ABSTRACTS OF PRESENTATIONS AT THE 28TH CONGRESS OF THE ISRAELI PHYTOPATHOLOGICAL SOCIETY

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A: CHEMICAL CONTROL

Management of Fusarium Crown Rot of Cucumbers in Greenhouses

Neta Mor,[1,*] Y. Meron,[1] M. Zaidan,[2] G. Sorkin,[3] R. Sadeh,[4] S. Shaanan[4] and J. Katan[5]

[1]*Extension Service, Ministry of Agriculture and Rural Development, Bet Dagan [*e-mail: netmor@shaham.moag.gov.il];* [2]*Milchan Bros., Tel Aviv;* [3]*Agrica-CTS, Tel Aviv;* [4]*Makteshim, Beer Sheva; and* [5] *Dept. of Plant Pathology and Microbiology, Faculty of Agricultural, Food and Environmental Quality Sciences, The Hebrew University of Jerusalem, Rehovot, Israel*

Fusarium crown rot of cucumbers is caused by *Fusarium oxysporum* f.sp. *radicis-cucumerinum* and has resulted in heavy losses to cucumbers in greenhouses, in winter and spring, in the Ahituv area of central Israel since 2002. The pathogen produces enormous amounts of macroconidia on the stems which become heavy sources of contamination. An integrated approach which deals with all sources of inocula has to be adopted for effective management of the disease. This should include sanitation, soil disinfestation, structural disinfestation (*e.g.* by solarization), crop rotation, tolerant cultivars, grafting and effective fungicides. In studies carried out during 2004 – 2005, it was found that the fungicides Octave and Mirage (prochloraz) are effective in preventing or controlling the disease, using three to five drench applications at rates of 0.1–0.2 ml or g per plant, at 14-day intervals starting 7–10 days after planting. However, application of Mirage before planting, as a single treatment, was not effective. The use of compost was not effective in suppressing the disease. Certain cultivars were more tolerant of the pathogen and their use enables reduction of the fungicide dose. Grafting on resistant rootstocks is very effective in preventing the disease. The fungicide Scholar (fludioxonil) is also effective in disease control. An optimal combination of various tools and approaches is necessary for obtaining lasting and effective control of the disease, with minimal use of fungicides. [*L*]

Priori Opti Broad-Spectrum Fungicide from Syngenta

Moran Siti & Luxembourg's Agricultural Team

Luxembourg Industries Ltd., Tel-Aviv, Israel [e-mail: moran@luxembourg.co.il]

The broad-spectrum fungicide Priori Opti (produced by Syngenta, Switzerland) contains two active ingredients, chlorothalonil and azoxystrobin, which are sold in Israel under the commercial names Bravo and Amistar, respectively. Priori Opti has the trans-laminar movement of azoxystrobin, combined with the excellent surface activity of chlorothalonil. The combination of these two active ingredients is responsible for the broad-spectrum activity on the major classes of pathogens and a high efficacy at different stages of disease development. Azoxystrobin belongs to the fungicide group known as Strobilurins, which have protectant and curative properties. The mode of action of this group is based on single-site inhibition of electron transport in the fungal mitochondria. Chlorothalonil is a protectant that has a multiple-sites mode of action, at Krebs cycle, making the development of resistance much less likely and thus Priori Opti is an excellent tool for resistance management. Priori Opti showed excellent efficacy in field trials in Israel on a variety of plant pathogens: *Alternaria dauci* (on carrots), *Alternaria solani* and *Phytophthora infestans* (on potatoes),

L = lecture; *P* = poster.

Stemphylium spp. and *Puccinia allii* (on garlic), *Ascochyta* spp. (on chickpeas and peas), and *Sphaerotheca fuliginea* and *Pseudoperonospora cubensis* (on cucumbers). [*L*]

Accelerated Degradation of Metam-sodium: Characterization and Mode of Action

Shachaf Triky-Dotan,[1,*] Miriam Austerweil,[1] Bracha Steiner,[1] D. Minz,[2] J. Katan[3] and A. Gamliel[1]

[1]*Inst. of Agricultural Engineering and* [2]*Inst. of Soil, Water and Environmental Sciences, ARO, The Volcani Center, Bet Dagan [*e-mail: shachafdotan@walla.com]; and* [3]*Dept. of Plant Pathology and Microbiology, The Hebrew University of Jerusalem, Faculty of Agricultural, Food and Environmental Quality Sciences, Rehovot, Israel*

Accelerated degradation in soil of methyl-isothiocyanate (MITC), the active ingredient of metam-sodium (MS), was documented in controlled environment studies and field experiments. The objective of the present study was to characterize the organisms which are responsible for the occurrence of accelerated degradation of MITC in soil. Under controlled conditions, accelerated degradation of MITC was induced in six agricultural soils. Inoculation of Rehovot natural soil (non-history) by mixing it with 10% of repeated MS-application ('history') history soil induced accelerated degradation in the mixed soil. MITC degradation was reduced in Rehovot history soil, which was sterilized as compared with non-sterilized soil. We developed a soil extraction method in order to concentrate the degrading organisms from the history soils. Mixing the soil extract from history soil with non-history soil generated accelerated degradation of MS. Accelerated degradation of MITC occurred in liquid culture of soil extract from history soil. Addition of several antibiotic compounds to a liquid culture containing soil extract from history soil did not reduce accelerated degradation of MITC. In contrast, heating of soil extract from history soil to 60°C for 2 h, eliminated the accelerated degradation. Initial results indicate that the DNA profile is different in history and non-history soil after MS application. [*L*]

Spatial Distribution and Control of Peanut Pod Wart

H. Caduri,[1] A. Gamliel,[1,*] Y. Cohen,[1] U. Zig[2] and Y. Peretz-Alon[2]

[1]*Inst. of Agricultural Engineering, ARO, The Volcani Center, Bet Dagan [*e-mail: agamliel@agri.gov.il]; and* [2]*Maon Regional Enterprises, D.N. Negev, Israel*

