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Opening Lecture

Genomic Sequencing of Fungi: On the Path to Understanding the Biology of Phytopathogens

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The fungal kingdom includes known pathogens, beneficial organisms, producers of secondary metabolites and model eukaryotic microorganisms. Obtaining the full genomic sequence of fungi establishes a comprehensive genetic database of the relevant organism. Web sites with full genomic sequences of several filamentous fungi, including *Magnaporthe grisea, Aspergillus nidulans, Coprinus cinereus* and *Fusarium graminearum,* are available. However, to date, the only full genomic sequence of a filamentous fungus published is that of *Neurospora crassa.* On the basis of available information, the genome size of filamentous fungi ranges from 30 to 40 Mbp and is comprised of approximately 10,000 genes. Based on the analysis of the full genome, filamentous fungi appear to have multiple environmental sensing modules. Evidence for the presence of genes encoding pathogenicity factors was also present in the saprophyte's genome. About 40% of the putative genes did not appear to have known structural homologues, a fact which stresses the need to advance the functional analysis of a large number of genes. Availability of the full genome sequences supports comprehensive gene expression experiments and comparative genomic sequence analyses. The information can be harnessed for development of diagnostic tools, elucidation of evolutionary and metabolic processes and identification of pathogenicity factors and potential antifungal targets. Genomic databases combined with genetic manipulations can be utilized to enhance the activity of beneficial filamentous fungi. *[L]*

A: CHARACTERIZATION OF PATHOGENS

Detection of *Clavibacter michiganensis* subsp. *michiganensis* in Tomato Seeds, Threshold Level and Disease Potential

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

Clavibacter michiganensis subsp. *michiganensis* (*Cmm*) is the causal agent of bacterial canker and bacterial wilt of tomato. It is considered to be the most important bacterial disease of tomato, causing substantial economic losses worldwide. *Cmm* is a quarantine organism under the European Union Plant Health legislation and in Israel. Infected seeds, seedlings and soil are the major sources for outbreaks of *Cmm* infection. There are no available commercial resistant lines; therefore the most effective way for disease control is by indexing tomato seeds for the presence of the pathogen and maintenance of appropriate sanitation conditions. The objectives of the present study were to evaluate several seed extraction procedures used for detection of *Cmm* in seed lots, determine the detection threshold of the pathogen in relation to seed sample size and disease incidence. Extraction methods that included grinding of the seeds were significantly better at detecting the pathogen in three different lots than methods that use only soaking. The detection threshold of *Cmm* in relation to seed sample size was determined by adding naturally infected seeds into samples of three different sizes (2,000, 5,000 and 10,000). *Cmm* was detected by agar plating assay on three media (CNS, mSCM, D_2ANX), and by direct and bio-PCR. In samples of 10,000 seeds containing one infected seed, *Cmm* could be detected only by bio-PCR and in only one replicate out of five. In samples containing five or ten infected seeds per 10,000 seeds, three of five and five of five replicates were detected, respectively. In samples of 5,000 seeds one infected seed could be detected in all five replicates only after adding a concentration step. A high correlation (R^2=0.7678) was found between artificially infected seeds and the disease rate. Seeds infected with fewer than 58 CFU g^{-1} did not cause disease, whereas lots with approximately 1,000 CFU g^{-1} caused disease in 78 plants out of 2,000. The results imply that the sensitivity of the current methods for detecting *Cmm* in seed lots permits a maximum sample size of 5,000 seeds. *[L]*

Use of *Botrytis cinerea* Marked Strains in Ecological and Population Studies

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Results of numerous works demonstrate that *Botrytis cinerea* is highly heterogeneous, and *Botrytis* inoculum can originate from many different sources with continuous gene flow between fields or greenhouses. We explored selenate, the toxic analog of sulfate, in order to obtain genetically marked strains suitable for ecological and population studies. The objectives of the current work were to (i) characterize the *B. cinerea* wild-type isolates for resistance/sensitivity to benzimidazole and dicarboximide fungicides; (ii) recover and characterize selenate-resistant sulfate nonutilizing (*sul*) mutants from *B. cinerea* strains; (iii) estimate the usefulness of strains marked with resistance to selenate and fungicides in ecological and epidemiological studies (focusing on survival and distribution of inoculum); and (iv) determine the VCG diversity in the Israeli population of *B. cinerea* using complementation between different *sul* mutants. Marked *B. cinerea* strains combined traits of fungicide resistance or sensitivity with resistance to selenate. In greenhouse experiments, more than 90% of plants showed *B. cinerea* infection, but only 10% to 30% plants were infected by the marked strain used as the source of inoculum. This result shows the importance of external inoculum in the epidemiology of gray mold. Mycelium of *B. cinerea* inside plant tissues remained viable for 90 to 120 days under field summer conditions, whereas conidia of *B. cinerea* remained viable up to 2 months under the same conditions. Complementary chromate-resistant and chromate-sensitive *sul* mutants were recovered and used for studying vegetative compatibility in *B. cinerea*. All tested *B. cinerea* strains were vegetatively self-compatible. Inter-strain vegetative incompatibility was common among *B. cinerea* strains. 'Bridging' strains were rather common among *B. cinerea* strains. The *B. cinerea* population in Israel consists of a network that is obtained by the 'bridging' strains. *[L]*

Subspecific Populations of *Colletotrichum coccodes*, the Causal Agent of Potato Black Dot: Molecular Characterization, VCG and Pathogenicity

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Potato black dot disease, caused by *Colletotrichum coccodes*, damages tuber quality and may reduce yield. The fungus, classified to the Deuteromycotina, lacks a known sexual cycle, where vegetative compatibility may serve as a means of genetic exchange among isolates. In a previous study, four multimember vegetative compatibility groups (VCGs) were characterized using nitrate auxotrophic (Nit) mutants. Our goals in this current study were to: (i) characterize a large population of isolates through VCGs and DNA fingerprinting and (ii) assess the degree of correlation between VCG assignment and pathogenicity to potato and the effect of temperature on radial growth rate. In the present study, 174 isolates originating from Israel and Europe were collected. Seventy-three isolates were assigned to the four previously reported groups (4.0%, 16.6%, 18.4% and 2.9% of all isolates) and 76 were assigned to four newly defined VCGs (4.0%, 5.2%, 27.6% and 8.6% of all isolates). Twenty-five isolates (14.4% of all isolates) could not be assigned to any of the major groups. Of the 38 isolates originating from Scotland, 34 were assigned to VCG7; this may indicate a possible correlation between VCG and geographical source in the *C. coccodes* population. The radial growth rate of 21 isolates representing the eight VCGs was determined at four temperatures (21°, 25°, 29° and 33°C); there was an indication of differences among several of the VCGs. Molecular characterization of 12 *C. coccodes* isolates representing four of the VCGs was conducted by RAPD PCR using five different primers; no difference was found in the molecular profile of these isolates. Aggressiveness of the isolates to potato is currently being examined in experiments on potted plantlets and tissue cultured plantlets. Potential correlations among VCGs and aggressiveness to potato, along with a primer able to differentiate among the VCGs, may enable the development of a reliable test to assess potential damage by *C. coccodes*. *[L]*

