ABSTRACTS OF PRESENTATIONS AT THE 27TH CONGRESS OF THE ISRAELI PHYTOPATHOLOGICAL SOCIETY

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A: PATHOGEN CHARACTERIZATION AND PROCESSES IN PATHOGENICITY

Occurrence of *Aspergillus carbonarius*, the Producer of Ochratoxin A, in the Vineyards of Israel and in Table Grapes after Harvest

A. Lichter,[1,*] L. Guzev,[1] A. Ovadia,[2] Tirza Zahavi,[3] S. Ziv[1] and N. Paster[4]

[1]*Dept. of Postharvest Science of Fresh Produce [*e-mail: vtlicht@volcani.agri.gov.il] and* [4]*Dept. of Food Science, ARO, The Volcani Center, Bet Dagan;* [2]*Agronomia Ltd., Gedera; and* [3]*Extension Service, Ministry of Agriculture and Rural Development, Qiryat Shemona, Israel*

Aspergillus carbonarius belongs to the *A. niger* group. Reports on its ability to produce the nephrotoxic and carcinogenic mycotoxin Ochratoxin A (OTA) raised the concern that its occurrence in vineyards might account for the presence of OTA detected in red wines in Europe. To address this issue, vineyards in several countries around the Mediterranean Basin were sampled during 3 years. In Israel, bunches without decay symptoms were sampled in ten vineyards of wine and table grapes at different stages of bunch development. The *Aspergillus* isolates on the surfaces of the berries were characterized morphologically, for production of OTA *in vitro*, and by molecular taxonomy for representative isolates. The following were the major findings: (i) The number of *Aspergillus* isolates present on the berries increased during the season. (ii) All the vineyards contained *A. carbonarius*. (iii) A large percentage of *A. carbonarius* isolates produced high levels of OTA. (iv) No OTA was identified in grape samples without decay symptoms. (v) More than one-third of the bunches that exhibited decay typical of black *Aspergillus* were infected with *A. carbonarius*. (vi) The infected part of the bunch contained OTA that did not translocate to the healthy section of the bunch. (vii) Application of fungicides in the vineyard did not achieve sufficient control of decay or fungal contamination of the berries. (viii) The composition of isolates on the berries did not change significantly after storage at 20°C or cold storage. (ix) Disinfecting the berries with ethanol was not effective in reducing the *Aspergillus* inoculum. (x) A correct dose of SO_2 during cold storage decontaminated the berries from *Aspergillus* isolates. Many of these findings related to wine grapes were also reported by other countries participating in this research and they clearly indicate that there is an actual risk of contamination of grapes and their products with OTA. Recently, EU regulations concerning the presence of OTA in wine have been implemented. Therefore, there is an urgent need to develop useful means to minimize the contamination of grapes and their products with *A. carbonarius* in order to eliminate OTA from grapes and their products. *[L]*

Expression of siRNA Targeted against TYLCV CP Transcripts Leads to Silencing of CP Gene Expression and Resistance to the Virus

A. Zrachya,[1,2] U. Ramakrishnan,[3] P.P. Kumar,[3] Yael Levy,[1] A. Loyter,[2] T. Arazi,[4] M. Lapidot[1] and Y. Gafni[1,*]

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

[1]*Dept. of Genetics [*e-mail: ygafni@volcani.agri.gov.il] and* [4]*Dept. of Ornamental Horticulture, ARO, The Volcani Center, Bet Dagan, Israel;* [2]*Dept. of Biological Chemistry, The Alexander Silberman Institute of Life Sciences, The Hebrew University of Jerusalem, Jerusalem, Israel; and* [3]*Dept. of Plant Biotechnology, School of Biotechnology, Madurai Kamaraj University, Madurai, India*

A major viral pathogen of tomato crops in many countries of the world is a geminivirus, the *Tomato yellow leaf curl virus* (TYLCV), a member of the genus *Begomovirus* which is transmitted by the whitefly *Bemisia tabaci*. TYLCV genome, unlike the genome of most plant viruses, is a circular covalently closed ssDNA molecule of 2,787 nucleotides. TYLCV genome encodes six open reading frames only one of which, *v1*, codes for the coat protein (CP) that represents a building block of the viral particle. TYLCV CP is absolutely essential for viral infection and movement. Mutations in this protein's gene were shown to interfere with systemic infection as well as particle formation and insect transmission. The TYLCV CP is the only known component of viral capsid. In addition, the CP plays a role in virus transport into the host cell nucleus, a crucial step in the viral life-cycle since it replicates and transcribes its genes there. In this study, we used short interfering double-stranded RNAs (siRNA) expressed under the control of the CaMV 35S promoter to target gene expression of the TYLCV CP. Transient assays done by agroinfiltration of the CP silencing construct followed by infiltration of fused GFP-CP (green fluorescent protein–coat protein) gene showed down-regulation of GFP expression in *Nicotiana benthamiana*. Transgenic tomato plants (cultivar 'Micro-Tom') expressing the siRNA targeted at the TYLCV CP gene, did not show disease symptoms for 7 weeks post-inoculation with the virus, whereas the control non-transgenic plants were sick within 2 weeks post-inoculation. The present study demonstrates for the first time that siRNA targeted at the CP of TYLCV can cause a delay in appearance of disease symptoms, thus enabling flowering and fruit production. *[L]*

Quorum Sensing Regulates Epiphytic Fitness and Virulence in *Pantoea agglomerans* pv. *gypsophilae*

L. Chalupowicz,[1,2] I. Barash,[2] M. Itkin[2] and Shulamit Manulis[1,*]

[1]*Dept. of Plant Pathology and Weed Research, ARO, The Volcani Center, Bet Dagan [*e-mail: shulam@volcani.agri.gov.il]; and* [2]*Dept. of Plant Sciences, Tel-Aviv University, Ramat Aviv, Israel*

Pantoea agglomerans (previously *Erwinia herbicola*) pv. *gypsophilae* (*Pag*) incites gall formation on *Gypsophila paniculata*. The pathogenicity of *Pag* is associated with an indigenous plasmid ($pPATH_{Pag}$) that is exclusively present in all pathogenic strains. Previous studies demonstrated that multiplication of *Pag in planta* is *hrp*-dependent and the bacterium is organized in cell aggregates within the growing gall. These observations led us to investigate the Quorum Sensing (QS) system in *Pag* and its contribution to epiphytic fitness and virulence of this bacterium. QS describes a regulatory mechanism which allows bacteria to sense the environment and coordinate the expression of genes in a population-dependent manner. It involves an interaction between a small diffusible molecule (*e.g.* acyl-homoserine lactone, AHL) and a transcriptional regulator. This system has not been identified previously in *Pag*. By using *Chromobacterium violaceum* CV026 mutant and *E. coli lux* reporters, we demonstrated that *Pag* synthesizes AHLs in which N-butyryl -D-homoserine lactone is the major signal identified by mass spectral analysis. We have cloned and sequenced the genes that encode for AHL biosynthesis: *pagI*, and the transcriptional regulator *pagR*.The two genes are linked, slightly overlapping and convergently transcribed. A mutant of *pagI* lacking production of AHL's signal showed a significant reduction in pathogenicity on gypsophila cuttings, mainly at an inoculum concentration of 10^6 cfu ml^{-1} and lower. In addition, a reduction of one order of magnitude in the *pagI* mutant population as compared with the wild type was observed on bean leaves. This result suggests that QS exerts an effect on epiphytic fitness of *Pag* as well. Recently we have identified *lux*-box-like motifs in the promoter regions of genes controlled by the Hrp regulon (*e.g. hrpL, hrpJ, hrpS*

and *pthG*). The involvement of QS in expression of these genes and virulence is under investigation. *[L]*

Effects of Sexual Reproduction on Aggressiveness and Population Structure of *Phytophthora infestans*

Shiri Lilach Klarfeld,[1] U. Gisi[2] and Y. Cohen[1,*]

[1]*Faculty of Life Sciences, Bar-Ilan University, Ramat Gan, Israel [*e-mail: ycohen@mail.biu.ac.il]; and* [2]*Syngenta Crop Protection Research Center, Stein, Switzerland*

Phytophthora infestans (Mont.) De Bary, the causal agent of late blight in potato and tomato, is a devastating disease worldwide. The annual production loss and cost of control measures have been estimated to be US$3.75 billion (GILB- http://gilb.cip.cgiar.org/index.php). *P. infestans* is a heterothallic oomycete capable of sexual reproduction when both mating types, A1 and A2, occur in close proximity. In recent years, late blight severity in Israel has increased, especially in tomato crops. Tomatoes are grown in Israel all year round, especially in net and plastic houses. The reason for the increased aggressiveness is not clear. It has been speculated that sexual recombination *via* oospores creates new progeny isolates with increased virulence and aggressiveness. Such isolates may dominate the population, with the oospores becoming a main source of inoculum. In order to test this hypothesis, we produced 33 crosses between four A1 and one A2 parental isolates of *P. infestans*. DNA-based molecular analyses with RAPD, AFLP and SSR showed that the progeny isolates were indeed F_1 hybrids. Some of the 300 F_1 progeny isolates possessed higher aggressiveness qualities, such as resistance to phenylamid fungicides, virulence factors and fitness parameters, when compared with their respective parents. These data suggest that sexual reproduction may alter the population genetics of *P. infestans* and consequently affect the epidemiology of late blight in tomato and potato. *[L]*

Effect of the Geminiviruses SLCV and WmCSV on Melon Plants

M. Lapidot*, Tali Sofrin, Amalya Abody, Lydia Cohen, Rachel Ben-Joseph, Z. Machbash and Limor Segev

*Dept. of Vegetable Research, ARO, The Volcani Center, Bet Dagan, Israel [*e-mail: lapidotm@volcani.agri.gov.il]*

Two new cucurbit-infecting viruses, *Squash leaf curl virus* (SLCV) and *Watermelon chlorotic stunt virus* (WmCSV) have become established in Israel during the last 2 or 3 years. Both viruses are transmitted by the whitefly *Bemisia tabaci* in a persistent and circulative manner, and are assigned to the genus *Begomovirus* within the family Geminiviridae. SLCV is found mainly in open-field squash plants, typically in the summer and autumn seasons, whereas WmCSV is found mainly in open-field watermelon plants. Both viruses have a host range within the Cucurbitaceae, and both viruses infect melon. In transmission experiments it was found that SLCV induces mild symptoms in infected melon plants, whereas WmCSV induces severe symptoms, including distinct yellowing and leaf senescence. Both viruses induce stunting of melon plants. To assay the effect these viruses have on yield components of melon, young melon seedlings were inoculated with both viruses *via* whitefly transmission. Following inoculation, the plants were grown in a net house. Plant yield was measured and compared with the yield of control non-inoculated plants. *[L]*