Peanut pod wart, which is caused by *Streptomyces scabies*, reduces the quality of exported in-shell peanuts. Preplant chemigation with formalin is employed commercially to control the pathogen using commercial irrigation systems (moving lines, sprinklers). In recent years there have been increased incidences of pod wart in fields even though fumigation was applied. The objective of the current study was to examine the possible factors which ameliorate the efficacy of formalin on pod wart control. The main hypothesis was that separate analysis of each of the possible factors is not practical. Therefore, we chose to use a 'data mining' approach with the aid of a Geographic Information System (GIS). Forty commercial peanut fields representing the spectrum of agricultural practices and fumigation procedures in Israel were sampled during 2003–2005. These fields encompass the possible relevant factors including soil type, inoculum potential and fumigant application methods. Sampling locations of each plot were incorporated into field maps using GIS. In each field non- fumigated plots were left as a control to represent the potential of disease onset. Differences in pod wart incidence and severity were observed among geographical regions and soil type. Higher disease incidence was observed in sandy soils compared with clay soils. The major factor which dominated pod wart control was the irrigation system which was used for the chemigation process. Moving irrigation lines were a more effective method than sprinklers. Soil rototilling prior to formalin application further improved the fumigation effect. From these findings a model was developed to analyze the contribution of each component to the success of

formalin fumigation. The model is based on four components: disease severity in non-fumigated plots, application method, soil type and soil preparation. Data mining and GIS can be useful tools to study disease spatial distribution of soilborne pests, and to analyze the factors which govern the effect of soil treatments on crop protection. [*L*]

Failure to Induce Resistance against Mandipropamid in *Phytophthora infestans*

Avia (Evgenia) Rubin,[1] D. Gotlieb,[1] Mariana Galperin,[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, Stein, Switzerland*

Mandipropamid (MPD, trade name Revus®) is a new foliar, anti-oomycete fungicide which, together with dimethomorph, iprovalicarb, and benthiavalicarb, belongs to the CAA (carboxylic acid amide) fungicide resistance class. Field resistance against CAAs was reported in *Plasmopara viticola* but not in *Phytophthora infestans.* Two types of studies were conducted in order to evaluate the potential of *P. infestans* to develop field resistance to MPD in the future: (*a*) artificial mutagenesis in the laboratory, and (*b*) selection pressure enforced in the field. In (*a*), sporangia of *P. infestans* sensitive to MFX (mefenoxam, Ridomil Gold) were exposed to UV light and/or chemical mutagens (*e.g.* ethyl methane sulfonate) and thereafter inoculated onto CAA- or MFX-treated potato leaves. In 48 independent experiments, several MFX-resistant mutants were obtained. All expressed stable resistance to MFX and were highly pathogenic to potato and tomato. Of the several CAA-resistant mutants obtained, none was stably resistant to CAAs. All lost resistance in one or a few transfer inoculations on potato leaves. Crosses made *in planta* (E. Rubin and Y. Cohen, *Plant Dis.,* 2006) between MFX-resistant mutants and CAA-resistant unstable mutants produced several stable MFX-resistant progeny isolates in F1 and F2 but no isolates with stable resistance against CAAs. In (*b*), potato and tomato crops in the open field or shade houses (six seasons, 2002–2005) were subjected to repeated sprays of CAAs at x0.5, x1, x2 or x4 the recommended dose and thereafter inoculated with multiple isolates of *P. infestans* or left to be naturally infected with late blight. Of approximately 300 isolates recovered in these experiments, none was resistant to CAAs (Y. Cohen *et al., Plant Pathol.,* in press 2007). All were as sensitive as the reference sensitive isolates. The data suggest that the likelihood for *P. infestans* to develop resistance to CAAs is rather low. [*P*]

Activity of Mandipropamid and Related Carboxylic Acid Amide Fungicides against *Phytophthora infestans*

Y. Cohen[1,*] and U. Gisi[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 Biology, Stein Ch 4332, Switzerland*

Mandipropamid (MPD, trade name Revus®) is a new foliar, anti-oomycete fungicide which, together with dimethomorph (DMM), iprovalicarb (IPRO), and benthiavalicarb, belong to the CAA (carboxylic acid amides) fungicide resistance class. The activity of MPD, DMM and IPRO against the various stages in the asexual life cycle of *Phytophthora infestans* was compared. None of the CAAs had an effect on zoospore release, zoospore motility, or zoospore encystment. However, they were strongly inhibitory of cystospore and sporangial direct germination. MPD was inhibitory at nM concentrations (0.0005 μg ml^{-1}), whereas DMM and IPRO were x10 and x100 less inhibitory, respectively. MPD and DMM induced major ultrastructural changes in cystospores during germination. CAAs had no direct killing effect on cystospores or sporangia. A 1-h exposure to CAAs allowed cystospores to germinate but induced malformation of germ-tubes. CAAs had a limited curative effect and minor effect on late blight lesion expansion or sporulation of *P. infestans* on already established lesions. Efficacy of preventive spray application to potato or tomato plants was fungicide- and dose-dependent, reflecting the relative efficacy of CAAs against spore germination. CAAs were similarly

effective against A1 and A2 isolates of *P. infestans*. as well as against metalaxyl-sensitive and -resistant isolates. MPD exhibited a significantly stronger trans-laminar efficacy against infection as compared with the two other fungicides. Epidemics induced in potato crops by multiple isolates of *P. infestans* were significantly better suppressed by MPD than by DMM or IPRO. MPD exhibited strong protection of the treated crops at one month after the last spray. It seems that three features contribute to the superior activity of MPD in the field: high activity against spore germination, strong trans-laminar efficacy and prolonged persistence on the crop. [*P*]

B: HOST – PARASITE INTERACTIONS

Botrytis cinerea Induces Senescence and is Inhibited by Autoregulated Production of Cytokinin

Dvora Swartzberg,[1,2] B. Kirshner,[3] Dalia Rav-David,[3] Y. Elad[3] and D. Granot[1,*]
[1]*Inst. of Plant Sciences,* [3]*Inst. of Plant Protection, ARO, The Volcani Center, Bet Dagan [*e-mail: granot@agri.gov.il]; and* [2]*Faculty of Life Sciences, Bar-Ilan University, Ramat Gan, Israel*

