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Opening Lecture

Integration of Cultural, Chemical and Biological Measures for the Control of Foliar Diseases of Vegetable Crops in Greenhouses

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In greenhouses, restricted ventilation and low light intensity – especially on the lower parts of the plants – result in a saturated atmosphere for too long periods each day and promote several fungal plant diseases such as gray, white and leaf molds and downy mildew. Manipulation of the greenhouse environment to prevent water-dependent pathogens can be practiced by farmers in non-heated greenhouses or in greenhouses partially heated during the cold winter. Alternative control methods are employed for the control of diseases promoted by high humidity. Some of the alternative control methods currently practiced in greenhouses are either passive or forced aeration, crop and growth medium management, coverage of the structure by films supplemented with additives – for the prevention of canopy wetness and to change light quality, and changing fertilization. This is in general effective against infection of leaves, flowers and fruits, but only partially so against stem infections, and not under conditions most favorable to the diseases. Biocontrol is another alternative control measure. The moderate effectiveness of the currently studied biocontrol agents (BCAs) calls for their integration with chemical and cultural control measures. The integration of BCAs and chemicals in the general management system is aided by the use of a forecaster to predict outbreaks of epidemics in the specific crop. According to the system (BOTMAN, developed by Y. Elad and D. Shtienberg), cultural measures and a BCA (Trichodex – *Trichoderma harzianum* T39) are the most important tools; chemical control is implemented only occasionally, as deemed necessary according to its potential effectiveness and the actual resistance of the pathogen population in the greenhouse.

The compatibility of chemical fungicides to be used in the greenhouse along with the BCA dictates which chemical can be used. In the future, the effect on insect pest control practices of measures aimed at plant diseases, and the effect of pest control measures on disease epidemics, should also be taken into consideration. *(L)*

A: BIOLOGY AND EPIDEMIOLOGY

Effects of Head Rot, Induced by *Rhizopus arrhizus*, on Sunflower Yield, Its Components and Quality

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Sunflower head rot is caused by the pathogen *Rhizopus arrhizus*. Initial symptoms of Rhizopus head rot are brown, sunken, water-soaked lesions on the back of the head. The spots gradually enlarge, and the interior of the head become soft. Typical symptoms may also develop on the achenes' peels and the taste of infected seeds may be bitter. Since the actual amount of loss induced by the disease in Israel is not known, a study was conducted with the objective of estimating the effect of Rhizopus head rot on sunflower yield, its components and quality.

The effect of Rhizopus head rot on yield was related to the crop growth stage at which infections occurred. Yield and its components (number of achenes per head and individual achene's weight) were reduced when infections occurred before the achenes started to change color from white to gray, but not when infections developed at later stages. When yield was reduced, loss in the number of achenes per head was greater than the reduction in individual achene's weight. A survey conducted in commercial fields revealed that disease incidence ranged from 5% to 25% by the end of the season. However, most infections occurred towards the end of the season, and thus the resultant yield losses did not exceed 3%. The conclusion is that as far as effects on yield are considered, it is not imperative to manage Rhizopus head rot.

Effects of the disease on yield quality were different. Infections at all crop growth stages induced the typical symptoms on the achenes' peels (which is an indication of bitterness in the seeds). The incidence of symptoms in commercial fields did not exceed 3%, but the economic consequences of this infection may be substantial, since the entire yield may be graded as of lower quality. Consequently, there are situations in which Rhizopus head rot should be managed to prevent reduction in yield quality. *(L)*

Oospore Production of *Phytophthora infestans* in Potato and Tomato Leaves

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Fungal, host and environmental factors affecting sexual reproduction of *Phytophthora infestans in planta* were studied. Intact as well as detached leaves were co–inoculated with sporangia of various combinations of A_1 and A_2 mating–type isolates; leaves were then incubated under various conditions and oospore production was estimated microscopically within whole, clarified leaflets. Some $A_1 + A_2$ isolate combinations were more reproductive than others, and some potato genotypes better supported oospore formation than others. Tomato usually supported more oospore formation than potato. To induce oospore formation, $A_1 + A_2$ sporangia were usually mixed at a 1:1 ratio; ratios

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

of 1:19 to 19:1, however, also allowed for abundant production of oospores. Optimal temperatures for sexual sporulation were in the range of 8–15°C but oospores were produced also at 23°C. Oogonia developed 5–6 days after sporangial co–inoculation and oospores after 8–10 days. Light had little effect on oospore formation in either tomato or potato leaves, provided that initial lesions were established under photoperiodical conditions. Although A_1 and A_2 sporangia were usually mixed before being inoculated onto leaves to obtain oospores, it was found that discrete A_1 and A_2 lesions produced on opposite sides of the midvein of tomato leaves also induced oospore formation in the midvein and in adjacent tissues. Oospores also were formed when the two halves of leaves were cut and separated at 3 days after sporangial co–inoculation, which corresponded to the appearance of late blight lesions. A continuous supply of moisture to the infected leaves was essential to oospore production. No oospores or oogonia were formed in severely diseased plants kept at 50–80% r.h. Such plants did allow for some oospore formation when kept continuously wet for 2 weeks in plastic boxes or tents. Detached leaves that floated on water supported the highest sexual sporulation. Under optimal conditions of wetness and temperature, as many as 100 oospores/mm^2 of tissue were recorded. *(L)*

B: NEW DISEASES

Plant Pathogens Little Known in Israel I. *Cylindrocladium* and *Calonectria*

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The genus *Cylindrocladium* comprises approximately 30 species, some known worldwide, *e.g. C. scoparium* and *C. parvum*, and some apparently limited to certain regions, such as *C. colhounii* in Mauritius and Japan, *C. clavatum* in Mauritius and Brazil, and *C. ilicicola* in Ireland. Some of the species, like *C. scoparium*, which attacks forest and fruit trees, vegetables and ornamental plants, are pathogenic to a wide range of hosts. The teleomorph, *Calonectria*, is known for some of the host species.

Extension advisers in Israel are not familiar yet with *Cylindrocladium* and *Calonectria*, or with the plant diseases caused by these fungi, which in itself is an indication of their relative scarcity here. Field diagnosis being thus excluded, diseased plants are referred to the diagnostic laboratory of our Services. *Cylindrocladium* spp. have been isolated in Israel (*vide* database of the Plant Disease Diagnostic Laboratory, PPIS) from diseased fruit trees (avocado, pear, peach, date palm, apple, nectarine), ornamentals (anemone, bottlebrush, callistemon, euonymus, ornithogalum, pittosporum, protea, rhododendron, rose, smoke-tree, wax flower) and vegetable crops (carrot, melon, potato, hot red pepper). The fungus was usually isolated from root- and stem base rots, except for one isolation from a branch canker of imported rose (kept in post-entry quarantine). *Calonectria* was isolated from a canker on a branch of pitanga. The holomorph (both *Calonectria* and *Cylindrocladium* stages) was observed on leaf spots of rhododendron and callistemon from a nursery.

The various anamorph isolates produced, in culture on PDA, characteristic conidiophores, conidia and chlamydospores which were sometimes in chains and sometimes formed sclerotium-like bodies. The culture from *Calonectria* isolated from pitanga was holomorphic, as were the cultures from the holomorphic infections on rhododendron and callistemon. Perithecia from different isolates were yellowish-brown, brown and dark orange. *(L)*

Anthracnose and Root Rot of Strawberry Caused by *Colletotrichum acutatum* in Israel

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Strawberry anthracnose occurred for the first time in Israel in 1995 and also in 1996, reaching epidemic proportions in nurseries and production fields. The species responsible for anthracnose was identified, using morphological and cultural characteristics, as *Colletotrichum acutatum*. *C. acutatum* was subsequently isolated from necrotic roots of stunted, chlorotic plants, which exhibited no symptoms of anthracnose. High levels of the pathogen from naturally infested field soil and perlite growth substrate, were quantified from the rhizosphere of diseased plants on a semi-selective iprodione-amended medium. Both foliar- and root-infecting isolates were equally pathogenic to strawberry, causing 95–100% plant mortality after inoculation on roots and foliage. In complementation (heterokaryon) tests using nitrate-nonutilizing (*nit*) mutants, 113 out of 115 isolates from different plant parts and locations belonged to a single vegetative compatibility group. Arbitrarily primed PCR (ap-PCR) of genomic DNA using four repetitive-motif primers produced nearly uniform banding patterns for 141 of the Israeli strawberry isolates from different sites, plots, plant tissues, and cultivars. Comparison of these band patterns with those of reference isolates from the USA confirmed that *C. acutatum* was indeed the species responsible for anthracnose on foliage and for necrosis of roots of strawberry in Israel. *(L)*

Detection of *Erwinia carotovora* subsp. *carotovora* in Crucifer Seeds

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An unusually high incidence of stem rot wilt and death of Crucifer plants such as cabbage, cauliflower and Chinese cabbage was observed during the last few years in Israel. The disease was found in fields treated with different soil fumigants. The soft rot bacterium *Erwinia carotovora* subsp. *carotovora (Ecc)* was consistently isolated from infected plants. The purpose of this work was to investigate whether soft rot bacteria occur also in seeds. Several lots of locally produced seeds and imported cabbage and cauliflower were examined for the presence of the pathogen. The bacteria were extracted from seed lots and isolated on a selective medium containing sodium polypectate.

Pectolytic bacteria were isolated from cabbage seeds imported from France and from cauliflower seed lots produced in Israel. Identification of the isolated bacterium was based on pathogenicity, and on biochemical and physiological characteristics. The bacteria are Gram-negative, motile, oxidase-negative, green-fluorescence-pigment-negative, and they ferment glucose rapidly. The strains were nonsensitive to erythromycin, did not reduce substances from sucrose or produce gas from glucose. They produced acid from lactose, trehalose, melibiose and cellobiose but not from maltose, α-methyl glucoside, palatinose and malonate. Indole and acetoin (3-hydroxy-2-butanone) production were negative. The strains hydrolized gelatin, tolerated 5% sodium chloride and grew at 37°C. Analysis of fatty acid profiles by gas chromatography showed a 0.95 similarity to *Ecc*. Tomato and pepper fruit rot tests were positive after 24 h. Inoculation of cabbage and cauliflower seedlings with different levels of inoculum produced soft rot symptoms after 24 h at 25°C and high humidity. This is the first report of direct isolation of *Ecc* from cabbage and cauliflower seeds. *(L)*

Detection of New Viruses in Ornamental Crops in Israel

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The intensification of ornamental production, the introduction of contaminated propagation plant material, and outbreaks of new insect biotypes all may play a role in the establishment and distribution of new viral diseases. In Israel, virus diseases are economically important due to the crop rotation practice. Viruses with a wide host range are well adapted to survive in successive crops of the same plant grown throughout the year. During the past 3 years many incidences of previously unknown virus diseases of ornamentals have been observed in Israel. During a survey of the incidence of virus infection in ornamentals, plant samples were collected on the basis of natural symptoms. Symptoms on suspected plants were variable and consisted of chlorotic spots, foliar necrosis, rings and line patterns, stem cankers, internal necrosis, and stunting. Plant samples were analyzed by host range, serology and electron microscopy.

