MEETING

ABSTRACTS OF PRESENTATIONS AT

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Invited Lecture

Biological Control of Postharvest Diseases of Fruits and Vegetables: Development and Commercial Implementation

S. Droby
Dept. of Postharvest Science, ARO, The Volcani Center, Bet Dagan 50250, Israel [Fax: +972-3-9683622; e-mail: samird@netvision.net.il]

The world trend is moving towards reduced pesticide use in fresh fruits and vegetables. In line with this trend, several physical and biological means have been evaluated as safer alternatives to chemical fungicides. Microbial antagonists for the control of postharvest diseases have received special attention, and have been investigated extensively. Most of the reported antagonists, yeasts, bacteria and filamentous fungi, occur naturally on fruit or other plant surfaces. Currently, there are only two biological products, Aspire^{TM} and Biosave 110 and 111, registered by the U.S. Environmental Protection Agency (EPA) and commercially available; they are both recommended for the control of postharvest rots of citrus and pome fruit. Additional microbial agents are being developed and some have reached semi- and full commercial testing and will be on the market in the near future. The implementation of biological control practices for postharvest diseases is lagging behind expectations, since the prospects for success are much higher than for foliar and soilborne diseases. Although commercialized biocontrol agents are few, there are several reasons for the limited use of this technology: the availability of cheap and effective fungicides; the reduced efficacy of biocontrol agents under commercial conditions; and the difficulty in accepting innovative control technologies, due to the changes involved. Microbial biocontrol agents of postharvest diseases have been criticized mainly for not providing as consistent, or broad-spectrum, control as synthetic fungicides. The first generation of biological control agents for postharvest spoilage has relied on the use of single antagonists. Perhaps it is unrealistic for us to expect disease control comparable to that achieved with synthetic fungicides, by the use of a single antagonist. It may be expected that the next generation of biologically based controls will utilize means of enhancing and perhaps synergizing control of postharvest diseases with antagonists. *(L)*

A: NONCHEMICAL ALTERNATIVES FOR METHYL BROMIDE AGAINST FUNGAL DISEASES

Invited Lecture

With Methyl Bromide and Without It

G. Kritzman
Inst. of Plant Protection, ARO, The Volcani Center, Bet Dagan 50250, Israel [Fax: +972-3-9685543; e-mail: kritzgio@netvision.net.il]

In the battle over the continued use of methyl bromide, the 'green' organizations have won. This victory exacts a heavy price to both agriculture and the environment. The decision of the Vienna

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

convention, *viz.*, gradual reduction of the use of methyl bromide until phaseout in the year 2005 and prohibition of it gave rise to two national committees: *a*, for better application of methyl bromide; and *b*, for finding substitutes for methyl bromide. Methyl bromide has some unique, attractive properties: a wide spectrum of activity, low price and easy application. That distinctiveness built up modern agricultural practice of 'insurance' treatments. The farmer does not make any efforts to identify the pests in his fields but, instead, before each crop he treats his ground with methyl bromide. This common situation of using methyl bromide as 'insurance' is no longer valid. From now on, the grower – with the extension services – will have to identify the pest and seek a solution. In my opinion, the world without methyl bromide will be neither cleaner from pesticides, nor any greener. *(L)*

Non-Chemical Approach to Soilborne Pest Management: Combining Organic Amendments with Soil Solarization

A. Gamliel,[1] Miriam Austerweil[1] and G. Kritzman[2]

[1]*Laboratory for Pest Management Research [Fax: +972-3-9604704; e-mail: agamliel@agri.huji.ac.il] and* [2]*Dept. of Plant Pathology ARO, The Volcani Center, Bet Dagan 50250, Israel*

Combining organic amendments with soil solarization is a non-chemical approach to improve the control of soilborne pests. Heating of soils covered with plastic film and amended with appropriate organic material, actuates a chain reaction of chemical and microbial degradation leading to the generation of toxic compounds in the vapor and liquid soil phases. Generation of toxic compounds is increased with temperature. These toxic compounds accumulate under the plastic mulch, and enhance toxic activity against soil flora and fauna, especially soilborne plant pathogens. The plastic mulch traps the volatile compounds and creates an atmosphere in the soil which enhances the degradation of the organic matter. The toxicity of the generated volatile compounds is expected to be higher under the high soil temperature prevailing during solarization. At the end of the process, the soil contains less pathogens, and different microflora, which may suppress renewed establishment of pathogens in this soil. Significant qualitative and quantitative differences in volatile compounds from heated and non-heated soil amended with plant residues (crucifers, and aromatic herb plants) or high nitrogen organic material (poultry manure, soymeal) were evident in both controlled-environment studies and field experiments. Microbial activity in heated or solarized soil amended with plant residues increased after the first 2 weeks of incubation, or solarization, as compared with non-amended soil. Apparently microbial activity is involved in pathogen control during solarization of amended soil. The effect of solarization of organic amended soil as a control method was tested under field conditions. Combining soil solarization with soymeal successfully controlled Verticillium wilt, *Rhizoctonia* and potato scab in two naturally infested soils. These treatments were effective in controlling cyst nematodes in a second sorghum crop without any additional treatment before the second crop. The control of the cyst nematodes benefited also a third wheat crop in the same plots, indicating the long-term effect of the combined treatment. *(L)*

Soil Solarization Using Sprayable Plastic Polymers to Control Soilborne Pathogens in Field Crops

Yael Skutelski,[1] A. Gamliel,[1] G. Kritzman,[2] I. Peretz-Alon,[3] E. Becker[4] and J. Katan[5]

[1]*Laboratory for Pest Management Research [Fax: +972-3-9604704; e-mail: agamliel@agri.huji.ac.il] and* [2]*Dept. of Plant Pathology, ARO, The Volcani Center, Bet Dagan 50250;* [3]*Hevel Ma'on Enterprises, Magen, M.P. Negev 85465;* [4]*NANA Assets and Management, Nir Oz 85122; and* [5]*Dept. of Plant Pathology and Microbiology, The Hebrew University of Jerusalem, Faculty of Agricultural, Food and Environmental Quality Sciences, Rehovot 76100, Israel*

Soil mulch for soil solarization using spray application of degradable polymers offers a feasible and cost-effective alternative to plastic tarps. The polymer is applied by spraying the desired

quantity. The sprayed polymer forms a membrane film, which maintains its integrity in soil and elevates soil temperature. Nevertheless, the membrane is porous and allows overhead irrigation. We developed a technology for the economic application of black polymer formulation, which is able to raise soil temperature and retain soil moisture under summer conditions (hot and dry). This process of solarization can significantly reduce populations of soilborne phytopathogenic fungi. The effect of soil solarization using sprayable polymers was tested in controlling soilborne diseases in potato and peanut crops. Soil solarization using sprayable mulches was effective in controlling Verticillium wilt in potato and peanuts. Combining solarization with formalin at reduced dosage (1000 l/h) was effective in controlling potato scab and peanut pod wart. The level of control of these diseases was similar to that obtained by solarization using plastic films. A long-term field study was established to test the effect of soil solarization combined with fumigants (formalin) or organic amendments (poultry manure and soymeal) on root diseases in potato and peanuts in continuous cropping. Solarization with sprayable polymers had a long-term effect in controlling *Verticillium* and *Rhizoctonia* in three successive potato and peanut crops. These combined treatments were successful in controlling potato scab and peanut pod wart after two or three successive crops. Combining soil treatment with appropriate crop rotation further improved pathogen control. *(L)*

Grafted Melon Plants – Current Status

R. Cohen,[1] M. Edelstein,[1] Carmela Horev,[1] Y. Burger,[1] J. Hershenhorn,[2] Shoshana Shriber,[1] S. Pivonia[3] and J. Katan[4]

[1]*Dept. of Vegetable Crops, [Fax: +972-4-9836936; e-mail: cron@netvision.net.il] and* [2]*Dept. of Weed Research, ARO, Newe Ya'ar Research Center, Ramat Yishay 30095;* [3] *'Arava R&D, Sappir Center, Central Arava 86825; and* [4]*Dept. of Plant Pathology and Microbiology, The Hebrew University of Jerusalem, Faculty of Agricultural, Food and Environmental Quality Sciences, Rehovot 76100, Israel*

The use of grafted plants has increased recently as one of the alternative methods enabling vegetable culture without soil disinfestation with methyl bromide. Watermelons grafted on *Cucurbita* rootstocks are grown commercially as a solution for the 'soil fatigue' phenomenon, and the yield is similar to or even higher than that of non-grafted watermelons. The research on grafting of melons is conducted in the 'Arava (southern Israel) and in the Bet Netofa Valley (northern Israel), with the purpose of combatting the wilt diseases caused by *Monosporascus cannonballus* and *Fusarium oxysporum* f.sp. *melonis*, respectively. The results achieved with melons grafted onto *Cucurbita* rootstocks have so far not been reproducible. In the 'Arava experiments, Monosporascus wilt incidence was significantly lower in grafted as compared with non-grafted plants. However, the efficacy of grafted melons in reducing disease incidence varied in different locations and growing seasons. In the Bet Netofa Valley experiments, *Cucurbita* rootstocks did not provide sufficient levels of protection against Fusarium wilt and the growth of grafted plants was inhibited. At this stage it is not clear whether the inhibition is due to the response of the rootstock to the pathogen, poor rootstock-scion compatibility, or infection of the grafted plant *via* adventitious roots originating from the scion. Melons grafted on melon rootstocks were investigated as well. Susceptible melons grafted onto *Fusarium*-resistant rootstocks did not collapse and yielded high quality melons as compared with total loss of the susceptible non-grafted control. In the 'Arava experiments, melon rootstocks exhibited the same efficacy in reducing *Monosporascus* incidence as the best *Cucurbita* rootstocks. Further attempts at obtaining grafted melon plants with acceptable resistances and yield should combine phytopathological and horticultural research, establishing customized growing practices for grafted melons. *(L)*

Grafting Sweet Basil as a Solution for Basil Wilt Disease

Evgenia Dor,[1] J. Hershenhorn,[1] D. Haimovich[2] and N. Dudai[2]

[1]*Dept. of Weed Research and* [2]*Dept. of Herbs and Spices, ARO, Newe Ya'ar Research Center, Ramat Yishay 30095, Israel [Fax: +972-4-9836936; e-mail: evgeniad@netvision.net.il]*