Botrytis cinerea is a non-specific, necrotrophic pathogen that attacks many plant species, including Arabidopsis and tomato. Senescing leaves are particularly susceptible to *B. cinerea* infection. We hypothesized that *B. cinerea* might induce senescence as its mode of action, and delaying leaf senescence might reduce *B. cinerea* infection. To examine this hypothesis, we followed expression of Arabidopsis *SAG12*, a senescence-specific gene, upon infection with *B. cinerea*. Expression of *SAG12* has been induced by attacks of *B. cinerea*, indicating that *B. cinerea* induces senescence. The promoters of *SAG12* as well as that of a second Arabidopsis senescence-associated gene *SAG13*, were previously analyzed in tomato plants and found to be expressed in a similar manner as in Arabidopsis plants (Swartzberg *et al.*, *Plant Biol.*, 2006). These promoters were used in tomato plants to drive expression of isopentyl transferase (IPT) that catalyzes the rate-limiting step in the biosynthesis of cytokinin, a senescence-inhibiting hormone. The induction of these promoters in tomato plants by *B. cinerea* infection was analyzed by expression of the reporter gene *GUS*. Both promoters exhibit high expression levels upon *B. cinerea* attacks on non-senescing leaves of transgenic tomato plants, supporting our conclusion that *B. cinerea* induces senescence as its mode of action. Expression of IPT under the control of the *SAG12* and *SAG13* promoters by *B. cinerea* resulted in suppression of *B. cinerea* infection, substantiating our prediction that delaying leaf senescence might reduce susceptibility to *B. cinerea*. This infection-suppressing effect was significantly enhanced by pretreating the plants with *Trichoderma harzianum* T39, a non-necrotrophic fungus that is used as a biocontrol agent against *B. cinerea*. [*L*]

Involvement of Viral Helper Component in *Zucchini yellow mosaic virus* Symptom Development in Cucurbits

Y.M. Shiboleth,[1,*] D. Leibman,[1] E. Haronsky,[1] T. Arazi[2] and A. Gal-On[1]
[1]*Dept. of Plant Pathology and Weed Science and* [2]*Dept. of Floriculture, ARO, The Volcani Center, Bet Dagan, Israel [*e-mail: shibolet@agri.gov.il]*

Zucchini yellow mosaic virus (ZYMV) is a potyvirus that infects cucurbits and causes severe disease symptoms. The viral Helper Component (HC) is its suppressor of gene silencing, among its other functions. HC causes the appearance of symptoms as demonstrated by transgenic plants expressing HC which exhibit virus-infection-like symptoms. An attenuated strain of ZYMV has been characterized and contains a single point mutation in the conserved FRNK motif in the central region of HC. In plants infected with potyviruses, there are abnormal levels of microRNAs (miRNA) and the complementary strand microRNA-star (miRNA*) accumulates. miRNA* is rapidly degraded in healthy plants. As miRNAs regulate key developmental genes, it is plausible that symptoms of

potyvirus-infected plants result from enhanced translation of these miRNA targets. In this study, a number of miRNA and miRNA* were found to be highly expressed in ZYMV-infected squash and melon plants at a time-point that was earlier than detectable symptoms. Considerable differences in miRNA and miRNA* levels were found between plants infected with the wild-type severe strain and the attenuated strain. These differences stem from a qualitative mechanism as the quantity of expressed HC was similar in both strains. It was found that both the severe and the attenuated HC are active as suppressors of gene-silencing. Thus, the attenuating mutation does not affect HC protein stability or its suppressor activity but reduces its effect on miRNA and miRNA* accumulation in the cell. In this study we found that HC binds siRNA and miRNA/miRNA* duplexes, and that the attenuating mutation lowers the binding affinity. Thus, it is possible that the mutation lessens active miRNA sequestration and permits near-normal regulation of miRNA target genes. [*L*]

Involvement of Volatile Compounds in the Pathogenicity of Green and Blue Molds on Citrus Fruit

A. Eick,[1] L. Cohen,[1] G. Rafael,[1] E. Lewinson,[2] N. Dudai,[2] R. Shapira[3] and S. Droby[1,*]
[1]*Dept. of Postharvest Science, ARO, The Volcani Center, Bet Dagan;* [2]*Dept. of Vegetable Crops, Newe Ya'ar Research Center, ARO, Ramat Yishay; and* [3]*Dept. of Food Science, Faculty of Agricultural, Food and Environmental Quality Sciences, The Hebrew University of Jerusalem, Rehovot, Israel [*e-mail: samird@agri.gov.il]*

Green and blue molds, caused by *Penicillium digitatum* and *P. italicum*, respectively, are the most damaging postharvest diseases of citrus. These fungi are host-specific for citrus and do not attack any other fruit or vegetable crops. Both pathogens are completely dependent upon injuries on the fruit surface, such as those occurring during harvest and subsequent treatments. Information on the physiological and biochemical basis of this host specificity is limited and conflicting. The main goal of this study was to elucidate the basis for host specificity of *P. digitatum* and *P. italicum* on citrus fruit. This was carried out by characterization of the biological activity of citrus volatiles on spore germination of *P. digitatum* and *P. italicum* as compared with non-host-specific pathogens, *P. expansum* and *Botrytis cinerea.* It was found that citrus volatiles had a stimulating effect on spore germination and growth of the specific pathogens, in the absence of nutrients. Germination rate was increased up to sevenfold by exposure to the volatiles of citrus peel discs. Citrus oils and single compounds, especially monoterpenes, were identified in citrus peel, and had a similar effect on all pathogens. We consider monoterpenes, and mainly limonene, which is the most abundant compound, as the most active and significant signaling compounds in the host recognition by the specific pathogens. [*L*]

Interaction of the Mango Bud Mite (*Aceria mangiferae*) with *Fusarium mangiferae*, Causal Agent of Mango Malformation Disease

Efrat Gamliel-Atinsky,[1,2,*] A. Sztejnberg,[2] M. Maymon,[1] E. Belausov,[3] E. Palevsky[4] and S. Freeman[1]
[1]*Dept. of Plant Pathology and* [3]*Microscopy Unit, ARO, The Volcani Center, Bet Dagan [*e-mail: efrat@volcani.agri.gov.il];* [2]*Dept. of Plant Pathology and Microbiology, The Hebrew University of Jerusalem, Faculty of Agricultural, Food and Environmental Quality Sciences, Rehovot; and* [4]*Dept. of Entomology, ARO, Newe Ya'ar Research Center, Ramat Yishay, Israel*