Calcineurin is Required for Sclerotial Development and Pathogenicity of *Sclerotinia sclerotiorum* in an Oxalic Acid-independent Manner

A. Harel, S. Bercovich and O. Yarden*

*Dept. of Plant Pathology and Microbiology, Faculty of Agricultural, Food and Environmental Quality Sciences, The Hebrew University of Jerusalem, Rehovot, Israel [*e-mail: oded.yarden@agri.huji.ac.il]*

Sclerotinia sclerotiorum is a necrotrophic, omnivorous plant pathogen with worldwide distribution. Sclerotia of *S. sclerotiorum* are pigmented, multihyphal structures that play a central role in the life and infection cycles of this pathogen. Calcineurin, a Ser/Thr phosphatase linked to several signal-transduction pathways, plays a key role in the regulation of cation homeostasis, morphogenesis, cell-wall integrity, and pathogenesis in fungi. We demonstrated that calcineurin expression in *S. sclerotiorum* is altered in a phase-specific manner during sclerotial development. Inhibition of calcineurin by FK506, cyclosporin A or inducible antisense calcineurin expression impaired sclerotial development at the prematuration phase and increased germination of preformed sclerotia. Induction of antisense calcineurin expression in *S. sclerotiorum* resulted in reduced pathogenesis on tomato and *Arabidopsis*. However, secretion of oxalic acid, a key virulence factor of *S. sclerotiorum*, was not altered. Inhibition of calcineurin conferred a reduction in cell wall β-1,3-glucan content and increased sensitivity to cell-wall-degrading enzymes and to the glucan synthase inhibitor caspofungin. Thus, calcineurin plays a major role in both sclerotial development and pathogenesis of *S. sclerotiorum* and most likely other phytopathogens. *[L]*

Gene Expression of *Colletotrichum gloeosporioides* Regulated by Alkalinization Stress

I. Miyara,[1] A. Sherman,[2] Hane Volpin[2] and D. Prusky[1,*]

[1]*Dept. of Postharvest Science of Fresh Produce [*e-mail: dovprusk@volcani.agri.gov.il] and* [2]*Dept. of Genomics, ARO, The Volcani Center, Bet Dagan, Israel*

Colletotrichum gloeosporioides is an important pathogen of tropical and subtropical fruits. During pathogen colonization the fungi alkalinize the host tissue by secreting a significant amount of ammonia. The alkalinization of the host tissue enhanced *pel*B expression, a gene encoding for pectate lyase that affects colonization of fruits, and possibly other genes that were not identified. As a first stage for identification of the genes expressed during the ammonification process and other environmental stress conditions, and their contribution to fungal pathogenicity, we prepared cDNA libraries of *C. gloeosporioides* grown at inducing pH *in vitro* and in semi *in vivo* on avocado fruits. The cDNA libraries were printed as a macroarray format and were used to determine the differential expression of genes under inducing and non-inducing conditions of colonization. The differential expression of *C. gloeosporioides* genes was considered in this work and will be used in the future for gene knockouts for identification of their functionality. *[L]*

Characterization of *Cucurbita moschata* and *C. maxima* Lines for Resistance to RNA Viruses Using Classical and Molecular Tools

G. Barkan,[1] Rivka Hadas,[2] M. Edelstein,[3] Diana Leibman[1] and A. Gal-On[1,*]

[1]*Inst. of Plant Protection [*e-mail: amitg@volcani.agri.gov.il],* [2]*Gene Bank for Agricultural Crops, Inst. of Plant Sciences, ARO, The Volcani Center, Bet Dagan; and* [3]*Dept. of Vegetable Crops, ARO, Newe Ya'ar, Israel*

Pumpkin (*Cucurbita maxima*) and tropical pumpkin (*C. moschata*) are relatively minor crops, whose agricultural importance is enhanced by their use as rootstocks. More than half of the watermelon seedlings in Israel are grafted onto pumpkin rootstock; therefore there is importance in the characterization of *C. moschata* Duchesne and *C. maxima* Duchesne lines resistant to multiple RNA viruses. Additionally, some of the viruses are soilborne, so that a sensitive scion could be protected by a resistant rootstock. Different viruses infect pumpkin and cause foliar damage and a decrease in agricultural product quality and quantity. For example, early infection with *Zucchini yellow mosaic virus* (ZYMV) can cause crop losses of up to 100% in pumpkin, due to a lack of suitable resistance sources. In this work new diagnostic tools, both molecular and immunological, have been developed for the identification of six abundant RNA viruses infecting cucurbits in the field in Israel. Sixty *C. moschata* and *C. maxima* accessions from different locations in the world were screened for resistance and tolerance to mechanical infection by the different viruses. Together with symptom-screening, we measured the accumulated virus level in different lines through RNA-hybridization and real-time PCR. We found that infecting with *Cucumber vein yellowing virus* (CVYV) did not cause disease symptoms in most of the lines. Virus presence could be found in a few lines only through RT-PCR. Lines which were infected with *Cucumber mosaic virus* (CMV) showed initial chlorotic spots symptoms on the inoculated cotyledons, but no sign of systemic viral movement. Inoculation with the potyviruses ZYMV and *Papaya ringspot virus-W* (PRSV-W) caused leaf-deformation, acute mosaic and significant damage in most lines. The accumulated virus levels of ZYMV and PRSV-W for most of the lines was high, but not homogenous. Lines that were inoculated with *Cucumber fruit mottle mosaic tobamovirus* (CFMMV) displayed chlorotic mosaic, yellowing and developmental damage in all the lines except for a single asymptomatic resistant line without detectable virus. No symptoms were detected when lines were inoculated with *Melon necrotic spot virus* (MNSV) by mechanical inoculation. In conclusion, in comparison with melon (*Cucumis melo*), which is sensitive to all six viruses, most of the lines tested were found to be sensitive to three viruses (ZYMV, PRSV-W and CFMMV), and resistant to CVYV, CMV and MNSV. *[L]*

Molecular Approaches for Identification of Novel Virulence-associated Genes from *Xanthomonas campestris* pv. *vesicatoria*

Dafna Tamir-Ariel*, N. Navon and S. Burdman

*Dept. of Plant Pathology and Microbiology, Faculty of Agricultural, Food and Environmental Quality Sciences, The Hebrew University of Jerusalem, Rehovot, Israel [*e-mail: tamird@agri.huji.ac.il]*

The establishment of bacterial pathogens in their host and the pathogens' ability to cause disease depend on a complex process that requires the coordinated activity of many bacterial genes, whose identity and mode of action are largely unknown. The 'In Vivo Expression Technology' (IVET) comprises promoter-trap methods directed to select *in vivo*-induced (*ivi*)-promoters, and is one of the most powerful means to identify genes that contribute to virulence, fitness and survival of bacterial pathogens. Most IVET-based studies have been carried out with bacterial pathogens of animals, and only few studies were performed with phytopathogenic and environmental bacteria. Here we report on the development of two IVET-based genetic approaches to identify novel genes from *Xanthomonas campestris* pv. *vesicatoria* (*Xcv*; bacterial spot disease of tomato and pepper) that are associated with pathogenicity. In the first IVET method, a library of fusions is cloned upstream of a promoterless *hrpA1* gene, and introduced into an *hrpA1* mutant that is unable to grow *in planta* and cause disease. The mutants carrying the different fusions are inoculated into a tomato cultivar that is susceptible to the wild-type strain. Fusions carrying *ivi*-promoters are then complemented for *hrpA1* and their pathogenic phenotype is restored. In the second variation, the library of fusions is created upstream of a promoterless resolvase, and introduced into a mutated strain that carries the resolvase target sites flanking an antibiotic resistance marker. Induction of promoters in the plant results in excision of the

antibiotic resistance marker. In the short term, our goal is to identify novel genes that are exclusively activated *in planta* and to understand their role in pathogenicity. Our long-term aim is to enrich our understanding of the gene machinery that promotes pathogenicity. *[L]*

A Polyphasic Approach to Assessing Genetic Diversity among *Xanthomonas campestris* pv. *campestris* Strains Using PFGE, AFLP, Rep-PCR and Integron Gene Cassette PCR

Angel Valverde,[1,2] T. Hubert,[1] A. Stolov,[1] A. Dagar,[1] J. Kopelowitz[3] and S. Burdman[1,*]
[1]*Dept. of Plant Pathology and Microbiology, Faculty of Agricultural, Food and Environmental Quality Sciences, The Hebrew University of Jerusalem, Rehovot, Israel [*e-mail: saulb@agri.huji.ac.il]; [2]Dept. of Vegetal Production, IRNASA-CSIC, Salamanca, Spain; and [3]Savyon Diagnostics Ltd., Ashdod, Israel*

Xanthomonas campestris pv. *campestris (Xcc),* the causal agent of black rot disease, is one of the most economically important diseases of crucifer crops worldwide. Several studies have suggested high heterogeneity among *Xcc* strains; however, knowledge of the extent of this genetic diversity is still limited. In this study, three molecular typing methods were used to investigate the diversity among *Xcc* strains. Using pulsed-field gel electrophoresis (PFGE), amplified fragment length polymorphism (AFLP) and repetitive sequence-based PCR (rep-PCR), we were able to divide 22 different strains into 11, 12 and 13 differentiated pattern types, respectively. These findings not only support the observed heterogeneity within *Xcc*, they suggest that variability at the genomic level in this pathovar is higher than previously estimated. PCR patterns obtained with integron-specific primers also confirmed a relatively high diversity among strains of this pathovar relative to other *X. campestris* pathovars. Although differences in aggressiveness were observed among different strains, pathogenicity assays using cauliflower and radish did not indicate a clear correlation between aggressiveness and haplotype affiliation. However, since most of the haplotypes consisted of single strains, further experiments should be performed to assess more accurately the correlation between haplotype affiliation and aggressiveness in different hosts. *[L]*

B: NON-CHEMICAL CONTROL AND ANTI-MICROBIAL COMPOUNDS

Root Colonization by *Trichoderma* and Its Effects on Root-knot Nematodes

Edna Sharon,[1,*] I. Chet,[2] M. Bar-Eyal[1] and Y. Spiegel[1]
[1]*Dept. of Entomology, Nematology Unit, ARO, The Volcani Center, Bet Dagan [*e-mail: vpshedna@volcani.agri.gov.il]; and [2]Dept. of Plant Sciences, The Weizmann Institute of Science, Rehovot, Israel*

