested on sunflower broomrape parasitizing sunflower and on Egyptian broomrape parasitizing tomato.

Sunflower - In greenhouse experiments, inoculation with *Foo* caused severe disease symptoms and up to 90% mortality of sunflower broomrape. *Fs* was less effective and reduced the number of inflorescences emerging above soil level by 60%. Inoculation with a mixture of *Fs* and *Foo* amplified disease symptoms and reduced the dry weight and the number of sunflower broomrape attached to the roots of the sunflower plant as compared with inoculation with *Foo* alone. The synergy factor (SF) calculated on the basis of dry weight or number of sunflower broomrape was 1.5, indicating a high synergistic effect between these two fungi. The same results were obtained in a field experiment.

Tomato - *Fs* demonstrated moderate activity toward Egyptian broomrape parasitizing tomato roots, whereas *Foo* caused no symptoms on the parasite. Their mixture prevented completely the damage *Fs* causes to the parasite. SF of the mixture of these two fungi based on dry weight or number of parasites was 0.4, indicating strong antagonistic effect between these two fungi against Egyptian broomrape parasitizing tomato. [L]

Use of Methyl Jasmonate for Suppression of Botrytis Rot in Various Cultivars of Cut Rose Flowers

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Gray mold on flower petals, caused by *Botrytis cinerea*, is a common disease of greenhouse-grown roses (*Rosa hybrida* L.), which develops rapidly after harvest and causes a significant reduction

in cut flowers value. Methyl jasmonate (MJ), known to induce plant defense responses, was examined for postharvest control of this disease in cut roses. Under laboratory conditions, pulsing of cut roses with 200 μM MJ for 24 h at 20°C provided six rose cultivars with systemic protection against *B. cinerea*. Spray application of MJ (>300 μM) to rose petals provided local protection, possibly by direct inhibition of *B. cinerea* spore germination and germ-tube elongation. Based on these results, a practical application of MJ was developed to meet growers' handling conditions, which included MJ pulsing (4 h at 20°C plus 20 h at 6°C) and spraying. The flowers were then packed and stored for 2 days at 6°C for air transport simulation, transferred subsequently to water cylinders placed at 20°C, and artificially inoculated with *B. cinerea* spore suspension. The optimal treatment under these conditions was pulsing with 350 μM MJ and spraying with 500 μM MJ, which neither increased ethylene production in petals nor was phytotoxic. This MJ treatment effectively suppressed gray mold development following natural and artificial infection in 11 rose cultivars. In yellow, orange and pink cultivars the MJ treatment also inhibited color fading. Collectively, our findings suggest a possible commercial application of MJ as a useful and environmentally friendly means for suppressing Botrytis rot in cut roses. [L]

Melons Grafted onto *Cucurbita* Rootstocks – View to the Future

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The use of grafted vegetables as one of the alternatives to soil disinfestation with methyl bromide is increasing in Israel. Watermelon (*Citrullus lanatus*) and melon (*Cucumis melo*) plants are grafted mainly onto *Cucurbita* rootstocks to lessen losses due to soilborne pathogens. The contribution of the rootstock to the grafted plant's resistance depends on the nature of the disease. In general, damage caused by non-specific root-rot pathogens such as *Rhizoctonia solani*, *Macrophomina phaseolina*, *Monosporascus cannonballus* and *Pythium* spp. are effectively reduced by using *Cucurbita* rootstocks. However, these rootstocks provide only partial protection from vascular diseases such as Fusarium wilt, in which case better protection can be achieved by grafting susceptible melons onto monogenic Fusarium-resistant melon rootstocks. The performance of the grafted plants depends not only on the rootstock but also on the scion response to pathogens and on the effect of the environment on disease development. The response of grafted and non-grafted melons of different cultivars to sudden wilt disease caused by the fungus *M. cannonballus* was evaluated in field trials conducted in the autumn and spring growing seasons. Significant differences in disease incidence were found among cultivars, between grafted and non-grafted plants, and between seasons. Grafting reduced plant mortality in the spring and autumn experiments but prevention of yield losses was more effective in the spring. More emphasis should be given to finding suitable rootstocks and adjusting agrotechniques for successful commercial cultivation of grafted melons in the autumn. [L]

Commercial Applications of *Metschnikowia fructicola* for the Biological Control of Postharvest Diseases of Sweet Potato

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Rhizopus and Fusarium rots, caused by *Rhizopus stolonifer* and *Fusarium* sp., respectively, are regarded as the major cause of postharvest losses in sweet potato, *Ipomoea batatas* (L.) Lam.