Impaired Purine Biosynthesis Affects Pathogenicity of *Fusarium oxysporum* f.sp. *melonis*

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The vascular wilt pathogen *Fusarium oxysporum* f.sp. *melonis* causes worldwide yield losses of muskmelon. In this study we characterized a UV-induced non-pathogenic mutant (strain 4/4) of *F. oxysporum* f.sp. *melonis*, previously identified as a potential biological control agent. During comparative analysis of vegetative growth parameters using different carbon sources, strain 4/4 showed a delay in development and secretion of extracellular enzymes, compared to the wild type strain. Amendments of the growth medium with yeast extract, adenine or hypoxanthine, but not guanine, complemented the growth defect of strain 4/4, as well as secretion and partial activity of cellulases and endopolygalacturonases, indicating that the strain is an adenine auxotroph. Incubation

of strain 4/4 conidia in adenine solution, prior to inoculation of muskmelon plants, partially restored pathogenicity to the mutant strain. As part of the characterization of pathogenicity factors of Fusarium wilt, a collection of approx. 2000 *Agrobacterium*-transformed mutants was generated and screened for pathogenicity on melon plants. At this stage, five putative impaired pathogenicity mutants are being characterized. *[P]*

B: PHYTOPATHOLOGICAL ASPECTS OF WATER RECYCLING SYSTEMS AND OF IRRIGATION WITH RECYCLED WATER

Adaptation of a Disinfection System, Based on Chlorination, to Eliminate Contamination of Greenhouse Drainwater with Soilborne Viruses

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The acute shortage in water supply for agriculture, the prohibition on the use of methyl bromide and the awareness of the need to prevent environmental pollution, encouraged the expansion of soilless greenhouse cultures and the recycling of the drainwater that is generated in these systems. Greenhouse cucumbers grown in Israel are affected by two soilborne viral pathogens. Cucumber fruit mottle mosaic virus (CFMMV) and *Cucumber leaf spot virus* (CLSV), which are known for their high survival in soil and water and therefore the re-circulation of drainwater may play a role in their spread in soilless media. The installation of a disinfection unit into the recycling system may help to prevent this process. This work was aimed at studying some parameters affecting the epidemiology of soilborne viruses in soilless cultures and at calibrating a chlorination system that is directed to prevent the accumulation of viral inoculum in the drainwater and the growth medium. An inoculum threshold of 10 μg ml^{-1} is required to produce CFMMV infection through the root system of cucumbers, as was determined in plastic pots using a purified preparation of CFMMV. The adsorption of purified CFMMV to soil, perlite and tuff was measured in columns, showing that perlite has the highest specific adsorption capacity while lower values were calculated for soil and tuff, respectively. The specific adsorption capacity of CFMMV in the three tested media was much higher than the inoculum threshold found in our lab experiments. Purified CFMMV inoculum survived in the tested media under greenhouse conditions for at least 4 months. Analysis of drainwater collected from a commercial greenhouse indicated the presence of infectious CFMMV and CLSV inocula, despite the application of chlorine into the circulated water. Lab tests showed that efficient disinfection of drainwater contaminated with CFMMV or CLSV at a concentration of 1 μg ml^{-1} is obtained by chlorination with 4 ppm hypoclorite for 4 h. The efficiency of chlorination in drainwater decreases dramatically in the presence of fertilizer ions. *[L]*

Relationship between Salinity and Disease Expression in Closed Soilless Growing Systems of Chive (*Allium schoenoprasum*)

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Chive (*Allium schoenoprasum* L.) plants were grown in four hanging, closed soilless growing systems at three levels of salinity: EC=1.0, 2.5 and 4.0; the fourth system involved irrigation with the

EC=2.5 solutions that were treated by slow sand filtration system (biological column). The filtration rate was 100 l^{-1} for a filter surface of 1 m^2. The main source of water for this experiment was pretreated water through a reverse osmosis membrane. At the beginning of the experiment, in each growing system two pre-inoculation plants were planted as a primary source of inoculum. The disease of chive root atrophy caused by *Fusarium* spp. was chosen for this experiment. During the season, we followed up the population of the pathogen in the irrigation solution, and the yield – which reflected the pressure of the salinity on the plants with or without the presence of the pathogen. The main results revealed that the optimum growth of chive plants was achieved under EC=2.5. Chive plants under EC=4 showed significant yield reduction. All of the pre-inoculation plants developed disease symptoms and their yield was low. During the growth season the pathogen was present in the irrigation water of all the systems except for that of the biological column. By comparing the two systems of EC=2.5 (with or without the biological column), the effect of the column on the yield was highly significant. It is concluded that two parameters have a strong effect on the chive yield: salinity and disease. *(L)*

Application of Treated Wastewater for Cultivation of Roses (*Rosa hybrida*) in Soilless Culture

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Scarcity of fresh water in most of the Mediterranean Basin countries makes treated urban waste water an unavoidable alternative water source for irrigation. In the present project we investigated the effect of irrigation with secondary treated waste water in a closed greenhouse under soilless cultivation, on rose cut-flower production and quality, and on the fate of microorganisms and fecal pollution indicators originating from the waste water, in the greenhouse environment and the cut-flower. In an experiment conducted at the Lakhish Experimental Farm rose plants grown in mineral (perlite) or organic (coconut fibers) soilless medium were irrigated with potable or secondary treated urban waste water, chlorinated according to the regulations of the Ministry of Health in Israel to the level of 0.5 ppm chlorine. During 36 months of exposure to the treated water, the visible appearance of the plants, cut-flower yield and postharvest performance were not affected by the irrigation treatments. Prior to chlorination, the treated water contained ~2 CFU per ml *Escherichia coli*. Chlorination reduced contents of *E. coli* in the irrigation water to levels below 0.2 CFU per ml. No *E. coli* were found in the greenhouse air, the soilless media, the plant tissue, or in the vase water of the cut-flower during vase life. The drainage solution of perlite-grown plants irrigated with the treated waste water contained higher total bacterial counts than plants irrigated with potable waters, but lower levels of fungi and yeasts. Total coliforms and bacterial counts in the soilless media were higher under irrigation with the treated waste water in comparison with potable water, and in the plant tissues fungi and yeast counts were higher under irrigation with the treated waste water. The application of chlorinated secondary treated domestic waste water under our experimental setup limited the survival of the microorganisms originating from the treated waste water, thus preventing health and environmental risk. *(L)*

Cucumber Root and Stem Rot Disease Caused by *Fusarium oxysporum* f.sp. *radicis-cucumerinum* and Its Prevention in Recirculating Nutrient Solutions