It has been suggested in the literature that the mango bud mite *Aceria mangiferae* plays an important role in the epidemiology of mango malformation caused by *Fusarium mangiferae.* Current work was designed to study the role of the mites in carrying fungal conidia, vectoring them into the primary penetration sites and possibly assisting fungal penetration and dissemination. Carrying: bud

mites were exposed to a gfp-marked fungal isolate. After 24 h the mites were removed and mounted for microscope observation. The gfp-fluorescing conidia were observed on the examined mites and did not seem to cling to any particular part of the mite body. Vectoring: agar plugs bearing either bud mites and/or gfp-marked pathogen were placed on a leaf near an apical bud on potted mango plants according to the following treatment design: (i) bud mites and gfp-marked pathogen; (ii) bud mites alone; (iii) gfp isolate alone; (iv) untreated control. Bud mites were found only in apical buds of treatments (i) and (ii) and gfp conidia were found in bud bracts only in treatment (i). Penetration: potted mango plants were inoculated with gfp-marked conidia in two treatments with or without the presence of bud mites. The frequency of infected apical buds was higher in the presence of bud mites. Dissemination: conidia and mite traps were placed in a diseased orchard for one year in order to determine a possible association between windborne bud mites and windborne conidia. No windborne bud mites bearing conidia were found on the traps, although high numbers of windborne conidia were detected. These results suggest that the mango bud mite can carry the pathogen conidia on its body, vector it to the apical bud and improve fungal penetration. However, it does not appear that the bud mites play a role in the aerial dissemination of conidia. [*L*]

Systemic Induced Resistance in Lettuce against Downy Mildew

Y. Cohen,* A. Baider, D. Gotlieb and Avia (Evgenia) Rubin
*Faculty of Life Sciences, Bar-Ilan University, Ramat Gan, Israel [*e-mail: ycohen@mail.biu.ac.il]*

Activation of the plant's own defense mechanisms by external stimuli is an efficient way to protect plants against pathogen attack (Y. Cohen, *Plant Dis.*, 2002). In the present study, we evaluated the potential of SAR (Systemic Acquired Resistance) compounds to induce resistance in lettuce plants against downy mildew caused by the oomycete *Bremia lactucae*. BABA (DL-3-amino-*n*-butanoic acid) was highly effective when applied as a foliar spray or as a soil drench. Bion (BTH) and sodium salicylate were ineffective, suggesting that BABA activates a salicylic acid-independent pathway of defense. Plants were highly protected when BABA was applied before or after inoculation. In the field, two sprays of BABA provided season-long protection against downy mildew. Microscope examinations revealed that sporangia of *B. lactucae* germinate freely on BABA-treated leaves, produce appressoria and penetrate into the epidermal cells. However, the primary and secondary vesicles produced in the epidermal cells became heavily encased with callose and, therefore, produced no intercellular hyphae. The invaded epidermal cells accumulate callose in their cell walls and lignin in cell walls and cytoplasm at a much later stage. Post-infection application of BABA induced callose encasement of haustoria, prevented further growth of the intercellular hyphae, and fungal sporulation. This is the first report on a SAR system in which the response of the pathogen precedes the response of the host. (*L*)

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The *Colletotrichum gloeosporioides pac*1 Gene is Required for Fungal Virulence in Avocado Fruits as Part of a Multi-factor Regulator on *pel*B

I. Miyara,[1,*] Hadas Shafran,[2] Eilat Servi,[1] Sarit Lavon,[1] Hilla Kramer Haimovich,[1] A. Sherman[2] and D. Prusky[1]
[1]*Dept. of Postharvest Science of Fresh Produce [*e-mail: itaymiyara@gmail.com] and* [2]*Dept. of Genomics, ARO, The Volcani Center, Bet Dagan, Israel*

Postharvest loss of avocado fruit due to the phytopathogenic fungus *Colletotrichum gloeosporioides* is a major economic problem. Conidium of the fungus penetrates cuticle of the fruit still

in the orchard during fruit growth and remains quiescent until fruit ripening. During storage, the fruit ripens and the fungus renews its development and dark brown decay is observed. During colonization, the pathogen alkalinizes the host tissue by secreting a significant amount of ammonia. The synergistic activities of tissue alkalinization and pectate lyase (*pel*B) gene activation and protein secretion are thought to be essential for full virulence of *C. gloeosporioides*. As many gene products with pH-sensitive activities are regulated by the PacC transcription factor in *Aspergillus nidulans*, we have functionally characterized a *pac*C gene homolog, *pac*1, from *C. gloeosporioides*. Mutants with loss-of-function alleles of the *pac*1 locus were created by targeted gene replacement. *In vitro* mycelial growth of these *pac*1 mutants was normal at the pH range of 4.5 to 7.0. Virulence in loss-of-function *pac*1 mutants was dramatically reduced and delayed in infection experiments with avocado fruits. However, mutants with loss-of-function *pac*1 showed only an 85% reduction of *pel*B transcript expression after 25 h and only a delay of PL secretion. Based on these results, *pac*1 appears to be necessary for the appropriate regulation of physiological processes for pathogenesis of *C. gloeosporioides*. [*L*]

Morphogenesis and Cell Cycle Coordination during Pathogenic Development in *Colletotrichum gloeosporioides*

Iris Nesher* and A. Sharon

*Dept. of Plant Sciences, Tel-Aviv University, Ramat Aviv, Israel [*e-mail: irisn@post.tau.ac.il]*

Colletotrichum gloeosporioides f.sp. *aeschynomene* (*Cga*) is a hemibiotrophic fungus pathogenic on the weed *Aeschynomene virginica*. Collego,® a mycoherbicide preparation used to control *A. virginica*, is produced from *Cga* conidia. The conidia are composed of a single cell with one haploid nucleus. We previously showed that *Cga* conidia can germinate in two different ways, depending on signals: 'pathogenic' germination, which is characterized by rapid nucleus and cell division and formation of a single germ tube, and 'saprophytic' germination, which is characterized by swelling of the conidium that precedes cell division, and formation of two germ tubes. Conidia that germinate in a 'pathogenic' mode cause severe disease, while those that germinate in a 'saprophytic' mode cause only mild symptoms. We used cell cycle blockers and morphogenesis inhibitors in order to test the interaction between cell cycle and morphogenesis during 'pathogenic' germination. Blocking of the cell cycle had no effect on morphogenetic changes during germination: cells that were treated with cell cycle blockers produced normal germ tubes, which elongated and even formed appressoria. Disruption of the actin filaments blocked germ tube formation, but there was no nuclear division in these cells and conidia remained as a single cell with a single nucleus even several hours after induction of germination. These results suggest that induction of pathogenic germination leads to morphogenetic changes, which are necessary for activation of the cell cycle. [*L*]