The fungus *Trichoderma* exhibits biocontrol activity against the root-knot nematode *Meloidogyne javanica.* The fungus – nematode interactions may include several direct and indirect mechanisms that may take place in the soil and in the plant roots. Parasitism of *Trichoderma* on nematode life-stages in association with root surfaces was examined *in planta,* on tomato plants. Interactions between the nematode and the fungus during root penetration by the nematode juveniles (J2) were demonstrated using *T. asperellum*-203 constitutively expressing GFP (green fluorescent protein) construct. The roots were colonized by the fungus, which had contact with the penetrating J2 and colonized their penetration holes. Parasitism of the fungus on nematode females within the roots, and on their laid out egg-masses, was examined on tomato roots grown in *Trichoderma*-treated soils from growth-chamber experiments. Females and egg-masses dissected from *Trichoderma*-treated roots were found to be colonized by the fungus. The results demonstrated the capability of *Trichoderma* to parasitize nematode life-stage on the roots and to interfere with the egg-producing process on the roots in soil. The role of induced systemic resistance was evaluated in a split-root system:

significant reductions in root penetration, nematode development inhibition within all life-stages, galling indices and subsequent retardation in reproductivity, were recorded in the root halves that were not directly treated with *Trichoderma*. The direct and indirect implications of fungal root colonization on nematode inoculation suggest that root colonization by *Trichoderma* might be a significant factor in nematode biocontrol. *[L]*

Antifungal Compounds from the *Momordica* Plant

Adi Yonas-Levi,[1] Y. Burger,[2] Ekaterina Gurski,[2] Carmella Horev,[2] U. Saar,[2] S. Gepstien[1] and R. Cohen[3,*]

[1]*Dept. of Biology, Technion – Israel Institute of Technology, Haifa; and* [2]*Dept. of Vegetable Crops and* [3]*Dept. of Plant Pathology and Weed Research, ARO, Newe Ya'ar Research Center, Ramat Yishay, Israel [*e-mail: ronico@volcani.agri.gov.il]*

Momordica charantia (bitter melon; Cucurbitaceae) is known in developing countries worldwide as a plant used in traditional medicine. *Momordica* (the accession Mom 53) was also found in the Arava Valley of southern Israel and herein is described the occurrence of antifungal activity in extracts from various accessions. *Momordica* plants tested at Newe Ya'ar are resistant to powdery mildew. *Momordica* extracts sprayed on melon and cucumber leaves infested with powdery mildew significantly inhibited disease development. Aqueous and organic extracts of *Momordica* inhibited the *in vitro* growth of *Macrophomina phaseolina, Alternaria alternata* and *Fusarium oxysporum* f.sp. *melonis*. There are differences in inhibition ability among the various *Momordica* accessions and also in the occurrence of antifungal activity in various extracts. For example, the small leaf cultivar group (especially accession Mom 53) exhibited antifungal activity in cold water extracts, but these extracts were heat-sensitive. However, no inhibitory activity was found in organic extractions from this group as compared with organic extracts expressing antifungal activity from other accessions. A typical pattern of inhibitory compounds was exhibited on TLC plates as compared with different patterns of non-inhibitory extracts. Preliminary experiments have revealed that some accessions of *Momordica* are resistant to whiteflies and some are resistant to nematodes. The identification of active compounds within the *Momordica* genetic diversity existing at Newe Ya'ar may serve as a basis for developing cultivars containing large quantities of desired chemicals. These plants might constitute a new crop supplying raw material for natural pesticide production. *[L]*

Use of the Endophytic Fungus *Meira geulakonigii* for Citrus Rust Mite Control

Z. Paz,[1,*] S. Burdman,[1] Aviva Gafni,[1] U. Gerson[2] and A. Sztejnberg[1]

[1]*Dept. of Plant Pathology and Microbiology [*e-mail: pazz@agri.huji.ac.il] and* [2]*Dept. of Entomology, Faculty of Agricultural, Food and Environmental Quality Sciences, The Hebrew University of Jerusalem, Rehovot, Israel*

The fungus *Meira geulakonigii* Boekhout, Scorzetti, Gerson & Sztejnberg was recently discovered and isolated from cadavers of the citrus rust mite (CRM) infesting 'Star Ruby' grapefruit (*Citrus paradisi* Macf.). CRM exposed to fungal conidia (10^8 ml^{-1}) in the laboratory suffered high mortality (>90%). In the field the application of conidia resulted in reduced fruit damage and high mite mortality (approximately 50%) of the CRM. A single application of conidia was as effective in mite control as repetitive monthly applications. In addition, the fruit damage level was similar in the treatments, and significantly lower than in the control. PCR analysis of total DNA extracted from untreated fruits and assayed with specific primers designed for fungal rDNA revealed the presence of *M. geulakonigii* and of *Cladosporium tenuissimum* inside the albedo. The latter fungus had no effect on CRM. In the laboratory, *M. geulakonigii* penetrated the albedo 3 days after its application and was detectable for more than 30 days. The fungus could not be detected inside leaves either in the laboratory or in the field. In addition, the CRM population on the fruit declined one week after

the colonization of the albedo by *M. geulakonigii*. These results support previous reports of fungal endophytes acting as bodyguards against pests. It is concluded that the fungus *M. geulakonigii* is an endophyte with the potential of serving as a biocontrol agent against the citrus rust mite. *[L]*

Planting between Separating Barriers Protects Crops from Soil Infection by Tobamoviruses

Y. Antignus,[1,*] L. Feigelson,[1] O. Lachman,[1] Malenia Pearlsman,[1] Orna Ucko,[2] Svetlana Dobrinin,[2] O. Hayman[3] and A. Koren[4]

[1]*Dept. of Virology, ARO, The Volcani Center, Bet Dagan [*e-mail: antignus@agri.gov.il];* [2]*Extension Service, Ministry of Agriculture and Rural Development, Bet Dagan;* [3]*Lidorr Chemicals, Tel Aviv; and* [4]*Hishtil Nurseries Ltd., Nehalim, Israel*

Tobamoviruses are soilborne, mechanically transmitted viruses without a known vector. This group of viruses is characterized by its high stability and long persistence in soil and drainage water. Presumably the transmission process of these viruses is associated with wounding and abrasions inflicted on the root system of transplanted plants and systemic movement of virus particles through the phloem. The technology of Speedling transplanting is widely used worldwide because of its horticultural advantages. However, abrasion of the root cone on the walls of the tray cells and during insertion into the soil, leads to wounding of the roots and thus exposes the plants to virus infection in *Tobamovirus*-infested soils. Our studies have determined that unwounded, newly formed roots that penetrated into an infested medium did not allow invasion by the virus. Field experiments have shown that the viral inoculum in soil could partially be eradicated by treatment with formaldehyde (Fordor), but viral inoculum that is carried in root debris is not affected by the treatment. On the other hand, lab and field experiments indicate that planting into virus-free substances such as condensed peat trays, perlite sleeves, tuff or compost serves as an efficient means to block soilborne epidemics of tobamoviruses in infested soils. *[P]*

Use of Pepper Rootstocks for Control of the Root-knot Nematode *Meloidogyne incognita*

Y. Oka,[1,*] N. Tkachi,[1] S. Shuker,[1] R. Levita,[2] S. Pivonia[2] and R. Ofenbach[2]

[1]*Nematology Unit, Gilat Research Center, M. P. Negev [*e-mail: okayuji@volcani.agri.gov.il]; and* [2]*Arava Research and Development, M. P. Arava, Israel*

Resistance of pepper species (*Capsicum annuum, C. baccatum, C. chinense, C. chacoense* and *C. frutescens*), cultivars and accessions to the root-knot nematodes (RKN) *Meloidogyne incognita* race 2 and *M. javanica*, and their graft compatibility with commercial pepper varieties as rootstocks, were evaluated in growth chambers and greenhouse experiments. Most of the plants tested were highly resistant to *M. javanica*, but susceptible to *M. incognita*. The *C. frutescens* accessions AR-96023 and DRO 8801 as rootstocks showed relatively high resistance to *M. incognita*. In *M. incognita*-infested soil in a greenhouse, AR-96023 supported approximately sixfold less nematode eggs per gram root and produced an approximately twofold greater yield compared with a non-grafted commercial variety. The commercial variety grafted on AR-96023 produced a yield as great as the non-grafted variety in the RKN-free greenhouse, in contrast to DRO 8801, which did not produce a high yield. Some resistant varieties and accessions used as rootstocks produced lower yields than that of the non-grafted variety in the non-infested greenhouse. Use of rootstocks with nematode resistance and graft compatibility may be effective for control of RKN on susceptible pepper. *[P]*

Interaction between *Fusarium oxysporum* f.sp. *orthoceras* and *F. solani* – Two *Orobanche cumana* Biocontrol Agents

R. Lati,[1] Evgenia Dor,[1,*] J. Hershenhorn[1] and J. Katan[2]

[1]*Dept. of Phytopathology and Weed Research, ARO, Newe Ya'ar Research Center, Ramat Yishay [*e-mail: evgeniad@volcani.agri.gov.il]; and* [2]*Dept. of Plant Pathology and Microbiology, Faculty of Agricultural, Food and Environmental Quality Sciences, The Hebrew University of Jerusalem, Rehovot, Israel*

Broomrapes (*Orobanche* spp.) are root parasitic plants causing severe damage and yield loss in vegetable and field crops in Israel, other countries in the Mediterranean region and Eastern Europe. Selective herbicides are unable to provide persistent, season-long control of these parasites. Thus, they are suitable targets for biocontrol. A few fungi showing potential as broomrape biocontrol agents were found: *Fusarium solani* (*Fs*) and *Fusarium oxysporum* f.sp. *orthoceras* (*Foo*) are two soilborne saprophytic fungi that cause severe disease symptoms on broomrape. Application of *Fs* and *Foo* together against sunflower broomrape (*O. cumana*) in sunflower provided effective control and a strong synergistic effect. The objective of this study was to clarify the interactions between *Fs* and *Foo* in the soil colonization phase and the saprophytic phase, and to find metabolites secreted by these fungi that affect each other. Survival of *Fs* and *Foo* was evaluated by applying them together or separately to sterilized or natural soil. When applied separately, both *Fs* and *Foo* developed better in sterilized soil. A negative developmental interaction was found, of each fungus alone or together in sterilized soil. Each fungus was inhibited by the presence of the other fungus. Both fungi developed better and had higher development rates when applied together in natural soil. These findings suggest a positive interaction between the two fungi in natural soil. The two fungi inhibited other soilborne fungi, reducing their population density. No significant difference was found in *Fs* and *Foo* survival ability when applied together or separately to natural soil, suggesting the equalization of the two interactions found: a negative interaction between *Fs* and *Foo* and a positive interaction of each on the natural fungal population in the natural soil. A bioassay of crude extract made from the growth media of *Fs* inhibited *Foo* growth as well as other soilborne fungi in petri dishes. The same results were obtained with *Foo* crude extract, which inhibited *Fs* growth and the other soilborne fungi, indicating the presence of antifungal metabolites. *[P]*