The common commercial treatment to prevent the diseases is the application of a fungicide, e.g. iprodione. However, iprodione was recently withdrawn from postharvest use in Israel. Public demand to reduce pesticide use, encouraged by greater sensitivity to environmental and health-related issues, has prompted in recent years research efforts focused on developing biological alternative management methods against postharvest diseases of various fresh commodities. The effectiveness of the recently registered biofungicide 'Shemer', based on a strain of the yeast *Metschnikowia fructicola* in controlling *Rhizopus* and *Fusarium* rots of sweet potato during postharvest storage, was evaluated in commercial packing houses. The biofungicide was applied to the sweet potato roots through a nozzle system fitted on the conveyor belt. In all trials, treatment with Shemer significantly decreased the level of decay in stored sweet potato roots when compared with the non-treated control. Disease incidence in treated roots stored up to 49 days at 12°C, ranged between 1% and 12% compared with 22–95% in the non-treated control. Shemer has been introduced in several packing houses in Israel as a commercial postharvest treatment to prevent the disease during handling and transit procedures. The use of Shemer offers a feasible biological control treatment which: (1) is effective against a wide range of pathogens; (2) is easily implemented in organic, conventional and integrated control systems; and (3) enables a significant reduction in toxic chemicals input. A review was presented of the results obtained to date in the commercial test trials, product application technology, and future outlook. [L]

Extracts of *Inula viscosa* Control Downy Mildew Caused by *Plasmopara viticola* in Grapevines

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Leaves of *Inula viscosa* were collected from the field, dried, and extracted with a mixture of acetone and n-hexane. The oily, water-insoluble paste obtained after evaporation of the solvents, was dissolved in acetone and used for the control of downy mildew in detached grape leaves, or emulsified and used for control of downy mildew in grapevines in the field. The pastes, derived from leaves collected at weekly intervals during April–August 2003 and dissolved in acetone, exhibited high efficacy in controlling *Plasmopara viticola* (downy mildew) in detached leaves of grapes in growth chambers. ED₅₀ values for the seven extracts ranged from 95.4 to 127.7 µg ml⁻¹ and ED₉₀ values ranged from 1257.8 to 1837.0 µg ml⁻¹, indicating no obvious effect of the harvest time of *I. viscosa* on activity against the pathogen. Field trials with selected shoots of grapevines were conducted at Bar-Ilan Farm in 2003. Results showed that a 34.4% emulsified concentrate of the extract provided excellent activity against the disease in the field. Leaves of the selected shoots were effectively protected against downy mildew up to 25 days after treatment and inoculation. Good efficacy of the formulated paste in controlling downy mildew lasted 2 weeks. The product also possessed some translaminar activity against downy mildew in grape leaves under field conditions. In two trials with whole grapevines conducted in 2002 and 2003 at Keramim, almost complete control of downy mildew was achieved with 0.5% of the extract. It appears that *I. viscosa* may be used as a herbal source for fungicidal preparations against downy mildew in grapes. [L]

New Applications of 'BioNem' and 'BioSafe' for Biological Control of Nematodes: Prospects and Opportunities

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BioNem and BioSafe, two biological nematicides based on a nematode-parasitic isolate of the bacterium *Bacillus firmus*, are registered as microbial nematicides in Israel for the control of root-knot nematodes (RKN) on cucumber and tomato crops. In the last year, the combined use of BioNem with soil solarization in IPM systems and a water-dispersible formulation of the product for the application through drip line irrigation equipment were evaluated in field trials. Soil solarization is an effective soil disinfestation method for controlling soilborne pathogens and weeds. However, plant parasitic nematodes have generally proved to be very difficult to control with soil solarization. Improved use of solarization for nematode control may therefore be achieved by coupling solarization with biological control agents. **BioNem combined with soil solarization:** Laboratory trials showed that application of BioNem (at the reduced dosage of 33% of the recommended rate) in combination with soil solarization resulted in improved control of *Meloidogyne* sp. nematodes, when compared with either treatment alone. In several field trials the combined treatment led to improved control of nematode damage also at critical times in the growing season. Thus, improved nematode control was achieved along with a reduction in the application rates of the bionematicide.

Water-dispersible formulation of BioNem for drip line application: Application of the new water-dispersible formulation of BioNem to a cucumber crop grown on tuff substrate heavily infested by root-knot nematodes (RKN), reduced the soil nematode population by 73% (3 months after application). In the treated plots, only 19% of the plants had a root system severely damaged by the RKN (galling index: 4–5, on a scale of 0–5) in comparison with 88% of the plants grown in the non-treated control. In additional trials, the application of the water-dispersible formulation of BioNem during the growing season led to a significant reduction in the nematode population and root damages on a tomato crop growing in sandy soil. [L]

Signum - A Broad-Spectrum Fungicide Containing the New Active Ingredients Boscalid and Pyraclostrobin