Development of a Scale for Evaluation of *Tomato yellow leaf curl virus* (TYLCV) Resistance Level in Tomato Plants

M. Lapidot,[1,*] R. Ben-Joseph,[1] Z. Machbash[1] and D. Levy[2]

[1]*Dept. of Vegetable Research, ARO, The Volcani Center, Bet Dagan [*e-mail: lapidotm@volcani.agri.gov.il]; and* [2]*Hazera Genetics, Mivhor, M.P. Lakhish Darom, Israel*

A scale of differential hosts was developed, which enables the determination and comparison of level of resistance to TYLCV expressed by resistant tomato plants. The scale is composed of seven different homozygous tomato genotypes that exhibit different levels of TYLCV resistance, ranging from fully susceptible to highly resistant. The differential hosts comprising the scale were inoculated with TYLCV under greenhouse conditions. Four weeks after inoculation, the plants were evaluated for disease symptom severity, and virus DNA titer was determined. The different genotypes were arranged on the scale according to the symptom severity score. They were then tested under

different environmental conditions, inoculated at different ages, and tested in a field experiment assaying TYLCV-induced yield reduction. While the symptom severity score of each individual resistant genotype changed with the environmental conditions, the relative position on the scale did not vary, except for one genotype. Thus, to evaluate disease resistance of a given tomato genotype, the genotype in question should be inoculated alongside the differential hosts comprising the scale, and within 4 weeks one can determine the relative level of resistance of the tested genotype. *[L]*

C: NON-CHEMICAL CONTROL

Reducing Virus Incidence in Potato Seed Tubers by Rapid Propagation: A Pilot Scheme in Kazakhstan

L. Sozinova,[1] A. Abay,[1] E. Ramankulov,[1] A. Nachmias[2] and G. Loebenstein[3,*]
[1]*National Biocenter, Astana, Kazakhstan;* [2]*Center for Potato Research in Hot Climates, Ofaqim, Israel; and* [3]*ARO, The Volcani Center, Bet Dagan, Israel [*e-mail: gad-talma@barak.net.il]*

The yield of potatoes depends to a large extent on the quality of the potato seed tubers, especially low rates of virus infection and the physiological age of the seed tuber. In Western Europe and the USA, schemes for producing high quality tuber seeds have been in operation for many years and the potato grower does not use his own crop for next years' planting. In many CIS countries, however, there is no good system for supplying tuber seeds of high quality. As a result, tuber seeds are highly contaminated by viruses and subsequently yields are low. For example, in a survey conducted during 1994–1996 in potato fields in Kazakhstan, which grows approximately 160,000 ha potatoes each year, high incidences of PVX (up to 70%), PVY (up to 100%), PLRV (up to 39%), PVS (up to 50%) and PVM (up to 85%) were observed. The average yield of potatoes in Kazakhstan is approx. 13.9 tons ha^{-1}. Seed potato propagation in Western Europe and the USA is done over several generations, with each generation having its tolerance allowance of virus and other diseases. Plant tissue culture methods are employed for the initial increase, and the second increase is generally done in a greenhouse. After this the tubers are multiplied in the field over six or seven successive generations, in areas where virus transmission by vectors is low. Since the rate of potato multiplication in the field is only tenfold per year, potato seed production requires large areas. In rapid propagation schemes, seed tubers are produced after as few as two or three field multiplications. The scheme adopted in Kazakhstan is described and first results to obtain 'certified' tuber seeds regarding virus incidence and yields, in comparison with local tubers, are presented. Thus, yields obtained in 2006 from the virus-tested tubers of several varieties ranged between 28.2 and 35.4 tons ha^{-1} (from small experimental plots), compared with 10.7 tons ha^{-1} in adjacent commercial fields. *[L]*

Efficacy of Biocontrol Agents in Powdery Mildew Suppression

O. Mendelsohn,* D. Rav-David, Y. Elad and D. Shtienberg
*Dept. of Plant Pathology and Weed Research, ARO, The Volcani Center, Bet Dagan, Israel [*e-mail: pelican1@netvision.net.il]*

Past experience revealed a large variability in the efficacy of biocontrol of powdery mildew, often caused by environmental conditions. A major constraint that was revealed is the partial efficacy of microbial antagonists under conditions of low or medium relative humidity. In order to expand the scope of biocontrol agents for a wide range of powdery mildews and environmental conditions, it is important to continue screening new candidates. Five isolates (two bacteria and three yeasts) with biocontrol potential were tested for their activity. One of the isolates (Y16) showed the best potential of powdery mildew biocontrol. Y16 is active under a wide variety of environmental conditions, including low relative humidity, and was consistently effective in the suppression of powdery mildew in adult plants of some important crops, *e.g.* cucumbers, tomatoes and wine grapes. In a field

experiment in a cv. 'Carignan' vineyard, control efficacy of Y16 was equivalent to that of commonly used chemical fungicides. When Y16 was fermented in a liquid medium, a high growth rate was reached at 25–30°C. Evaluation of possible control mechanisms revealed that Y16 antagonistic activity is based on induced resistance. Conidiation was the most affected stage in the disease cycle, and dead cells were also active. For a higher control efficacy, Y16 should be sprayed prior to disease onset; subsequent sprays should be applied at short intervals, up to twice a week. That is probably due to population decline or low aggressiveness of the control mechanism. The activity of Y16 under low relative humidity is an important advantage. Thus, Y16 can be implemented with other control measures in an integrated pest management program. *[L]*

Integrated Management of Gray Mold (*Botrytis cinerea*) in Lisianthus

L. Shpialter,[1,*] D. Rav David,[1] I. Dori,[2] L. Ganot,[2] D. Shmuel,[2] E. Matan,[2] Y. Messika,[3] M. Bruner,[3] Y. Nishri,[3] I. Mor[3] and Y. Elad[1]