Tomato spotted wilt virus was detected in *Eustoma russellianum, Aster ericoides, Asclepias tuberosa*, New Guinea *impatiens, Zinnia, Lobelia* and *Cestrum purpureum.* Iris yellow stripe tospovirus was detected in *Hippeastrum.* The appearance of these viruses, previously unreported in Israel, is correlated with the none too distant arrival in the region of the important vector *Frankliniella occidentalis*, the western flower thrips. Cucumber mosaic virus was detected in *Aconitum, Dipladenia, Zebrina* and *Anigozanthos.* Petunia vein clearing virus was detected in vegetatively propagated *Petunia.*

The detection of tospoviruses in Israel and the widespread distribution of thrips capable of transmitting the virus, constitute severe potential threats to the ornamental industry. A strict quarantine policy should be adopted to prevent the establishment and distribution of new viral pests in this country. *(L)*

C: VIRUS AND PHYTOPLASMA DISEASES

Use of a Segment from the Coat Protein of Maize Dwarf Mosaic Virus (MDMV) to Inhibit the Spread of Other Potyviruses by Aphids

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Research was conducted to develop a new approach for the control of virus spread by vectors. This approach was investigated with regard to potyviruses, which is the largest group of plant viruses, with very significant economic importance. The method is based on the procedure employed for competition in aphid transmission of MDMV. In this research we showed that the N-terminal region of the coat protein (CP) competes against and inhibits the virus spread by aphids. The method has several advantages when transgenic plants are produced: the incorporation of only a small fraction of the viral genome reduces the chance of disturbance to the host genome expression. In addition the possibility of heterotransencapsidation is eliminated, since only one-quarter of the coat gene is involved. Nevertheless, this CP segment may influence the aphid transmission of other potyviruses; two viruses were examined for such a possible effect. The competition in transmission of Johnson grass mosaic virus (JGMV) by aphids membrane-fed with 10% sucrose solution containing 1 mg/ml fused protein of the N-terminal MDMV CP bound to maltose-binding protein (MBP), was tested. The carrier MBP by itself had no effect. When the three amino acids DAG were deleted from the N-terminal region, the polypeptide lost its competitive effect against JGMV, similarly to the observation with MDMV. These results indicated a competive effect of the N-terminal CP in phylogentically closely related viruses of the sugarcane mosaic potyvirus subgroup.

An additional virus tested was potato virus Y (PVY), infecting tobacco. In all the experiments performed, no competition between the N-terminal MDMV CP and PVY transmission was observed, although in some experiments with a low rate of transmission, it seemed like there was partial

competition. These experiments indicate the possibility of different attachment sites for different groups of viruses in the aphid stylet. Alternatively, another mode of action may be involved, where the binding of the virus to the aphid stylet is helper-component-mediated. The N-terminal region of only closely related potyviruses may interfere in this mediation and not affect phylogenetically distant viruses of this group, which have a different N-terminal structure. This work constitutes a first step toward understanding the interaction between specific sites on the virion coat and its vector. *(L)*

Synergism between Cucumber Mosaic Virus (CMV) and Zucchini Yellow Mosaic Virus (ZYMV) in Cucurbits

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CMV and ZYMV cause devastating epidemics in cucurbits worldwide. CMV belongs to the cucumoviridae, infecting a wide range of crops, whereas ZYMV – a member of the potyviridae – is limited to infection of cucurbits. The two viruses are transmitted by aphids in a non-persistent manner, and they can be found together in the same plant in the field. Mixed infection of the two viruses in growth chamber experiments led to collapse and death of squash seedlings, and produced more severe symptoms in cucumbers and melon varieties than of either virus separately. The accumulation levels of CMV in a mixed infection were higher than infection with CMV alone. ZYMV RNA and coat protein accumulated to levels similar to that resulting from the infection of ZYMV alone. The phenomenon of synergism occurred with mixed infections of different strains of ZYMV and CMV and also in separate infection with the two viruses. The synergistic effect was tested in CMV-tolerant cucumber (*Cucumis sativus* L., cv. 'Delila') and melon (*Cucumis melo* L., cv. 'Revigal'). Mixed infection of ZYMV and CMV of these cultivars showed a very high accumulation of CMV RNA and viral encoded proteins compared with infection with CMV alone, whereas the symptoms were similar to those following infection with ZYMV alone. A similar synergistic effect was found with a mixed infection with watermelon mosaic virus (WMVII; phylogenetically close to ZYMV) and CMV. The possibility of synergism between CMV and other potyviruses is being studied. *(L)*

Effect of Tomato Yellow Leaf Curl Virus (TYLCV) on Yield of Commercial Cultivars, and New Breeding Lines Tolerant to the Virus

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TYLCV is one of the most devastating viruses affecting tomatoes. It is a monopartite geminivirus, transmitted by whiteflies. The best way to reduce TYLCV spread is by breeding for resistance. The resistance level of two new tomato breeding lines which exhibit a very high level of tolerance to the virus, TY-172 and TY-197, was examined in a field test. The new breeding lines were compared with the TYLCV-tolerant commercial cultivars 8484 (Hazera Seed Co.), 3761 (A. B. Seeds), 'Fiona' (Sluis & Groot) and 'Tyking' (Royal Sluis); as a susceptible control we used cv. 5656 (Hazera Seed Co.). The plants were inoculated in a growth chamber at the first leaf stage at a very high inoculation pressure, using nearly 200 viruliferous whiteflies per plant. After a short recovery period, the plants were transplanted in the field. Non-inoculated plants of the same cultivar or line which were pre-exposed to non-viruliferous whiteflies, served as controls. The inoculated

plants were compared with the control, non-inoculated plants, in terms of total yield and fruit weight and number. Development of disease symptoms and virus accumulation in the inoculated plants were monitored as well. There were substantial differences in the level of tolerance exhibited by the various cultivars and breeding lines, with TY-172 and TY-197 expressing the highest level of tolerance. The results demonstrate the ability of the various tolerant tomato cultivars and lines to inhibit the effects of the virus upon inoculation at high pressure and at a very early stage of plant development. *(L)*

Sensitive Detection of Potato Leafroll Virus (PLRV) with a Nonradioactive Riboprobe

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PLRV occurs worldwide wherever potatoes are grown, causing severe crop losses. It is transmitted by aphids in a persistent-circulative manner. Potatoes are generally propagated by tubers; therefore planting infected tubers will transmit the virus to the next growing season. It is thus of major importance to test the planting material for viruses in general and particularly for PLRV. Today ELISA is used to detect PLRV in leaves, but assay of PLRV in non-sprouted tubers is erratic. Detection of PLRV in dormant tubers would therefore save time and expense.

We have developed a highly sensitive method to detect PLRV using a nonradioactive RNA probe (Riboprobe). This probe is based on a nucleotide sequence of the PLRV genome. Reverse transcription–polymerase chain reaction (RT-PCR) was used to amplify a fragment of approximately 2000 bp for preparing a digoxigenin-labeled complementary RNA probe. The probe detected PLRV at a concentration of 1 pg/ml, compared with 2 ng/ml by ELISA. This highly sensitive method enabled detection of PLRV in infected leaves at a dilution of 1:10,000, and in dormant potato tubers at dilutions of 1:10–1:100. Results are obtained in 2 days, with cost estimates similar to those of ELISA. *(L)*

Molecular and Biological Characterization of Melon Necrotic Spot Virus (MNSV) Infecting Melons and Watermelons in Israel

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MNSV has been reported to be a major pathogen of melon crops worldwide. It belongs to the carmoviruses family, has an isometric particle 30 nm in size, and its distribution in nature is by mechanical and seed transmission, as well as by the soil fungus *Olpidium* sp. Three strains of the virus have been described: the Japanese, infecting melons systemically; the Californian, with a slightly different host range; and the European, infecting systemically cucumbers but not melons.

In the winter of 1992 we observed the collapse of melons grown in plastic houses in the southern part of the Arava Valley. This phenomenon started as yellow spots on plant tops which later spread systemically and turned into necrotic lesions which spread and ultimately caused the plant's death. Disease incidence was in many cases close to 100% and resulted in total loss of the yield. The virus isolated from the diseased plants was characterized and found identical to the description of the melon strain of MNSV. During the last 2 years we have observed collapse of watermelons grown in winter in the area between Elat and Kikar Sedom. The disease affected watermelons grown both in plastic houses and in the open field. Infection was accompanied by severe necrotic reactions on

leaves, stems and fruits and the economic damage was very severe. The virus that was isolated from the infected plants has a close serological relationship to MNSV but was distinguished by its inability to infect melons systemically. The molecular weight of the capsid protein of this isolate was slightly lower than that determined for the melon strain. We suggest that the virus isolated from watermelons is a strain of MNSV. *(L)*

A New Disease of Anemone Caused by Mixed Infection of Phytoplasma and Virus-Like Particles

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Anemone is a popular ornamental grown in Israel for cut flower production and as a garden plant, the latter for both export and local use. The crop is gaining economic significance and its growing areas are expanding. In 1996, 20 million tubers were exported, compared with 5 million in 1994. Anemone is highly susceptible to viral diseases. Seven viruses infecting anemones have been detected worldwide, and are a major threat to the production and quality of the crop. During surveys conducted in Israel in 1995, the following viruses were detected: cucumber mosaic virus (CMV), turnip mosaic virus (TuMV) and tobacco necrosis virus (TNV). Losses can be particularly severe in plants infected with more than one virus.