Arabidopsis IQD1, a Novel Calmodulin-Binding Nuclear Protein, Stimulates Glucosinolate Accumulation and Plant Defense

Maggie Levy,[1,*] Qiaomei Wang,[1,#] R. Kaspi,[2] M.P. Parrella[2] and S. Abel[1]

[1]*Dept. of Plant Sciences and* [2]*Dept. of Entomology, University of California-Davis, Davis, CA, USA.* [*]*Current address corresponding author: The Otto Warburg Minerva Center for Biotechnology in Agriculture, Faculty of Agricultural, Food and Environmental Quality Sciences, The Hebrew University of Jerusalem, Rehovot, Israel [e-mail: levym@agri.huji.ac.il];* [#]*Current address: Dept. of Horticulture, Zhejiang University, Hangzhou, China*

Glucosinolates are a class of secondary metabolites with important roles in plant defense and human nutrition. To uncover regulatory mechanisms of glucosinolate production, we screened *Arabidopsis thaliana* T-DNA activation-tagged lines and identified a high-glucosinolate mutant caused by overexpression of *IQD1*. A series of gain- and loss-of-function *IQD1* alleles in different accessions correlates with increased and decreased glucosinolate levels, respectively. We demonstrated that an *IQD1*-GFP fusion protein is targeted to the cell nucleus and that the recombinant *IQD1* binds to calmodulin in a Ca^{2+}-dependent fashion. Analysis of steady-state mRNA levels of glucosinolate pathway genes indicates that *IQD1* affects expression of multiple genes with roles in

glucosinolate metabolism. Histochemical analysis of tissue-specific *IQD1::GUS* expression reveals *IQD1* promoter activity mainly in vascular tissues of all organs, consistent with the expression patterns of several glucosinolate-related genes. Interestingly, overexpression of *IQD1* reduces insect herbivory, which we demonstrate in dual-choice assays with the generalist phloem-feeding green peach aphid (*Myzus persicae*), and in weight-gain assays with the cabbage looper (*Trichoplusia ni*), a generalist-chewing lepidopteran. In contrast, the specialist mustard aphid (*Lipaphis erysimi*) chooses to lay its larva on plants with high *IQD1* expression and high glucosinolate level. *IQD1* overexpression also causes enhanced resistance to the necrotrophic fungus *Botrytis cinerea*, as evidenced by significantly smaller lesion size. As *IQD1* is induced by mechanical stimuli, we propose *IQD1* to be a novel nuclear factor that integrates intracellular Ca^{2+} signals to fine-tune glucosinolate accumulation in response to biotic and abiotic challenges. *[P]*

BioNem WP – Biological Nematicide in Flower Crops

I. Shamai,* M. Lazare, Daphna Blachinsky, Jana Antonov, A. Bercovitz, Katia Feldman, Nataly Markov, M. Prengler and M. Keren Zur

*Agrogreen Minrav Group, Qiryat Minrav – Tech Park, Ashdod, Israel [*e-mail: michell@agrogreen.co.il]*

The high susceptibility of ornamentals to root-knot nematodes (RKN) dictates intensive chemical control. Infested planting material, poor crop rotation and repetitive use of nematicides cause higher disease severity and lower control efficacy. Irrigation by water recirculation worsens the problem. 'BioNem WP' is a biological nematicide based on the bacterium *Bacillus firmus*. The product is applied through the irrigation system or by drenching, preferably pre-planting. In perennial crops, 'in-season' applications are effective too. BioNem WP is registered in Israel for use in cucumbers, tomatoes, peppers, eggplants, peaches and herbs. The efficacy of BioNem WP was examined in different ornamentals. In *Asclepias tuberosa*, one in-season application of BioNem WP reduced the RKN population in the soil, and the nematode damage to the roots (galling index) was lowered by 50% compared with the untreated plots. *A. tuberosa* seeds were sown in RKN-infested soil. In plots treated with BioNem WP, the extent and viability of the emerging plants were significantly higher ($P<0.1$) than in the chemical-treated and the non-treated plots. *Echinops* rhizomes infested with RKN were planted in pots. The RKN population in the soil and in the roots was reduced significantly ($P<0.05$) after dipping the rhizomes in BioNem WP suspension. A similar pre-plant treatment in a commercial field increased plant viability compared with the non-dipped plants. The RKN (*M. hapla*) population developed in roses (var. 'Rose Beauty') grown in tuff in a greenhouse and irrigated with a recirculating water system. A single application of BioNem WP maintained the RKN population at a low level for several months. These examples demonstrate the suitability of BioNem WP for controlling RKN in various floriculture situations. *[P]*

Development of Natural Therapeutic Products and Preservatives from Citrus Peels

Janeta Orenstein* and Uzi Afek

*Dept. of Postharvest Science of Fresh Produce, ARO, Gilat Research Center, M.P. haNegev, Israel [*e-mail: janaor@volcani.agri.gov.il]*

Is it possible to turn a by-product such as citrus peels into a multi-functional product? Treatment of citrus peels with a unique and natural biotechnological process results in an aqueous extract which produces broad-spectrum anti-microbial substances. The extract inhibits *in vitro* pathogenic fungi and bacteria. Chemical analysis of the aqueous extract identified four compounds: scoparone, umbeliferon, scopoletin and ascoletin. These compounds, also known as phytoalexins, contribute to the resistance of citrus against pathogens. The products are used in the food, cosmetics and pharmaceutical industries. Our results aroused the interest of Israeli and international cosmetic

companies. The product contains active ingredients that can supply therapeutic care for various skin disorders, such as acne, athlete's foot, herpes and skin rashes. It also accelerates the healing of wounds including diabetic ulcers. Microbiological tests showing that this product can function as a natural active ingredient as well as a natural preservative have inspired additional interest among food and cosmetic producers worldwide. *[P]*

The Role of Phytohormones in Basal Resistance and *Trichoderma*-induced Systemic Resistance to *Botrytis cinerea* in *Arabidopsis thaliana*

Nadia Korolev*, Dalia Rav-David and Y. Elad

*Dept. of Plant Pathology and Weed Research, ARO, The Volcani Center, Bet Dagan, Israel [*e-mail: vpptlg@volcani.agri.gov.il]*

Thirty-nine mutants of *Arabidopsis thaliana* and their parental ecotypes were tested for resistance/susceptibility to *Botrytis cinerea* and ability to develop *Trichoderma*-mediated induced systemic resistance (ISR). Ecotype Colombia-0 (Col-0) was relatively resistant to *B. cinerea*, and *Trichoderma harzianum* T39 application at sites spatially separated (root application) from the *B. cinerea* inoculation (leaves) resulted in reduction of gray mold symptoms. Ecotypes Wassilewskija-4 (Ws-4), Nossen-0 (No-0) and Landsberg-0 (Ler-0) had a low level of basal resistance to *B. cinerea* and were found to be blocked in their ability to express ISR. Mutants derived from ISR non-inducible ecotypes displayed a ISR non-inducible phenotype, whereas ISR inducibility of mutants derived from ISR-inducible genotype Col-0 depended on the type of mutant. Thus, salicylic acid (SA)-impaired mutants derived from Col-0 were ISR-inducible, whereas jasmonic acid/ethylene (JA/E)-impaired mutants of the same origin were ISR-non-inducible. SA-impaired mutant *npr1-5* derived from No-0 was ISR-non-inducible. SA-impaired mutants kept the basal level of resistance to *B. cinerea*, whereas most of JA/E-impaired mutants were highly susceptible, confirming that resistance of *A. thaliana* to *B. cinerea* is mediated by the JA/E-pathway, whereas the role of an SA-mediated pathway is minor. ABA- and GA-impaired mutants were highly susceptible to *B. cinerea* and showed ISR-non-inducible phenotype irrespective of line of origin. Auxin-related mutants of *Arabidopsis* originating from Col-0 mostly kept the basal level of resistance to *B. cinerea* and were ISR-inducible, as was the highly susceptible mutant *axr1-3*. Mutants resistant to both auxin and E and an auxin-resistant mutant originating from Ler-0 were ISR-non-inducible. Most of *Arabidopsis* genotypes unable to express *Trichoderma*-mediated ISR against *B. cinerea* exhibited enhanced disease susceptibility towards this pathogen. Trichodex treatments enhanced the growth of *Arabidopsis* plants regardless of genotype or ISR inducibility. *[P]*

Overexpression of Aminotransferase Genes from a Susceptible Melon Confers Resistance against Downy Mildew

I. Benjamin,[1] Marjana Galperin,[1] D. Kenigsbuch[2] and Y. Cohen[1,*]

[1]*Faculty of Life Sciences, Bar-Ilan University, Ramat Gan [*e-mail: ycohen@mail.biu.ac.il]; and*
[2]*Dept. of Postharvest Science of Fresh Produce, ARO, The Volcani Center, Bet Dagan, Israel*

Downy mildew caused by the oomycete pathogen *Pseudoperonospora cubensis* is a devastating foliar disease of cucurbits worldwide. The wild melon PI 124111F (PI) is highly resistant to all pathotypes of *P. cubensis*. The resistance is controlled genetically by two partially dominant, complementary loci and expressed as a hypersensitive response reaction. Previously, we have demonstrated that the resistance of PI to *P. cubensis* is controlled by the enzymatic resistance (*eR*) genes *At1* and *At2* which constitutively encode the photorespiratory peroxisomal enzyme glyoxylate aminotransferase. We showed that glyoxylate-aminotransferase activity is 3.3-fold higher in the resistant melon (PI) than in the susceptible melon, independent of infection by the pathogen. The reason for higher enzyme activity in the resistant plant compared to the susceptible plant is not

known. Here we report that the genes *At1* and *At2* are present in the genome of the susceptible melon lines Hemed and AY with only minor differences in the amino acid deduced alignment compared with *At1* and *At2* from the resistant PI. However, expression of either *At1* or *At2* in the susceptible melon Hemed was much lower than in the PI, suggesting a regulation at mainly the transcriptional level. *At1* and *At2* from Hemed were separately introduced into another susceptible melon (BU21/3) and overexpressed with the aid of CaMV35S promoter. The transgenic plants overexpressing either *At1* or *At2* exhibited enhanced activity of glyoxylate aminotransferase and remarkable resistance against *P. cubensis*. It may be concluded that resistance/susceptibility of melon against downy mildew is controlled by the activity of glyoxylate aminotransferase. This activity is regulated at the transcriptional level of the enzyme. *[P]*

C: CHEMICAL CONTROL

Signum - New Registrations in Israel for the Control of *Peronospora* spp., *Botrytis cinerea*, *Sclerotinia* spp., *Cercospora* spp. and *Stemphylium* spp.