[1]*Dept. of Plant Pathology and Weed Research, ARO, The Volcani Center, Bet Dagan;* [2]*R&D South, Besor Experiment Station, M.P. Negev; and* [3]*Extension Service, Ministry of Agriculture and Rural Development, Bet Dagan, Israel [*e-mail: olenas01@pob.huji.ac.il]*

Gray mold that is caused by the fungus *Botrytis cinerea* severely affects lisianthus. The fungus infects the stem bases of whole plants and plant stubs that are left after flower harvesting. Dense canopy and leaf rosette close to the ground prevent adequate air movement and disease control. This research was conducted to study factors that affect gray mold of lisianthus in integrated management of the disease. Conditions that influence the disease severity were tested in a controlled growth chamber; different control methods were tested under commercial conditions at the Besor Experiment Station. Optimal conditions for disease development were relative humidity above 85% and a temperature range of 18–22°C. Disease developed on wounds even at r.h. as low as 65%. High temperatures delayed disease development. Chemical fungicides applied before infection with *B. cinerea* suppressed disease under controlled conditions, whereas calcium had a significantly lesser effect. Only pyrimethanil, fenhexamide and iprodione effectively suppressed gray mold when applied after infection. Additionally, when these products were applied as drenches at different concentrations onto potted plants, different disease suppression levels were achieved. Calcium fertilization was ineffective. Microclimate management by greenhouse soil cover with polyethylene, burying the drip irrigation system, plant density reduction, increased-calcium fertilizations and chemical fungicides were examined under commercial conditions. The polyethylene cover and buried drip irrigation significantly reduced the humidity in the greenhouse and suppressed gray mold on the stem base or plant stubs. Decreased planting density resulted in significantly lower disease levels However, this did not result in a high frequency of healthy plants as in the higher plant density treatment. Calcium fertilization did not significantly suppress the disease, whereas sprays of calcium nitrate exhibited some disease suppression. The tested fungicides fenhexamide and pyrimethanil or their use in alternation with iprodione were most effective. An integrated gray mold control system is being eveloped based on the above positive results. *[L]*

Commercial Applications of 'Shemer' for the Control of Pre- and Postharvest Diseases

Daphna Blachinsky,* M. Lazare, I. Shamai, J. Antonov, A. Husid, A. Bercovitz, Katia Feldman, B. El-ad, M. Prengler and M. Keren-Zur

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

Shemer is a biofungicide based on the yeast *Metschnikowia fructicola*. The mode of action of this yeast is believed to be mainly through competition, with no involvement of antibiotic

substances, and therefore has a minimal effect on the environment. The commercial product (water-dispersible granules) is stable under ambient storage, and can be applied through spray or drench application systems in the field or in packinghouses. Shemer has been registered in Israel and tested under commercial field and packinghouse conditions in several crops including strawberries, sweet potatoes, grapes and citrus. Over the past 2 years the effectiveness of the product has been evaluated under commercial field and packinghouse conditions against several new pathogens of fruits and vegetables. Shemer treatments in commercial packinghouses significantly reduced the development of *Rhizopus stolonifer* on peaches, *Botrytis cinerea* on pepper and *Sclerotinia sclerotium* on carrots in pilot-scale tests. Application of Shemer in the field proved useful also in protecting fruits after harvest, when postharvest treatments are not practiced. Weekly applications of Shemer on strawberry reduced rot development in the field and also the incidence of gray mold (*B. cinerea*) and Rhizopus fruit rot (*R. stolonifer*) during storage. In northern Italy, application of Shemer 24 h before harvest of soft berries, reduced significantly the number of decayed berries caused by *B. cinerea*. In Chile, Shemer was found effective in reducing Botrytis rot in table grapes. In most of the trials, the level of decay control was comparable to that of the most common chemical fungicides currently used by the industry, leading to the registration of the product in Israel. Integration of Shemer with other environmentally safe approaches, including physical means (hot water wash, heat, modified atmosphere) or a variety of mild chemicals (food grade additives), increased the treatment efficacy. [*L*]

SG 101: Environment-Friendly Agent for Controlling Scab on Potato

L. Tsror (Lahkim),[1,*] M. Hazanovsky,[1] N. Ben Yehuda[2] and M. Lavee[2]
[1]*Dept. of Plant Pathology, ARO, Gilat Research Center, M.P. Negev [*e-mail: tsror@agri.gov.il]; and* [2]*Pimi Marion, Agro Clean Technologies, Hutzot Alonim, Israel*

Common scab on potato, caused by *Streptomyces* spp., can result in severe damage to potatoes. The increased prevalence of this disease, especially in the Ma'on region of Israel, is attributable to several factors, among which are: sensitive crops such as peanuts and radish being grown in short rotation; soils that are conducive to the disease; and the absence of effective eradication methods. The objective of the present study was to evaluate SG 101, a stabilized formulation of hydrogen peroxide agent (3.5–7% H_2O_2), as a seed treatment for the reduction of common scab on potato. Field trials were conducted during 2006 at Gilat (loess soil) and Halutza (sandy soil). Heavily infested seed tubers (cv. Désirée) were sprayed with a low volume of SG 101 and planted in these two sites. A significant reduction in the incidence of common and russet scab on progeny tubers was observed in SG 101 treatments. These findings indicate the high potential of SG 101 as a short-term treatment for controlling scab. However, the potential long-term advantage of using SG 101-treated seed tubers is reduction of *Streptomyces* spp. in soils. Further studies of the potential use of SG 101 as a furrow treatment and its effect on other seedborne pathogens are being conducted. [*L*]

Biocontrol of Root-knot Nematodes by a Predatory Nematode

Meira Bar-Eyal,[1] Y. Oka,[1,2] Y. Spiegel[1] and Edna Sharon[1,*]
[1]*Dept. of Entomology, Nematology Unit, ARO, The Volcani Center, Bet Dagan [*e-mail: vpshedna@volcani.agri.gov.il]; and* [2]*Gilat Research Center, M.P. Negev, Israel*