Sweet basil (*Ocimum basilicum*) is a major crop in the fresh herbs industry in Israel. The main limiting factor in basil growing is the sweet basil wilt disease caused by the fungus *Fusarium oxysporum* f.sp. *basilici*. The common management practice to control the disease is methyl bromide, which will soon be banned, and solarization, an expensive method which requires time and special conditions. Resistant basil varieties do exist, but are of poor commercial value, whereas susceptible varieties often have high commercial value. In such situations, grafting of sweet basil can serve as a practical approach. This study focused on grafting susceptible basil varieties with high commercial value on resistant stocks with low commercial value as a way to escape disease. Experiments were conducted in the greenhouse in pots and on detached bedding and in the field at the Newe Ya'ar Research Center. Under these growth conditions, mortality of the susceptible non-grafted sweet basil variety 'Arba' was high (65–100%). The same mortality level was observed when resistant and susceptible plants were grafted on the susceptible stock. The resistant variety 'Nufar' had a high level of resistance toward the disease. Mortality of the non-grafted resistant plants, as well as of resistant and susceptible plants grafted on resistant stock, did not exceed 10%. The dynamics of fungal infestation differed between the two varieties. Fungal hyphae in susceptible plants spread quickly and after 2 weeks was found throughout the root system and up to the first two true leaves. The hyphae in resistant plants could be found only at the upper third of the root system. Results of the field experiment indicated that grafting had no effect on foliage biomass of yield quality. Foliage weight of resistant plants grafted with susceptible scions was the same as that of non-grafted susceptible plants. No differences in the height, nodes number, essential oil composition and leaf area could be detected between grafted and non-grafted plants from the same variety. It is concluded that susceptible basil plants grafted on resistant basil stocks are protected from sweet basil wilt disease without any loss in yield quantity or quality. Therefore, grafting of sweet basil should be considered as an optional method to control the disease. *(L)*

B: CHEMICAL ALTERNATIVES TO METHYL BROMIDE

Application of Dazomet (Basamid) for Soil Disinfestation

Yael Cornfeld,[1] A. Gamliel,[1] A. Grinstein [deceased], Miriam Austerweil,[1] Bracha Steiner,[1] L. Klein[2] and J. Katan[3]

[1]*Laboratory for Pest Management Research, ARO, The Volcani Center, Bet Dagan 50250;* [2]*Dead Sea Bromine Group, Be'er Sheva 84101; and* [3]*Dept. of Plant Pathology and Microbiology, The Hebrew University of Jerusalem, Faculty of Agricultural, Food and Environmental Quality Sciences, Rehovot 76100, Israel*

One of the potential alternatives to soil fumigation for disinfestation is Basamid (dazomet) granular. Basamid granular is a micro-granular dazomet formulation. It does not contain halogens, which can be an advantage with regard to environmental impact. Basamid granular is a relatively inert solid material, which undergoes degradation to the active agents when incorporated in soil. The breakdown of dazomet is rapid in moist aerobic soils, resulting in methyl*iso*thiocyanate (MITC) as the major breakdown product (98%) and other products (CS_2, HCHO, H_2S and NH_3) in very low amounts. The main objective of the current study was to develop application technologies to enable its future use for soil disinfestation in semi-arid and irrigated regions. Basamid movement in soil was studied in soil columns. The fumigant was applied to the top 5 cm of soil columns and leached with 100 ml aliquots of water at 15-min intervals. Leaching immediately after application resulted in partial control of fungal propagules in the top 5 cm. Effective pathogen killing was achieved when leaching was delayed to 8 h after application. Analysis of leachates at the bottom of the column

showed a linear increase in MITC as leaching was delayed. MITC generation was very low during the first 2 h following application. Therefore, sufficient time should be allowed for the accumulation of a toxic level of MITC. This phenomenon is of importance when Basamid is to be incorporated in soil by overhead irrigation. The lag time for MITC generation enables irrigation and incorporation in soil with minimal escape of MITC from the soil before plastic mulch is laid on the treated plot. The disinfestation activity of Basamid was tested in a small-plot study. Activity was significantly reduced when soil temperatures decreased in winter. When the maximum soil temperature dropped below 20°C, only partial control was achieved even at a depth of 30 cm. This indicates the need for higher dosages of Basamid when soil temperatures are low, or to apply the fumigant only when soil temperatures are favorable. *(L)*

ForDor, an Economical Substitute for Methyl Bromide

G. Kritzman,[1] Z. Bar,[2] O. Himan,[3] B. Kirshner,[1] I. Dornai,[1] V. Pekarski,[1] Miriam Kimchi[1] and M. Bar-Eyal[1]

[1]*Inst. of Plant Protection, ARO, The Volcani Center, Bet Dagan 50250 [Fax: +972-3-9683543; e-mail: kritzgio@netvision.net.il];* [2]*Smadar Agriculture Direct Marketing Ltd., Ashqelon 78103; and* [3]*Dor Chemicals Ltd., Haifa 26110, Israel*

Methyl bromide, a broad-spectrum pesticide for the control of insect pests, nematodes, weeds and plant pathogenic fungi, must be phased out by 2005. Possible alternatives to methyl bromide as a soil treatment are those treatments that have at least some of the activity of methyl bromide. Various formulations of ForDor (marketed by Smadar Ltd., Ashqelon, Israel) have proven effective against a wide range of bacterial, fungal and nematode pathogens and are economical alternatives to methyl bromide. They are *(a)* friendly to the environment, rapidly converting to CO_2 and H_2O without residues; *(b)* applied easily to the desired soil depth or media; *(c)* able to control a wide spectrum of pests (bacteria, fungi and nematodes); *(d)* an economical substitute for methyl bromide; and *(e)* recommended for pathogen-free quality seeds production. ForDor controls the following in peanuts: pod wart disease (*Streptomyces* spp.), *Pseudomonas* spp., *Pythium* and nematodes; in carrot: *Pythium* spp., scab (*Streptomyces*) and nematodes; in tomatoes: *Clavibacter, Xanthomonas* spp., *Pseudomonas* spp., *Erwinia* spp. *Xanthomonas* spp., *Verticillium, Fusarium, Pythium* and nematodes; and in potatoes: *Streptomyces* spp. (potato scab), *Pseudomonas* spp., *Erwinia* spp., *Pythium, Verticillium, Fusarium* and nematodes. *(L)*

Soil Fumigation with Telopik (C35) Prior to Potato Production

Leah Tsror (Lahkim), Orly Erlich, M. Aharon, Sarah Leviush-Mordechai, Y. Cahlon, A. Hadar, Y. Cohen, L. Klein and I. Peretz-Alon

Dept. of Plant Pathology, ARO, Gilat Experiment Station, M.P. Negev, 85280 Israel [Fax: 972-7-9926337; e-mail: tsror@bgumail.bgu.ac.il]

Potato early dying syndrome caused primarily by *Verticillium dahliae* and also by *Colletotrichum coccodes*, causes up to 50% yield loss in susceptible cultivars. These pathogens produce on decaying potato stems masses of sclerotia that infest the soil. One of the means of control is soil fumigation. The objective of the present study was to evaluate the efficiency of Telopik (C35) (61% 1,3-dichloropropene and 35% chloropicrin) in controlling soilborne pathogens, prior to potato growing. Two field experiments were conducted in autumn 1998, at Gilat and Besor. In the Gilat experiment, the incidence of *Verticillium*-infected plants and the incidence of dry stems carrying *V. dahliae* microsclerotia were significantly reduced by C35 and methyl bromide treatments. Colonization levels of the fungus in the control were significantly higher than in all fumigation treatments. The yield obtained in the control was 22% lower than in the other treatments. At Besor, the incidence of infected plants was reduced by treatments as follows: 55–30% with Fordor 37 (37% formaldehyde),

7.5% with Bromopik (70% methyl bromide and 30% chloropicrin), and zero with methyl bromide and C35. The incidence of infected dry stems was also significantly reduced. Yields obtained from plots treated with Bromopik, C35 or methyl bromide were significantly higher than those treated with Fordor and in the control. In summary, C35 effectively reduced *Verticillium* and thus can be used as a soil fumigant prior to planting potato. *(L)*

C: ALTERNATIVES FOR METHYL BROMIDE AGAINST NEMATODES

Chrysanthemum coronarium as 'Green Manure' to Control Plant-Parasitic Nematodes

Meira Bar-Eyal, Y. Spiegel, M. Mor and Edna Sharon

Dept. of Nematology, ARO, The Volcani Center, Bet Dagan 50250, Israel [Fax: +972-3-9604180; e-mail: vpspigl@netvision.net.il]

Chrysanthemum coronarium, which grows wild in Israel during the winter and spring months, was used as 'green manure' to control plant-parasitic nematodes, which cause much damage to agricultural crops. *Chrysanthemum* application to soil infested with the root-knot nematode, *Meloidogyne javanica*, resulted in a significant reduction in galling indices and an increment in the fruit and top fresh weights. Experiments were conducted in pots in the greenhouse as well as in microplots, where the plants (fresh or dry) were chopped, incorporated in soil, and left for one month before planting. The activity of plants from different botanical families was compared with that of *C. coronarium*, which evinced the highest activity against the root-knot nematode. Hatching of *M. javanica* eggs, exposed *in vitro* to extracts of *Chrysanthemum* leaves, was significantly reduced compared with water controls. Second-stage juveniles (J2) exposed to the extracts were immobilized and 100% mortality was observed after 24 h. Primary characterization of the activity on J2 revealed a low molecular weight (< 3 kDa), heat resistance, and an optimum at pH 5. Acetone extract of dry *Chrysanthemum powder*, applied to root-knot nematode-infested soil, showed higher nematicidal activity than a water extract treatment, indicating the possible involvement of lipophilic components. *Chrysanthemum* may be used in organic or integrated pest management as a cheap and safe measure for nematode control, and to reduce the use of harmful chemicals. *(P)*

BioNem: A Biological Nematicide

M. Keren-Zur, J. Antonov, A. Bercovitz, A. Chusid, A. Grimberg, K. Feldman, G. Kenan, N. Markov and M. Revhun

Minrav – The Biological Division, Jerusalem 91291, Israel [Fax: +972-2-6733265; e-mail: mitab@inter.net.il]

The forced phaseout of methyl bromide has heightened the need for alternatives to soil fumigation. Possible alternatives that are known today can serve only as partial solutions and are not efficient in controlling nematodes, especially root-knot nematodes (*Meloidogyne* spp.). 'BioNem' is a biological nematicide based on a non-pathogenic isolate of the bacterium *Bacillus firmus*, formulated with non-toxic additives. A specially designed formulation ('BioSafe') that contains no synthetic ingredients, is available for organic farming. BioNem and BioSafe were proven effective in a large number of commercial field trials. These trials were focused mainly on the control of root-knot nematodes in vegetables. BioNem is suitable for integrated pest management and can be combined with fungicides and herbicides. BioNem can also be applied after soil fumigation. The application of the bionematicide after soil solarization is an option to chemical soil fumigation. *(P)*