Z. Dagan

Agricultural Department, Agan Chemical Manufacturers Ltd., Ashdod, Israel [e-mail: ziv.d@agan.co.il]

Signum is a fungicide produced by BASF containing the new active ingredients boscalid 26.7% and pyraclostrobin 6.7% in a wettable granule formulation. Boscalid is an analide compound and pyraclostrobin is a strobilurin compound. Both active ingredients inhibit fungal respiration but act on different sites on the electron transport chain in the inner membrane of the mitochondria. Signum has shown good efficacy in the field for the control of *Alternaria dauci* in carrots and *A. macrospora* in cotton; *Sphaerotheca fuliginea* and *Pseudoperonospora* spp. in cucurbits; *Leveillula taurica* in eggplant and pepper; *L. taurica* and *Cladosporium fulvum* in tomato; and *Didymella rabiei* in chickpeas. Further field trials showed the efficacy of the product for the control of *Botrytis cinerea* in tomatoes, peppers, eggplants, marrows, ruscus and strawberries and for the control of *Cercospora* spp. in celery, at the application rate of 0.75 kg ha^{-1}; for the control of *Rhizopus* spp. and *Aspergillus* spp. in grapes at the application rate of 0.1% and 0.15%, respectively; and for the control of *Stemphylium* spp. in garlic, onions and chives at the application rate of 0.5 kg ha^{-1}. In addition, dipping carrot roots in Signum 0.1% solution for 30 sec prevented *Sclerotinia sclerotiorum* infection in storage. Signum shows good biological efficacy on pathogenic fungi from a number of taxonomic classes: Phycomycetes (*Rhizopus* spp. and *Pseudoperonospora* spp.), Ascomycetes (*L. taurica*, *Sphaerotheca* spp. and *S. sclerotiorum*) and Deuteromycetes (*D. rabiei*, *Alternaria* spp., *Aspergillus* spp., *B. cinerea*, *C. fulvum* and *Cercospora* spp.). Signum's wide spectrum of activity allows the farmer to control with one spray more than one disease in a crop, such as *Uncinula necator*, *Pseudoperonospora* spp. and *Rhizopus* spp. in grapes; or *L. taurica*, *B. cinerea*, *Alternaria* spp. and *Cladosporium fulvum* in tomatoes. BASF, the manufacturer of Signum, demonstrated the product's efficacy for the control of *Rhizoctonia solani. [L]*

Vydate-L – Preventing Damage in Carrot Caused by Migratory Nematodes

N. Mogilner,[1,*] S. Glidai,[1] O. Levi,[1] E. Ben-Nun,[2] E. Shlevin[2] and Y. Oka[3]

[1]*Milchan Bros. Ltd., Ramat Gan [*e-mail: ela@milchanbros.co.il]*; [2]*Kibbutz Saad, haNegev; and* [3]*Gilat Research Station, ARO, M.P. haNegev, Israel*

Nematodes can be a major problem in carrot production worldwide. Carrot is damaged by two major groups of nematodes: root knot nematodes – *Meloidogyne* spp., and migratory nematodes – *Longidorus, Pratylenchus* and *Paratylenchus* spp., etc. Management of nematodes is important for profitable carrot production. The important group abundant in the Sharon region of Israel is root

knot nematodes, whereas in the Negev region it is the migratory group. Vydate-L (oxamyl 24% SL), produced by DuPont, belongs to the carbamates. It is approved for use as an insecticide, acaricide and nematocide on various annual and perennial crops. Over the last 5 years we have carried out numerous trials on carrot in the northern Negev, Sharon, and Bet She'an Valley. These trials proved that a single spray application of Vydate-L, at the early post-sowing stage, eliminates and decreases the typical damage caused by nematodes. Studies carried out in Michigan State University (USA) showed that damage to *Longidorus* spp. is caused to the carrot over a fortnight after germination. Based on trials carried out in Israel, Vydate-L was approved for commercial soil broadcast spray application at the rate of 10 – 20 l ha^{-1}, in 300 l water ha^{-1}, at early post-sowing treatment (from sowing date to 10 days after sowing). Activation and penetration of Vydate-L into the soil layer is achieved by the routine germination sprinkler split irrigations. Vydate-L provides a nematocidal or nematostatic activity, related to the concentration rate of oxamyl in the soil. Acidifying the spray solution improves the stability of oxamyl for the short term in the rootstock zone. In trials and observations carried out in Israel, a higher rate of 40 l ha^{-1} was tested as well, and no phytotoxic adverse effect was recorded on vegetative growth or on yield components. The activity of Vydate-L in alkaline soil (pH >8) is relatively short. Residue tests did not detect any oxamyl residues in carrot roots 90 days after application. *[L]*

Folicur Application for Reducing *Sclerotium rolfsii* Pod Rot in Peanuts

Michal Reuven,[1,*] O. Rabinowich,[2] G. Shay,[2] Yael Nevo,[3] N. Ragolsky[3] and Y. Ben-Yephet[1]

[1]*Dept. of Plant Pathology and Weed Science, ARO, The Volcani Center, Bet Dagan [*e-mail: michalre@volcani.agri.gov.il]; [2]Extension Service, Ministry of Agriculture and Rural Development, and [3]Field Crops Team, Upper Galilee, Israel*

In recent years, peanut yield losses from pod rotting due to *Sclerotium rolfsii* Sacc. increased significantly in soils of the Hula Valley, which have a high peat content. We studied the effect of Folicur (tebuconazole) on inhibiting sclerotial germination in the laboratory and on pod rot caused by the fungus in the field. In the laboratory experiments Folicur persistence and microbial degradation in peat and mineral soil from the Hula Valley was compared with that in sandy loam from Bet Dagan. One application of Folicur to soil inhibited sclerotial germination for 7 months. Ten repeated applications of Folicur did not reduce its efficiency in inhibiting sclerotial germination. These results indicate that Folicur is a stable fungistat, with low microbial degradation, in the three soils tested. Field experiments were carried out in a 0.1-ha plot naturally infested with sclerotia (*ca* 43 sclerotia per kg of peat soil). The first experiment investigated the effect of two, four and six Folicur applications per season on fungal growth and pod rot. Folicur was applied through the sprinkler irrigation system, 1000 ml ha^{-1} in each application. Folicur treatments reduced the fungus appearance around the plant crown by 25%, a similar rate as compared with the control, but did not reduce pod rot. The second field experiment studied the effect of three different methods of Folicur application at the same dose of 2000 ml ha^{-1}, on the proportion of diseased pods: (i) incorporating the dose into the top 15 cm soil layer, (ii) two applications *via* the irrigation system, and (iii) combined application of half the dose into the soil and the other half *via* the irrigation system. The lowest pod rot proportion – 20% – was observed in the combined application, as compared with 33%, 29% and 37% in incorporation into soil, two applications *via* sprinklers, and control, respectively. Peanut pod rot caused by *S. rolfsii* was best controlled when Folicur was both incorporated into the soil and applied *via* the irrigation system. *[L]*

Preventing the Development of Rotting Causal Agents in Summer Onion

H. Yunis,[1,*], D. Sarid[2], A. Omeri[3] and O. Naot[1]
[1]*Extension Service, Ministry of Agriculture and Rural Development, Bet Dagan [*e-mail: yhisham@shaham.moag.gov.il]; [2]Agricultural Dept., Agan Chemical Manufacturers Ltd., Ashdod; and [3]Lidorr Chemicals Ltd., Ramat HaSharon, Israel*

The fungi *Aspergillus niger* and *Fusarium oxysporum* f.sp. *cepea* (*Foc*) are known as important causal agents of onion rotting during storage. They are apparently seedborne and can cause infections during germination and during the early seedling development stages. These fungi are also soilborne and might therefore also cause direct infection during the various later growth stages of summer onions. Soilborne pests in general, and onion-flies in particular, may assist in increasing the chance of successful infestation with *Aspergillus* and *Fusarium*. After infestation the fungi are maintained either in the onion basal area (*Foc*) or between the onion scales (*A. niger*), where they remain latent until harvesting. *A. niger* may appear on the infested onion scales in the field in the form of black mycelium and black spores, but its main damage occurs during storage. Onions that are infested with *Foc* rot during harvest and become black-colored 'mummies'. Infested onions that do not show any typical disease symptoms can develop onion rot caused by either *Aspergillus* or *Fusarium* during storage, rendering them unmarketable. It was found that chemical treatments applied in the field during crop growth can markedly and significantly reduce these rotting diseases during storage (with a concomitant increase in marketable onions up to 100%). However, early season treatments – to the seeds or applied at the early growth stages – were less effective (if at all) than those applied during the second half of the season. 300 g l^{-1} pyrimethanil (as Mythos SC300) showed very good efficacy in controlling onion rot caused by *A. niger*. 500 g kg^{-1} trifloxystrobin (as Flint WG 50%) had an effect against *A. niger* and when applied at a high dosage (200 g a.i. ha^{-1}) it had a significant effect against *Foc*. 67 g kg^{-1} pyraclostrobin combined with 267 g kg^{-1} boscalid (as Signum WG 33.4%) showed some efficacy in preventing rot. All the tested treatments proved to be safe for the crop. *[L]*

Mutagenesis of *Phytophthora infestans* for Resistance against Dimethomorph and Mefenoxam

Evgenia Rubin,[1] Tehila Hadad,[1] D. Gotlieb,[1] U. Gisi[2] and Y. Cohen[1,2,*]
[1]*Faculty of Life Sciences , Bar-Ilan University, Ramat Gan, Israel [*e-mail: ycohen@mail.biu.ac.il]; and [2]Syngenta Crop Protection Research Center, Stein, Switzerland*