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In closed soilless growing systems there is a risk of spreading root or stem pathogens throughout the greenhouse. Treatment of the irrigation nutrient solution is therefore important. The aim of this experiment was to investigate the spread of the pathogen *Fusarium oxysporum* f.sp. *radicis-cucumerinum* from four sources of inocula: planting of a few diseased seedlings, naturally infested plant debris, *Fusarium* spores in the drainage water and airborne spores during the growing season. The experiments were carried out at two locations: (i) an isolated greenhouse at En Gedi located in the Dead Sea region of Israel; and (ii) the R&D Lakhish Experiment Farm in the central region of Israel. The experiment at En Gedi included six nutrient solution treatments, and solar heat pasteurization, UV irradiation, and two biofilter systems (slow filtration). Cucumber var. 'Socrates', regular seedlings and seedlings grafted on pumpkin rootstock were grown under recirculating drainage water on two growth media, namely, volcanic ash supplemented with compost or perlite crumbs. At Lakhish, the plants were grown on perlite crumbs and the experiments included three systems of water treatments: biofilter, chlorination and electrodes. The most efficient system for spreading the pathogen was on common seedlings grown on perlite without any water treatment, the source of the pathogen being spores in the drainage water. Under these conditions 98% of the plants became diseased and ~85% were dead within 70 days after planting. Approximately 60% of the grafted plants were not affected by the disease; the others were infected through adventitious roots of the scion. The presence of organic material in the growth media significantly decreased the severity of the epidemic. Solar heat pasteurization, UV and biofilter with high organic amendment were efficient in controlling the spread of the pathogen, whereas in the untreated controls more than 90% of the plants were destroyed. In the Lakhish experiment, the slow sand filtration prevented the spread of the pathogen. Treating the water with chlorine or electrodes resulted in toxic effects to the plants and in insufficient control of the pathogen. *(L)*

C: RESISTANCE

Screening of Grape Rootstocks for Resistance to the Nematode *Xiphinema index*

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Replanting of vineyards in nematode-infested soil often results in considerable economic damage to the newly planted grapes. The means for reducing nematode populations are limited; therefore, using nematode-resistant or -tolerant rootstocks is one of the preferred means of control. The ecto-parasitic nematode *Xiphinema index* feeds mostly on root-tips and causes damage; it can be found in all regions where grapes are grown. This nematode attacks mainly trees and bushes and has a life cycle of 4–6 months. It is also a vector of the grapevine fanleaf nepovirus. Nine grapevine rootstocks (Cabernet Sauvignon grafted), some with known resistance to root-knot nematodes, were screened for resistance to *X. index*. Experiments were conducted with naturally infested soil (20 nematodes per 100 g soil) in 10-*l* containers in a greenhouse at 26±2°C with a 14-h photophase; there were nine replicates for each rootstock. Nematode juveniles and females were extracted and counted from soil samples 8, 12 and 15 months after planting. Nematode reproduction rates represented the resistance or susceptibility level of the rootstocks. 'Salt Creek' (Ramsey), 'Paulsen 1103', '101-14 Mgt' and 'Ruggeri' were susceptible; 'Richter 110' and 'Harmony' rootstocks expressed some resistance; 'Freedom' and '161-49c' were resistant; and 'VR039-16' was highly resistant. *[L]*

Induced Resistance in Plants Treated with Solarized Soil or *Trichoderma*

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Both soil solarization and *Trichoderma* reduce the incidence of many diseases due to their direct detrimental effect on pathogens. In the present work, we studied induced systemic resistance following solarization and/or *Trichoderma* treatments. In our system, only the roots were in contact with the solarized soil or *Trichoderma* preparation, while the foliage was inoculated with pathogens. Strawberry, cucumber and common bean grown on treated soil or coconut growth substrate showed significant reduction in disease after leaf inoculation with *Botrytis cinerea* or with *Sphaerotheca fuliginea* (cucumber only), thereby indicating induced resistance. For example, the percentage disease coverage on foliage of cucumber caused by *S. fuliginea* was 25.0, 12.8, 11.4 and 12.7 in nontreated, solarization, *Trichoderma* and combined treatments, respectively. Attempts are being made to find the relationship between indigenous populations of microorganisms in the rhizosphere and disease control. For this purpose we use a molecular approach based on 16s-rDNA and denaturating gradient gel electrophoresis (DGGE). The above-mentioned treatments resulted in changes in the DGGE patterns of soil and substrate populations. Induced resistance by soil solarization corresponds with previous studies demonstrating physiological changes in foliage of plants growing in solarized soil, and with higher populations of fluorescent pseudomonads (known as resistance inducers) in the rhizosphere of the plants in solarized soils. *[P]*

D: VIROLOGY

Invited Lecture

Natural Resistance Mechanisms to Viruses in Plants

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During evolution most plants became resistant to most pathogens. We distinguish between **Active resistance**, where R genes and signal transduction are involved; and **Passive resistance,** for example lack of components needed for viral replication or translocation.

The following resistance mechanisms were considered:

1. Gene silencing. The majority of the viruses cannot survive in plants because of post-transcriptional gene silencing (PSTG) and viral RNA degradation.
2. Inability to move through the plasmodesmata.
3. The necrotic local lesion and caspase-like proteases. Caspase inhibitors induce systemic movement of TMV; Salicylic acid (SA). Treatment with SA reduces the spread of TMV.
4. The N gene. Codes for a 131 kDa protein, belongs to the TIR-NBS-LRR of the R genes. The N protein starts a signal transduction cascade, where more proteins are produced that inhibit virus replication.
5. Induced resistance: Is a gene silencing mechanism involved, whereby siRNA suppresses viral RNA ahead of the infection?

6. 'Green Islands' and possible involvement of PSTG.
7. Inhibitors of viral replication from resistant tissues: from tobacco NN (IVR), from induced resistant tissue and from 'Green Islands' tissue.
8. The IVR protein has tetratricopeptide repeat (TPR) domains and leucine-rich repeats (LRR) indicative of protein-protein interactions. TPR motifs are present in interferons.
9. The IVR gene was cloned (clone NC330) and used for transforming susceptible tobacco. Plants with varying degrees of resistance were obtained. Some of the TMV-resistant plants were also resistant to *Botrytis cinerea. [L]*

Cucumber leaf spot virus (CLSV), a New Soilborne Virus Disease of Greenhouse Cucumbers in Israel

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Cucumber plants grown in greenhouses showed mosaic-type symptoms and vein banding on leaves; plant growth was not significantly affected and fruits remained symptomless. A virus with isometric particles 30 nm in size was isolated from infected tissues. Similar infectious particles were found in drainage water in the greenhouse. The infecting virus could not be identified on the basis of host range and symptoms' patterns. In order to identify the virus pathogen, cDNA clones were prepared from purified virus particles and their nucleotide sequences were compared with a library of viral sequences in the GenBank. The tested sequence was shown to have a high degree of identity (but not complete) with a *Cucumber leaf spot virus* (CLSV) sequence (95%). It has lower identity (66%) with a *Pothos latent virus* (PoLV)-type member of the same Aureusvirus genus and 48% with *Cucumber necrosis virus* (CNV) belonging to the Tombusviridae family. The infecting virus was thus identified as a variant of CLSV. The nucleotide sequences of the individual ORFs within the virus genome were analyzed. The variability between the CLSV isolates was higher at regions encoding the virus replicase and CP genes while the 3'-end region was more conserved. It may be hypothesized that the observed differences in host range and symptomatology of the new CLSV isolate originated from the variability at the CP and Rep genes. *[L]*

The *Tomato yellow leaf curl virus*-resistant Tomato Line TY-172, Inhibits Virus Replication but not Virus Translocation