β-aminobutyric Acid-Induced Resistance in Plants against Nematodes

Y. Oka,[1] Y. Spiegel[2] and Y. Cohen[3]

[1]*Dept. of Nematology, ARO, Gilat Experiment Station, M.P. Negev 2, 85280;* [2]*Dept. of Nematology, ARO, The Volcani Center, Bet Dagan 50250 [Fax: 972-3-9604180; e-mail: vpspigl@netvision.net.il]; and* [3]*Faculty of Life Sciences, Bar-Ilan University, Ramat Gan 529000, Israel*

The ability of several chemicals, including salicylic acid, dichloro*iso*nicotinic acid, benzothiadiazole and aminobutyric acids, to induce resistance in plants against nematodes was tested. Only the β-isomer of aminobutyric acid (BABA) induced resistance in plants against nematodes. Treatment of tomato plants with BABA by foliar spray (2,000 ppm) or soil drench (500 ppm) reduced the root damage caused by the root-knot nematode *Meloidogyne javanica*, and the number of nematode eggs on the roots. BABA also reduced the number of nematode juveniles penetrating roots, and inhibited nematode development. Feeding sites of the nematode in tomato plants treated with BABA were small and vacuous. Radioactive BABA applied on tomato leaves translocated to young shoots and the root system, and accumulated in the galls. BABA also reduced the number of egg masses of a root-knot nematode (*Meloidogyne* sp.) on cereals. Treatment of wheat and barley with BABA induced resistance against cereal cyst nematodes, *Heterodera avenae* and *H. latipons*. Soil drench of wheat with 125 ppm BABA reduced the number of *H. latipons* cysts by 93%, whereas 500 ppm BABA was needed to obtain a similar reduction of *H. avenae* cysts on wheat. Although these treatments did not affect the invasion and feeding site formation of these cyst nematodes, the nematode development in BABA-treated cereal plants was inhibited. These results suggest that BABA is a potential nematode control agent based on its novel mode of action. *(L)*

D: CHEMICAL DISEASE CONTROL

Rational management of *Didymella rabiei* in Chickpea by Integration of Genotype Resistance and Curative Application of Fungicides

D. Shtienberg,[1] H. Vintal,[1] S. Brenner,[2] A. Yaniv[3] and B. Retig[2]

[1]*Dept. of Plant Pathology [Fax: +972-3-9683688; e-mail: danish@netvision.net.il] and* [2]*Dept. of Field Crops, ARO, The Volcani Center, Bet Dagan 50250; and* [3]*Lidorr Chemicals Ltd., Ramat haSharon 47100, Israel*

Various aspects of the integration of genotype resistance to chemical control of Ascochyta blight (caused by *Didymella rabiei*) in chickpea were examined in field experiments conducted from 1993 to 1999. Four commercially available chickpea cultivars that represent a range of resistance to *D. rabiei* were used. The efficacy of chemical control in a highly susceptible cultivar was significantly ($P<0.01$) related to the conduciveness of the environment to the pathogen: adequate (>80% control) disease suppression was achieved when conditions supported mild epidemics, but insufficient (<20%) control was achieved when conditions supported severe epidemics. The contribution of genotype resistance to disease suppression in a moderately susceptible cultivar varied from <10% when conditions supported severe epidemics, to *ca* 60% when conditions supported mild epidemics. Integration of chemical control resulted in 95% control when conditions supported mild epidemics, but only 65% control when conditions supported severe epidemics. The existing level of resistance in a moderately resistant cultivar resulted in 70% control when conditions supported severe epidemics; fungicides improved control efficacy significantly to >95%. Under mild epidemics, genotype resistance alone provided >95% control. The level of genotype resistance available in a highly resistant cultivar was sufficient to suppress the disease adequately under all conditions, even without

application of fungicides. The possibility of relying on curative, rather than prophylactic, application of fungicides was tested in the greenhouse and in four field experiments. A curative treatment (*i.e.*, after rain or overhead irrigation events) was found to suppress the disease as effectively as regular application of fungicide sprays, and required fewer sprays. In two experiments the interaction between genotype resistance and chemical control under various amounts of irrigation applied *via* overhead sprinklers (as a simulation of rain) was tested. It was found that both the level of genotype resistance and the quantity of water should be taken into account in deciding whether to apply a curative spray. *(L)*

Efficacy of Polar, a Polyoxin B Compound, in Controlling Powdery Mildews in Apple and Nectarine Trees, and in Grapevines

M. Reuveni,[1] H. Cohen,[2] Tirza Zahavi[3] and A. Venezian[4]

[1]*Golan Research Institute, University of Haifa, Qazrin 12900 [Fax: +972-6-6961930; e-mail: mreuveni@research.haifa.ac.il];* [2]*Morag, Qazrin 12900;* [3]*Extension Service, Ministry of Agriculture and Rural Development, Zefat 13100; and* [4]*Tivonchem Ltd., Haifa 31333, Israel*

Field experiments were conducted to evaluate the efficacy of Polar, a polyoxin B compound, in controlling powdery mildews in commercial orchards and a vineyard during the years 1997–99 in the Golan region of Israel. Foliar sprays of 0.0125–0.05% (v/v) suspensions of Polar, sulphur, the new strobilurin fungicide kresoxim-methyl, and sterol inhibitor (SI) fungicides inhibited development of the powdery mildew fungi on leaves, fruits and leaves, and fruit clusters of field-grown apple trees, nectarine trees, and grapevines, respectively. The effectiveness of polyoxin B in controlling powdery mildews on apple trees and grapevines was similar to that of the standard treatment with the SI fungicides or sulphur. Applications of Polar on nectarine trees provided better control of *Sphaerotheca pannosa* (Wallr.) Lev. than did the SI fungicides. Alternating treatment with polyoxin B with the SI fungicide myclobutanil enhanced the inhibitory effect against *Podosphaera leucotricha* on apples and provided the best control of powdery mildew. Applications of Polar were not phytotoxic to plant tissue. Polar seems to be more effective against mycelial growth and sporulation of powdery mildew fungi than against germination of conidia. Polar at 1, 10 and 100 mg/*l* inhibited germination of *Uncinula necator* conidia by 52.5%, 76.6% and 100%, respectively. A foliar spray of 100 mg/*l* suspension of Polar on greenhouse-grown grapevine leaves prior to inoculation with *U. necator*, provided >90% protection against powdery mildew development. Spraying of Polar (100 mg/*l*) on mildewed leaves bearing sporulating colonies of *U. necator* suppressed the fungus. The inhibitory effectiveness of Polar makes it a good candidate for the control of powdery mildews on grapevines, apple trees and, especially, nectarine trees. *(L)*

Flint – A New Mesostemic Product of the Strobilurin Group for Control of Powdery Mildew

D. Baum, Y. Vilder, Ch. Kuraby, R. Rulf, A. Fried, A. Beracha and J. Baum

C.T.S. Ltd., Agricultural Department, Tel Aviv 61000, Israel [Fax: +972-3-9225964; e-mail: esti@cts.co.il]

'Flint' 50 WG (active ingredient: trifloxystrobin) is a new fungicide of the strobilurin group, which is a synthetic derivative of strobilurin A and Oudemansin (natural strobilurins extracted from wood-decaying fungi). The strobilurins interfere with the electron transfer in the mitochondria. Flint, a mesostemic fungicide, has a high affinity with the plant surface and is absorbed by the waxy layers of the plant. It re-distributes at the plant surface by superficial vapor movement and re-deposition. It penetrates plant tissue, has translaminar activity and there is little or no transport within the vascular system of the plant. Flint interferes with the fungus's life cycle at different growth stages. It serves mainly as a protectant, except for powdery mildew, against which it has some curative activity. Flint

is absorbed rapidly (within 1–2 h) into the waxy layer and is not washed off by rain or irrigation. Flint has been tested and found very effective for powdery mildew control. At very low rates, it achieved a high control level of powdery mildew not found in any of the DMI (dimethylation inhibitor) group of fungicides (triazoles and others). In addition, it was found to be effective against *Leveillula taurica* in tomato, pepper, eggplant and paprika, but required higher rates for control of powdery mildew due to the different modes of infection of powdery mildew and *L. taurica*: powdery mildew is very active on the surface of the plant, which has the highest rate of Flint, and *L. taurica* is active in the plant tissue, where Flint concentrations are much lower. *(L)*

Activity of Trifloxystrobin (Flint), a New Strobilurin Fungicide, against Foliar Diseases in Fruit Trees and Grapevines

M. Reuveni,[1] R. Rolf[2] and Tirza Zahavi[3]

[1]*Golan Research Institute, University of Haifa, Qazrin 12900 [Fax: +972-6-6961930; e-mail: mreuveni@research.haifa.ac.il];* [2]*C.T.S. Ltd., Petah Tiqwa 49517; and* [3]*Extension Service, Ministry of Agriculture and Rural Development, Zefat 13100, Israel*

Trifloxystrobin (Flint; Novartis), a new strobilurin mesostemic fungicide, is active against a wide range of fungal plant pathogens. The present study showed that it is highly effective in controlling powdery mildews on leaves, fruit and leaves, and bloom clusters of field-grown apple, nectarine and mango trees, respectively; rust disease on leaves of plum trees; and powdery and downy mildew in grapevines. In the majority of the field experiments conducted during 1998–99, trifloxystrobin (at a concentration of 0.01–0.015% v/v) was superior to demethylation inhibitor (DMI) fungicides and sulphur in controlling powdery mildews on each crop, and provided 95–100% protection compared with non-treated trees. Trifloxystrobin effectively inhibited rust on plum trees and provided 98% protection compared with the control. Laboratory experiments conducted to study how and by which physical mode of action trifloxystrobin is active against both grape powdery and downy mildew pathogens, revealed that a concentration of 0.1 μg of trifloxystrobin WG 50 per ml completely inhibited sporangial germination of *Plasmopara viticola.* Trifloxystrobin was very effective against the conidial germination of *Uncinula necator in vitro*, and inhibited mycelial growth and sporulation of both fungi *in vivo.* It showed strong prophylactic and local activity in intact plants and detached leaf disks. Trifloxystrobin had translaminar activity, but failed to translocate *via* the vascular system. It provided the best control against powdery mildew on fruit clusters under heavy infection pressure and was similar to metalaxyl-Cu treatment in controlling downy mildew on leaves of field-grown grapevines. The inhibitory effectiveness of trifloxystrobin renders it eminently suitable for inclusion in integrated control programs against foliar diseases in orchards and vineyards, and as a component of resistance-avoidance strategies. *(L)*