Dimethomorph (DMM) and mefenoxam (MFX, the active enantiomer of metalaxyl) are anti-oomycete fungicides effective against downy mildews and late blight. Field resistance against metalaxyl was reported in several oomycetes including *Phytophthora infestans* and *Plasmopara viticola*, whereas field resistance against DMM was reported in *P. viticola* but not in *P. infestans*. Here, we report on a mutagenesis study in which the development of resistance in *P. infestans* against DMM and MFX was compared. Mutagenesis was induced by exposing detached or attached sporangia (isolates 28, 96, and AD) to UV light (254 or 302 nm), MNNG (methyl-nitro-nitrosoguanidine), NMU (nitroso-N-methylurea) or ethidium bromide (EB) (0.5– 50 μg ml^{-1}) for 30 or 60 min. Treated and untreated (control) sporangia were thereafter mixed with water, DMM or MFX and inoculated onto potato or tomato leaves. MNNG, NMU and EB produced no resistant mutants. In four out of five experiments with UV and in three out of five experiments with UV+EB (totalling approx. 300 million sporangia), late blight lesions developed in leaves treated with up to 50 μg ml^{-1} of either DMM or MFX, whereas non-irradiated, control sporangia produced lesions in leaves treated with up to 0.5 and 0.1 μg ml^{-1} of DMM and MFX, respectively. Resistance against MFX remained stable upon

repeated sporangial inoculations onto leaves treated with either water or MFX, whereas resistance against DMM declined within a few transfers. Resistance against DMM persisted longer on water-treated leaves than on DMM-treated leaves. In an attempt to stabilize the resistance against DMM, A1 and A2 putative mutants were co-inoculated onto tomato leaves and the oospores produced were used in perlite – tomato cultures to obtain 16 F1 and 168 sib F2 progeny isolates. Stable resistance to MFX was observed among the segregating F2 offspring isolates, but no progeny isolates with stable resistance to DMM were obtained. Field experiments conducted during 2003 – 2005 at Bar-Ilan Farm, Israel, showed that enforced selection pressure, prophylactic or curative, imposed by DMM (500 – 1000 μg ml^{-1}, 2 – 4 sprays per season) on mixed isolates of *P. infestans* (60 – 200 isolates) infecting potato or tomato crops, produced no resistant isolates against DMM. It seems that in contrast to MFX, against which stable resistance developed instantly, stable resistance against DMM is unlikely to develop. *[L]*

Invited Lecture presented by M. Bar-Joseph

The Ecology of Infections of Fruit Trees with Viroids in the Near East

M. Bar-Joseph*, O. Cohen and O. Batuman
*The S. Tolkowsky Laboratory, Dept. of Virology, ARO, The Volcani Center, Bet Dagan, Israel [*e-mail: mbjoseph@gmail.com]*

Viroids are sub-viral pathogens that are readily transmitted by grafting and mechanically from infected to healthy plants. These means of transmission have often been used to explain why many of the old-clone citrus varieties were infected with citrus viroids. The main questions that were addressed by us are as follows:

(i) What could be the reason for the common finding of multiple species of viroids infecting old-clone citrus trees in the Near East? Thus, for example, most old line Shamouti sweet orange trees in Israel are infected by at least five different CVds.

(ii) What could have caused the wide dissemination of viroid infections among fruit trees and vines of this region?

(iii) Are viroids new pathogens or have they cohabited with many of the local domesticated plants for long periods?

(iv) After years of considering citrus viroids as potential components of citrus management, mainly for dwarfing, why was the policy changed so that now we attempt to eliminate all viroids from bud wood source trees?

(v) We have shown that viroids are effectively adsorbed and transmitted from infected to healthy citrus trees by goat horns, and pointed out the potential use of this information for developing new tools for viroid sampling and detection. *[L]*

D: HOST – PARASITE INTERACTIONS

Tomato apical stunt viroid (TASVd), a Pathogen of Greenhouse Tomatoes in Israel is Seedborne and Transmitted by Bumble Bees

Y. Antignus,* Malenia Pearlsman, O. Lachman and L. Feigelson
*Dept. of Virology, ARO, The Volcani Center, Bet Dagan, Israel [*e-mail: antignus@agri.gov.il]*

Tomato apical stunt viroid (TASVd) was reported as a devastating pathogen of greenhouse tomatoes in Israel. This isolate shares 92% and 99% identity with the Ivory Coast-type strain and an Indonesian strain, respectively. No information is available regarding the epidemiology of this viroid complex. The results of the present study indicate that TASVd is not transmitted by aphids (*Myzus persicae*) or whiteflies (*Bemisia tabaci*) nor through root infection in infested soil. The results

suggest that the viroid is present in the embryonic tissues of tomato seeds originating from TASVd-infected plants. Transmission rates through seeds may reach 80% when plants are infected early. Moreover, it has been confirmed that bumble bees can transmit the viroid from infected tomato source plants to healthy plants. Based on these findings it is suggested that the primary spread of the viroid in greenhouse tomatoes is by seed transmission, and the secondary distribution is enhanced by mechanical contact with workers' infested hands and tools as well as by the pollination activity of bumble bees. *[L]*

Pepper Plants: Symptomless Hosts and Reservoirs of *Tomato yellow leaf curl virus* (TYLCV)

M. Lapidot,[1,*] Lydia Cohen,[1] Rachel Ben-Joseph,[1] Tracy A. Sherwood[2] and Jane E. Polston[2]

[1]*Dept. of Vegetable Research, ARO, The Volcani Center, Bet Dagan, Israel [*e-mail: lapidotm@volcani.agri.gov.il]; and* [2]*Dept. of Plant Pathology, University of Florida, Gainesville, FL, USA*

Five *Capsicum* species were tested for their susceptibility to *Tomato yellow leaf curl virus* (TYLCV) and the mild strain of TYLCV (TYLCV-Mld). TYLCV was able to infect 30 out of 55 genotypes of *C. annuum*, one out of six genotypes of *C. chinense*, one out of two genotypes of *C. baccatum*, and one out of one genotype of *C. frutescens*, but was unable to infect the one genotype of *C. pubescens* tested. This is the first evidence for the susceptibility of *C. baccatum*, *C. chinense* and *C. frutescens* to TYLCV. Unlike TYLCV, TYLCV-Mld was unable to infect *C. chinense*. No host differences were observed between the Israeli and Florida isolates of TYLCV. None of the *Capsicum* species showed symptoms after infection with TYLCV or TYLCV-Mld. TYLCV was detected in fruits of *C. annuum*, but whiteflies were unable to transmit virus from fruits to plants. Whiteflies were able to transmit both TYLCV and TYLCV-Mld from infected pepper plants to tomato plants. These data demonstrate the ability of some genotypes of pepper to serve as reservoirs for the acquisition and transmission of TYLCV. *[L]*

The Importance of Locule Susceptibility to *Alternaria alternata* in Core Rot Development in Red Delicious Apples

J. Niem,[1] I. Miyara,[1] M. Reuveni,[3] Y. Ettedy,[1] M. Fleishman[2] and D. Prusky[1,*]

[1]*Dept. of Postharvest Science of Fresh Produce [*e-mail: dovprusk@volcani.agri.gov.il] and* [2]*Dept. of Horticulture, ARO, The Volcani Center, Bet Dagan; and* [3]*Golan Research Institute, University of Haifa, Qazrin, Israel*

Alternaria alternata is the main fungal pathogen responsible for moldy-core in apple cultivars of the Red Delicious group. Spores presumably infect young fruits through the open calyx tube, and the growing mycelia reach the seed and carpel wall during fruit development and storage. Moldy-core is characterized by the growth of the mycelia within the locules, with or without penetration into the mesoderm. The disease may become invasive and lead to a slow, dry rot confined to the flesh immediately surrounding the core. By investigating resistant ('Golden Delicious') and sensitive ('Red Delicious') species, we tried to follow the mechanism of fruit resistance. There was a difference between the two species in the ability of the pathogen to colonize the carpel wall, which was higher in the sensitive species. There was a high level of cellulase production by the fungi growing on the sensitive cultivar. These results showed that the carpel wall is an important physical barrier against fungal colonization and disease development. *[L]*

Characterization of the *Pantoea agglomerans* Population in Israel

D. Weinthal,[1,2] I. Barash,[2] L. Valinsky,[3] M. Panijel[2] and Shulamit Manulis[1,*]
[1]*Dept. of Plant Pathology and Weed Research, ARO, The Volcani Center, Bet Dagan [*e-mail: shulam@volcani.agri.gov.il]; [2]Dept. of Plant Sciences, Tel-Aviv University, Tel Aviv; and [3]Central Laboratories, Israel Ministry of Health, Jerusalem, Israel*

Pantoea agglomerans (formerly *Erwinia herbicola*) is widespread in nature as an epiphyte or endophyte on many different plants. Some strains of this species have evolved into gall-forming pathogens, including *P. agglomerans* pv. *gypsophilae* (*Pag*) causing galls on gypsophila or HR (hypersensitive reaction) on beet; and *P. agglomerans* pv. *betae* (*Pab*) eliciting galls on gypsophila and beet. The pathogenicity of *Pag* or *Pab* is dependent on a plasmid (pPATH$_{Pag}$ or pPATH$_{Pab}$) harboring a pathogenicity island (PAI). The PAI accommodates *hrp* gene cluster, type III virulence effectors, IAA and cytokinin biosynthetic genes and IS elements (insertion sequences). The ultimate goal of this study is to understand how the plasmid-born PAI has emerged and is distributed among various pathogenic strains/pathovars of *P. agglomerans*. Initial results indicated that the two plasmids contain an identical origin of replication (*oriV*) that belongs to the IncN-R46 incompatibility group. To find out whether the pPATH$_{Pag}$ is distributed in a heterogeneous population, the diversity of the pathogenic strains was investigated using the AFLP (amplified fragment length polymorphisms), Rep-PCR (repetitive extragenic palindromic PCR) and PFGE (pulsed field gel electrophoresis) methods and analyzed by the 'Fingerprinting II software'. Results obtained suggested the presence of a divergent population of pathogenic *P. agglomerans* composed of three distinct groups: *Pag* serotype I, *Pag* serotype II and *Pab*. pPATH plasmids present in pathogenic strains differed in size and restriction sites pattern. The pathogenicity island of pPATH is conserved in the two *Pag* groups but diverged from that of the *Pab* group, suggesting a differential natural selection on these two pathovars. pPATH$_{Pag}$ was shown to be a non-conjugative plasmid and therefore could not account for its distribution among diverse *Pag* strains by mobilization. The common *oriV* of pPATH$_{Pag}$ and pPATH$_{Pab}$, as well as the presence of many similar virulence genes and IS elements in the plasmid-born PAIs of both plasmids, suggest that they had evolved from a common origin and in the same plasmid. Presumably this plasmid was initially conjugative and mobilized into diverse strains but lost its conjugative capacity at later stages of evolution. *[L]*