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Signum is a fungicide produced by BASF containing the new active ingredients boscalid 26.7% and pyraclostrobin 6.7% in a wettable granules formulation. Boscalid is an analide compound that inhibits the enzyme succinate ubiquinone reductase (Complex II) in the respiratory electron transport chain in the inner membrane of the mitochondria. Boscalid is a systemic compound that is rapidly absorbed by the sprayed plant tissues. Pyraclostrobin is a semi-systemic strobilurin compound that inhibits respiration by inhibiting electron transport in Complex III. Signum inhibits fungal growth by cutting off the source of energy and by eliminating the availability of chemical building blocks necessary for the synthesis of essential cellular compounds. Both active ingredients suppress fungal spore germination, inhibit germ tube growth and appressorium formation (preventing infection), as well as inhibiting mycelial growth and spore formation (curative effect). Signum has been classified as a reduced risk pesticide by the U.S. Environmental Protection Agency. The product has good residual activity and is active against a broad spectrum of pathogenic fungi. Extensive field-testing in Israel has shown effective control of *Alternaria* spp., *Botrytis* spp., powdery mildews, downy mildews, *Cladosporium* spp., *Cercospora* spp., *Rhizopus* spp., *Ascochyta*, *Aspergillus* spp. and *Sclerotinia* spp. Recently Signum received approval for registration in Israel for the control of the following diseases: *Alternaria dauci* in carrots and *A. macrospora* in cotton at 0.4 kg ha⁻¹, *Leveillula taurica* in eggplant and pepper and *L. taurica* and *Cladosporium fulvum* in tomato at 0.75 kg ha⁻¹. Registration is expected shortly for control of *Alternaria solani* in potatoes, *A. citri* in citrus, *Didymella rabiei* in chickpeas, *Sphaerotheca fuliginea* and *Pseudoperonospora* spp. in cucurbits, and *Plasmopara viticola*, *Aspergillus* spp. and *Rhizopus* spp. in grape. [L]

Calirus™ - A Safe Fungicide for Controlling Root and Foliar Diseases

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CalirusTM is a new, patented product for controlling root and foliar diseases in a wide range of crops. It combines two components, metal ions (zinc and copper) and phosphite (PO₃), which act together in synergy. The product can be applied by drenching, spraying or *via* the irrigation system and its mode of action includes a direct inhibition of pathogens as well as induced resistance in plants. In efficacy trials, CalirusTM has been proven effective in controlling root and foliar diseases caused by *Pythium aphanidermatum* and *F. ultimum* (in vegetables), *P. violae* (in carrot), *Phytophthora infestans* (in potato and tomato), *P. citrophthora* (in citrus seedlings), *Plasmopara viticola* (in grape), *Bremia lactucae* (in lettuce), *Pseudoperonospora cubensis* (in cucurbits), *Alternaria* sp. (in citrus trees) and *Plasmodiophora brassicae* (in crucifers). The product also contributed to the partial control of diseases caused by *Rhizoctonia solani* (in cucumber), *Botrytis cinerea* (in strawberries), *Uncinula necator* (in grape), and others. In addition, CalirusTM exhibited growth-stimulating activity. For example, it substantially increased the number of rootlets of citrus seedlings, as well as the fresh weight, stem diameter and number of flowers of pepper plants. CalirusTM is nontoxic, nonpersistent and environmentally friendly. It is presently under registration procedures in the U.S.A. (EPA) and in Israel. In Israel it is being registered initially for limited use in soil-less cultivated crops and to control diseases caused by soilborne pathogens. It is predicted that marketing in Israel will commence in the second half of 2004. [L]

Detoxification of Mycotoxins by Probiotic Bacteria

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Mycotoxins are secondary metabolites produced by molds that occur mostly in agricultural produce and processed foods. The mycotoxins are highly toxic to humans and animals and most of them exhibit, *inter alia*, acute, sub-acute, chronic, carcinogenic and teratogenic properties. The health risk caused by mycotoxins, their stability during food processing and other stages, along with the potential to use them in bio-terrorism, have placed mycotoxins at the very top of public health concerns. In the last two decades attempts have been made to improve the population's health status by modulating the indigenous intestinal flora by live microbial adjuncts, designated probiotics. The overall aim of our research is to develop a protective system against mycotoxin intoxication, by using probiotics capable of degrading mycotoxins that will act in the small intestine. We have focused our research on two widespread mycotoxins: patulin, which is found mainly in apples and pears and their products, and the trichothecene T-2, which appears mainly in cereals. A strain of the bacterium *Lactobacillus plantarum* capable of degrading patulin was isolated by us from a fermented sausage. Patulin degradation was found to be pH- and temperature-dependent with an optimal degradation rate at pH 7.0 and 37°C. HPLC analyses revealed a degradation product that elutes (as indicated by the retention time) prior to the intact patulin. The toxicity of the degradation product, as determined by a bioassay with *Escherichia coli*, was two orders of magnitude less than that of patulin. The gene *Tri101* encoding to acetyltransferase, known to inactivate T-2, was isolated from *Fusarium graminearum* and cloned in a lactic acid bacteria expression vector. Gene expression in bacteria was monitored by SDS-PAGE. A bioassay based on sensitive yeast cells revealed a decrease in toxicity upon incubation of T-2 with lactic bacteria expressing *Tri101*. Use of genetically modified probiotics may lead to a novel means of mycotoxin detoxification. [L]