E: PHYSIOLOGY AND RESISTANCE

Invited Lecture

Molecular Solutions to Phytopathological Problems

A. Sharon

Dept. of Plant Sciences, Tel-Aviv University, Tel Aviv 69978, Israel [Fax: +972-3-6409380; e-mail: a55555@post.tau.ac.il]

The study of plant diseases at the molecular level has helped us to evolve new concepts of the way plant diseases develop; specific roles have been assigned to certain molecules and corresponding genes have been isolated. The new information and genes, together with advanced genetic engineering and molecular techniques, can now be used to perfect advanced solutions for pest management. Indeed, pest-resistant transgenic plants comprise more than half of the commercial and

field releases of genetically modified plants. An overview of the current status of genetically modified organisms for better pest control was given, together with specific examples. A large number of crop plants with genetically engineered insect and herbicide resistance are already commercially available. Bacteria-, nematode- and fungi-resistant transgenic plants are in advanced stages of field trials. Modifications of beneficial microorganisms by genetic engineering have also shown promising results. Transformation of weed and insect biocontrol fungi has been used to enhance virulence as well as to introduce reporter and pesticide resistance genes to the fungus. Biological control of the chestnut blight disease has been obtained by field release of a genetically modified fungal isolate. *(L)*

Cell-Wall-Degrading Enzyme Involvement in Black Spot Disease Caused by *Alternaria alternata*

D. Eshel,[1] J.M. Lorang,[2] Ruth Ben-Arie,[1] A. Dinoor[3] and D. Prusky[1]

[1] *Dept. of Postharvest Science of Fresh Produce, ARO, The Volcani Center, Bet Dagan 50250, Israel [Fax: +972-3-9683622; e-mail: esheld@agri.huji.ac.il];* [2] *Dept. of Botany and Plant Pathology, Oregon State University, Corvallis, OR 97331-2902, USA; and* [3] *Dept. of Plant Pathology and Microbiology, The Hebrew University of Jerusalem, Faculty of Agricultural, Food and Environmental Quality Sciences, Rehovot 76100, Israel*

Alternaria alternata causes black spot symptoms in persimmon fruits following prolonged storage at -1°C. An *A. alternata* transformant of a wild-type isolate expressing green fluorescent protein (GFP) showed that infection of fruit during storage can occur by production of an appressorium-like structure or through wounds. Preharvest treatment with gibberellic acid (GA_3) induced resistance of the fruit and prevented fruit softening. GA_3 did not affect germination of the spores on the fruits but did reduce colonization rate. Endoglucanase secretion in the presence of cell walls extracted from GA_3-treated fruits was 40% lower than with cell walls of untreated fruits. To determine the expression of cellulases during symptoms development, we used RT-PCR (reverse transcriptase-polymerase chain reaction). Five glycosyl hydrolase genes were cloned from the fungal plant pathogen *A. alternata;* these genes were expressed during the first 4 days after infection of persimmon fruits. An endoglucanase from *A. alternata*, with Mw 41 kDa, was purified from a culture-inducing medium. Purified endoglucanase was able to cause black spot symptoms on the fruit, similar to those caused by *A. alternata.* The present results suggest that symptoms caused by *A. alternata* in persimmon are dependent on the capability to secrete endoglucanase in developing lesions. *(L)*

The Cellulolytic Activity of *Fusarium proliferatum* and Its Efficacy in Controlling Grape Downy Mildew

U. Yechye and A. Sztejnberg

Dept. of Plant Pathology and Microbiology, The Hebrew University of Jerusalem, Faculty of Agricultural, Food and Environmental Quality Sciences, Rehovot 76100, Israel [Fax: +972-8-9466794; e-mail: abraham@agri.huji.ac.il]

A sporulating colony of *Plasmopara viticola*, the causal agent of grape downy mildew, covered with hyphae of *Fusarium proliferatum*, was found in 1991. A single spore isolate from that *F. proliferatum*, named G6, shows antagonistic traits against fungi belonging to the Oomycotina, including *P. viticola.* The antagonistic potential of G6 against grape downy mildew was demonstrated in laboratory experiments, and reduced the amount of sporangia by 80–97%. This study presents results of greenhouse experiments on grape plants, and shows cellulolytic activity of G6 as a possible mode of action of antagonism. In greenhouse trials performed on grape saplings, cv. 'Sultanina', a considerable reduction in *P. viticola* activity was found in plants treated with the G6 isolate, as

compared with untreated controls, expressed as inoculum reduction of the downy mildew pathogen. Ten percent of the downy mildew lesion area was actively producing sporangia after G6 treatment, in contrast to 68% of the area in the untreated control. *P. viticola* belongs to the group Oomycotina which are characterized by a cell wall containing cellulose. We investigated the possibility that the parasitism of *F. proliferatum* involved extracellular cellulases. The fungus was cultivated on liquid medium, extracellular proteins leached to the medium were precipitated, and after partial cleaning these proteins were separated by gel electrophoresis. An activity assay was carried out to verify cellulolytic activity. A band of approximately 50 kDa was shown to have a high level of enzymatic activity. The results of the greenhouse experiments are an additional step towards the possible development of a significant biocontrol method for grape downy mildew, a major disease of grapes. Our protein assay opens a window towards understanding the mode of *F. proliferatum* parasitism and to the possible interference of cellulases in the parasitism events. *(L)*

Glucanase Activity and Genetic Transformation of *Fusarium proliferatum*, a Biocontrol Agent of Grape Downy Mildew

S. Bakshi,[1] O. Yarden[1,2] and A. Sztejnberg[1]

[1]*Dept. of Plant Pathology and Microbiology and* [2]*Otto Warburg Center for Agricultural Biotechnology, The Hebrew University of Jerusalem, Faculty of Agricultural, Food and Environmental Quality Sciences, Rehovot 76100, Israel [Fax: +972-8-9466794; e-mail: yardeno@indycc1.agri.huji.ac.il]*

The *Fusarium proliferatum* G6 isolate shows antagonistic traits against fungi belonging to the *Oomycotina*, and among them *Plasmopara viticola*, the causal agent of grape downy mildew. At temperatures lower than 18°C (which are favorable for growth of *P. viticola*) there is a negative correlation between the temperature and growth capacity and antagonism of the G6 isolate. Following the exposure of *F. proliferatum* G6 microconidia to UV radiation, and screening for growth at low temperatures, an isolate (designated 1505) that excelled in linear growth rate at low temperatures (13–18°C) was obtained. In a pathogenesis test performed at temperatures ranging from 13 to 25°C, we found that isolate 1505 significantly lowered the number of sporangia that were released from *P. viticola* in comparison with the effect of the original isolate (G6). We determined the activity and abundance of extracellular lytic enzymes of isolates G6 and 1505. The isolates were grown in liquid media and glucanase enzyme activity secreted to the medium was analyzed with different substrates. The activity of extracellular exo-β-1,4-glucanase and carboxymethylcellulase (CMCase) was significantly higher in isolate 1505 than in isolate G6. The presence of extracellular enzymes is in agreement with the assumption that lytic enzymes are involved in mycoparasitism. There is a possibility that the high activity of these enzymes in isolate 1505 may be related to the higher antagonistic activity of this isolate compared with isolate G6. In the course of this study we modified a protoplast-based method for DNA-mediated transformation of *F. proliferatum*. Resistance to an antibiotic (hygromycin B) was conferred to the fungus and a reporter gene (GUS) was successfully integrated into the fungal genome. We intend to take advantage of transformed isolates for the analysis of mycoparasite–pathogen–plant interactions. *(L)*

Weakening and Delayed Mortality of *Fusarium oxysporum* f.sp. *niveum* by Heat Treatment: Flow Cytometry and Growth Studies

M.P. Assaraf,[1] C. Ginzburg[2] and J. Katan[1]

[1]*Dept. of Plant Pathology and Microbiology, The Hebrew University of Jerusalem, Faculty of Agricultural, Food and Environmental Quality Sciences, Rehovot 76100; and* [2]*Dept. of Ornamental Horticulture, ARO, The Volcani Center, Bet Dagan 50250, Israel*

The survival of *Fusarium oxysporum* f.sp. *niveum* with time following heat treatment, was studied using fluorescent staining, flow cytometric, physiological and microscopic assays. Exposure

of germinating conidia to sublethal and lethal temperatures from 36 to 42°C for 60 min, followed by rhodamine 123 staining and flow cytometry, revealed increasing levels of fluorescence. This increase reflects a change in mitochondrial membrane potential indicating a weakening induced by stress. Fluorescent staining with fluorescein diacetate, acridine orange, propidium iodide and FUN-1 using flow cytometry and germination assessments showed that viability of conidia or germinating conidia of *F. oxysporum* f.sp. *niveum* exposed to heat decreases with increasing temperature. Viability was further reduced 13 and 24 h after treatment. However, viability was higher than that observed with the longer assay of the plate count method, suggesting delayed mortality of the heat-treated germinating conidia. This was verified by subculturing single germinating conidia that had been previously heated, and by light, fluorescent and scanning electron microscopic visualization. Programmed cell death was not observed in *Fusarium* heat-treated conidia or germinating conidia, based on the detection of plasma membrane phosphatidylserine translocation, cell-cycle measurements, detection of DNA fragmentation and microscopic visualization for the appearance of apoptotic bodies. The findings of this study suggest that propagules which survived the heating and are apparently alive, may undergo irreversible detrimental processes, eventually leading to their death by yet unidentified mechanisms. These findings have implications for the development of methods of control which use heat, such as soil solarization and heat treatment of seeds and fruits. *(L)*

Dynamics of the *Fusarium moniliforme* Population in Cornfields during the Growth Season

Marjana Galperin and D. Kenigsbuch

Dept. of Life Sciences, Bar-Ilan University, Ramat Gan 52900, Israel [Fax: +972-3-5351824; e-mail: kenigsd@mail.biu.ac.il]