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TY-172, a resistant tomato host of *Tomato yellow leaf curl virus* (TYLCV), is known to inhibit normal viral infection by remaining symptomless after infection. The mechanism by which this happens was addressed by inoculation of selected leaves of TY-172 and a susceptible tomato plant with TYLCV using whiteflies and clip cages, and comparing the amounts of new viral DNA produced in resistant and susceptible plants at the inoculation site over time. At each time point, the amount of new viral DNA in TY-172 was much lower than that in the susceptible host. This reduction was due to the reduction in ssDNA, since the amount of viral dsDNA was the same in both resistant and susceptible hosts. This suggests that TY-172 interferes with TYLCV replication, and that the inhibition is at the second stage of the replication cycle where dsDNA is formed. In order to determine whether the resistance of TY-172 affects viral long-distance movement, the appearance of viral DNA

at the plant apex was monitored. Viral DNA appeared at the plant apex at the same time, 48 h after inoculation, in both the susceptible and resistant plants. The viral DNA accumulation level at the plant apex was the same in both hosts until 96 h after inoculation, after which time a greater amount of viral DNA was found in the susceptible host. These results suggest that TY-172 interferes with the production of ssDNA in the viral replication cycle but not with the long distance movement of viral DNA. *[L]*

E: BIOLOGICAL CONTROL

Epidemiological Aspects of Biocontrol of Dodder by *Colletotrichum*

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Some epidemiological aspects of biocontrol of dodder by *Colletotrichum* were studied. Three factors are reported: (i) number of inoculations (one *vs* two); (ii) number of moisture periods after inoculation (one *vs* several); and (iii) size of dodder infestation. The host plant for studying the first two factors was *Dichondra*, grown in flats. The dodder was inoculated by spraying with spore suspensions. Disease incidence was determined by casting a net of squares over the flats and counting the squares with disease. Subsequently, disease severity was recorded for every square. The average number of infections/square was derived from disease incidence using the logit transformation. The Area Under Disease Incidence Progress Curve (AUDIPC) was calculated. Disease incidence and severity did not differ significantly between treatments. It seems, therefore, that a single inoculation may be sufficient for establishing an epidemic, and biocontrol may be possible even in regions where the wetness is limited. Another factor tested was the size of dodder infestation. This was studied in the field on dodder parasitizing ornamental *Senecio*. The dodder was inoculated with spore suspension in the evening and covered with plastic bags for the night. Disease development in small dodder infestations was slow. Sixteen days after inoculations only 10% of the dodder was diseased. In medium-sized dodder infestations 67% of the dodder was infected, while in the large-sized dodder infestations 98% of the dodder was totally destroyed. The differences between small-sized and large-sized infestations were significant. It was unexpected that it would be more difficult to control small-sized dodder infestations. This may be explained by the relatively larger green host area around the infestation, into which dodder may escape from the infection by *Colletotrichum*. On the other hand, in the large-sized dodder infestation there is not much green tissue of *Senecio*, into which the dodder could escape. *[L]*

Inhibition of Phytopathogens by the Fungi *Acaromyces ingoldii, Meira argovae* and *Meira geulakonigii*

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The fungi *Acaromyces ingoldii (Ai), Meira argovae (Ma)* and *Meira geulakonigii (Mg)* secrete secondary chemicals (SC) that inhibit plant pathogens. This antagonistic activity was assayed against several phytopathogenic fungi. The effect on the pathogens was tested in petri dishes with potato-dextrose agar containing the SC that had been secreted by the three antagonistic fungi. The germination of the conidia of *Penicillium digitatum* was completely inhibited (100%) in dishes that contained the SC of *Ai, Ma* and *Mg*. The mycelial growth of *P. digitatum* with the SC of *Ai* was

completely inhibited, whereas with the SC of *Ma* and *Mg* mycelial growth was slow and sparse, in comparison with the control. The SC of *Ai* completely inhibited the sclerotia germination of *Sclerotinia sclerotiorum* and of *Sclerotium rolfsii.* In the treatments with the *Ma* and *Mg* secretions the germination time of the sclerotia was prolonged and the mycelia were very sparse. The former phytopathogen produced no sclerotia and only few were observed in the *S. rolfsii* cultures. Partial inhibition of germination and sparse mycelial development of other phytopathogens, namely, *Alternaria solani, Fusarium mangiferae* and *Colletotrichum gloeosporioides,* were observed in plates separately containing the SC of the three fungi. Oranges inoculated with conidia of *P. digitatum* one week after being inoculated with the fungus *Mg,* showed only half the disease rate of the control oranges. The composition of the secreted SC and their mode of action are still unknown. The uses of these antagonistic fungi in biological control have the potential to reduce the damage of several fungal pathogens in different crops. *[L]*

Vectoring a Biocontrol Agent by Honeybees to Strawberry Flowers Results in Biocontrol of Fruit Gray Mold

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A previous study revealed that biocontrol of strawberry fruit gray mold (*Botrytis cinerea*) by spraying the *Trichoderma* preparation is effective only when very frequent applications are given or a high dose is used (S. Freeman *et al.* (2004) *Eur. J. Plant Prot.* 110:361-370]. The ability of bees to carry powder from the beehive to flowers can be harnessed for biological control purposes, by using them to transfer inoculum of fungi and bacteria from the hive to flowers. The present study involved the biocontrol preparation of *Trichoderma harzianum* T39 and honeybees for treating flowers of strawberry plants against *B. cinerea* infection and development of flower and fruit gray mold. A newly developed 'Triwaks' dispenser was fit to the hives and found effective in loading honey bees with *T. harzianum.* Bees leaving hives equipped with the Triwaks dispenser were loaded with up to $1.45*10^5$ CFU *T. harzianum* T39 per bee [A. Bilu *et al.* (2004) *Biocontrol Sci. Technol.* 14:607-617]. During two successive seasons, ten beehives were placed at the edge of a commercial strawberry field and the biocontrol powder was added to their dispensers on rainless days from mid-December until mid-March. Experimental plots were located 25–50 m from the hives for disease evaluation in treatments that consisted of *Trichoderma* delivered by bees, chemical botryticide sprays, and their combination. *Trichoderma* population on flowers was evaluated in plots placed up to 200 m from the hives. Visiting of bees in control plots was prevented by nets spread on control and chemical treatments. The nets proved not to alter the humidity in the canopy level as compared with uncovered plots where bees were allowed to visit. The *Trichoderma* level on flowers was $>10^4$ CFU/flower up to 100 m from the hives. This level was found in earlier experiments necessary for the control of strawberry gray mold. Thus, bees proved to be a potential means for the delivery of the biocontrol fungus to the infection site of *B. cinerea.* During the period of gray mold development, bee-delivered *T. harzianum* T39 was found to be similar to or better than season-long sprays with chemicals (fludioxonil+cyprodinil and pyrimethanil). It is concluded that the delivery of *Trichoderma* by bees to strawberry flowers is effective in strawberry fruit gray mold control and can be integrated in an IPM scheme for this crop. However, a suitable formulation needs to be developed for this purpose. *[L]*