Root-knot nematodes, *Meloidogyne* spp., are sedentary root endoparasites; polyphagous species such as *M. incognita* and *M. javanica* cause great damage to agriculture worldwide. In order to reduce the use of chemicals, attempts have been made to develop alternative measures for nematode control, including biocontrol agents. In this study, a predacious nematode that can feed on various life-stages of *M. incognita* and *M. javanica* was found. This nematode is currently under identification; it is probably a new species belonging to Diplogasteroidea. This work evaluated the potential

of the predacious nematode as a biocontrol agent against root-knot nematodes and predator–prey interactions were studied. Predacious nematodes that were introduced into *M. javanica*-infested soil, before the introduction of tomato transplants, reduced root galling indices. Introducing the predacious nematode into *M. javanica*-infected tomato roots in axenic cultures reduced the second-stage juveniles' (J2s) population. Predacious capabilities on both *M. incognita* and *M. javanica* life-stages were observed: the predators were attracted towards the prey and could feed on egg masses, and on separated eggs – which did not contain the gelatinous matrix enveloping the egg masses; J2s were also attacked. The nematode reproduced on bacteria in nutritional medium and each female produced approximately 60 individuals at 25°C. The life cycle was completed within 8 days and the presence of males was not necessary for reproduction. The results suggest that the predatory nematode has the potential to serve as a biocontrol agent against root-knot nematodes; it depends on the development of appropriate mass production and conservation methods. [*L*]

Disease Suppressiveness by Composts: Its Involvement in Organic Farming and Mechanisms

A. Yogev,[1,2,*] Y. Hadar,[1] M. Raviv,[2] S. Medina,[2] J. Katan[1] and R. Cohen[2]
[1]*Dept. of Plant Pathology and Microbiology, The Hebrew University of Jerusalem, Faculty of Agricultural, Food and Environmental Quality Sciences, Rehovot; and* [2]*Dept. of Vegetable Crops, ARO, Newe Ya'ar Research Center, Ramat Yishay, Israel [*e-mail: honeyb@mgamla.co.il]*

In organic farming, compost is applied yearly in large quantities. In previous studies we found that various composts induce soil suppressiveness towards soilborne pathogens. The objective of the present study was to assess the level of suppressiveness in soils with a history of organic farming. We collected pairs of soil samples from various sites: with a history of organic farming *vs* soil from conventional farming. The pairs were from adjacent sites. It was found that in many but not all cases, soil suppressiveness was greater in the organic soils than in the corresponding conventional ones. Suppressiveness was correlated with biological activity, which was assessed by calorimetry. The possibility that compost induces resistance in plants was considered. Growing plants in composts, transferring them to sand, and inoculating them did not reveal induced resistance. However, when the split-root approach was used, using grafted plants, induced resistance to Fusarium wilt in melon was evident. It is concluded that compost has the potential to suppress diseases and that various mechanisms are involved. [*L*]

Induced Soil Suppressiveness to Plant Diseases by Herbs and Soil Solarization

E. Klein,[1,2] A. Gamliel[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; and* [2]*Laboratory for Pest Management Research, Inst. of Agricultural Engineering, ARO, The Volcani Center, Bet Dagan, Israel [*e-mail: agamliel@agri.huji.ac.il]*

Solarization of soil amended with herb crop residues improves pathogen control and induces changes in the soil microflora. The objectives of this study were to evaluate the effect of a combination of herb-amendments and soil-solarization on soil suppressiveness, *i.e.*, reduced disease onset following post-treatment inoculation of a soilborne pathogen. Suppressiveness to *Fusarium oxysporum* f.sp. *radicis-cucumerinum* (*Forc*) and to *Meloidogyne javanica* was induced by amending Rehovot soil with herb residues (1% w/w), and incubating it for 4 weeks, under field conditions or in a controlled laboratory system. Then, the soil or cucumber transplants were inoculated with eggs of *M. javanica* or conidia of *Forc*, respectively. Disease incidence of inoculated plants in soil which was previously amended was reduced by 60%, compared with inoculated plants in nonamended soil. The most potent herb residues to induce soil suppressiveness were *Diplotaxis tenuifolia* (wild rocket),

Artemisia dracunculus (tarragon), and *Salvia officinalis* (sage). Root galling in tomato or basil plants, caused by *M. javanica*, was suppressed by 50% in soil which had been amended with wild rocket as described. Soil suppressiveness to *Forc* following the incorporation of herb residues was also evident in two more soils, En Tamar and Bet Dagan, which differ in their physical and chemical properties. The capability of soil to suppress cucumber crown and root rot was evident even after three repeated inoculations and plantings of cucumber seedlings in the same soil. Volatile compounds which are generated and released into the soil during the decomposition of the residues probably play a role in inducing soil suppressiveness. In contrast, induced resistance of the host plant does not seem to play a role in this phenomenon. The role of soil microorganisms in induced soil suppressiveness is being studied. [*L*]

Resistance of Wild Tomato Species to Powdery Mildew Caused by *Oidium neolycopersici*

Ina Jolkowsky and Y. Cohen*

*Faculty of Life Sciences, Bar-Ilan University, Ramat Gan, Israel [*e-mail: ycohen@mail.biu.ac.ili]*

Powdery mildew is an important disease of tomato, especially in crops growing in greenhouses or shade houses. The aim of the present study was to select for powdery mildew resistance in cultivated tomato lines or cultivars. Tomato plants were grown in the greenhouse and artificially inoculated with conidia of *Oidium neolycopersici.* None of the 200 lines and commercial cultivars inoculated was resistant. We therefore looked for resistance in wild tomato species. The following wild tomato species (obtained from A. Lebeda, Palacky University, Czech Republic) were found highly resistant: *Lycopersicon cheesmanii* 522, *L. hirsutum* 1391, *L. hirsutum* 1731, *L. hirsutum* 1738, *L. parviflorum* 1322, *L. parviflorum* 2133, *L. pennellii* 1302, *L. pennellii* 1657 and *L. pennellii* 2560. Our lines of *L. pimpinellifolium* (*e.g.* 3707, 3708) were susceptible. Some wild species were immune, showing no symptoms, whereas others produced a hypersensitive response a few days after inoculation. Microscope examinations revealed that resistance of the immune species was associated with inhibition of conidial germination on the leaf surface. Epicuticular waxes (ECW) were extracted from leaves of immune species by momentary dipping in n-hexane and applied to susceptible tomato leaves. Such leaves exhibited resistance to the disease, whereas leaves treated with n-hexane were susceptible. Thin layer chromatography analysis of the ECW revealed that different compounds are responsible for conidial germination inhibition in different species. Crosses were made between *L. esculentum* and wild tomato species in order to transfer resistance to high quality tomato inbreds. [*P*]