Fusarium moniliforme Sheldon commonly infects a wide range of crops throughout the world and is a major parasite of the Gramineae, particularly in tropical and subtropical regions. In corn, this fungus can cause stalk rot, leaf spot, ear and kernel rot, damping-off and seedling blight. Infected seeds that are planted may increase the incidence of seedling blight and contribute to systemic infection of plants. Moreover, seedlings can be readily infected by *F. moniliforme* from infected soil debris. Corn kernels, as they mature, may become either externally or systemically infected. The effect of the fungicide Octave (prochloraz), irrigation and fertilizers on the intensity of the appearance of *F. moniliforme* in corn plants and corn field soils was studied in a cornfield in northern Israel during 1998/99. The effect of Octave was estimated using naturally infected seeds and artificially infected seeds (*nit* mutant) in an attempt to evaluate the contribution of seed infection on penetration and colonization of corn plants and kernels with *F. moniliforme*. The results showed that watering and fertilizers did not influence the dynamics of the soil and plant population of *F. moniliforme* and did not reduce disease severity associated with this fungus. The fungicide Octave essentially limited the soil population of *F. moniliforme* in two experiments, as well as plant population of fungus caused by artificial infection. The contribution of seed infection to kernel infection was ~50% and showed seed transmission of *F. moniliforme*. *(P)*

Identification and Characterization of Reduced Pathogenicity Mutants of *Colletotrichum gloeosporioides* Obtained by REMI

N. Yakoby,[1,2] R. Zhou,[1] Ilana Kobiler,[1] A. Dinoor[2] and D. Prusky[1]

[1]*Dept. of Postharvest Science of Fresh Produce, ARO, The Volcani Center, Bet Dagan 50250 [Fax: +972-3-9683622; e-mail: prusky33@netvision.net.il]; and* [2]*Dept. of Plant Pathology and Microbiology, The Hebrew University of Jerusalem, Faculty of Agricultural, Food and Environmental Quality Sciences, Rehovot 76100, Israel*

Restriction enzyme-mediated integration (REMI) was used to generate tagged pathogenicity mutants of the phytopathogenic fungus *Colletotrichum gloeosporioides*. The fungus attacks a

wide variety of tropical and subtropical fruits. REMI makes it possible to generate a range of tagged pathogenicity mutants *via* transformation of a linear plasmid, in the presence of a restriction enzyme (*Xba*1), which contains a selectable marker (hygromycin resistance). Following seven transformations, 3500 hygromycin-resistant isolates were subjected to a virulence assay by point inoculation on the mesocarp and pericarp of avocado fruits cv. 'Fuerte'. Fourteen isolates showed different degrees of virulence on avocado fruits and two isolates, Cg-M-142 and Cg-M-1150, were further characterized. Cg-M-142 was found to generate appressoria on avocado pericarp like the wild-type isolate Cg-14, unlike Cg-M-1150 – which did not generate appresoria even after 96 h. Cg-M-142 showed reduced maceration on avocado pericarp and mesocarp in comparison with Cg-14, whereas Cg-M-1150 generated much-reduced maceration on mesocarp and no symptoms on the pericarp. Southern blot analysis of Cg-M-142 and Cg-M-1150 showed REMI integration at the *Xba*1 site with differences in the number of tandem integrations. Isolate Cg-M-1150 showed, in addition, another ectopic integration. Plasmid rescue analysis of Cg-M-142 and Cg-M-1150 harbored the native transformation plasmid and plasmids with the genome-flanking region that are currently being sequenced for genotype analysis. *(L)*

Host Specificity and Survival of *Colletotrichum acutatum* from Strawberry

Sigal Horowitz,[1,2] A. Sharon[2] and S. Freeman[1]

[1]*Dept. of Plant Pathology, ARO, The Volcani Center, Bet Dagan 50250 [Fax: 972-3-9683543; e-mail: freeman@netvision.net.il]; and* [2]*Dept. of Plant Science, Tel-Aviv University, Tel Aviv 69978, Israel*

Anthracnose is one of the major fungal diseases of strawberry (*Fragaria* x *ananassa*) occurring worldwide. In Israel, the disease is caused by the species *Colletotrichum acutatum* Simmonds. The pathogen causes crown rot and produces lesions on immature petioles and stolons and thus limits production of strawberry plants. It is common to find a single botanical species of *Colletotrichum* that infects multiple hosts, whereas several *Colletotrichum* species or biotypes may be associated with a single host. In this work we examined the host range and specificity of *C. acutatum* from strawberry. Five isolates of *C. acutatum*, obtained from infected strawberry, were used to inoculate five botanical species: pepper, eggplant, tomato, bean and strawberry. Inoculated plants were maintained in humid conditions by overhead irrigation under greenhouse conditions. The fungus survived and proliferated in these plants over a 3-month period without causing any symptoms, except on strawberry. Colonization of the pathogen in these plant tissues was confirmed by a diagnostic test based on polymerase chain reaction (PCR) with specific primers for *C. acutatum*. Microscopic observation of tomato tissue, that was artificially inoculated with the pathogen, revealed appressorial formation on the leaf surfaces. Five isolates possessing conidia similar to those of *C. acutatum* were isolated from the weeds *Conyza* sp. and *Vicia* sp. which were growing near naturally infected strawberry plants in the open field. Microscopic examination of *Conyza* sp. revealed abundant acervuli with masses of conidia. These isolates caused typical anthracnose symptoms on strawberry and survived on the weed hosts without causing visible disease symptoms. Molecular characterization of these isolates by ap-PCR grouped them within the subpopulation of *C. acutatum* from strawberry. Artificial inoculation of various fruits and vegetables with *C. acutatum* from strawberry produced disease symptoms, indicating the potential for cross-infection by this subpopulation on other hosts in addition to strawberry. *(P)*

Genetic Variation and Virulence of *Verticillium dahliae* from Different Hosts in Israel

Nadia Korolev,[1] J. Katan[2] and Talma Katan[1]

[1]*Dept. of Plant Pathology, ARO, The Volcani Center, Bet Dagan 50250 [Fax: +972-3-9683543]; and* [2]*Dept. of Plant Pathology and Microbiology, The Hebrew University of Jerusalem, Faculty of Agricultural, Food and Environmental Quality Sciences, Rehovot 76100, Israel [e-mail: gamliel@agri.huji.ac.il]*

A total of 742 isolates of *Verticillium dahliae* were recovered from 15 host plant species (chrysanthemum, cotton, eggplant, olive, peanut, pepper, potato, tomato, rose, watermelon, and five weed species) and soil, from 73 locations throughout Israel. Isolates were assigned to genetically isolated groups (vegetative compatibility groups - VCGs) by complementation tests between *nit* mutants using Israeli and international tester strains. Four VCGs (1, 2A, 2B, and 4B) were identified and correlated with host and geographical origin. VCG1 (37 isolates) originated from cotton from the north-eastern region of Israel; VCG2B (252 isolates) was recovered mainly from cotton in the northern part of the country, while some strains were isolated from chrysanthemum, eggplant, olive, pepper and tomato. VCG4B (414 isolates) was widespread over the Negev and was recovered from 14 host plant species (with most isolates from potato). The minor VCG2A (39 isolates) was scattered throughout the country and was often recovered from tomato, olives and eggplant. Seventy-three isolates originating from different VCGs, hosts and locations were tested for pathogenicity to cotton, eggplant and okra using the root-dip method. Three pathotypes were defined according to pathogenicity of isolates to cotton ('Acala SJ2'): defoliating (D), defoliating-like (DL), and non-defoliating (ND). D-pathotype was represented by VCG1 cotton isolates; DL-pathotype was represented mainly by VCG2B cotton isolates from the Yizre'el Valley; ND-pathotype (mostly VCGs 4B and 2A) was recovered from 15 species and was common mainly in potato fields in the Negev. Using eggplant as the second test-plant enabled us to sub-divide DL and ND pathotypes into sub-pathotypes (DL1, DL2, ND1, ND2 and ND3). Okra was extremely susceptible to all pathotypes, with slightly greater susceptibility to D as compared with DL and ND pathotypes. The local populations of *V. dahliae* associated with major crops such as cotton or potato were homogeneous for their VCG and pathotype composition (except a new focus of Verticillium wilt in the Hula Valley, where both VCGs 1 and 2B were found), whereas strains from other hosts often represented mixed populations or varied from site to site in the same region. *(P)*

Is Induced Resistance Reversible?

Alona Ovadia and Y. Cohen

Faculty of Life Sciences, Bar-Ilan University, Ramat Gan 52100, Israel [Fax: +972-3-5354133; e-mail: coheny@ashur.cc.biu.ac.il]

DL-3-aminobutyric acid (BABA) applied as a foliar spray or soil drench protects crop plants against diseases caused by airborne fungi (*e.g. Phytophthora infestans* in tomato, *Peronospora tabacina* in tobacco, *Plasmopara viticola* in grapes), soilborne fungi (*e.g. Fusarium oxysporum* f.sp. *melonis* in melon) and nematodes (*e.g. Meloidogyne javanica* in tomato and cucumber). None of these pathogens is inhibited by BABA *in vitro*. Other isomers of aminobutyric acid are inactive. BABA, when applied as either a foliar spray or soil drench, induces the accumulation of pathogenesis-related (PR)-proteins in tomato. In tobacco, however, the accumulation of PR-proteins was induced by a foliar spray but not by a stem injection or soil drench. The NahG mutant of tobacco is protected by BABA against downy mildew as well as the wild-type indicating a salicylic acid-independent pathway. The ethylene-synthesis inhibitor STS did not compromise activity of BABA. However, co-application of BABA with L-proline (or gamma-aminobutynic acid, GABA) did compromise the activity of BABA, suggesting competition for transport into the host and/or pathogen. L-proline or GABA failed to reverse the activity of BABA if applied post-infection. BABA inhibited the uptake of ^{14}C-L-proline into grape and tomato leaf discs but not into mycelia of *P. infestans*, suggesting a differential competition for plant *vs* fungal transporters. In contrast, L-proline reduced the uptake of ^{14}C-BABA into *P. infestans* but not into tomato leaf discs, reaffirming differential transport systems in the host and the fungus. We propose that BABA induces resistance *via* various mechanisms, depending on the host/pathogen system. *(L)*

Development of a System to Detect Tomato Mosaic Virus (ToMV) in Commercial Tomato Seed Lots

Rivka Hadas,[1] Malenia Pearlsman,[2], Tanya Gefen,[1] O. Lachman,[2] Esther Hadar[3], Galit Sharabany[3] and Y. Antignus[2]

[1]*Official Seed Testing Laboratory (Fax: +972-3-9683889) and* [2]*Dept. of Virology, ARO, The Volcani Center, Bet Dagan 50250 [e-mail: vpantig@agri.gov.il]; and* [3]*Hazera Quality Seeds Ltd., Shiqmim 79837, Israel*