Phytoplasma-associated diseases have been detected in Israel in various crops. Anemone plants showing virescence symptoms, *viz.*, stunting and flower malformation, were observed recently in the Sharon and the Galilee growing areas. The plants were tested for the possible presence of phytoplasma-like organisms. The high losses indicate the involvement of more than one causal agent. All efforts to transmit the disease by mechanical inoculation from naturally infected anemone to various host plants, failed. In ultrathin sections of sieve tubes, very high numbers of phytoplasma-like organisms were observed. In neighboring phloem parenchymal cells large masses of double-enveloped, virus-like particles were observed. These particles were localized in the mitochondria, causing the degradation of both the interior and the exterior membranes. Cytopathological effects were observed in the cellular structure. Particles' shape was similar to that of closteroviruses. We were unable to isolate virus particles from the infected cells. The possible interaction between phytoplasma and the virus was discussed. *(L)*

Phytoplasma-Associated Yellows Disease of Carrot in Israel

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A disease affecting carrots in Israel, causing great losses – especially in storage, was observed in the Bet She'an Valley in 1996. Symptoms associated with the disease are: adventitious shoots, cracking of the main root, formation of hairy roots on the main root, and various soft rots that develop mainly in storage. This syndrome resembles "yellows" or "witches' broom" described in some countries. Plant pathogenic phytoplasma was observed by electron microscopy in sieve tube elements of different diseased carrot tissues, such as leaf and root. Phytoplasma was detected using polymerase chain reaction (PCR). Phytoplasma-specific primers (16S rRNA – universal primers) were used for PCR with DNA extracted from different plant parts. A second set of group-specific primers was also employed to detect the type of phytoplasma in carrots. The relatedness of phytoplasma detected in the different plants was determined by restriction-fragment-length polymorphism analysis using four restriction enzymes. The association of phytoplasma with this disease has been demonstrated and was found to be related to the Aster Yellows group. *(P)*

Tylenchulus semipenetrans, the Citrus Nematode, and *Xiphinema brevicolle* are Present in the New Citrus Plantations in the Northern Negev

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Citrus seedlings, purchased from 13 citrus nurseries located in the central part of Israel, have been planted in the northern Negev (southern part of Israel) since 1993, in areas where citrus had never been planted before. In 1996 we started to screen citrus nematodes in these areas in samples taken from an area of 75 ha. Fifty-four percent of the plantations were not infested with plant-parasitic nematodes, whereas the rest were infested with the citrus nematode, *Tylenchulus semipenetrans*: 22% at a low infestation level (up to 500 second-stage juveniles [J2] per g root); 18% at a critical level (up to 4,000 J2/g root); and 6% at a very high level (up to 20,000 J2/g root). Such a high population level just 3 years after planting, may cause much damage, because of the microorganisms which follow the nematodes' penetration to the root. These secondary pathogens induce slow decline of the trees – a phenomenon that has already been observed in these plantations. Agrotechnical conditions such as aerated soil, drip irrigation and optimal temperature during most of the year, accelerate the increase in the nematode population. Nematode-infested citrus trees are more susceptible to stress conditions such as dryness and salinity; therefore, the relatively high-saline water which is used for irrigation may also contribute to the severity of the damage incurred by the nematodes.

In addition to the citrus nematode, the plantations are infested with other ectoparasites, such as *Xiphinema brevicolle* (20 nematodes/g soil), which was found in 3% of the area screened. The optimal conditions mentioned above for the development of the citrus nematode, are suitable also for that of this ectoparasite species. Under greenhouse conditions, after 8 months, top fresh weight of young citrus seedlings (sour orange, *Citrus aurantium*) planted in *X. brevicolle*-infested soil (5 specimens/g soil) was 48% less than that of citrus grown in non-infested soil.

Surveys revealed the presence of *T. semipenetrans* and *X. brevicolle* in 12 of the 13 citrus nurseries checked. This strengthened our hypothesis that these two nematode species had been brought to the plantation area along with the seedlings. *(L)*

Isolation and Characterization of a Collagen Gene from the Root-knot Nematode *Meloidogyne javanica*

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The root-knot nematode *Meloidogyne javanica* is a sedentary endoparasite which spends most of its life cycle inside roots. This plant-parasitic species undergoes a dramatic and unique change in gross morphology during its development. The nematode's surface is comprised of a multilayered cuticle, which consists mainly of collagen proteins. Isolation of collagen genes from pre-parasitic and parasitic stages of *Meloidogyne* and characterization of their expression pattern should provide a better understanding of the affiliation between the alteration in collagen expression and the gross morphological and biochemical changes of the cuticle, during the nematode's singular transformation. We have identified, cloned and characterized the first cuticular collagen gene, *Mjcol-3*, of the plant-parasitic nematode *M. javanica*. The gene putatively encodes a 32.4 kDa collagen protein, including a propeptide which possesses a subtilisin-like protease cleavage site. The basic

structure of *Mjcol-3* predicted-protein sequence is highly similar to the *Caenorhabditis elegans dpy-7* gene, possessing 65.9% identity between the two gene sequences. Compared to *dpy-7*, the putative *Mjcol-3* protein possesses a shorter carboxy-terminus. This nonconserved feature may indicate differences in the contribution of *dpy-7* and *Mjcol-3* collagens to the structure of the cuticle. The gene is developmentally regulated: transcripts are found mainly in pre-parasitic developing eggs, less so in parasitic fourth-stage juveniles, and to a much lesser extent in young females. *(L)*

Scanning Electron Microscope Observations on Banana Roots Infested with the Spiral Nematode (*Helicotylenchus multicinctus*)

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Helicotylenchus multicinctus is a migratory endoparasitic polyphagous nematode attacking and causing heavy damage to banana roots in many banana-growing countries, including Israel. The nematode invades the banana root and burrows cavities in the superficial layers of the root cortical parenchyma, where it feeds on the cells, develops, and lays eggs. The infected areas are indicated on the root surface as dark elongated lesions. The hatching juveniles may either stay in the root and enlarge the lesion, or leave it and look in the soil for another root to attack. Banana roots moderately to heavily infested with the spiral nematode, as evaluated by lesion density, were sampled in a 3-year-old plantation in the Jordan Valley. Root sections were fixed, dehydrated and embedded in polyethylene-glycol blocks and the infected sites were hand-sectioned with a razor blade. Scanning electron microscope observations revealed nematodes and their eggs in cavities formed due to parenchymal cell destruction. A close association between the nematodes and the mycelium of various fungi inhabiting the banana root tissues was also observed. It seems that the spiral nematode enhanced the invasion of the fungi into the banana roots, thus accelerating their decay. *(P)*

E: SOILBORNE AND WATERBORNE DISEASES

Verticillium Wilt of Paprika Pepper

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Wilt, stunting and early death of paprika pepper plants observed recently in Kibbutz Lahav in the northern Negev were caused by *Verticillium dahliae*, and resulted in a 22% reduction in the dry matter yield. *V. dahliae* isolated from paprika pepper was found to differ morphologically from isolates originating from potato. Its growth rate on PDA was slower than that of the potato isolate, but microsclerotia formed earlier. Pathogenicity tests with *V. dahliae* isolates originating from paprika pepper demonstrated its aggressive virulence toward paprika pepper. Symptom severity obtained in three inoculated paprika cultivars ranged from 3.7 to 4.9 (on a scale of 0 to 5). This isolate also caused stunting in all three cultivars, resulting in plant height reduction of 43% to 62%. Colonization levels were 35-fold higher in stems than in leaves. In contrast, *V. dahliae* isolated from potato did not cause any disease symptoms or stunting in paprika pepper and could not be detected in stems or leaves. The pepper isolate was also more aggressive than the potato isolate toward potato and eggplant, as expressed in higher disease incidence, symptom severity and colonization level of the fungus. These findings suggest that a buildup of the virulent isolate might seriously affect potato crop production in fields where potato and paprika pepper are major alternative crops, and other crops may be affected as well. *(L)*

Dissemination in Space and Survival in Time of *Fusarium oxysporum* f.sp. *radicis-lycopersici*

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Tomato crown and root-rot, caused by *Fusarium oxysporum* f.sp. *radicis-lycopersici* (*F.o.r.l.*), is a potentially severely destructive disease. The purpose of this study was to determine whether *F.o.r.l.* is a polycyclic pathogen, namely, if it has the ability to complete several disease cycles per growing season, or is it a monocyclic pathogen, namely, has one cycle per growing season. It is currently assumed that most soilborne pathogens are monocyclic in nature, whereas foliar pathogens are polycyclic. Such characterization of pathogens has important epidemiological consequences for disease management.

The development of the disease caused by *F.o.r.l.* was followed in a naturally infested field in the years 1995 and 1996. The data were analyzed statistically using geostatistics, in order to determine the probability that disease in one plant is related to infection of neighboring plants. Variogram functions, by which the changes in variance around the focus of the disease were quantified, provided evidence that during the season the disease spreads in the field to a distance of a few meters. In another set of experiments, inocula of *nit* mutants of *F.o.r.l.*, which are resistant to chlorate ($KClO_3$), were buried near tomato plant roots. These mutants can grow on chlorate media and therefore can be identified specifically. This technique enabled us to follow the pathogen's movement in time and space under natural conditions. The *F.o.r.l.* mutant succeeded in infecting plants at a distance of 1.5 m along the row from the inoculum source, resulting in typical disease symptoms. The spread of the pathogen along the rows was *via* root-to-root contact.

Our results suggest that *F.o.r.l.* is a polycyclic pathogen. This is a deviation from the classic pattern of nonzoosporic soilborne pathogens, which are considered to be monocyclic. *F.o.r.l.* produces macroconidia on the surface of infested plants. These conidia may provide another mechanism of dispersal, contributing to the polycyclic behavior of the pathogen.The dissemination mechanisms, including aerial dispersal of macroconidia, are being studied. *(L)*

Cavity Spot Disease in Carrots: Host–Pathogen Relationships and Control

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Cavity spot disease in carrot is caused by fungi of the genus *Pythium* spp. Disease symptoms include dark brown cavity lesions of elliptical shape. These spots reduce the marketable quality of the crop; thus control measures are needed for achieving pathogen-free carrots. Oospores of *Pythium* spp. survive in soil and provide the initial inoculum. Infection of carrots occurs in infested soil at an early stage of growth (50 days after planting). The pathogen is attracted to root exudates and excretes pectolytic enzymes which degrade the carrot cell wall, produces haustoria-like organelles, penetrates the tissue, and advances in the matrix between the cell wall. Inoculation of carrots was conducted with several species of *Pythium* to confirm their pathogenicity. Slow-growing *Pythium* species caused cavity spot. They invade the carrot tissue 3–4 days before a local hypersensitivity reaction by the plant sets in, which confines the cavity spots. Plant hypersensitivity reaction to pathogen infection in the infected cells includes increased activity of the enzymes phenylalanin ammonium lyase and polyphenol oxidase, increased amounts of lignin, phenols, phytoalexins, and

pathogenesis-related proteins. Carrot infection at an early stage of crop growth results in reduced crop yields. Application of metalaxyl gave effective control of cavity spot in field experiments. *(L)*

Identification of the *Pythium* Species Isolated from *Gypsophila paniculata* and Other Hosts in Israel

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Sixty-six isolates of *Pythium* were collected from ten hosts comprising nine botanical families: 37 isolates from *Gypsophila paniculata* (Caryophyllaceae), seven from *Protea leucadendron* (Proteaceae), five from *Aconitum carmichaelli* (Ranunculaceae), five from *Pelargonium domesticum* (Geraniaceae), three from *Rosa* sp. (Rosaceae), three from *Lycopersicon esculentum* (Solanaceae), two each from *Anigozanthos* sp. (Amaryllidaceae) and *Lisianthus russellianum* (Gentianaceae), and one each from *Gerbera jamesonii* (Compositae) and *Capsicum annuum* (Solanaceae). Identification of the *Pythium* species was accomplished by (i) microscopic observation of fungal structures developing on a grass-water medium, (ii) growth pattern on agar culture media and (iii) hyphal growth following incubation at 40°C. Fifty-seven isolates were identified as one of the following seven species: *Pythium aphanidermatum, P. irregulare, P. myriotylum, P. ultimum, P. macrosporum, P. sylvaticum* and *P. torulosum*; nine isolates of *Pythium* could not be identified to the species level. Of 37 isolates collected from *G. paniculata*, 60% were *P. aphanidermatum*, 19% *P. irregulare*, 17% *P. myriotylum* and 2% *P. ultimum*. The remaining 20 isolates collected from eight other hosts consisted of: 25% *P. aphanidermatum*, 15% each of *P. myriotylum, P. ultimum, P. macrosporum* and *P. sylvaticum*, 10% *P. irregulare* and 5% *P. torulosum*. *P. aphanidermatum* was the most prevalent species in *G. paniculata*, as well as in other hosts. In pathogenicity tests on *G. paniculata*, cucumber or tomato, all *Pythium* isolates were similarly pathogenic to these hosts. *(P)*

Plant Pathogen Persistence in Reclaimed Water Used for Irrigation

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The great population increase in Israel and the rapid development of intensive agriculture necessitate large amounts of water. Israel is located on the edge of desert land, so it is natural that water of low quality will have to be used in agriculture for irrigation. Particular consideration has been given to pathogenic organisms and hazardous chemicals which are related to public health, and criteria and regulations have been formulated for the use of all kinds of water for irrigation. In sharp contrast to this need, almost no studies have been published on the presence of plant pathogens in water for agricultural uses. The aim of this research was to obtain quantitative data on the bacterial and fungal plant pathogens in some of the main sources of water for irrigation and to provide tools and formulate regulations for the use of irrigation water free of phytopathogens.