'BioNem WD', a Unique Bionematicide for Perennial Crops

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Many perennial crops, including flowers, deciduous orchards, bananas and vineyards are damaged severely by nematodes. Volatile nematicides are not suitable for application during the growing season, whereas use of non-volatile nematicides necessitates numerous applications. Such a control regime results in environmental damage, resistance development and biodegradation leading to decreased efficacy. 'BioNem WD' is a bionematicide based on the naturally occurring bacterium *Bacillus firmus*. The preparation is applied through the irrigation system or by pre-plant spraying. BioNem WD is registered in Israel for cucumbers and tomatoes and was found effective in various other crops. Pre-plant as well as mid-season application of BioNem WD decreased root-knot nematode populations in the soil. In perennial crops the control of nematodes in the deeper layers of the soil is crucial. Sprinkler application of BioNem WD caused a 62% and 76% reduction of the root-knot nematode population at depths of 10–30 cm and 30–50 cm, respectively. Drip irrigation of BioNem WD in the winter in a peach orchard reduced 65% of the root-knot nematode population which remained at a low level until the following autumn. The effect of a spring application was slightly lower. In another peach orchard trial, BioNem WD was compared to a chemical standard (Rugby Super). BioNem WD reduced 65% of the root-knot nematode population and kept it at this level for 8 months until the following autumn. Rugby Super also decreased the population but the effect lasted for 3 months only. BioNem WD caused a significant increase (17%) in the peach yield compared with untreated and Rugby Super-treated plots. BioNem WD decreased the root-knot nematode population also in vineyard soil and in yellow pitaya. These findings prove that the preparation is a safe and efficient measure for nematode control of annual and perennial crops. *[L]*

Colletotrichum Disease Development Along Dodder Strings, from Spot Inoculation

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The specific pathogen of dodder, *Colletotrichum*, is a potential biocontrol agent. We have examined the extension of the diseased area along the strings of dodder. The host for the dodder was an ornamental *Senecio*. The dodder was inoculated by placing a 1 mm^2 square of sporulating culture on the string in an area where the string was not attached to the host plant. The plants with the inoculated dodder were kept in a moist chamber, at 20°C for 22 h. The length of the inoculated strings and the length of the diseased area were measured daily. The diseased area extended at the rate of 4 mm day^{-1}. The rate of growth of inoculated strings was 25 mm day^{-1} and of the non-inoculated it was 34 mm day^{-1}. Conclusion: the disease of dodder dries up the strings and slows down the growth of healthy-looking strings. Microscopic examination of the diseased strings revealed that the pathogen does not grow within the string as far as the symptoms. It reached only 10–20 mm from the inoculation spot, even when the disease extended as far as 140 mm. In the first 10 mm, mycelium and chlamydospores were observed and also many acervuli. In the next 10 mm, along the strings, in some of the replicates, the mycelium was poor and only a few acervuli were observed. An assumption to be tested is whether toxins and/or enzymes might be involved in disease development beyond the growth

of the pathogen. The pathogen persists in dry strings, probably due to chlamydospores produced in them. *[P]*

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F: HOST–PATHOGEN RELATIONSHIPS

Invited Lecture presented by D. Prusky

Regulation of the Synthesis and Metabolism of the Preformed Antifungal Compounds in Avocado Fruits by *Colletotrichum gloeosporioides*

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The preformed (Z,Z)-1-acetoxy-2-hydroxy-4-oxo-heneicosa-12,15-diene (AFD) is the most active antifungal compound in avocado; it affects the quiescence of *Colletotrichum gloeosporioides* in unripe fruit. Fungal pathogens may modulate the level of the compound by affecting the synthesis and metabolism of the molecule, which is a fatty acid derivative. Genes encoding for Δ^{12} fatty acid desaturase and a very-long-fatty-acid elongase were hypothesized to take part in the biosynthesis of AFD and their expression pattern and enzymatic activity were determined in relation to the content of AFD. High expression of those genes was detected in young fruits, where the level of AFD was highest and when inoculated with *C. gloeosporioides* or treated with 1 mM H_2O_2. Pathogenic fungi may also modulate the metabolism of AFD by degrading the flavonoid epicatechin that inhibits the avocado enzyme which decomposes AFD. Extracts of laccase enzyme obtained from decayed tissue or culture media were fully capable of degrading epicatechin within 4 and 20 h, respectively. Isolates of *C. gloeosporioides* with reduced laccase activity and no ability to metabolize epicatechin showed no pathogenicity on ripening fruits. The present results offer a new perspective on the capability of *C. gloeosporioides* to modulate the level of the AFD by either inducing its synthesis in unripe fruits or its catabolism in ripening fruits. *[L]*

A Mutation in the NirA-like Transcription Factor Causes Lack of Pathogenicity in *Colletotrichum acutatum* on Strawberry

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A nonpathogenic REMI mutant of *Colletotrichum acutatum,* designated Ca-5, was isolated whereby in the absence of an external nitrogen source it exhibited extended germ tube growth prior to appressoria formation on solid surface and strawberry leaf. Ca-5 exhibited restricted hyphal growth and did not cause lesions on plants but grew necrotrophically when inoculated directly onto wounded sites. The deduced amino acid sequence of the REMI-impaired gene product, designated Nir1, is highly similar to the *Aspergillus nidulans* NirA protein, a transcriptional regulator of nitrogen metabolism. Inoculation of leaves with wild type or Ca-5 conidia in the presence of a nitrogen source resulted in massive epiphytic hyphal production, appressoria formation and rapid symptom development. The nutritional status of *C. acutatum* at an early stage of colonization and appressoria formation was assessed by following the expression of nitrate reductase (NR) and

glutamine synthetase (GS) in different media. Under all growth conditions there was no effect on GS; however, NR was induced by nitrate and repressed by a rich medium. In addition, NR transcription increased at the appressoria stage, indicating that nitrogen starvation constitutes a cue for regulation of appressoria development. Our results suggest that nitrogen starvation stimulates synchronous preinfection development which is lacking in Ca-5. *[L]*

Strawberry Powdery Mildew: Conditions for Its Development and Suppression

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The fungus *Sphaerotheca macularis* f.sp. *fragariae* is the causal agent of strawberry powdery mildew which occurs on leaves, flowers and fruits. The pathogen can cause severe damages in greenhouse crops as well as in low tunnel crops and in nurseries. Microclimatic conditions favoring or limiting disease are not well known; however, this knowledge is essential for efficient control. We found that optimal conditions for conidia germination are temperatures between 15 and 25°C and high (75–97%) relative humidity (r.h.). High irradiation inhibits germination and germ tube elongation. Conidiation of *S. macularis* at 70–85% r.h. was higher than at 95% r.h. Surprisingly, a percent of the conidia retained ability to germinate up to 5 months. At the optimal conditions of 20°C and 75% r.h., a disease cycle was completed within 4 days and severity was maximal. Temperatures of 10° and of 30°C, combined with a r.h. >95% and high irradiation, restricted the disease under controlled conditions. A good correlation was found between the response of conidia germination and disease severity to most of the above-mentioned parameters. Control agents sprayed on the strawberry crop at high frequency are able to reduce disease incidence and severity. The combination of control agents with host resistance was tested under commercial-like conditions. It was found that the most prominent factor that contributes to disease reduction is host resistance/tolerance. Thus, durable resistant strawberry cultivars with suitable horticultural characteristics as a major means of control may solve the powdery mildew problem. *[L]*