The highly active international seed market has enhanced the rapid spread of seedborne pathogens. Viruses within the tobamovirus group belonging to the category of seedborne viruses attack important vegetable crops. These viruses spread very fast due to their high stability, long survival and the ability to be transmitted mechanically. The goal of this work was to develop a reliable and sensitive system for the detection of ToMV (a member of the tobamovirus group) in tomato seeds. Detection of the virus by serology and molecular methods is efficient and reliable; however, in contrast to bioassays, it does not provide information on the viability of the virus particles. We compared the sensitivity of a bioassay to the well-known ELISA test and found that the two methods were similar in sensitivity, allowing the detection of virus titer as low as 100 ng/ml. This procedure enabled us to detect one infested seed within a mixture of 500 healthy seeds when samples were of highly infested seed lots. However, when infestation level is lower, the detection sensitivity is reduced by a factor of five. Significant differences of sensitivity were found within the different test plants, the most suitable test plant for the identification of ToMV in tomato seeds being *Nicotiana tabacum* cv. *xanthi* N.N. We have determined the standards and requirements for reliable detection of ToMV in commercial seed lots. Based on our results, in order to identify an infestation level of 0.2% it is necessary to test a total of 2000 seeds (ten replicates of 200 seeds per seed lot). To test the infectivity of the virus in positive samples, it is recommended to use a bioassay. *(L)*

A New Strain of Pepper Mild Mottle Virus (PMMV) Overcoming Resistance Conferred by L4 Alleles

Y. Antignus, O. Lachman and Malenia Pearlsman

Dept. of Virology, ARO, The Volcani Center, Bet Dagan 50250, Israel [Fax: +972-3-9604180; e-mail: vpantig@agri.gov.il]

Four alleles for resistance to pathotypes of PMMV were described in the past and designated L1–L4. The resistance conferred to pepper by this set of alleles is continuously overcome by newly appearing PMMV virus strains which were designated P0, P1, P2, P3 according to the resistance sources that they overcome. PMMV is a member of the tobamoviridae differing serologically and biologically from TMV (tobacco mosaic virus) and ToMV (tomato mosaic virus) of this group. During the growth season of 1999 we found pepper (var. 'Cuby') plants of the L4 genotype showing severe virus symptoms. These plants exhibited severe mosaic and ringspots on their leaves; the fruits were distorted with blisters on their surface. The virus has a host range similar to the formerly described PMMV pathovars but the symptoms induced by this virus were significantly more acute. Rigid virus particles 300 nm in length and characteristic of the tobamo group were isolated from infected tissues. Serological analysis excluded the possibility that infection was caused by TSWV (tomato spotted wilt virus), CMV (cucumber mosaic virus) or PVY (potato virus Y) which may induce a plant reaction similar to the above described symptoms. However, a strong ELISA reaction was obtained when virus preparations were reacted with specific antiserum against PMMV. Primers representing a specific region in the PMMV coat protein were used for its amplification by RT-PCR

(reverse transcriptase polymerase chain reaction). DNA products of this reaction were further used for restriction analysis, enabling us to distinguish between different strains of PMMV, including the above described new virus isolate. Based on these results and Koch's postulates, we suggest that the virus isolated from pepper plants of the L4 genotype is a newly emerged strain of PMMV that should be designated P1,2,3,4. *(L)*

A New Disease of Greenhouse Tomatoes in Israel Caused by Tomato Apical Stunt Viroid (TASVd)

Y. Antignus,[1] O. Lachman,[1] Malenia Pearlsman,[1] R. Gofman,[1] O. Zeidan[2] and M. Bar-Joseph[1]

[1]*Dept. of Virology, ARO, The Volcani Center, Bet Dagan 50250 [Fax: +972-3-9604180; e-mail: vpantig@agri.gov.il]; and* [2]*Extension Service, Ministry of Agriculture and Rural Development, Bet Dagan 50250, Israel*

A new disease of greenhouse-grown tomatoes appeared recently at several locations in the coastal region of Israel. It was found in both regular and cherry tomato varieties. The characteristic symptoms of the disease include shortening of the internodes in the apical part of the plant, and general stunting and degeneration. Young leaves remain small and thin or show downward leaf curling and crinkling, depending on the variety and time after infection. Old leaves may show distortion, yellowing and brittleness. Disease spread in commercial greenhouses is rapid and leads to heavy loss of yield. When plants are inoculated under laboratory conditions at an early growth stage they remain stunted, have a bushy appearance, and show significant sprouting of new growth points along the cardinal stem. Incubation time for symptom appearance under these conditions is approximately 2 weeks. The disease is easily transmitted by mechanical inoculation and by grafting and this is probably the reason for the characteristic spread patterns of the disease along the crop rows. Viroid nucleic acid was extracted from infected tomato plants and separated on polyacrylamide gel, giving a specific band with a size of ~360 nt. The purified viroid nucleic acid served to produce DNA clones by RT-PCR (reverse transcriptase-polymerase chain reaction. The sequenced clone has 363 nt with 93% homology to the published sequence of tomato apical stunt viroid (TASVd) that was first described from the Ivory Coast. Sequence comparison of viroid isolates from two different locations in Israel showed identity except for one base mismatch. These results indicate that the viroid isolated from tomatoes in Israel is an Israeli variant of TASVd. *(L)*

G: BIOLOGY AND EPIDEMIOLOGY

Colletotrichum: Host Specificity and Pathogenicity on Selected Crops

S. Freeman

Dept. of Plant Pathology, ARO, The Volcani Center, Bet Dagan 50250, Israel [Fax: 972-3-9683543; e-mail: freeman@netvision.net.il]

Colletotrichum and its teleomorph *Glomerella* are considered major fungal plant pathogens worldwide. Several *Colletotrichum* species or biotypes are known to cause disease in a single host such as do *C. acutatum, C. fragaria* and *C. gloeosporioides* in strawberry. It is also common to find a single species infecting multiple hosts such as *C. gloeosporioides* on avocado, strawberry, apple, peach and other species. Identification of *Colletotrichum* species responsible for disease is vital for implementing effective control strategies and for breeding for resistance. Traditional methods, vegetative compatibility, and various molecular techniques are being used for identifying species in *Colletotrichum* populations on almond, anemone, avocado, statice and strawberry crops that are cultivated in close proximity in Israel. Cross-infection potential was evident between two species, *C. gloeosporioides* [including representatives of distinct subpopulations from almond (Israel), apple

(US), avocado (Israel) and mango (Israel)] and *C. acutatum* [from anemone (Israel, Australia), apple (US), peach (US) and strawberry (Israel)], on a variety of fruit and plant species in artificial inoculations. Molecular analyses proved that rDNA analyses including ITS (internal transcribed spaces) sequencing, are reliable for taxonomic identification of certain *Colletotrichum* taxa, whereas repetitive DNA elements, A+T-rich DNA RFLPs, and arbitrarily-primed PCR are useful methods for determining subpopulation diversity within species. Subpopulations within *C. acutatum* and *C. gloeosporioides* have been characterized, but their taxonomic status is unclear. Multiple genotypes of *C. acutatum* exist, as well as isolates of the species appearing on multiple hosts. Although clonal populations have been observed (*e.g.* on strawberries in Israel), the contribution of the sexual stage to genetic diversity within *C. acutatum*, similar to that hypothesized for *C. gloeosporioides*, still needs to be addressed. *(L)*

Development and Maturation of Perithecia of *Didymella rabiei* on Chickpea Straw

Ephrath Gamliel,[1] D. Shtienberg,[1] H. Vintal[1] and A. Dinoor[2]

[1]*Dept. of Plant Pathology, ARO, The Volcani Center, Bet Dagan 50250 [Fax: +972-3-9683688; e-mail: danish@netvision.net.il]; and* [2]*Dept. of Plant Pathology and Microbiology, The Hebrew University of Jerusalem, Faculty of Agricultural, Food and Environmental Sciences, Rehovot 76100, Israel*

Didymella rabiei is the perfect stage of *Ascochyta rabiei*, the causal agent of Ascochyta blight in chickpea. The disease is one of the main constraints to chickpea production in the Mediterranean area. The sexual fruiting bodies, the perithecia, develop on chickpea straw during the autumn and winter and mature in early spring, together with the growth of the new chickpea crop. Factors affecting the development and maturation of perithecia were studied in a controlled environment and in the field. Both mating types of the fungus were detected in all chickpea production areas in Israel, indicating that potentially, the development of the perfect stage is feasible. Effects of temperature on the development and maturation of perithecia were determined on artificially inoculated pieces of chickpea stems. Stem pieces were exposed to three periods (of 3 weeks each) of varying temperatures (between 5 and 20°C). In the first period they were exposed to one temperature, transferred to another temperature in the second period, and then to a third temperature for the last period. Mature perithecia developed only if the stem pieces were exposed to 5–15°C in the first period, 5–10°C in the second period and 5–20°C in the third period. In a second set of experiments different scenarios of wetting/drying alternations of the stem pieces were tested. Mature perithecia did not develop on stem pieces that remained dry for the duration of the experiment but only on those that were wet, at least for some of the time. However, significantly more perithecia developed on stem pieces that were exposed to wetting/drying alternations than on those that were kept continuously wet for the duration of the experiment. *(L)*

Dissemination of *Fusarium oxysporum* f.sp. *radicis-lycopersici* – the Causal Agent of Fusarium Crown and Root Rot in Tomatoes – in Soil, Roots, Air and Seeds of a Non-Host Plant

Yael Rekah,[1] D. Shtienberg[2] and J. Katan[1]

[1]*Dept. of Plant Pathology and Microbiology, The Hebrew University of Jerusalem, Faculty of Agricultural, Food and Environmental Quality Sciences, Rehovot 76100 [Fax: +972-8-9466794; e-mail: gamliel@agri.huji.ac.il]; and* [2]*Dept. of Plant Pathology, ARO, The Volcani Center, Bet Dagan 50250 Israel*

The tomato crown and root-rot disease caused by *Fusarium oxysporum* f.sp. *radicis-lycopersici* (*Forl*) is a devastating disease. In a study carried out recently it was demonstrated that *Forl* is a polycyclic pathogen in nature, and can spread from diseased to healthy plants during a growing

season. In the present study, the means of the pathogen's dissemination in time and space were studied. *Forl* was found to spread along the rows in the field during the growing season. In greenhouse experiments it was demonstrated that the pathogen spreads *via* root-to-root contact, from diseased to adjacent healthy plants, and not by self-growth in the soil. Large numbers of *Forl* macroconidia produced on the stem surface of diseased plants are aerially disseminated in fields and greenhouses and may infect healthy plants. It was demonstrated that infection occurs either by soil infestation followed by root infection, or by direct infection of the foliage. Disease incidence of foliar-inoculated tomato plants reached 85–100% of the inoculated plants. A widespread wild plant (*Tamarix nilotica*) which has become a common weed in fields in the southern part of Israel is also involved in the dissemination and survival of the pathogen. Roots of the weed are colonized by the pathogen, enabling it to proliferate and survive hostile conditions. Seeds of *T. nilotica*, contaminated with propagules of *Forl*, are spread in the field during the pathogen's conidiation period and assist in the dissemination of the pathogen. In summary, *Forl* is a unique soilborne pathogen. The various means of dissemination enable it to complete several disease cycles during a crop's growing season, a trait which is usually attributed to foliar pathogens. These characteristics require reconsideration of the management strategies currently used for its suppression. *(L)*