Our results indicated the presence of major plant pathogens such as *Erwinia* spp., *Pseudomonas* spp., *Pythium* spp., *Fusarium* spp. and others in reclaimed wastewater of the third line of the National Water Carrier conducting water from the coastal area of Israel to the arid areas in the south of the country; aqueducts conducting irrigation water in the Bet She'an Valley; Lake Kinneret; and in recirculating water in greenhouses. During 25 months of bi-weekly sampling, large fluctuations in the tested pathogens were observed. In the third line of the Carrier, total bacterial population

(cfu/ml) varied from 10^2 to 10^6; plant pathogenic *Pseudomonas* or *Erwinia* spp. from 0 to $\sim 2 \times 10^3$; *Fusarium* spp. from 0 to 10^2; and *Pythium* spp. from 0 to 3.5×10^2. The same organisms were found at the other locations. Most of the tested pathogens were able not only to survive and be transmitted by the water, but also to multiply. Their potential as the primary source of inoculum, and the threshold needed to initiate disease in plants, were studied. *(L)*

F: CONTROL OF SOILBORNE DISEASES BY SOLARIZATION AND/OR FUMIGATION, COMPOSITING OR FUNGICIDES

Sprayable Plastic Polymers for Soil Solarization

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Soil solarization is a modern approach to soil disinfestation. It consists of solar heating of plastic-mulch-covered moist soil during the hot summer months. Solarization is of particular importance as a soil disinfectant in today's intensive agriculture, since the system is simple to apply and does not necessarily involve hazardous chemicals or complicated machinery. Nevertheless, applying plastic films for soil mulch requires special equipment and involves specific procedures. The film edges should be buried to hold them in place, and in continuous-mulch treatment the sheets are glued together. It is not possible to change the mulching pattern during work and the capacity of area coverage is limited. Plastic mulches must be removed from the fields before crop growth or after harvest; the process is expensive and labor-intensive. Plastic residues are often left in the field and can adversely affect agricultural practices and the machinery used for growing future crops.

Soil mulch for soil solarization using spray application of degradable polymers offers a feasible and cost-effective alternative to plastic tarps. The sprayed polymer forms a membrane film which maintains its integrity in soil and elevates the soil temperature. Nevertheless, the membrane is porous and allows overhead irrigation. Under experimental conditions, certain spray mulches were able to raise soil temperature and retain soil moisture under summer conditions in Israel. Soilborne populations of phytopathogenic fungi were significantly reduced by this process of solarization. It was as effective as solarization using plastic film in controlling Verticillium wilt and common scab in potato, and peanut pod wart in peanut during two consecutive crops. *(L)*

Heat Mortality of Pathogen Propagules as Affected by Moisture During Space Solarization

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Dry thermal inactivation of microorganisms is less effective than moist. Solarization of the greenhouse structure, which is a complementary disease management approach to eliminate aerial residual inoculum in the greenhouse space and structure, is a dry process. Therefore, detailed information on the effect of prewetting of the pathogen propagules on their thermal inactivation is needed, in order to improve the efficiency of dry heating. It was found that prewetting treatments reduced the sensitivity to heat of multicellular propagules such as sclerotia of

Sclerotium rolfsii, macroconidia of *Fusarium oxysporum* f.spp. *radicis-lycopersici, lycopersici* and *basilici*. The converse (increased heat sensitivity) was observed with unicellular propagules, such as chlamydospores of *F. oxysporum* f.spp. *radicis-lycopersici* and *melonis*. Cycloheximide suppressed heat tolerance of prewetted sclerotia of *S. rolfsii*, which indicates the possible involvement of a metabolic process (*e.g.* heat shock proteins). Scanning electron microscope (SEM) observations revealed cracks in the rind of heated *S. rolfsii* sclerotia, whereas untreated sclerotia appeared smooth and with fewer cracks. Prewetting treatments reduced significantly the number of cracks on heated sclerotia. The results of this study indicate that improvement of greenhouse sanitation by space solarization can be achieved under the proper conditions. *(P)*

Dry Heat Mortality of Pathogen Propagules: Experimental and Numerical Studies

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Greenhouse space solarization, by closing the structure, is complementary to soil disinfestation for the elimination of residual inoculum surviving on the greenhouse structure. The goal is sanitation to avoid reinfestation of soil and plants by the surviving inoculum. Closing the greenhouse during the summer, raises air temperatures under Mediterranean conditions to 55–65°C; this is accompanied by a decrease in relative humidity, resulting in dry heating. Dry heating reduces control effectiveness as compared with wet heating. However, previous studies have shown that if heating time is extended, we can achieve control of a variety of pathogens. Our aim in this study was to develop a dynamic model to predict rate of pathogen mortality using climatological data from a closed greenhouse.

Two pathogens were tested: sclerotia of *Sclerotium rolfsii*, and soil naturally infested with *Fusarium oxysporum* f.sp. *radicis-lycopersici* (*F.o.r.l.*), incitant of crown and root rot of tomatoes. Under practical agricultural conditions some of these propagules remain on the greenhouse structure, with the potential of functioning as a source of contamination. With both pathogens, a series of survival tests were performed at several different constant temperatures. These tests were conducted at a temperature range of 44–67.5°C, in order to specify reaction order and constants of thermal inactivation for each tested temperature and pathogen. Thermal survival curves were verified by two methods: by adjusting to the Arrhenius model and by the 'boxcars' method. In addition, dynamic models developed for fluctuating temperature regimes in both methods were validated with observed survival data. We found that the thermal survival curve of the fungus *F.o.r.l.* (which has unicellular resting structures, chlamydospores) obeys first-order kinetics and has an activation energy value of 60,000 cal/g-mol. *S. rolfsii* sclerotia, which are multicellular structures, do not obey first-order kinetics. Dynamic models for fluctuating temperatures were developed and are in the course of validation, in an attempt to predict mortality rates of various pathogens in the process of greenhouse space solarization. *(L)*

Combining Solarization and Fumigants at Reduced Dosage for Effective Control of Soilborne Pathogens: Controlled Environment Study

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Chemical fumigants have been of great help to agricultural production for many years. However, certain fumigants, such as methyl bromide (MB), were found to cause environmental pollution, and even pose the threat of potential atmospheric ozone depletion. Thus, alternatives for effective control of soilborne pests and diseases are necessary: soil solarization is a non-chemical approach to controlling such pests. It has both advantages and limitations, the latter including occupation of the soil for 4–6 weeks and climate dependency. Moreover, solarization does not control all pathogens. Combining solarization with fumigants can improve pathogen control and extend it to more pests and for use under less favorable climatic conditions. Moreover, such a combination may enable us to reduce fumigant dosages and further minimize environmental risks.

The effect of a combination of a sublethal dosage of MB with heating for a short period on killing of fungal propagules was tested in controlled environment containers. Sublethal heating of propagules of *Sclerotium rolfsii* and *Fusarium oxysporum* f.sp. *basilici*, followed by their exposure to a sublethal dosage of methyl bromide, resulted in effective control of the propagules. Incubation of the treated propagules in natural soil for 7 days further decreased their viability. Apparently, the soil microflora contributed to the killing of fungal propagules, which were weakened by the heat and MB. The effect of a sublethal dosage of MB and short solarization on killing of fungal propagules was tested in the field. Fumigation with MB at 15 g/m^2 combined with solarization for 8 days resulted in effective killing of propagules of *S. rolfsii, F.o. basilici* and *F.o. melonis. (L)*

Combining Solarization and Fumigants at Reduced Dosage for Effective Control of Soilborne Disease in the Field

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Soil solarization is a modern approach to soil disinfestation. It consists of solar heating of plastic-mulch-covered, moist soil during the hot summer months. It is simple to apply and does not necessarily involve hazardous chemicals. However, the procedure requires the occupation of the soil for 4–6 weeks and is climate-dependent; moreover, solarization does not control all pathogens. Thus, improving control by solarization might enable the use of solarization under a wider range of conditions, and might even shorten the solarization period necessary for pathogen and pest control. Solarization combined with reduced dosages of methyl bromide (20 g/m^2), metham-sodium (30 ml/m^2) and formalin (250 ml/m^2) was tested to control crown rot of tomato and sudden wilt of melon in the southern part of Israel. The soils in this region are heavily infested with the causal pathogens, and solarization alone is not effective as a control method. Fumigants were applied to the soil surface through drip irrigation lines which were placed under a plastic mulch. Methyl bromide and metham-sodium at reduced dosages combined with solarization, gave effective control of crown rot of tomato and sudden wilt of melon; formalin combined with solarization was not effective in controlling these diseases. A combination of solarization and reduced rates of fumigants can be a feasible alternative treatment to improve control of pathogens and minimize environmental risks.*(L)*

Effect of Reduced Dosage of Methyl Bromide and Metham-Sodium in Controlling Fusarium Wilt of Carnation

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The effect of methyl bromide (MB) and metham-sodium (MS) on controlling Fusarium wilt in carnations caused by *F. oxysporum* f.sp. *dianthi* was studied in a naturally infested field. MB was applied using the hot gas method, at 70 g/m^2 under 35 μm low-density polyethylene or 35 g/m^2 under gas-tight film ('Barromid', Plastopil Hazorea, Kibbutz Hazorea, Israel) and MS at 300 ml/m^2 or 150 ml/m^2 applied with sufficient water to wet the soil profile down to 60 cm at field capacity. Non-fumigated plots were used as the control. Efficiency of pathogen control was determined by (*a*) counting viable propagules which survived fumigation in soil samples; and (*b*) disease incidence in carnation plants in fumigated as compared with control plots. Propagule counts in soil samples from 5, 20, 40 and 60 cm depths were below detection level in the fumigated plots, as compared with 10,000 propagules/g soil in the non-fumigated plots. In soil samples collected from each plot (fumigated and non-fumigated) at three depths: 0–20, 20–40 and 40–60 cm, 15% of the total counts at each depth in control plots, survived in the fumigated plots. Cuttings of a highly susceptible cultivar ('Citronella') were planted in the field following the fumigation treatments. Disease incidence recorded 180 days after planting was 95%, 40%, 33%, 28% and 37% for the non-fumigated, MS 150 ml/m^2 and 300 ml/m^2, MB 35 g/m^2 and 70 g/m^2 treatments, respectively. In additional experiments with MS, propagule counts were below the detection level in soil samples taken from a depth of 60 cm following treatment with 100, 200 and 300 ml/m^2, as compared with 10,000 propagules/g soil in the non-fumigated plots. In another experiment, disease incidence in carnation plants (cv. 'Hermon') was 100%, 16% and 10% for the non-fumigated, 150 and 300 ml/m^2 treatments, respectively.