Characterization of the Ascochyta-like Complex of Wild *Cicer judaicum*, an Annual Relative of Chickpea

O. Frenkel,[1,*] D. Shtienberg,[2] A. Sherman[3] and S. Abbo[1]
[1]*Dept. of Field Crops and Genetics, Faculty of Agricultural, Food and Environmental Quality Sciences, The Hebrew University of Jerusalem, Rehovot [*e-mail: omerfre@yahoo.com]; [2]Dept. of Plant Pathology and Weed Research and [3] Dept. of Genomics and Bioinformatics, ARO, The Volcani Center, Bet Dagan, Israel*

Ascochyta blight is a significant disease in many legumes. In the eastern Mediterranean Basin sympatric legume cropping and wild *Cicer* populations have existed since the dawn of agriculture. However, there are no reports on the occurrence and frequency of Ascochyta blight in wild chickpea populations in the region. Our objectives were to isolate and characterize the complex of Ascochyta-like species found on *Cicer judaicum*, an annual relative of domesticated chickpea. A survey of Ascochyta-like diseases in several *C. judaicum* populations across central Israel was conducted during 2003 – 2005. Pathogens were isolated from infected plant parts and characterized. The most common type had an incidence of up to 62% in some areas. It caused lesions at the collar region of the plants, as well as on leaves and stems. The pathogen was identified as *Phoma pinodella*, one of

the causal agents of the Ascochyta disease in peas. The second type, with an incidence of up to 9%, caused black and gray canker on the stems and branches, rarely killing the whole plant. This type was identified as *Didymella rabiei*, the causal agent of Ascochyta blight in domesticated chickpea. Molecular comparisons of the spacers from the rDNA sequences of the two pathogen types revealed 99.5 – 100% homology between the first type and *P. pinodella*, and 100% homology between the second type and *D. rabiei*. In aggressiveness studies, the *D. rabiei* isolates caused severe infections on both *C. judaicum* and *C. arietinum* (50% and 60%, respectively). *P. pinodella* induced symptoms of lesser severity on both *Cicer* species (8% and 15%, respectively). On domesticated and wild pea, *P. pinodella* isolated from *C. judaicum* induced brown spots on leaves and lesions on the stems. In conclusion, *C. judaicum* is a host for Ascochyta blight pathogens and may serve as a source of initial inoculum for both domesticated chickpea and pea. *[L]*

Nematodes in Fruit-Tree Nurseries in Israel

Evgeny Kozodoi* and Ella Gomberg

*Plant Protection and Inspection Services (PPIS), Ministry of Agriculture and Rural Development, Bet Dagan, Israel [*e-mail: evgenik@moag.gov.il]*

Nematode-induced diseases of fruit trees can cause decreased yield and eventually possibly premature death. This is especially true in Israel, where climatic conditions favor nematode development. Sources of nematode infestation of orchards are either infested seedlings or soil with a pre-existing nematode population. Thus, a new orchard should be planted in clean soil, using nematode-free seedlings. Handling and sanitation of seedlings in nurseries are important factors in the production and release of seedlings to the environment. A survey conducted from 1997 to 2005 showed that most seedlings grown in detached bags were nematode-free, whereas open-field grown seedlings were not. Jewish 'Halakha' dictates that fruit-tree seedlings be grown (i) with their roots touching the earth, or (ii) at least having the earth 'in sight' (unobstructed view between the roots and ground). Obviously, the first option allows for easy passage of nematodes and other disease agents from the ground to the seedlings, while the second one is both religiously correct and phytosanitarily safe. The survey emphasized the importance of the planting medium with regard to nematode infestation. Olive seedlings grown in bags filled with natural soils (sand, sandy red loam, loam) were infested to various degrees, whereas seedlings in bags filled with potting materials (tuff, peat, coconut fibers) were invariably clean. During the 'Shmita' year of 2000, only deciduous seedlings in detached bags with potting materials were sold. All the seedlings checked were free of nematodes, which concurs with the above finding. In addition, it was found that gall-nematodes were the most frequent in seedlings of stone-fruits, olive and subtropical trees; *Pratylenchus* spp. were most frequent in pome-fruit seedlings; and the citrus nematode most frequent in citrus seedlings. *[L]*

Sources of Initial Inoculum and the Spatial and Temporal Dispersal of *Phytophthora infestans* in the Northern Negev of Israel

D. Shtienberg,[1,*] Y. Elad,[1] H. Vintal,[1] Yafit Cohen,[1] Y. Cohen,[2] Evgenia Rubin[2] and U. Adler[3]

[1]*Dept. of Plant Pathology and Weed Research, ARO, The Volcani Center, Bet Dagan [*e-mail: danish@volcani.agri.gov.il];* [2]*Faculty of Life Sciences, Bar-Ilan University, Ramat Gan; and* [3]*Extension Service, Ministry of Agriculture and Rural Development, Bet Dagan, Israel*

Late blight, caused by *Phytophthora infestans*, is the most destructive foliar pathogen of potatoes and tomatoes in Israel and elsewhere. The most important measure used for disease management – chemical control – is applied on an individual plot basis. In most cases disease suppression is adequate and the losses induced by the disease are minimal, if any. However, when environmental

conditions interrupt the regular spray schedule (*e.g.* prolonged rain events) or when effective fungicides are lacking (*e.g.* in organic production), uncontrolled epidemics may drastically reduce yield. In most cases the source of initial inoculum in the northern Negev region of Israel is outside the specific potato field or the tomato greenhouse. We hypothesized that coping with the initial sources of *P. infestans* on a regional basis would reduce the potential threat of late blight epidemics in that region, and thus in individual plots. The first step towards this goal is to identify the sources of initial inoculum and to quantify the spatial and temporal dispersal of the disease. To accomplish this goal, a comprehensive survey was conducted in the autumn seasons of 2004/05 and 2005/06 in the Besor region of the northern Negev of Israel, an area of *ca* 1000 km^2. The location of diseased plots and the time of disease observation were determined weekly (using GPS). Mapping the data enabled us to identify the sites of disease origin and how it spread in time and space. In most cases disease originated in plots where potatoes had been grown in the previous season and the fields were infected with late blight. Thus, it was concluded that infected volunteer potato plants are the main initial source of *P. infestans* in the Besor region. Analyses of the data suggested that only a small number of initial inoculum sites (five to eight sites per season) exist each season. This information will be used in the following season and attempts will be made to destroy volunteer plants emerging in infected potato fields before they can infect the new, adjacent crops. *[L]*

E: DIAGNOSTICS (DETECTION METHODS) AS A BRIDGE BETWEEN RESEARCH AND THE FARMER

Two *Peronospora* Species Causing Downy Mildew of Carnation and Gypsophila (Caryophyllaceae) in Israel

I.S. Ben-Ze'ev,* Genya Elkind and Edna Levy
*Plant Protection and Inspection Services (PPIS), Ministry of Agriculture and Rural Development, Bet Dagan, Israel [*e-mail: israelb@moag.gov.il]*

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Development and Application of a Diagnostic Tool for Simultaneous Detection of Four Quarantine Viruses by Combining DIG-labeled Multiplex RT-PCR and Macroarray DNA Hybridization

H. Tager,[1] S. Morin,[2] R. Gofman,[1] E. Teverovsky,[1] F. Akad[3] and M. Zeidan[1,*]
[1]*Plant Pathogens Diagnostic Service, Plant Protection and Inspection Services (PPIS), Bet Dagan [*e-mail: zeidanm@moag.gov.il]; [2]Dept. of Entomology and [3]The Otto Warburg Biotechnology Center, Faculty of Agricultural, Food and Environmental Quality Sciences, The Hebrew University of Jerusalem, Rehovot, Israel*

The risks of introducing severe plant viruses into Israel by importing plant propagation material can include causing compounded damages directly to the farmers and to Israel cropping systems. Therefore, preventing the introduction of such unwanted immigrants constitutes the most effective and environmentally friendly solution. To achieve this goal, it is necessary to carry out laboratory tests for virus-free certification of plant propagation materials. For specific detection, serological and/or nucleic acid-based methods are routinely used. Frequently, detection by serological methods is hampered by a low concentration of virus particles in plant tissue and background problems which intensify the need for a more reliable and sensitive detection method. A PCR-based detection system is most sensitive and greatly reduces the testing time needed. However, the high costs of PCR-based detection tests and repeated smears in amplification products prohibit its routine application. Moreover, certification of virus-free propagation material necessitates testing for several virus species. Therefore, simultaneous detection of several viruses in one test is most

important and necessary. In this work we report on developing and applying a diagnostic tool for simultaneous detection of four quarantine viruses: *Raspberry ringspot nepovirus (RRSV), Strawberry latent ringspot sadwavirus (SLRV), Tomato bushy stunt tombusvirus (TBSV)* and *Tobacco ringspot nepovirus (TRSV).* For this purpose dried plant material infected with these viruses was purchased from DSZM center in Germany according to import license conditions issued by the PPIS. Specific primers for the four viruses were designed and used to specifically amplify, and clone partial virus cDNAs. Species-specific cDNAs products obtained by PCR were immobilized onto nylon membrane in pre-assigned format to generate a macroarray. Subsequently, hybridization was carried out with DIG-labeled multiplex-RT-PCR products amplified from the infected tissue to facilitate the detection and identification of the plant virus(es) by reverse dot blot hybridization. The specificity of this diagnostic tool was tested on four different mixes of dried plant material infected with combinations of three out of the four viruses. The sensitivity of this method for detection of all four viruses reached several picograms of plant RNA. In comparison with ELISA tests for sap-dilutions preparations in healthy plant material, the molecular tool was several-fold more sensitive. This method was adopted by the PPIS for routine testing and used for surveying bulb and flower crops fields and nurseries in Israel. The results of this survey, which included 241 samples from 16 fields and nurseries, showed that RRSV, TRSV, TBSV and SLRV do not exist in Israel. *[L]*

Identification of *Phytophthora* Species by Morphology and Molecular Biology Methods

Edna Levy*, Genia Elkind, M. Zeidan, Emma Teverovsky and I.S. Ben-Ze'ev
*Plant Protection and Inspection Services, Ministry of Agriculture and Rural Development, Bet Dagan, Israel [*e-mail: ednal@moag.gov.il]*