Cross Pathogenicity of *Fusarium oxysporum* f.sp. *cucumerinum* on Melon Plants

Daniella Cafri,[1] Y. Katan[1] and Talma Katan[2]

[1]*Dept. of Plant Pathology and Microbiology, The Hebrew University of Jerusalem, Faculty of Agricultural, Food and Environmental Quality Sciences, Rehovot 76100 [Fax: +972-8-9466794; e-mail: gamliel@agri.huji.ac.il]; and* [2]*Dept. of Plant Pathology, ARO, The Volcani Center, Bet Dagan 50250, Israel*

Formae speciales in the wilt pathogen *Fusarium oxysporum* are considered specific to single hosts. A few cases of cross pathogenicity between different *formae speciales* that cause diseases in plant species belonging to the Cucurbitaceae have been reported, although the results are inconsistent or even contradictory. We evaluated the pathogenicity of isolates of f.sp. *cucumerinum (Focu)* isolated from diseased cucumber plants in Israel, to cucumber – which is the specific susceptible host (suscept), and to melon – which is considered a nonsuscept. Some but not all isolates of *Focu* suppressed growth of young and old melon plants and even caused typical Fusarium wilt symptoms. Moreover, a significant positive correlation was obtained between *Focu* virulence to cucumber and virulence to melon. In contrast, f.sp. *melonis (Fom)* did not cause disease in cucumber plants. These findings do not conform with the classical definition of *forma specialis*. Disease symptoms and their severity varied with isolate and inoculum concentration, plant age, plant density and drought stress level. When different horticultural types of melon carrying the same monogenic resistance to *Fom* were compared, 'Galia'-type melon was more resistant to cross inoculation with *Focu* than was the 'Ananas'-type. Thus, specific genes for resistance to *Fom* (*Fom*1 and/or *Fom*2) were not effective when the plants were cross-inoculated with *Focu*. Instead, the reaction of melon plants to cross inoculation seems to depend on the genetic background of the plants. These findings should be considered when classifying *F. oxysporum* pathotypes and may also have practical implications for crop rotation, breeding of resistant cultivars, and selection of resistant rootstocks for cucurbit plants. *(L)*

Resistance to Downy Mildew, *Pseudoperonospora cubensis*, in Cucumbers

Ludmila Petrov,[1] Karin Boogert,[2] Lanina Sheck,[1] A. Baider,[3] Evgenia Rubin[3] and Y. Cohen[3]

[1]*Agrogene Ltd., 85252 M.P. Negev, Israel [Fax: +972-7-9922203];* [2]*Dept. of Horticulture, Wageningen Agricultural University, Wageningen 6700, the Netherlands; and* [3]*Faculty of Life Sciences, Bar-Ilan University, Ramat Gan 52900, Israel [e-mail: coheny@ashur.cc.biu.ac.il]*

Downy mildew caused by the oomycete fungus *Pseudoperonospora cubensis* (Berk. et Curt.) Rost. is a major disease of cucurbits in temperate regions of the world. Chemical control of the disease often fails due to the prevalence of fungicide-resistant isolates. It is therefore imperative to develop genetically resistant inbred lines for agriculture. Such inbreds were developed in *Cucumis melo* and *Cucumis sativum*. In melon PI 124111F, resistance to downy mildew is controlled by two incompletely dominant genes, *Pc-1* and *Pc-2;* and in melon PI 124112 by two incompletely dominant genes, *Pc-1* and *Pc-3*. In cucumber, Van Vliet and Meijsing identified a single recessive gene, *dm*, for resistance to downy mildew. This gene was traced back to 'Poinsett', which in turn originated from PI 197087. Doruchowski and Lakowski-Ryk showed that resistance in Wisconsin 4783 is controlled by three recessive genes, *dm-1, dm-2* and *dm-3*. Susceptibility in SMR-18 is governed by the dominant gene *Dm-3* and two complementary genes, *Dm-1* and *Dm-2*. The purpose of this study was to screen several cultigens of cucumber for resistance to downy mildew and to determine the mode of inheritance of this resistance in order to produce a resistant inbred line that would serve as a parent for producing F_1 hybrids with increased resistance to downy mildew. Two inbred lines and five F_1 hybrids of cucumber resistant to downy mildew, were evaluated for resistance in growth chambers and the field. Best-performing plants were selfed and S_4 plants were crossed with a high-yielding, parthenocarpic, susceptible partner. Resistance in J-13, a derivative of Wisconsin 2843, was found to be controlled by incompletely dominant gene(s). Resistance was expressed as small, chlorotic, water-soaked lesions with negligible sporulation of the fungus. No breakdown of this resistance was observed at 12°C. *(L)*

Verticillium dahliae on Olives

Leah Tsror (Lahkim),[1] A. Levin,[2] Marina Hazanovsky,[1] Orly Erlich,[1] M. Aharon,[1] U. Yogev[2] and A. Gamliel[3]

[1]*Dept. of Plant Pathology, ARO, Gilat Experiment Station, M.P. Negev, 85280 [Fax: 972-7-9926337; e-mail: tsror@bgumail.bgu.ac.il];* [2]*Kibbutz Revivim, M.P. Haluza; and* [3] *Inst. of Agricultural Engineering, ARO, The Volcani Center, Bet Dagan 50250, Israel*

Verticillium dahliae causes economic damage to olives at all its production sites. In 1998 the disease, characterized by desiccated leaves and branches, and/or defoliation, was detected in Israel at Revivim. Disease incidence in cv. 'Barnea' increased from 20% to 40% within a year. Recently the disease has also been observed on cv. 'Picual' (disease incidence increased from 22% in Jan. 1999 to 30% in Aug. 1999) and on cv. 'Souri' (22% in July 1999). Among 1200 symptomatic trees monitored in August 1999, 47.8% were Barnea, 25.6% Picual and 26.6% Souri. The average first yields of Picual were 42.7 and 8.7 kg/tree in healthy and symptomatic trees, respectively; those of Barnea were 41.5 and 21.4 kg/tree in healthy and diseased trees, respectively. The fungus was isolated from branches at the top, middle, and bottom of both diseased trees and healthy ones. Recovery of the fungus was more successful in autumn–winter (61%) than in summer (31%). Several isolates were classified into vegetative compatability groups 4B and 2A. In order to reduce the spread of the disease and minimize its damage, field experiments were conducted in summer 1998/99. Disease incidence of infected trees treated with sprayable black degradable plastic mulch (Ecotex) was lower (50%) than in those treated with plastic sheets or the control (70%), 8 months after solarization. Disease severity index was also reduced in the latter treatment, especially after the dry branches were cut. *(L)*

Decline of Perennial *Limonium* Cultivars Caused by *Colletotrichum gloeosporioides*

S. Pivonia,[1] Rachel Levita,[1] G. Tzuberi,[1] Mali Zachs,[2] Aida Zviebil,[3] Y. Nizani[3] and S. Freeman[3]

[1]*Arava R&D and* [2]*Extension Service, Ministry of Agriculture and Rural Development, Sapir, M.P. Central Arava 86825; and* [3]*Dept. of Plant Pathology, ARO, The Volcani Center, Bet Dagan 50250, Israel [Fax: 972-3-9683543; e-mail: freeman@netvision.net.il]*

Limonium is cultivated in Israel as a perennial ornamental crop. The phenomenon of *Limonium* wilting and mortality of plants was first observed in 1995 in the Arava desert region of southern Israel. Plant death occurred initially on a limited scale but has spread since to many growing areas. Wilting and death of plants occurs at elevated temperatures, mainly from June until October. Typical symptoms are: outward curling of mature leaves; necrosis of sepals; wilting of young shoots following pruning; necrosis at the base of the crown; and stem cankers containing masses of orange pigmented conidia, appearing only in areas with high relative humidity. Koch's postulates were fulfilled confirming that the fungus *Colletotrichum gloeosporioides* is the causal agent of wilting and death of plants. The fungus was readily isolated from the crowns of wilted plants. Plants transplanted into infested soil wilted and died at a high rate. Eight fungicides were tested *in vitro* for control of the pathogen. Four compounds (prochloraz-Mn, fluazinam, cyproconazole and bromoconazole) showing efficacy *in vitro*, were used for further testing under field conditions. Application of prochloraz-Mn (0.2%) at 10-day intervals was the most effective treatment for reduction of wilting and death of plants. Below-ground drip irrigation caused a significant reduction in disease incidence compared with above-ground conventional drip irrigation. Amplification of DNA from isolates of *Colletotrichum* infecting *Limonium* using PCR-specific primers revealed that the isolates belong to the species *C. gloeosporioides*. However, the population structure was diverse, with at least five different genotypes being observed using arbitrarily primed PCR. The perfect stage *Glomerella cingulata* was discovered in infected *Limonium* tissue in nature, possibly contributing to genetic diversity of the pathogen population. *(L)*

Gray Cotton: Identification of the Causal Agent and the Factors that Affect Its Severity

Carmit Nave,[1] D. Shtienberg,[1] H. Vintal,[1] Y. Nitzani,[1] G. Kritzman,[1] A. Ficher,[2] E. Gat[3] and A. Dinoor[4]

[1] *Inst. of Plant Protection [Fax: +972-3-9683688; e-mail: danish@netvision.net.il] and* [2] *Inst. of Soils, Water and Environmental Sciences, ARO, The Volcani Center, Bet Dagan 50250;* [3] *Cotton Production and Marketing Board, Herzliyya 46103; and* [4] *Dept. of Plant Pathology and Microbiology, The Hebrew University of Jerusalem, Faculty of Agricultural, Food and Environmental Quality Sciences, Rehovot 76100, Israel*