In conclusion, reduced half-strength dosages of MB or MS were as effective as higher dosages in controlling *F. oxysporum* f.sp. *dianthi* in soil and in reducing disease incidence in carnation plants. *(P)*

Differential Suppression of Cucumber Damping-Off among Three Pythium Species in Composts at Different Temperatures

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The suppressiveness of compost amendments to pre-emergence damping-off of cucumber incited by isolates of *Pythium aphanidermatum*, *P. myriotylum* and *P. irregulare* was studied in growth chamber experiments. Experiments were designed to examine the effects of temperature (20, 24, 28 and 32°C), compost type (municipal biosolids compost and leaf compost) and compost dose on suppression of diseases caused by different *Pythium* isolates from different hosts. In dose-response experiments, the optimal dose for disease suppression in each compost was 80 mg/cm^3 soil. Disease incidence induced by each of the three *Pythium* species and the level of compost suppressiveness were each temperature-dependent. For example, *P. aphanidermatum* and *P. myriotylum* caused damping-off at each of the four temperatures tested, whereas *P. irregulare* was virulent only at 20 and 24°C. Municipal biosolids compost was suppressive only at the lower temperatures (20 and 24°C), whereas the leaf compost was suppressive at these, as well as at higher temperatures (28 and 32°C). Both composts significantly suppressed damping-off caused by *P. irregulare* at 20°C (85% suppression in the municipal biosolids compost and 60% by leaf compost) and at 24°C (*ca* 60% suppression in each of the two composts) and by *P. myriotylum* at 20°C (*ca* 60% suppression). The leaf compost was suppressive to *P. myriotylum* also at 32°C (52% suppression). Municipal biosolids compost significantly suppressed damping-off caused by *P. aphanidermatum*

(56% suppression) at 20°C, whereas the leaf compost was suppressive only at 28 and 32°C (*ca* 40% suppression). In experiments with a variable temperature cycle [32°C day (14 h) and 22°C night (10 h)], only *P. aphanidermatum* and *P. myriotylum* were pathogenic to cucumber seedlings. Under these conditions, the leaf compost significantly suppressed damping-off caused by *P. aphanidermatum* (20% suppression) but not that caused by *P. myriotylum*. Municipal biosolids compost was not suppressive under these conditions. In experiments where the two composts were combined, there was no increase or decrease in suppressiveness over the levels observed with each component singly. There was a significant difference in pathogenicity among isolates of each of the three *Pythium* species, among isolates derived from the same host, and between isolates from different hosts. *(P)*

Chemical Control of *Monosporascus*, the Causal Agent of the Root Rot and Vine Decline Disease of Melons

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The root rot and vine decline disease (sudden wilt) of melons has been recognized in the Arava Valley of southern Israel for many years. When a severe epidemic develops, the entire yield may be destroyed. The primary causal agent of the disease is the fungus *Monosporascus* sp. In artificial inoculations, *Pythium aphanidermatum* and *Fusarium solani* were capable of inducing similar wilt symptoms, but to a lesser extent. Soil disinfestation with methyl bromide is a common method by which the disease is controlled. However, since the use of this fumigant will be prohibited from the year 2010, there is a need to find alternative measures for disease management.

Of 30 fungicides tested, fluazinam (Frownside) was the most effective in inhibiting *Monosporascus* mycelial growth in culture. It was very effective also against *P. aphanidermatum*. The efficacy of Frownside was evaluated in the Arava in two field trials. The first trial was conducted in spring 1996, in microplots artificially inoculated with vegetative mycelium of *Monosporascus*. The second trial was conducted in autumn 1996, in naturally infested soil. Treatment with Frownside significantly reduced disease incidence in both trials throughout the growing season. Disease incidence by melon harvesting time was reduced by 86% and 23% in the spring and autumn trials, respectively.

In order to establish an adequate disease management practice, integrated control should be employed. Disease management should include, in addition to improved application of the chemical, use of tolerant melon cultivars and grafted melon plants. *(L)*

G: CONTROL OF DISEASES BY VARIOUS NONPESTICIDAL PROCEDURES; INTEGRATED CONTROL

Extracts of *Inula viscosa* for Controlling Plant Diseases on Fresh and Dry Postharvest Products

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Several tests were conducted to determine the effect of a boiling water extract of *Inula viscosa* in controlling plant diseases. Adding extract of *I. viscosa* to PDA caused a long delay in the development of various species of pathogenic fungi. In two of the fungi, *Botrytis cinerea* and *Rhizopus stolonifer*, sporulation was delayed up to 12 days after inoculation. A direct correlation was found between the concentration of the extract in PDA and the delay in fungus development. A comparison of extracts of several parts of the plant (*viz.*, young leaves, old leaves, stem, roots and flowers) which were dried with hot air for 5 days prior to extraction, revealed that the greatest inhibitory activity was achieved by a boiling water extract of mature leaves; that of young leaves was significantly lower. Extracts of roots and flowers gave only insignificant inhibition. A 10-min dip of groundnut seeds in a boiling water extract of *I. viscosa* reduced significantly the infection of *R. stolonifer*, *B. cinerea*, *Aspergillus niger* and *Aspergillus flavus*, in comparison with untreated seeds. *I. viscosa* extracts significantly inhibited seed germination of various crops. They also inhibited development of *R. stolonifer* and *B. cinerea* on postharvest grapes and tomatoes. There was no significant difference between plants collected at 18 different locations in Israel. Since among 12 pathogenic fungi *R. stolonifer* was found to be the most susceptible to the *I. viscosa* extract, this fungus was used for bioassays of the extract activity. *(L)*

Local and Systemic Control of Powdery Mildew (*Leveillula taurica*) on Pepper Plants by Means of Foliar Spray of the Fertilizer Monopotassium Phosphate (MKP)

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Powdery mildew caused by *Leveillula taurica* (Lév.) Arnaud is a major disease and a limiting factor of field- and greenhouse-grown peppers. Foliar spray of 1% solution of the fertilizer monopotassium phosphate (MKP, KH_2PO_4) on the lower leaves of greenhouse-grown peppers provided local and systemic protection against *L. taurica*, as compared with control plants. MKP gave 100% control of powdery mildew on treated lower leaves and 50% of systemic protection on upper leaves. This followed a single application on lower leaves of plants which had been exposed on the same day or up to 48 h post-treatment to natural infection by heavily diseased plants. However, application of MKP 1–3 days before the exposure to the source of inoculum was less effective in controlling powdery mildew. MKP suppressed powdery mildew as judged by inhibition of the development of new symptoms, as well as sporangial production of the fungus on infected tissue. Microscopic examination showed destruction of both hyphae and conidial-structures on MKP-treated leaves. MKP was less effective than a fungicide treatment in controlling powdery mildew on greenhouse-grown plants. Alternating the phosphate fertilizer with the systemic fungicide, however, enhanced the inhibitory effect against the mildew and results were similar to those achieved with the commercial fungicide treatment. The alternation treatment, in which the number of fungicide applications was reduced by 50%, offers new possibilities for disease control and reduction of pesticide usage. Phosphate solutions were not phytotoxic to plant tissue and did not affect the yield, as compared with the commercial treatment. However, lower yields were recorded in the control (untreated) plots, due to mildew infection on leaves. Our findings indicate that MKP spray may be employed as an alternative practice for the control of powdery mildew in peppers. *(L)*

Effect of Modified Atmospheres for the Control of Black Spot, Caused by *Alternaria alternata*, on Stored Persimmon Fruits

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Modified atmosphere packaging (MAP) of persimmon resulted in the accumulation of acetaldehyde to a level of 80 μg/ml, of ethanol to a level of 900 μg/ml, and of CO_2 up to 30%. When fruits were stored at -1°C for 4 months in such atmospheres, the incidence of black spot disease caused by *Alternaria alternata* was reduced. The effects of each of these gases were examined in order to determine their individual involvement in the inhibition of *A. alternata* development during storage. When *A. alternata*, grown at 20°C on PDA or inoculated into persimmon fruit, was exposed for 24 h to different levels of each volatile, acetaldehyde was the most fungistatic but only at higher concentrations than those that accumulated under MAP; CO_2 was moderately inhibitory at concentrations from 10% up to 60%, whereas ethanol had no effect. Similar inhibitory effects were obtained with acetaldehyde, at 620 μg/ml, or 30% CO_2, when *in vitro* cultures of *A. alternata* and infected fruits were exposed for up to 2 weeks at 20°C, but 1000 μg/ml ethanol had an only transitory inhibitory effect under these conditions. From our analysis of the effect of concentration *vs* time for each gas accumulating in MAP, it is suggested that the increasing concentration of CO_2 during storage is the principal factor in the inhibition of black spot disease development. *(L)*

BOTMAN for the Integrated Control of Foliar Pathogens in Vegetable Greenhouse Crops

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A decision support system named BOTMAN was developed for the management of diseases in vegetable greenhouses. The BOTMAN system provided recommendations for the control of diseases caused by *Botrytis cinerea* and leaf mold (*Fulvia fulva* = *Cladosporium fulvum*) on tomato and of white mold (*Sclerotinia sclerotiorum*) and *B. cinerea* on cucumber. According to the instructions obtained from the BOTMAN system, chemical fungicides or a biocontrol agent (Trichodex = *Trichoderma harzianum* T39) are applied during the growth season. The decision whether to apply a biological or a chemical agent is taken before each spray according to the following criteria: The suitability of weather conditions for disease development (4 days future weather forecast), the severity of the diseases, the susceptibility of the pathogen to fungicides and their relative efficacy, the nature of the greenhouse and the crop. When weather is expected to be extremely promotive to the pathogens (heavy rains, optimal temperatures), the greenhouse is poorly ventilated and the inoculum density is high, then the use of highly effective chemicals is recommended. Under other conditions, moderately effective fungicides are used and when conditions are expected to be moderately conducive to development of the diseases, the use of the biocontrol agent is recommended.