Quarantine regulations enforce detection of *Phytophthora cinnamomi* in several imported ornamental plants. Identification includes isolation of the fungus in the antibiotic-rich P_5ARP[H] medium, on which its mycelium has a characteristic coralloid shape and is devoid of sporangia. If such mycelium is observed it is further subcultured on carrot agar medium, where it produces characteristic sporangia and releases zoospores. Recently a new type of *Phytophthora,* called *P. ramorum,* was identified. This fungus causes severe damage to oaks, pine trees and ornamental plants. Routine tests on imported rhododendrons for detection of *P. cinnamomi* revealed a type of *Phytophthora* weakly coralloid which produces on P_5ARP[H] medium a multitude of sporangia, some releasing zoospores and some germinating to produce smaller, secondary sporangia. The ellipsoid, papillate sporangia averaged 25 x 50 μm in size and the average length/width ratio resembled in shape and secondary proliferation the newly described *P. ramorum.* Verification for the morphological identification was continued with molecular biology methods. Conventional PCR was performed on DNA extracted from the fungus mycelium with two pair primers specific for *P. ramorum.* In addition, the sequence of the ITS region was checked using universal primers for fungi. The conventional PCR products did not show the typical fragments for *P. ramorum.* Sequencing of the ITS region gave 97% similarity with *Phytophthora citrophthora.* These results emphasize the need for and importance of combining morphological and molecular methods in the identification of *Phytophthora* species. *[L]*

Susceptibility of Melon Plants to Fusarium Wilt is Affected by the Type of Growth Medium

R. Cohen,[1,*] Carmella Horev,[2] U. Saar,[2] Anat Yogev,[3] Y. Burger,[2] M. Raviv[3] and Z. Geller[4]

[1]*Dept. of Plant Pathology,* [2]*Dept. of Vegetable Crops and* [3]*Dept. of Ornamental Horticulture, ARO, Newe Ya'ar Research Center, Ramat Yishay [*e-mail: ronico@volcani.agri.gov.il]; and* [4]*Extension Service, Ministry of Agriculture and Rural Development, Givat Ada, Israel*

Selecting resistant plants out of segregating plant populations is a critical stage in the process of breeding for resistance. Escape plants, showing no symptoms, are an obstacle to continued progress in breeding. The relationship between growth medium and disease development has so far been investigated mainly in terms of disease suppression, whereas the breeder is interested in enhancing disease development in order to minimize the number of escapees. In studies investigating disease suppression by composts, it was found that peat (serving as an organic control) enhanced disease development compared with inorganic media such as perlite and sandy soil. The objective of the present research was to study the peat – *Fusarium* system in order to establish a better procedure for the selection of resistant plants and to investigate the mechanism enhancing disease development. The possible effect of plant nutrition on disease incidence was studied. The contents of all macro- and micro-elements tested were similar except for that of iron, which was sevenfold higher in plants grown in sand as compared with its content in melon seedlings grown in peat. However, disease development was not affected by adding iron to the inoculation system. The influence of the medium on disease development is often attributed to microorganisms' activity in the rhizosphere. This is probably not the case with peat. The same rate of disease incidence was evident in inoculated plant populations grown in sterile and natural peat compared with the elimination of suppression effects in sterile compost. The pH level in peat from different origins tended towards acidity, which was expressed in different levels of phytotoxicity. The possible direct and indirect effect of pH on disease development is currently being investigated. *[L]*

Analyses of Compost Ascomycetes Populations Associated with Suppression of *Sclerotium rolfsii*

M. Danon,[1,*] S. Zmora,[2] Y. Chen[2] and Y. Hadar[1]

[1]*Dept. of Plant Pathology and Microbiology [*e-mail: danon@agri.huji.ac.il] and* [2]*Dept. of Soil and Water Sciences, Faculty of Agricultural, Food and Environmental Quality Sciences, The Hebrew University of Jerusalem, Rehovot, Israel*

Composts have long been recognized as facilitating biological control of soilborne plant pathogens. Composts can serve as an ideal food base for biocontrol agents and offer an opportunity to be introduced into and become established in soils. In this study we found that mature biosolids compost (yard waste and sewage sludge) is suppressive to germination of the sclerotia of *Sclerotium rolfsii* on compost plates, and can also suppress the disease development in beans. Microscopic observations revealed that sclerotia placed on suppressive compost were attacked by mycoparasites. Using denaturing gradient gel electrophoresis (DGGE) of PCR-generated DNA fragments, fungal populations from compost and sclerotia surface were followed for 5 days during sclerotia germination or colonization by the mycoparasites. The sclerotia microenvironment is selective for some of the fungal populations from the compost. For example, *Geomyces* species were found to be involved in the mycoparasitism of *S. rolfsii.* There was a time-dependent population shift on the sclerotia surface. We found that a prolonged curing process reduced and even eliminated the suppressive quality of

compost. Compost curing reduced DOC that was correlated with loss of suppression within less than 4 months of curing. A dramatic shift of Ascomycetes populations as a consequence of compost curing was observed both in compost and in sclerotia samples. This research was aimed at studying the mechanism of disease suppression by means of identifying the populations of antagonists of *S. rolfsii* and their environmental interactions. *[L]*

Prevention and Control of Fusarium Crown Rot in Cucumber in Greenhouses

Anat Yogev,[1,*] M. Raviv,[1] R. Cohen,[1] N. Ganayim,[2] M. Aba-Tuama,[2] H. Yunis,[2] Y. Hadar[3] and J. Katan[3]

[1]*ARO, Newe Ya'ar Research Center, Ramat Yishai [*e-mail: honeyb@mgamla.co.il];* [2]*Extension Service, Ministry of Agriculture and Rural Development, Bet Dagan; and* [3]*Dept. of Plant Pathology and Microbiology, Faculty of Agricultural, Food and Environmental Quality Sciences, The Hebrew University of Jerusalem, Rehovot, Israel*

Fusarium crown rot in cucumber is caused by *Fusarium oxysporum* f.sp. *radicis-cucumerinum* and is a relatively new disease in Israel. The pathogen is a soilborne fungus which also produces macroconidia on the stems and these become an additional source of pathogen dispersal and contamination of the greenhouse structure. We adopted a holistic management approach for controlling all sources of inocula before, at and after planting. A pathogen-free perlite medium became infested when it was placed in a greenhouse close to an infested medium, further demonstrating the effective dispersal of this pathogen and the contamination capacity of the greenhouse structure. Structural solarization for 25–30 days, achieved by closing the greenhouse and thereby raising air temperatures to 60–70°C, was effective in controlling inocula on the greenhouse structure of this pathogen, as well as of *Didymella*. Grafting on resistant rootstocks was highly effective in controlling the disease and the use of grafted cucumbers as an intermediate crop reduced disease incidence. The use of a compost consisting of a mixture of separated cow manure and tomato residues was effective in suppressing the disease in a perlite medium, for three consecutive seasons. Chemical control based on the use of prochloraz was effective, but we regard this tool as an interim solution. It was concluded that integrated management, including effective soil or medium disinfestation, is necessary for ensuring a healthy crop. *[L]*

Effects of Boron, Included in Irrigation Water, On the Response of Plants to Pathogens

G. Sorkin,[1] U. Yermiyahu,[2] D. Shtienberg[1,*] and Y. Kapulnik[3]

[1]*Dept. of Plant Pathology and Weed Research [*e-mail: danish@volcani.agri.gov.il] and* [3]*Dept. of Field Crops and Natural Resources, ARO, The Volcani Center, Bet Dagan; and* [2]*Dept. of Soil Chemistry and Plant Nutrition, ARO, Gilat Research Center, M.P. haNegev, Israel*

Boron is an essential element for plants in a narrow range of concentrations and is involved in several physiological processes in the plants. In response to pathogen attack, plants activate local and systemic response. Systemic acquired resistance (SAR) is induced by a prior infection or treatment with chemical inducers, and improves the plant's ability to cope with a broad range of pathogens. In previous studies it was found that potato fields irrigated with recycled water, which contains higher concentrations of boron than drinking water, were less susceptible to *Phytophthora infestans* (the causal agent of late blight). Application of boron together with fungicides in lower concentrations increased the control efficacy of the latter. Furthermore, treating tomato leaves with boron systemically, reduced late blight development. The aim of this work was to examine the effect of boron in irrigation water on plant response to pathogens. In tomatoes, boron's effects on late blight were concentration-dependent: when boron was introduced in the irrigation water at low concentrations (up to 1.5 mg l^{-1}), increasing boron concentration was associated with an increase in

late blight severity. At a boron concentration of >2 mg l^{-1}, an increase in its concentration resulted in a decrease in disease severity. In another series of experiments it was found that boron induced SAR reaction in tomatoes (against *P. infestans*) and tobacco (against TMV). Boron toxicity was not observed in these experiments. PR-1a gene expression was examined in tobacco callus cells. Next, effects of boron on expression of SAR genes were studied in tomatoes and tobacco. Boron locally enhanced the expression of PR-1a and chitinase and systemically enhanced the expression of ETR1, a receptor for ethylene, in tomato. Results of the current study suggest that boron, at sub-phytotoxic concentrations, affects the response of host plants to pathogens. *[L]*

Molecular Look at the Rhizosphere Effect

D. Minz,[1,*] S.J. Green,[1,2] E. Inbar,[1,2] M. Offek[1,2] and Y. Hadar[2]

[1]*Inst. for Soil, Water and Environmental Sciences, ARO, The Volcani Center, Bet Dagan [*e-mail: minz@volcani.agri.gov.il]; and* [2]*Dept. of Plant Pathology and Microbiology, Faculty of Agricultural, Food and Environmental Quality Sciences, The Hebrew University of Jerusalem, Rehovot, Israel*

The rhizosphere effect is a well known phenomenon. Molecular tools of microbial ecology currently allow us to study the effect and forces involved with it without the bias introduced by the need to cultivate bacteria from the rhizosphere. Examination of the root effect on bacteria in its vicinity demonstrates that different groups of bacteria react differently to the distance from the root, and some show no reaction to it. Addition of compost to soils and potting mixes resulted in a substantial shift in the community composition of soil and plant-associated bacteria. Compost amendment interfered with the 'rhizosphere effect' and had a major effect on microbial communities in the rhizosphere and on the roots. This effect was reduced with increasing proximity to the root. These molecular tools allow us to monitor the effect that introduced bacteria has on the community in the rhizosphere. Introduction of *Streptomyces* derived from damping-off suppressive composts had a tremendous effect on the bacterial community in the cucumber rhizosphere. *[L]*