The syndrome of discolored, gray cotton has increased in prevalence and intensity in recent years. The chemical property of discolored fibers is altered and their quality is 5–10% lower than that of an equivalent uninfected crop. Research was initiated to identify the causal agent(s) of the syndrome and to determine the environmental conditions that promote its formation. Twenty-one different species of fungi were isolated from cotton samples taken from fields around the country, the most common being two species of *Aspergillus: A. fumigatus* and *A. niger*. Their involvement in the syndrome was verified by completion of Koch's postulates. Naturally discolored fibers were inspected under light and scanning electron microscopes. It was found that fungal mycelia grew on the fibers and sometimes even within the fiber cavities. When fibers were placed in humid conditions, the fungi developed and sprouted abundantly, and dark-colored hyphae and spores covered the fibers externally. These fungi contaminate also fibers of seemingly non-infected cotton. The environmental conditions governing the development of *Aspergillus* spp. on the fibers were studied under controlled environment conditions. The most important factor was the duration of fiber wetness and the number of wetting event, in an alternating wet/dry regime. In the field, the cause of wetness is dew, which is formed at night. The significance of fiber wetness for the development of the fungi on the fibers was examined in field experiments conducted in a hot and dry region (Bet She'an Valley). Overhead irrigation with small quantities of water (to simulate dew) resulted in fiber discoloration, indicating that the fungi contaminated the fibers naturally, but that their development was restricted by the dryness. *(L)*

Phytopathological Aspects of Recirculating Irrigation Water in Greenhouses

G. Kritzman,[1] Y. Dishon,[2] D. Silverman,[3] B. Kirshner,[1] I. Dornai,[1] V. Pekarski,[1] Miriam Kimchi,[1] M. Raviv,[1] B. Bar-Yosef[1] and J. Katan[4]

[1]*ARO, The Volcani Center, Bet Dagan 50250 [Fax: +972-3-9683545; e-mail: kritzgio@netvision.net.il];* [2]*Kibbutz En Gedi, Dead Sea;* [3]*Extension Service, Ministry of Agriculture and Rural Development, M.P. Bet She'an 10900; and* [4]*Dept. of Plant Pathology and Microbiology, The Hebrew University of Jerusalem, Faculty of Agricultural, Food and Environmental Quality Sciences, Rehovot 76100, Israel*

The spread of the bacterium *Xanthomonas vesicatoria (Xv)* was assessed in a recirculating hydroponics system (En Gedi system) with a pepper crop, in an experimental greenhouse at En Gedi. In one experiment the inoculum was injected into the recirculating irrigation water, and in the second experiment the inoculum source was infected plants. The water was disinfested by UV irradiation at ~150 mW/cm^2.s. In both experiments, disinfestation with UV resulted in a decrease in the *Xv* population in the irrigation water and in the stem tissue. Similar results were obtained with *Erwinia carotovora* pv. *carotovora* and *Pythium aphanidermatum*, which appeared spontaneously in the system. The spread of *P. aphanidermatum* was also assessed in the experimental greenhouse at En Gedi. The source of the inoculum was infected plants. Disinfestation was carried out by UV irradiation or by Slow Sand Filtration (SSF), with three types of plant growth medium: hydroponics, volcanic scoria (tuff), or tuff mixed with 40% compost. At En Gedi, symptoms of growth retardation in pepper plants were observed only in the hydroponics system. In the hydroponics system with UV irradiation, the plants were less developed than those in the tuff systems, but far more developed than in the hydroponics system without UV irradiation. In the hydroponics system, root infection by *Pythium* was 100%, compared with 6–40% in the tuff system. In the hydroponics system the roots are in continuous contact with irrigation water, whereas in the tuff system irrigation takes place only once or twice a day and for short periods. We assume that the addition of compost had a suppressive effect on the pathogen. In the hydroponics system the UV irradiation was effective only in the early stages but did not prevent the outbreak of the disease later in the season. In the tuff system we found no advantage to either of the disinfestation methods. However, analysis of the water did show a reduction in pathogen population with UV irradiation and with SSF, compared with the untreated control. The importance of plant pathogens in roses growing in re-used water was also discussed. *(L)*

Integration of Strategies for Controlling Root Rot in Avocado in Israel

Miriam Zilberstaine

Extension Service, Ministry of Agriculture and Rural Development, Hadera 38100, Israel [Fax: +972-6-6377815; e-mail: mirzil@netvision.net.il]

Avocado root rot caused by the fungus *Phytophthora cinnamomi* Rands causes the death of avocado trees (*Persea americana* Mill.) and is a major economic problem for the commercial cultivation of avocado worldwide. In Israel the fungus was first isolated in autumn 1982 in a small number of orchards and in imported ornamentals. Drastic and immediate measures were adopted to stop the spread of the disease in Israel. The late Dr. Y. Pinkas and his associates conducted a comprehensive nursery survey, following which all infected avocado plants in the nurseries were destroyed. A policy of destroying any orchards where infected trees were found was also adopted. This measure slowed the spread of the fungus in Israel. During 16 years research was focused mostly on the development of rootstocks resistant to the fungus (horticultural selection and testing of resistant rootstocks). In addition, steps were taken to stop the spread of the disease to other orchards, and methods were studied for rehabilitating infected mature groves, including the evaluation of chemical eradication formulations. Seventeen rootstocks resistant to the disease were seleeted originating from three genetically diverse sources. The first group was from Israeli rootstocks known for their

superior production (a product of J. Ben-Ja'acov's selection program) and includes VC28, VC49, VC55, VC66 and VC69. Rootstocks were also selected from Ben-Ja'acov's germplasm collection and include VC207, VC218, VC225, VC239, VC241 and VC256. The final group of resistant rootstocks arose from surviving trees in an infected orchard at Giv'at Hayyim and includes VC801, VC803, VC804, VC811 and VC812. All these rootstocks are being tested in commercial orchards in Israel. Additionally, these materials have been sent to South Africa and California for evaluation in those production areas. In producing orchards where infection has been detected, certain actions need to be taken in order to *(a)* halt the spread of the disease from the affected orchard to neighboring groves; and *(b)* chemically treat the infected trees. In this study, the efficacy of two materials was tested, 'Foli-O-Fos 400' (produced by U.I.M. Agrochemicals, Australia; m-dKP) and 'Canon 50' (produced by Luxembourg, Israel). In trials conducted with nursery plants and in a bioassay, the two materials were found to be equally effective. *(L)*

Colonization Dynamics and Population Structure of Bacterial Epiphytes of Tomato Leaves

E. Jurkevitch and G. Shapira

Dept. of Plant Pathology and Microbiology, The Hebrew University of Jerusalem, Faculty of Agricultural, Food and Environmental Quality Sciences, 76100, Israel [Fax: +972-8-9466794; e-mail: jurkevi@agri.huji.ac.il]

Bacterial populations found on leaves of greenhouse and field-grown tomato plants (*Lycopersicon esculentum*) were quantified and pure cultures of isolated colonies identified. Higher microbial populations (up to 10^5 cfu g^{-1}) obtained by dilution plating were measured from extracts of greenhouse, overhead-irrigated plants than from soil-irrigated plants (10^3 cfu g^{-1}). Identification by fatty acid methyl-ester analysis (FAME) of 216 and 114 strains isolated from overhead-irrigated plants grown in a vegetable garden and from greenhouse-grown plants, respectively, and of 83 strains originating from soil-irrigated field-grown plants and identified with the BIOLOG system, revealed that in each case, populations were dominated by a few genera. Denaturing gradient gel electrophoresis analysis of single and bulk leaflets from dry and humid leaves of greenhouse-grown plants revealed similar profiles and a few dominant bands on a highly diverse background. Bacteria extracted from washed leaflets were concentrated and sprayed on greenhouse-grown, soil-irrigated plants without prior cultivation. More than 10^5 cfu g^{-1} were sustained for 4 days on these plants as compared with sterile buffer-sprayed leaves, which sustained fewer than 10^4 cfu g^{-1}. Randomly selected colonies isolated from leaves sprayed with the bacterial extracts were identified by FAME, but no significant enrichment for any strain could be detected. However, isolates of saprophytic species detected at high frequencies in these experiments and in plants grown outdoors sustained various levels of colonization up to 4 days after inoculation, indicating differential epiphytic fitness. The non-epiphytic bacteria *Escherichia coli* and *Azospirillum brasilense* disappeared from the leaf surface within the same experimental period. *(L)*

Fire Blight in Loquat – Etiology and Control

Miriam Zilberstaine,[1] Shulamit Manulis,[2] Frida Kleitman[2] and S. Levi[3]

[1]*Extension Service, Ministry of Agriculture and Rural Development, Hadera 38100 [Fax: +972-6-6377815; e-mail: mirzil@netvision.net.il];* [2]*Dept. of Plant Pathology, ARO, The Volcani Center, Bet Dagan 50250; and* [3]*Growers' Organization for Field Experiments, Central Region, Hadera, Israel*

Fire blight, caused by the bacterium *Erwinia amylovora*, is a devastating disease of pomaceous fruit trees. In autumn 1994 in Israel, a severe outbreak of the disease occurred on loquat (*Eriobotrya japonica*), a subtropical evergreen tree of the Rosaceae family. Little is known about the etiology and epidemiology of the disease in loquat, since most studies have been conducted on pear and apple

trees. The aims of the present work were to study the disease etiology in loquat trees, survival and distribution of the bacteria, susceptibility of various varieties of loquat to *E. amylovora*, and means of disease control. The experiments were conducted on trees of the varieties 'Akko-1', 'Zrifin', 'Yehuda' and 'Zikim', grown in containers under insect-proof net, and in commercial orchards. Inoculations with 10^5–10^7 cells/ml were done on flowers and wounds (caused by thinning the flower clusters). First symptoms of ooze drops appeared 6–10 days after inoculation, on young trees grown in containers. Symptoms of browning were observed after 15–21 days in young trees and after 30 days in orchard trees. Flowers were less susceptible to infection than were wounds. The wounds were susceptible to infection up to 24 h after thinning the flower cluster in young trees and up to 48 h after in orchard trees. Studies of survival and spread of *E. amylovora* in loquat trees showed that the bacteria spread rapidly from the site of infection to the stem, in both vertical directions up to 50 cm from the inoculation site. The bacteria survived in the tree branches, under natural Israeli conditions, no longer than 150 days. The variety Zikim was found to be the least susceptible; and Akko-1 the most. Several bactericides were examined in artificially and naturally infected trees. Results demonstrated that 0.15% 'Starner' (oxolinic acid 20% WP) was most effective in controlling the disease in loquat, when sprayed during the first 24 h after inoculation.

By title only

Use of N-Viro soil, a limed sludge product, to reduce *Fusarium* and other soilborne fungi. P. Fine, A. Gips, M. Reuven, R. Rosenberg, V. Solopanov, I. Kolesnic and Y. Ben-Yephet