BOTMAN was tested in 12 experiments in the seasons of 1993–1996. On the average, *ca* ten sprays were applied in each experiment in the standard treatments (alternation of various fungicides on a calendar basis). In plots treated according to BOTMAN, four chemical sprays and six biological sprays were applied. Respective control of white, leaf and gray mold was 55%, 65% and 70% in the standard treatment, and 63%, 60% and 64% in treatments according to BOTMAN recommendations. The difference between these two treatment regimes was insignificant. Trichodex contributed significantly to the control achieved by the fungicides in BOTMAN. By relying on this system for management of diseases, 60% of the chemical sprays were saved. *(L)*

Steam Treatment for Reducing Postharvest Decay in Potato and Carrot

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Potato and carrot are leading crops grown for export, the local market and food processing. Decay caused by bacteria and fungi is one of the major problems associated with storage and shelf life of these crops. In Israel and worldwide, continual efforts are made to reduce the use of chemicals in agriculture, making it imperative to find alternative methods of prolonging the storage and shelf life of vegetables. In a study aimed at finding effective methods, the results indicated that steam treatments considerably decrease decay incidence in stored potato and carrot. For potato, the optimal treatment consisted of exposing the tubers to steam for 4–6 seconds. After 60 days of storage at 8°C and an additional 14 days of shelf life at 24°C, decay incidence in the steam-treated tubers was 0.5%, compared with 8% in the control. Overexposure to steam (more than 10 seconds) damaged the tissue and increased decay. The optimal treatments for carrot were exposure to steam for 3 seconds or dipping in 0.1% Rovral (iprodione) plus 0.02% chlorane (a commercial postharvest treatment of carrot). After 1 month of storage at 0.5°C plus 7 days of shelf life at 24°C, decay incidence was 4% and 2% in the 3-second steam treatment and the commercial treatment, respectively, whereas in the nontreated control it was 47%. The incidence of decay in carrots overexposed to steam (5 seconds or more), which damaged the tissue, was greater than in the control. *(L)*

H. DISEASE CONTROL BY FUNGICIDES

Control of *Botrytis cinerea* in Cut Rose Flowers by Means of Vapors of Pyrimethanil

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Botrytis blight (*Botrytis cinerea* Pers. ex Fr.) causes severe damages to rose flowers. Latent infection which develops during storage and shipping may result in severe economic losses. The use of the fungicide pyrimethanil (as Mythos 300SL) was examined for control of Botrytis blight of cut rose flowers. It was found that development of the disease on petals can be prevented by vapor action of pyrimethanil. Initially the fungicide was found to be active in closed humidity chambers when it was applied to the walls of the containers and not directly on the petals. Later the fungicide was sprayed (0.2%, 100 μ drops, 1000 drops/cm^2) on the paper and boxes which are used for packing rose bunches (20 flowers/bunch; 20 bunches/box). Following treatment, to simulate transport conditions, flowers were kept for 2 days at 2–3°C followed by 12 days of incubation at 20°C and high relative humidity to induce the appearance of Botrytis blight. In spite of the conditions very conducive for disease development, the vapor treatment reduced blight development by 50%. Larger scale experiments are being conducted with flowers sent through the regular marketing chain. *(L)*

Ohayo (Fluazinam), a Fungicide for the Control of *Botrytis* and Soil Diseases

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Ohayo is a product of ISK, Japan, and belongs to the pyridinamine group. It is formulated as an emulsifiable concentrate containing 500 g a.i./l fluazinam. Ohayo inhibits the process of oxidative phosphorylation and is effective against a wide range of plant pathogens. The product is highly effective, especially in prophylactic applications, is not systemic, but withstands rain and irrigation, and has a long-lasting effect. It has been tested for the past few years for control of diseases in vegetables, field crops and vineyards, and was found effective against various diseases and safe for the tested crops. Ohayo is registered in Israel for the control of *Phytophthora infestans* in potatoes, and *Botrytis cinerea* in vines. When sprayed at the rate of 0.1% it showed high efficacy in prevention of Botrytis rots on bunches before harvest: in trials conducted in wine grapes in Ramat HaGolan, with Ohayo sprayed at 0.1% throughout the crop season (starting from the flowering stage), there was a reduction of >60% in the infestation level of *Botrytis* in the bunches, in comparison with the untreated plots. In another trial in Ramat HaGolan, in which it was sprayed at 0.1% at the flowering stage, and afterwards at the end of the growing season, there was a decrease of >70% in the number of infested bunches in comparison with untreated plots, and a decrease of 90% in the level of infestation per bunch. A single spray with 0.1% Ohayo in vineyards at the end of the growing season, led to a 70% decrease of bunch decay in storage. When it was sprayed four times towards the end of the season, and after rains, decay was prevented almost totally. After 7 weeks' storage, the treated bunches were free of disease, compared with 80% decay in the untreated bunches.

In trials where potato seeds were dipped in 0.5% Ohayo solution, the level of *Rhizoctonia solani* infestation at harvest was 1.5%, compared with 11% in the untreated plots. In another trial, Ohayo 1% + Nebijin (flusulfamid) 2% was sprayed at ULV on potato seeds before sowing. At harvest only 3% *Rhizoctonia* infestation was recorded in the treated plots, in comparison with 74% infestation in the untreated ones. ULV sprays of Ohayo 0.5% and 2% on peanut seeds caused a significant decrease in plant collapse in the field. Approximately 15% of the seedlings collapsed in the untreated plots, as a result of *Aspergillus niger* infestation, whereas in the treated areas only 2.5% plants collapsed.

Ohayo was very effective in preventing soil disease caused by *Monosporascus* in melons grown in the Arava Valley. The product, applied through the drip irrigation system at the rate of 1.5 *l*/ha, reduced plant collapse by 96% in comparison with standard treatment, in which benomyl and propanocarb were given in drench application. *(L)*

Copman – A Formulation for the Control of Diseases in Potatoes

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Copman (=1066), an exclusive development of Jewnin-Joffe Industry Ltd., Israel, and of Urania Agrochem, Germany, contains two active ingredients: 22.3% mancozeb – for the control of fungal diseases; and 33.5% copper, as copper hydroxide – for the control of bacterial diseases. The proportions between these two ingredients were determined according to a series of laboratory tests of formulations in different proportions. Copman (WP) was the most efficacious formulation; its WP contains special stickers in addition to the active and inert ingredients.

Potatoes are attacked by *Phytophthora infestans*, by *Alternaria solani*, and by bacterial diseases, of which *Erwinia* spp. is the most important. For the control of *P. infestans* and *A. solani*, protectants, especially dithiocarbamates, are widely used. For the control of bacteria, copper products are added. Copman was tested in potatoes for 2 years, in autumn and in spring, in different areas throughout Israel. The plots were sprayed either with a knapsack or with a commercial sprayer. In all the trials conducted, Copman at 30 kg/ha was efficient in the prevention of *P. infestans* and of *A. solani*. In locations where *Erwinia* spp. were present, they too were efficiently controlled.

In addition to the product's efficacy and convenience, its use reduces the amount of toxicants released into the environment. *(L)*

The Use of Protectant and Systemic Fungicides to Control Powdery Mildew in Fruit Trees and Vines

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The intensive use of EBI (ergosterol biosynthesis inhibitor) fungicides for powdery mildew control during the last few years may have facilitated the appearance of fungal resistance. To reduce this selection pressure, treatments with Karathane (dinocap) and Karamat (dinocap + fenbuconazole) have been examined. Karathane has curative and protective activity and fairly short persistence; Karamat exerts a triple effect due to the systemic penetration of fenbuconazole (Indar).

Field trials have been conducted at different locations in Israel during the last 5 years, in fruit trees and vines, to compare the efficacy of eight different EBI fungicides: Anvil (hexaconazole), Atemi (ciproconazole), Dorado (pyrifenox), Folicur (tebuconazole), Indar (fenbuconazole), Ofir (penconazole), Systhane (myclobutanil) and Vectra (bromuconazole) and the protectant (90% W.P.) sulfur product, Gofrativ – with that of Karathane and Karamat. Sequential treatments of Karathane and Karamat were given at concentrations from 0.03% to 0.08% and at a frequency of 7 to 16 days, the frequency of spraying depending on the crop and the infection level. In most of the trials, no significant difference was found between the antifungal effect of Karathane or Karamat and the systemic fungicides. Therefore, the integration of this type of protectant fungicide is strongly recommended for control of powdery mildew in fruit trees and vines in order to avoid or delay the development of fungal resistance. *(P)*

Variation in Sensitivity of *Venturia inaequalis* to DMI and Strobilurin Fungicides

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Venturia inaequalis (scab) can cause heavy infections of apple fruit and leaves. In Israel it is prevalent in orchards in two regions: in the coastal plain and internal valleys, planted with cv. 'Anna'; and the Golan region, planted with introduced cultivars such as 'Red Delicious' (RD). Leaves of RD that overwinter on the floor of the orchard produce perithecia routinely and ascospores serve as the primary inoculum every spring in the Golan region. In contrast, ascospores of *V. inaequalis* were not detected in leaves of Anna that overwinter on the floor of the orchards of the coastal plain. In Anna orchards, leaves infected with scab from the previous autumn and early winter (October through January), and still hanging on the trees, serve as the source of conidial inoculum causing primary scab infections of the newly emerging buds. Frequent fungicide spray treatments are required to avoid heavy crop losses. During the early 1980s demethylation inhibitor (DMI) fungicides were introduced for the control of apple scab. DMIs remain the main class of fungicide for the control of apple scab and powdery mildew; consequently the pathogen population has been and continues to be widely exposed to these fungicides.