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Effects of Growth Regulators and Pruning on the Susceptibility of Pear Trees to *Erwinia amylovora*

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Fire blight, caused by *Erwinia amylovora*, is the most devastating disease of pears in Israel and elsewhere. The first infections occur usually in the flowers, and then the bacteria progress in the host tissues, from annual to perennial branches, towards the trunk. In a previous study it was demonstrated that sorbitol content in annual wooden branches is a reliable measure for host response to pathogen invasion. The rate of disease progression in perennial branches was related to the rate of changes

in sorbitol content in annual branches. This relationship was similar and significant in trees with different growth habits (high or low vigor) and in both seasons when infection occurred (spring or autumn). In this study we examined the effects of growth regulators and pruning, which are common horticultural practices, on the rate of disease progression and on the sorbitol content. Treatments were implemented to trees with high and low growth vigor. Effect of the treatments varied in trees with high and low vigor and also within each group, according to the phenological stage of the trees (dormancy break, fruit set, regrowth after fruit set). In some cases the treatments increased the rate of disease progression (and the changes in sorbitol content) whereas in others they decreased. Next we tested the hypothesis that the pathogenicity of *E. amylovora* is governed by changes in host physiology (that could be estimated in terms of sorbitol content) leading to expression of genes encoding for pathogenesis proteins. A construct between the *hrpE* promoter and the reporter gene *inaZ* was prepared. The construct was transformed to *E. amylovora* and the level of *inaZ* expression was measured. It was found that the activity of the reporter gene was significantly higher in pruned pear seedlings inoculated with the transformed bacteria, as compared with its activity in non-pruned seedlings. In addition, spraying with a growth regulator significantly decreased the expression of *hrpE in vivo. [L]*

Influence of Growth Media Composition on Growth and Enzyme Activity of Broomrape Biocontrol Agents *Fusarium oxysporum* f.sp. *orthoceras* and *Fusarium solani*

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Fusarium oxysporum f.sp. *orthoceras* (*Foo*) was isolated in Bulgaria from *Orobanche cumana*-diseased inflorescences and was found to be highly pathogenic and specific to this parasite. *Fusarium solani* (*Fs*) was isolated in Israel from diseased Egyptian broomrape inflorescences. The fungus demonstrated high potential as a biological control agent against Egyptian broomrape (*O. aegyptiaca*). The objective of the present project was to optimize growth conditions of the two fungi in order to obtain a high biomass and high level of spore production. The influence of media composition on fungi growth was tested in liquid growth media containing extract or macerate of broomrape inflorescences in various concentrations as compared with minimal media containing sucrose. Increasing broomrape extract concentration in the media decreased fungi biomass. Fungi biomass when grown in media containing broomrape macerate increased with the increase of broomrape macerate concentration from 3 to 24 g l^{-1}. Spore number was higher in media containing broomrape macerate than in media containing broomrape extract. The highest *Foo* spore number and CFU were found in growth media containing broomrape macerate at a concentration of 12 g l^{-1}. In the same media, *Fs* produced a very high level of spores, but with low CFU counts, indicating low spore viability. Filtrates of *Foo* and *Fs* growth media containing substrates for the enzymes polygalacturonase, polygalacturonide lyase, pectinase, protease, pectin methyl esterase and cellulase were analyzed. *[L]*

Two Biological Types of Garlic Leaf Rust (*Puccinia allii*)

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The cause of garlic rust disease is the fungus *Puccinia allii*. This fungus has a worldwide distribution, can devastate garlic fields and attacks also other species of *Allium*. In Israel, wild leek

(*Allium ampeloprasum*) can suffer heavy attacks and serve as a primary focus for the distribution of the disease in cultivated fields. The fungus is macrocyclic and produces all stages of its life cycle on *Allium* plants. The teliospore in the debris of the previous year germinates after the autumn rains and produces **four** basidiospores which, in turn, infect young leaves of a new crop. Haploid pycnia develop, and cross fertilization between pycnia of different mating types occurs. The resulting aecia with aeciospores infect garlic leaves and produce uredinial sori that are responsible for the future epidemic. Recently, an aggressive variant of this rust caused the devastation of most garlic fields in California. This variant has a shortened life cycle and lacks the pycnial and aecial stages. The teliospore produces only **two** basidiospores, and haploidy is confined to the promycelium. We have compared isolates collected in Israel, Turkey, and Europe with the Californian type and found additional differences, indicating that the latter belongs to a different subspecies. *[L]*

The Appearance of a Highly Virulent Race of Barley Leaf Rust as a Result of Crossing Two Avirulent Isolates

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Israel is located in the main region of origin of barley cultivation. *Hordeum spontaneum*, the wild ancestor of cultivated barley, is widespread throughout the country. Barley is an important crop in developing counties, mainly for animal feed; in other areas it is used for beer production as well as animal feed. Obligate parasites of barley have also evolved in our region. Barley leaf rust caused by *Puccinia hordei*, one of the pathogens, causes severe economic damage to barley production. The fungus has a complex life cycle, with barley as the main host and species of the geophyte *Ornithogalum* as alternate hosts. In mixed populations of *H. spontaneum* and the alternate host, we find highly virulent races in the pathogen and resistant plants that have evolved in the host species. Such a highly virulent race, with virulence on gene Pa7 in barley cv. 'Cebada Capa', was found for the first time in the world in Israel in 1976. A year later, this virulence was reported in Morocco. To date, it has also been reported all over the United States. In our research carried out under controlled greenhouse conditions, two avirulent isolates of the fungus were crossed. One of the 14 descendants of this cross was found to be virulent on Cebada Capa. These findings indicate that the sexual stage of the fungus can serve as a warning sign for new dangerous virulence. To detect such recombinations and prevent damage to cultivation, prebreeding of resistant cultivars should comprise intercrosses between putative parents that exhibit resistance. *[L]*

G: NON-CHEMICAL MEANS OF CONTROL

Synergistic Effect of Ethanol Dip and Modified Atmosphere on Prevention of *Botrytis* Rot of Table Grapes

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Grape storage requires stringent control of gray mold caused by *Botrytis cinerea*. The commercial practice is dependent on sulfur dioxide as a fumigant, which is applied by various means with well known advantages and disadvantages. Many alternative technologies were developed over the years, some of them with limited efficacy, others with low applicability and mostly without significant practical demand. Modified atmosphere (MA) of table grapes suffers from a narrow threshold between control of gray mold and damage to the berries and stems due to a high level of carbon dioxide within the film-enclosed package. We demonstrated in the past that dipping table

grapes in ethanol after harvest has a very pronounced effect in preventing decay. However, ethanol does not leave a protective residue within the grapes so it is not expected to prevent latent infections from developing decay nests during prolonged storage. However, when grapes of cv. 'Superior' were treated with ethanol and then subjected to a MA using plastic films (Xtend®), we achieved an additive effect and observed persistent control of gray mold without injury to the grapes. The advantage of this plastic film was mainly in its water conductance, which prevented accumulation of free water that is often the limiting factor in MA packaging. This combination results in greater decay control, which is a requirement for commercial applicability. If an undesirable aftertaste developed within the fruit due to the MA, one day of exposure to ambient air was sufficient to dissipate it. Taken together, these results demonstrate the commercial potential of this method. *[L]*

Verticillium Wilt as a Limiting Factor in Production of Grafted Eggplants

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The use of grafted vegetables increased in recent years all over the world, including the Mediterranean. Using grafted plants serves as a protective tool against a wide range of soilborne pathogens and enables expansion of the range of crops that can be grown in infested soils. Commercial eggplant cultivars have no resistance against the pathogen *Verticillium dahliae*, which causes Verticillium wilt, and therefore grafting on a resistant rootstock may enable growing eggplants in soils infested with this pathogen. Small-scale experiments were conducted in which susceptible eggplants were grafted on *Solanum torvum* – a wild species with high tolerance to the disease as a rootstock, or on tomato rootstocks (Beaufort and 4402) resistant to *V. dahliae*. In these studies, carried out at several locations in Israel, the grafted plants gave higher yields and growth was improved, compared with the non-grafted susceptible plants. However, grafting did not reduce disease incidence in the eggplants. In comparison, the tomato resistant rootstocks provided full protection against this pathogen to grafted tomato plants. Additional experiments carried out at the Faculty of Agriculture revealed that all rootstocks used do not prevent the pathogen's movement from the root to the susceptible scion, although disease severity in the scion differs according to the rootstock used, indicating a rootstock–scion interaction that influences disease severity. Different combinations of rootstocks and scions will be examined in the future. *[L]*

Interactions between Grafted Tomato Plants and *Fusarium oxysporum* f.sp. *lycopersici*

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Grafting has become a popular method to control soilborne diseases in tomato culture, especially in protected horticulture in East Asia, Europe, as well as Israel. Interactions between *Fusarium oxysporum* f.sp. *lycopersici* race 2 and tomato transplants of various genetic makeup were studied in greenhouse experiments. Following controlled inoculation, resistant rootstocks provided high to complete protection to susceptible scions, whereas the reciprocal combination resulted in severe disease symptoms of the resistant scions. In the latter combination, disease symptoms were evident 4–5 days earlier than those of inoculated intact (non-grafted) susceptible plants. It was evident that the rootstock genotype played a substantial role in the colonization of the inoculated plants and in disease expression. A heterozygote rootstock provided incomplete protection to a susceptible

scion (10–35% plants with visible symptoms), whereas all susceptible plants grafted on a dominant homozygote rootstock remained healthy. The levels of pathogen colonization of grafted and non-grafted inoculated plants were similar. Cross-grafted plants, namely, plants consisting of two sets of rootstocks and scions of different genotypes, which were inoculated with *Fusarium*, indicated that only a combination consisting of resistant rootstocks and scions provides protection against the pathogen. When the resistant rootstock in the cross-grafted plants was removed, the protection provided to the grafted plant was nullified. Phytopathological and physiological aspects of grafted plants should be considered further in future studies. *[L]*

Timorex – A New Organic Tea Tree Oil-based Product for Controlling *Rhizoctonia* in Potato

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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 industry. Its use against plant pathogens has not been investigated. Recently, together with the Biomor company, we have developed a new organic formulation – Timorex – containing 66% tea tree oil, effective against a broad spectrum of plant diseases in vegetables, herbs, grapevines and fruit trees, with no phytotoxicity to plant foliage. Infection of potato tubers by *Rhizoctonia solani* causes a significant reduction in yield. Its control in conventional management is achieved by the use of effective fungicides. In field trials conducted in 2003–04 the efficacy of Timorex was examined by spraying it on the soil during seed sowing and by preplant spraying of the seeds at low volume. Timorex applied during sowing reduced the percentage of *R. solani* infection in tubers, compared with controls. Spraying of Timorex at low volume inhibited *R. solani* infection in tubers. In all experiments Timorex was still less effective than conventional fungicides. No significant differences were found among treatments on the effects on growth and yield. The mode of action of Timorex is not clearly understood, but it acts as a protectant against a wide range of fungi by inhibiting spore germination, mycelial growth and sporulation and as a curative treatment by suppression of mildewed tissue. *[L]*

Timorex – A New Organic Tea Tree Oil-based Product for Controlling Grape Powdery Mildew

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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 industry. Its use against plant pathogens has not been investigated. Recently, the Biomor company has developed a new formulation – Timorex – containing 66% tea tree oil 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 Timorex at concentrations of 0.001% and 0.01% provided 50% and 65% inhibition, respectively, in conidial germination of *Uncinula necator*. A concentration of 0.1% completely inhibited germination. Growth chamber tests on potted plants revealed that Timorex at a concentration of 0.5–1% effectively controlled powdery mildew when applied as a prophylactic treatment and suppressed the fungus, as indicated by reduction of visible colonies when sprayed on mildewed leaves bearing sporulating colonies of *U. necator*. Field trials conducted in 2003–04 showed that Timorex at a concentration of 0.5–1% controlled powdery mildew and was as effective as or better than sulfur when applied at 7-day intervals. Spraying at 14-day intervals was less effective. Timorex does not harm natural enemies and other beneficial insects and can be used as a replacement for sulfur in both organic and conventional growth. The mode of action is not clearly understood, but it acts as a protectant against a wide range of fungi by inhibiting spore germination, mycelial growth and sporulation and by suppression of the fungus on mildewed tissue. *[P]*

Quantitative Aspects of Thermal Inactivation of Soilborne Pathogens Under Structural (Dry) and Soil (Wet) Solarization

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Solarization is a nonchemical approach for disinfestation. Solarization efficacy is governed by diurnal fluctuations in climatic conditions. However, most of the information on quantifying the thermal inactivation of pathogens is collected under laboratory constant temperature conditions. Modeling solarization efficacy is essential for predicting the effective level of pest control. By modeling, we integrate the partial effects of varying temperatures on pest control, either empirically or by utilizing thermal inactivation data under constant temperatures. Structural solarization is carried out by closing the greenhouse during the hot season. Air temperature inside the structure can reach a maximum of more than 60°C, while the relative humidity (r.h.) could drop to 15%; structural solarization is therefore considered as a dry heating. Low humidity leads to reduced effectiveness of thermal inactivation. Thus, a model of structural solarization should consider both diurnal fluctuating temperatures and r.h. values. Applying a modified Weibull model based on the above considerations, we obtained good agreement between calculated and observed control (expressed by R^2) of *Fusarium oxysporum* f.sp. *radicis-lycopersici (Forl)* and *Sclerotium rolfsii (Sr)*. The R^2 values for *Forl* ranged in most experiments from 0.86 to 0.98 and for *Sr* from 0.82 to 0.94. Soil solarization, on the other hand, is carried out in wet soil; hence, the model should consider only diurnal temperature fluctuations. Pathogen kinetic data under constant temperatures were used to develop the equations in which the temperatures were normalized to degree-hours (NDH). The rate of pathogen control was positively correlated with heating intensity, as expressed by NDH above certain temperatures. The derived R^2 values between NDH and pathogen survival during soil solarization at the 10 to 40 cm depth were 0.53 to 0.86 for *Forl*, and 0.82 to 0.95 for *Sr*. At greater soil depths (40 cm), the R^2 values were lower for *Forl*, suggesting a possible involvement of additional factors, *e.g.* biocontrol. The above modeling approaches can provide tools for quantifying solarization effectiveness under various climatic conditions. *[L]*