Single-spore isolates and scab samples from the two regions in Israel have been compared with samples from the UK including isolate E1, obtained in 1949 and therefore never exposed to DMI fungicides. Most of the isolates from Israel that showed reduced sensitivity to DMIs were from the

RD orchards in the Golan Heights; only one such isolate was obtained from an Anna orchard: it was less sensitive *in vitro* but sensitive *in vivo*. Isolates with reduced sensitivity to DMI fungicides have been found also in the UK. Isolates with reduced sensitivity to myclobutanil were also found to be less sensitive to other DMI fungicides in both seedling and agar-media tests. Two strobilurin analog fungicides (a new class of fungicides which inhibits the cell respiration of fungi) – azoxystrobin (AS) and kresoxim methyl (KM) – have already been tested in Israel and found to be very effective for control of apple scab. The sensitivity of single-spore isolates was tested on media amended with AS or KM. These *in vitro* tests showed a range of sensitivities to strobilurins among Israeli and UK isolates. Seedlings inoculated with single-spore isolates or populations of *V. inaequalis* and tested with AS or KM, are being used to forecast the risk of resistance to the strobilurins. *(P)*

I: PHYSIOLOGY, GENETICS AND RESISTANCE

Effect of Day Length on Black Dots of Potato

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Potato black dot caused by the fungus *Colletotrichum coccodes* (Wallr.) S.J. Hughes, infects leaves, stems, roots and tubers, leading to early plant death and, as a consequence, reduced yields. The response of four potato cultivars to *C. coccodes* was tested under field conditions during spring and autumn, the main growing seasons. The results indicated that in autumn, disease severity and fungal colonization levels were increased, and the final yield was lower than during the spring season. This occurred in cvs. 'Nicola', 'Cara' and 'Désirée'; only in the case of one cultivar, 'Alpha', was yield reduced in spring 1995. Similar results were obtained in experiments which exposed potato to Verticillium wilt. In controlled growth chambers where illumination length was the only variable, the results were similar. Growth conditions of 8:16 L:D increased disease severity in roots and stems, enhanced fungal colonization levels, and caused rapid formation of sclerotia, as compared with 16:8 L:D conditions. These effects of daylength were observed in four potato cultivars: Désirée, Agria, Alpha and Nicola. Effects of different lighting regimes were also observed in potato micropropagants of the same cultivars grown in test tubes under aseptic conditions in growth chambers. *(P)*

Induced Susceptibility of Avocado Fruits to *Colletotrichum gloeosporioides* by Methyl Jasmonate

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Avocado anthracnose caused by *Colletotrichum gloeosporioides* is the most important postharvest rot of avocado. The pathogen infects fruit during the growth period but remains quiescent until harvest. Freshly harvested fruit is free of visible disease symptoms, but decay lesions develop rapidly during fruit ripening and softening. Quiescence of *C. gloeosporioides* infection in unripe avocado fruit has been suggested to be dependent on the presence of fungitoxic concentrations of the preformed antifungal compound 1-acetoxy-2-hydroxy-4-oxo-heneicosa-12,15-diene (*diene*). Its concentration in unripe avocado peel fruit decreased about tenfold during ripening; then disease symptoms became evident when it decreased to subfungitoxic concentrations. The *cis,cis* 1,4-pentadiene structure present in the *diene* configuration suggests that lipoxygenase (LOX) can oxidize

the compound. Increased LOX activity in extracts of avocado peel during fruit ripening accounts for the decrease in the *diene* level and the appearance of disease symptoms. Fruit susceptibility is dependent on avocado LOX activity, which under normal ripening conditions is regulated by a natural inhibitor with antioxidative activity, present in the avocado peel and identified as epicatechin.

It was observed that methyl jasmonate enhanced a LOX-responsive activity in the peel of avocado cv. 'Fuerte' which resulted in a faster decline in the level of the antifungal *diene* and an earlier development of *C. gloeosporioides* decay. The increment in LOX activity occurred following enhanced expression of mRNA LOX. For this purpose we isolated and characterized a 2.85 Kb full-length avocado cDNA clone encoding an avocado LOX. Present results suggest that fruit susceptibility might be affected directly by the regulation of LOX activity, since no effect of methyl jasmonate was observed on epicatechin levels during the induction of fruit susceptibility. *(L)*

Induction of Antifungal Compound Formation in Idioblasts of Avocado Mesocarp by Ethylene Treatment

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Resistance of avocado fruits to *Colletotrichum gloeosporioides* is related to the presence of the antifungal compound 1-acetoxy-2-hydroxy-4-oxo-heneicosa-13,14-diene in the peel of unripe fruits. The mesocarp of unripe fruits is susceptible to fungal attack since the antifungal compound is compartmentalized in specific idioblasts (oil cells). Treatment of freshly harvested avocado fruits with 40 $\mu l/l$ ethylene enhanced the level of the antifungal *diene* in the peel of unripe fruits. Exposure of isolated mesocarp and pericarp tissue to ethylene enhanced the level of the antifungal *diene* in the mesocarp only. In this tissue, 85% of the antifungal *diene* is compartmentalized in idioblast cells. We isolated and exposed them to ethylene. The level of the antifungal *diene* increased after 3 h of exposure. The increase in the level of the antifungal *diene* by ethylene was affected by the temperature and the length of exposure to ethylene. Ethylene treatment strongly increased the incorporation of [^{14}C]-malonyl CoA by idioblasts into a compound that co-chromatographed with the antifungal *diene*. Interestingly, the efflux of the labeled compound from the idioblast increased when the cells were incubated in the presence of mesocarp lipids. It is suggested that the ethylene can directly enhance the synthesis of the antifungal *diene* in idioblasts and affect the export of this compound to oily fractions of the fruits. *(L)*

Quantitative Genetic Analysis of Host–Parasite Relationships Using Frequency Distribution Curves of Offspring

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The genetic control of host–parasite relationships in oats and crown rust was studied. Two parameters of quantitative interaction were used: LP – the length of the latent period, and IE – the infection efficiency. Quantitative genetic control of traits may be determined by analyzing frequency distribution curves of the offspring in several generations. These distribution curves describe the expression of quantitative parameters in the offspring. The distribution curves are characterized by their symmetry or asymmetry, different width-to-height ratios, presence or absence of parental types, and transgressive segregation. The shape of the curves indicates dominance and/or recessiveness

of traits, frequency of genes, heritability, the effect of environmental factors, and provides a rough estimate of the number of genes involved.

LP and IE of parental rust cultures as well as of F_1 and F_2 cultures, were determined on three varieties of oats and the curves were drawn for each parameter on each variety. LP and IE were measured also for the parental oat varieties and their F_1 and F_3 generations, tested with the two parental rust cultures. Based on the frequency distribution curves, the conclusions about the genetic control of the LP parameter are: *(a)* In the pathogen control is polygenic, but with a small number of genes involved. Short LP is dominant, at least in several resistance genes; aggressiveness is therefore dominant. *(b)* In the host, there is a single major gene with susceptibility dominant. Several minor genes are expressed in F_3. Host susceptibility (short latent period) is dominant. The conclusions regarding the genetic control over IE are: (i) In the parasite control is polygenic, but with a small number of genes, and no dominance. It may be that the frequency of some of the alleles reducing the IE is higher than that of those promoting it. Some nongenetic factors are also involved. (ii) In the host, a few genes are involved in the genetic control of IE and resistance is partially dominant.

We are aware that the information derived from frequency distributions is not complete. Numerical values and accurate data are missing. To complete the general analysis, calculations based on performance are needed. *(L)*

Utilization of *Hordeum vulgare* subsp. *spontaneum* for Control of Barley Diseases in Ecuador

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Scarcity of resistance sources to important diseases in numerous crops has prompted the search for resistance genes in wild progenitors and relatives of crops in the latter's areas of origin. Israel is located in the center of origin and genetic diversity of *Hordeum vulgare* subsp. *spontaneum*, the ancestor of cultivated barley. The two taxa are cross-compatible. Israel abounds in wild *Ornithogalum* species - the alternate host of barley leaf rust incitant *Puccinia hordei*, which, as proven by our studies, has a wide host range. The prolonged co-evolution of the *Hordeum* main host – *Ornithogalum* alternate host – *P. hordei* pathogen, has resulted in the appearance of numerous types and levels of host protection matched by a wide pathogenicity range in *P. hordei*.

In the Andean mountains of South America, barley is the most important cereal crop cultivated the year round. It serves for human consumption and as animal feed. Susceptibility to leaf rust and stripe (yellow) rust, *P. striiformis*, of Ecuadorian commercial barley cultivars necessitates the introduction of resistance factors to these diseases into breeding programs. In a joint research project between Tel-Aviv University and INIAP, populations of *H. vulgare* subsp. *spontaneum* were selected for resistance to *P. hordei* of the hypersensitive and the slow rusting types. Meanwhile 24 selections combining hypersensitive resistance to leaf rust and stripe rust were crossed with Ecuadorian commercial barley cultivars susceptible to both rusts. Plants belonging to the F_5 generations of hybrids have shown resistance to leaf rust and some of them demonstrated also resistance to stripe rust. *(L)*

The Production of Auxin in Transgenic Fungi and Its Influence on Fungal Pathogenicity

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As natural plant pathogens, fungi hold potential as biocontrol agents of weeds in cultivated fields. Highly virulent fungal pathogens may rapidly and efficiently eliminate large weed populations, while natural host specificity ensures that non-hosts are left untouched. Many weed pathogens have been identified, but most are either not specific enough (cultivated plants are killed) or not virulent enough (weed control is incomplete) to be effective as biocontrol agents. The insertion into and high-level expression of virulence genes in fungi may help boost the virulence of specific weed pathogens, rendering many fungal species better candidates for weed control. Among the many potential virulence factors are plant hormones. Phytopathogenic bacteria produce such hormones during the course of infection and they are known to contribute to disease development.

In this study we tested the potential of the plant hormone indole-3-acetic acid (IAA) to enhance fungal virulence. The fungus *Cochliobolus heterostrophus* was transformed with the IAA biosynthesis genes *iaaM* and *iaaH* of the bacterium *Pseudomonas savastanoi*. Transgenic isolates of opposite mating type containing either *iaaM* or *iaaH* were crossed and progeny obtained. Transformants that produce indole-3-acetamide (IAM) and/or IAA were identified and their pathogenicity to host (corn) and non-host (pea) plants was tested. Isolates that contained both genes and produced IAA showed increased virulence on corn, whereas IAA had no effect on the pathogenicity of the fungus to pea. These results indicate that fungal virulence may be enhanced without affecting host specificity. Superior biocontrol agents with the desired level of virulence and host range may be obtained through similar methods. *(L)*

Effect of Soil Solarization on Endogenous Cytokinins and Gibberellins in Tomato

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Soil solarization, a non-chemical and environmentally sustainable method of soil disinfestation, promotes plant growth even in the absence of major pathogens. This phenomenon was termed increased growth response (IGR), and is initiated by a number of biotic and abiotic soilborne factors. In the tomato, IGR is recorded first in the shoots and subsequently also in the roots. Yet the early expression of the IGR in the shoots depends on a signal from the roots, the main plant organ in direct contact with the soil. Using soybean-callus bioassay and radioimmunoassay, we demonstrated higher cytokinin (CK)-like activity in root extracts from 15-day-old plants grown in solarized *vs* non-solarized (control) soil. However, no consistent differences of measurable CKs between the two treatments were observed for either xylem sap or leaf extracts. Combined gas chromatography–mass spectrometry analyses of gibberellins (GAs) in extracts of the pooled shoot tips and true leaves of 7-day-old transplants revealed a higher level of GA_1 and a lower level of GA_{19} in plants from solarized soil compared with the control. The former was the dominant potentially active GA in leaves at this age. Six to seven days later, the endogenous concentrations of GA_1 and GA_3 (the latter being the dominant active GA at this age) and, to a much lesser extent, also of GA_{19} and GA_{20} (precursors of GA_1 and GA_3), were higher in leaves from plants grown in solarized soil than in control. Changes in the two active GAs, GA_1 and GA_3, were highly correlated with the IGR in leaves; no consistent trend was observed, however, for GA_4 or GA_8.