Control of Root-knot Nematodes Using Ammonia-releasing Fertilizers

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Ammonia is known to have nematicidal activity, but its efficacy is greatly affected by soil pH. High concentrations of ammonia are more likely to occur under alkaline conditions (pK_a = 9.3), since the relative amounts of ammonia and ammonium, which is not nematicidal, are pH-dependent. Application of cement kiln dust (CKD) from cement industries at concentrations of 0.1% and 0.2% (w/w) in combination with chicken litter (0.1%, 0.2% and 0.4%, w/w) or ammonium sulfate (50 and 100 mg NH_4-N kg^{-1}) to *Meloidogyne javanica*-infested soils greatly reduced the number of recovered *M. javanica* juveniles in the laboratory, and the tomato root galling index in a growth chamber, whereas sole applications of these materials did not or only moderately reduced the nematode infection to tomato plants or the number of vital nematode juveniles recovered from the soil. Similar results were also obtained by using lime-stabilized sludge (N-Viro Soil) at concentrations of 0.5%, 1.0% and 2.0% (w/w) in combination with ammonium sulfate. The main effect for the interactions of CKD or N-Viro Soil and chicken manure or ammonium sulfate in nematode suppression was significant. Application of CKD or N-Viro Soil temporarily increased the soil pH and, therefore, ammonia (NH_3) concentrations in the soil. In a field microplot experiment, ammonium sulfate in combination with N-Viro Soil greatly reduced the root galling index and increased the shoot weight of tomato plants. Combinations of ammonia-releasing fertilizers, either organic or chemical, with alkaline soil-amendments, may serve as a nematode control method in sustainable agriculture. *[L]*

Suppression of Soilborne Fungal and Bacterial Pathogens by Composts

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The ability of cattle manure-based composts, mixed with various sources of carbonaceous wastes (straw, orange peels and dry tomato or pepper plants) to suppress *Fusarium* pathogens of tomato, basil, melon and cucumber was tested in greenhouse experiments. Plant roots were inoculated with conidia of the relevant pathogen, or exposed to a natural inoculum, and the seedlings were planted in growth media containing 50% perlite and 50% tested compost, or peat as a conducive control. The composts significantly suppressed these diseases as well as tomato bacterial disease caused by *Clavibacter michiganensis*. *Fusarium* conidia of a natural inoculum (produced on stems of tomato or basil) were added to the composts or peat and their viability with passage of time was assessed. The conidia population declined with time more rapidly in the composts, compared with peat, indicating the presence of suppressing factors in the compost. Similar results were obtained with *C. michiganensis*. Gamma irradiation of the composts nullified their suppressive effect, as tested with *F. oxysporum* f.sp. *melonis*. Further incubation for 24 h of the previously irradiated composts resulted in rapid reproduction of the surviving bacteria, accompanied by restoration of the suppressive capacity. Suppressive composts should be considered as components in integrated disease management programs. *[L]*

Chemical Control of Fusarium Crown Rot and Didymella Diseases in Cucumbers Grown in Greenhouses

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Outbreaks of Fusarium crown and root rot of cucumbers were recorded during the winter and spring of 2002 and 2003, at Ahituv in the central part of Israel, as well as at other sites. This pathogen produces an enormous amount of macroconidia on the stems, which are dispersed and become an additional source of contaminating inoculum. Didymella blight caused by *Didymella bryoniae* in greenhouse cucumbers is also of concern to the growers. The latter pathogen attacks the aerial parts of the plant, including fruits. Field experiments for Fusarium control with Bavistin (carbendazim) demonstrated satisfactory results during 2002 and 2003, but less good control in 2004 experiments. The use of Octave (prochloraz) in 2004 experiments resulted in good control of Didymella blight, as well as of Fusarium in greenhouses with low disease incidence. Further studies will attempt to determine the optimal dose and application intervals. Control of the above mentioned diseases requires a comprehensive and integrated approach which should take care of all inoculum sources, by means of sanitation, structural solarization or chemical disinfestation of the greenhouse, use of fungicides, soil disinfestation, crop rotation, grafting and other nonchemical and chemical means. *[L]*

Cyflufenamid – A New Active Ingredient for the Control of Powdery Mildew

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Cyflufenamid (code name NF-149) in a 50 g l^{-1} EW formulation is a new fungicide developed by Nippon Soda, registered in Japan in 2003 for the control of powdery mildew in vegetables and in the advanced stages of registration in Europe for powdery mildew control in cereals. Cyflufenamid inhibits the infection process of powdery mildew by preventing haustorium formation, haustoria development, growth of secondary hypha and conidiospore formation. The product does not inhibit spore germination, germ tube elongation and appressoria formation. The mode-of-action has not been completely elucidated but there is no cross resistance between cyflufenamid and other classes of fungicides. It has been proposed that the mode-of-action will be novel. The product demonstrates excellent persistency, and good translaminar and vapor-phase activity but is not systemic. The product demonstrates good curative efficacy but is recommended to be sprayed preventatively. Field trials in Israel showed good control of *Sphaerotheca fuliginea* in cucurbit crops (melon, watermelon, cucumber and marrow), of *Leveillula taurica* in solanaceous crops (tomato, pepper, eggplant) and of *Uncinula necator* in grapes. Another unique characteristic of cyflufenamid is the low application rate (5–10 g a.i. ha^{-1}). The manufacturer has recommended tank mixing cyflufenamid with DMI fungicides in all spray applications, in an attempt to prevent the development of fungicidal resistance. *[L]*

Quantitative Damage of the *Squash leaf curl virus* (SLCV) and Leaf Silvering Effect in Squash

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Large whitefly populations severely attack autumn sown squash in Israel. This causes serious damage to the crop, which is expressed by leaf silvering and a pronounced reduction in the quality and quantity of the yield. For the last 3 years whiteflies have been diagnosed as the vector of a new virus that has not been known previously in squash in Israel. This virus has been identified as the *Squash leaf curl virus* (SLCV). In the present trial an effort was made to define the quantitative damage of the above described problems. We determined the relative contribution of different methods for controlling the virus transmission and the leaf silvering phenomenon. These methods included mulching the squash seedbed with yellow colored polyethylene sheets and various chemical treatments (with imidacloprid, thiacloprid and spiromesifen) both separately and in combination. The time of appearance and disappearance of the virus symptoms was influenced by the soil mulching with yellow tarps. The virus symptoms appeared and disappeared a week later in the plants of mulched plots as compared with plants growing in the bare soil. No meaningful effect has been found of any of the treatments in reducing the level of virus infestation as observed by the visual symptoms on the plants. These symptoms disappeared without any relation to the treatments that were applied, including the plots that were not treated at all. In addition, we found no influence of the symptomatically expressed virus level on the final crop yield. On the other hand, the mulch and chemical treatments had a positive influence on reducing and/or preventing the leaf silvering phenomenon. The combination of both soil mulching and chemical treatments provided the most successful results. In addition, prevention of leaf silvering contributed most significantly to the yield increase, starting from a certain leaf silvering severity level. *[L]*