Our results suggest the involvement of rootborne CKs and of leaf GA_1 and GA_3 in the IGR. The CKs may be the signal that initiates the early expression of the IGR in shoots from tomato seedlings grown in solarized soils, and the GAs may further promote it. *(L)*

Diversity, Distribution and Properties of *Verticillium dahliae* Vegetative-Compatibility Groups (VCGs) in Israel

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Vegetative compatibility analysis has been used to reveal genetic diversity in anamorphic populations of several fungi, including the important vascular wilt pathogen *Verticillium dahliae*. Several hundred isolates of *V. dahliae* from 12 host-plant species and soil, at 41 sites in Israel, were tested for vegetative compatibility using nitrate-nonutilizing (*nit*) mutants. An improved chlorate–containing medium (WAC) which was developed, enabled us to generate *nit* mutants from all the isolates tested. Three VCGs were found and identified as VCG2A, VCG2B and VCG4B by using reference strains of the OARDC international system: 26 isolates were assigned to VCG2A, 127 isolates to VCG2B, and more than 300 isolates to VCG4B. All VCG2B isolates were recovered from the northern part of Israel, and all VCG4B isolates – from the south. Isolates of the minor VCG2A were scattered among the two major VCGs, ranging from 8% (north) to 3% (south) of the isolates tested. VCGs did not correlate with host-plant of origin: all crops from the south (cotton, potato, eggplant, tomato, peanut, and weeds) were infected with VCG4B and seldom with VCG2A, whereas all crops from the north (cotton, eggplant, chrysanthemum, and weeds) were infected with VCG2B and sometimes with VCG2A.

Isolates belonging to different VCGs differed in some properties, including morphology, response to temperature, and virulence. Different pathotypes were found among 32 isolates, using cotton ('Acala SJ-2') and eggplant ('Black Beauty') as differentials. Most VCG4B and VCG2A isolates, irrespective of their origin, induced weak-to-moderate disease on both differentials, with somewhat stronger foliar symptoms on eggplant. On the other hand, several pathogenicity patterns were revealed among VCG2B isolates: all cotton isolates caused severe foliar symptoms, heavy stunting and often death of inoculated cotton plants. Two VCG2B isolates from eggplant resembled VCG4B in their pathogenicity, whereas two others caused severe disease on eggplant and weak or no symptoms on cotton. *(P)*

K: MOLECULAR BIOLOGY

Characterization of *Puccinia* Species by Teliospore Fatty Acids

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Fatty acid (FA) profiles obtained by gas-liquid chromatography (GLC) are used increasingly as a chemotaxonomic tool for bacterial identification and systematics. This tool has proved to be useful with fungi grown in standardized axenic cultures, but there are still only few publications on its use for fungal taxonomy. In the early 1960s Tulloch and Ledingham, compared the FA compositions of different spore stages of *Puccinia* species and concluded that they were too similar to be useful for classification purposes.

We analyzed the FA composition of uredospores, teliospores, aeciospores and pycniospores of several species of *Puccinia* from the Tel-Aviv University rust collection, using the GLC methods and software of MIDI (Microbial ID Inc., Newark, DE, USA). Our results indicate that within one

species each spore type has its own FA profile, distinct from the profiles of other spore types. The study was continued with teliospores, as this is the only preservable spore type common to both macrocyclic and microcyclic rusts. Two or more analyses of the same isolate, carried out at different dates, usually produced FA profiles which clustered within two euclidean units or less, as known for different analyses of the same bacterial strain. FA profiles obtained by averaging these of 15 or more con-specific isolates were characteristic to that species, even though the isolates originated in different countries and continents. After having produced a small library with profiles of five *Puccinia* species, additional isolates were correctly identified at the specific/subspecific level, using the numerical taxonomy programs of the MIDI software.

Two FAs, linolenic (18:3 *cis* 9,12,15) and oxiraneoctanoic acid (18:0 *cis* 9,10 epoxy), detected in substantial quantities in rust teliospores by Tulloch and Ledingham were not detected in this study. Analysis of extracts by GLC-MS, using similar GC parameters, revealed traces of up to 1% of 18:0 *cis* 9,10 epoxy. It has been shown by Muller *et al.* (1994) that different oil extraction methods from fungi differ in the recovery percentage of specific FAs, yielding different FA profiles. Despite this method-induced lack of two FAs, the profiles obtained using the MIDI system were distinct enough to allow their use for identification of species and possibly for systematic purposes as well. *(L)*

Molecular Diagnosis of Garlic (*Allium sativum*) Viruses in Israel and Evaluation of Tissue Culture Methods for Their Elimination

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Garlic (*Allium sativum* L.) sets no fruit or seeds and can be propagated only vegetatively. Garlic is infected by many different viruses which cause significant yield losses. There are many discrepancies as to the identification and characterization of these viruses in the literature. Most of them are of RNA genome, from the families Potyviridae, Carlaviridae, and Carla-like unclassified viruses. There is also proof for the existence of viruses of other families.

In Israel the main garlic cultivar grown is 'Shani', in which viral symptoms can be seen. It is impossible today to relate specific symptoms to a specific virus. Antibodies for identifying specific viruses have not been developed. There are no proven virus-free or single-virus-infected plants in Israel, necessary for completing Koch's postulates. In recent years a large number of garlic virus gene sequences have been published. This information facilitated the construction of primers for Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR), and probes for Northern blot analysis of a number of garlic viruses thought to be present in Israel. Single step RT-PCR has proven very useful in identifying local viruses. Immunocapture-RT-PCR has also been developed and found satisfactory. Various clones of Poty-, Carla- and Carla-like viruses have been obtained. The nucleotide sequences and the resulting amino-acid composition of the viruses in Israel were found to be similar, but not identical, to published data, necessitating sequencing of local strains to allow specific primer synthesis for PCR use. To obtain virus-free garlic, cv. Shani plants were grown from meristem and callus culture, either from heat-treated or untreated *in-vitro*-grown plants, or directly from cloves, with or without a ribonuclease preparation. The evaluation of the results of these treatments will be made by the molecular methods developed. The integration of these methods will enable the propagation of virus-free garlic cv. Shani in Israel. *(L)*

Mango Malformation – Visualization of *Fusarium subglutinans* in Infected Flowers and Branches by GUS Transformants

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Although more than 100 years have elapsed since mango malformation was first described, the development of *Fusarium subglutinans* – the causal agent of the disease – within malformed tissues is still obscure. The reason for such lack of information is due partly to the dispute among researchers concerning the identity of the causal agent. The unusual nature of the disease, characterized by a unique disease syndrome, reflects drastic hormonal-like changes which contribute to the dispute. In order to trace and follow the fungus in mango tissues, the GUS (β-glucoronidase) reporter gene was used. Virulent wild-type isolates of *F. subglutinans* were transformed with a plasmid containing both GUS and the hygromycin reporter genes. The transformants did not lose their virulence and after artificial inoculations typical disease symptoms developed. The fungus was viewed microscopically in flowers and in developing vegetative buds, cleared by chloral hydrate. The presence of the pathogen in different plant organs, including a preference for colonization around certain sites, was demonstrated and discussed. A method for quantification of transformants expressing GUS within infected mango bud and flower tissues is currently being developed. *(L)*

Isolation of a Gene Coding for an IVR-like Protein

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We have reported that localization of TMV in tobacco is associated with a 23K protein (IVR), that inhibited several plant viruses. This protein was also found in induced-resistant tissue of Samsun NN. Using polyclonal antibodies to IVR, a clone (NC330) was isolated from a cDNA library inserted into lamda ZAP vector. This clone had 1016 bp with an open-reading frame of 597 bp. Comparing the amino acid sequence with sequences in gene banks revealed similarity with two antiviral proteins from yeast SKI and SKI3. We subcloned NC330 into the protein expression vector pET22b and a specific band of approximately 22K was observed in immunoblots. This expression protein reduced TMV replication in biological tests. The NC330 clone hybridized with RNA from induced-resistant tissue of Samsun NN but not from non-induced tissue of Samsun NN, nor from infected or non-infected tissue of Samsun. These results were confirmed using Reverse Transcriptase – Polymerase Chain Reaction (RT-PCR). Hybridization of PCR products from genomic DNA of Samsun NN and Samsun using specific primers resulted in two bands, of approximately 3 Kb and 1 Kb. We suggest that at least two genes are involved in localization: one coding for the antiviral protein IVR, and the second the N-gene as a regulator. The results of the hybridization of the NC330 probe with RNA and genomic DNA support this suggestion. *(L)*

Isolation of a Pathogenicity Gene from the pPATH Plasmid of *Erwinia herbicola* pv. *gypsophilae*

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Erwinia herbicola pv. *gypsophilae (Ehg)* induces gall formation on gypsophila. Our previous studies demonstrated that pathogenicity of *Ehg* is associated with a plasmid (pPATH). This plasmid harbors a cluster of genes encoding IAA and cytokinin production. Insertional inactivation of IAA or cytokinin biosynthetic genes caused a reduction in gall size but did not eliminate gall formation. The pPATH contains at least six copies of a newly identified insertion sequence, IS1327 of the IS6 family.

A cluster of genes with high homology to the type III secretion system was isolated from the pPATH. Insertional mutants of these genes revealed non-pathogenic mutants. Tn3-spice mutagenesis of the pLA150 cosmid clone derived from the pPATH resulted in detection of additional pathogenicity loci. One locus of 3.2 kb was identified immediately downstream to the phytohormone's biosynthetic genes and was flanked by two copies of IS1327. Sequence analysis of the 3.2 kb fragment revealed one open-reading frame of 1.9 kb with no homology to any known genes. Insertional mutants spanning the 3.2 kb fragment eliminated gall formation on either gypsophila cuttings or seedlings. All mutants have been restored to pathogenicity *in trans* by the 3.2 kb clone. The ice nucleation activity of the mutants, using the reporter transposon Tn3-spice, was quantified by a droplet technique. The insertional mutants gave detectable levels of ice nucleation activity. The ice nuclei activity *in planta* was considerably higher than *in vitro*. Further studies are aimed at isolating the protein encoded by this gene and elucidating its function. The possible secretion of the protein through the type III secretion system will be investigated. *(L)*

By title only

Monitoring of the grape mildew, *Uncinula necator*, in the vineyard
S. Ovadia and D. Shtienberg

The use of products of the triazole group to control the grape mildew, *Uncinula necator*, in the vineyard
S. Ovadia, Daphna Blachinsky, R. Hefez and Z. Ben-Arie

Prevention of the grape mildew, *Uncinula necator*, in the vineyard
S. Ovadia, R. Hefez, Daphna Blachinsky and Z. Ben-Arie