Resistance to Anilinopyrimidines and Other Fungicides in *Botrytis cinerea* in Israel

Nadia Korolev,[1,*] M. Mamiev,[1] Tirtza Zahavi[2,#] and Y. Elad[1]

*Dept. of Plant Pathology, ARO, The Volcani Center, Bet Dagan [*e-mail: vpptlg@volcani.agri.gov.il]; and [2]Extension Service, Ministry of Agriculture and Rural Development, Qiryat Shemona, Israel [#e-mail: tirtzaz@yahoo.com]*

Gray mold (*Botrytis cinerea*) is a highly damaging disease of numerous greenhouse- and field-grown crops in Israel. Chemical application remains an important means of control, although it is impeded by the development of pathogen resistance. This research was undertaken to monitor the resistance to newly introduced anilinopyrimidine, hydroxyanilide, phenylpyridinamine and phenylpyrrole fungicides, as well as to the older benzimidazole and dicarboximide fungicides. More than 400 *B. cinerea* isolates from five hosts at 14 locations throughout Israel were tested. Isolates resistant or less sensitive to fluazinam and pyrimethanil were found with a frequency of approximately 10% from greenhouses in Ahituv and Besor; a few isolates were recovered also from vineyards in Ortal and Sha'al treated with the target fungicides, and from some fields of ruscus.

Resistance to fludioxonil and fenhexamid was very rare (~0.5%). Resistance to benzimidazoles and dicarboximides was widespread among field and, especially, greenhouse isolates, but had different patterns of distribution. It was maximal among cucumber isolates from greenhouses in Ahituv: 95% of isolates were resistant, in spite of the absence of benzimidazole fungicide treatments for at least the last 2 years. It could be concluded that the resistant population in Ahituv had become established during the previous period of intensive benzimidazole treatments. There was a low occurrence of benzimidazole-resistant isolates in the greenhouse in Besor among *Lisianthus* isolates (4%), also in the absence of target treatments in the last few years. Isolates moderately resistant to dicarboximide fungicides were found widespread among both greenhouse and field isolates in most places. Isolates with multiple resistance to several fungicides were recovered. [*P*]

Wheat Wild Relative Populations: Resistance to Foliar Diseases

J. Manisterski,* P. Ben-Yehuda and S. Ezrati
*Institute for Cereal Crops Improvement, George S. Wise Faculty of Life Sciences, Tel-Aviv University, Ramat Aviv, Israel [*e-mail: cereal@post.tau.ac.il]*

Modern breeding is in constant need of new genes. Wild relatives of cultivated crops own a vast gene pool, due to their large genetic diversity. Consequently, their use in breeding is increasing significantly. Israel is located in the center of origin of cereal crop plants, where natural populations have coevolved with their natural pathogens. Destruction of natural habitats in this heavily populated part of the world enhances the erosion of genetic diversity. In order to preserve this gene pool, collecting and conserving representative samples from different populations of the wild relatives is extremely important. The Harold and Adele Lieberman Germplasm Bank, located at the Institute for Cereal Crops Improvement (ICCI) of Tel-Aviv University, conserves seed of cereal crop relatives still growing wild in Israel. Evaluation for disease responses plays a major part in the characterization of this collection. This work represents a study of resistance to wheat foliar diseases: leaf rust, yellow rust and stem rust in wild wheat (*Triticum turgidum* var. *dicoccoides*) and *Aegilops* spp. from the section Sitopsis, in the seedling and adult stages. Resistances to these three foliar diseases were found in *Ae. longissima*, *Ae. sharonensis* and *Ae. speltoides* at different proportions between species and among different populations within each species. Low proportions of resistant accessions were found in *T. dicoccoides*. Results of the extensive resistance study, together with detailed passport data of the accessions, are stored in the gene bank database, and will be presented in the future on the ICCI's website. [*P*]

D: IDENTIFICATION AND DIAGNOSTICS OF PATHOGENS

Detection of *Botrytis* in Grapes by Antibodies, qPCR and GFP

M. Celik, T. Kaplunov, Y. Zutchi, S. Ish-Shalom, S. Lurie and A. Lichter*
*Dept. of Postharvest Science, ARO, The Volcani Center, Bet Dagan, Israel [*e-mail: vtlicht@agri.gov.il].*

The fungus *Botrytis cinerea* is the major cause of decay in table grapes during storage and the severity of decay is directly related to the level of contamination by the fungus. There is a wide array of methods to prevent decay during storage and in many cases it is possible to employ simple methods to prevent postharvest decay. However, when inoculum level is high, more stringent methods must be employed. To date there are no efficient means available to forecast the storage potential of grapes. The objective of this study was to test the feasibility of quantifying *Botrytis* in artificially inoculated grapes and to follow disease progression during storage. Var. 'Superior' grapes were

inoculated with a strain of *Botrytis* that contains the green fluorescent protein (GFP) and the course of disease was followed visually during 4 days at 20°C or 4 weeks in cold storage. The GFP protein enabled efficient qualitative detection of the inoculated fungus. Antibodies for rapid detection of *Botrytis* yielded positive results only in the late stages of storage. The amount of *Botrytis* in the tissue was also determined using specific primers in quantitative PCR and the amount of fruit DNA was normalized with grape-specific primers. The results demonstrate positive identification of the fungus at all storage time points and an increase in the amount of the fungus during storage. These results demonstrate the sensitivity of the qPCR method in characterization of *Botrytis* inoculation of grapes and in other agricultural produce. *[L]*

Discrimination of Soilborne Pathogens *via* Fourier Transform Infrared Attenuated Total Reflectance (FTIR-ATR) Spectroscopy

R. Linker[1,*] and L. Tsror (Lahkim)[2]

[1]*Dept. of Civil and Environmental Engineering, Technion–Israel Institute of Technology, Haifa [*e-mail: linkerr@tx.technion.ac.il]; and* [2]*Dept. of Plant Pathology, ARO, The Volcani Center, Bet Dagan, Israel*

Soilborne pathogens cause severe damage to agricultural crops. Rapid, reliable and inexpensive methods for identification of pathogen infestation at an early stage are essential for efficient application of fungicides and bactericides. Fourier transform infrared (FTIR) spectroscopy is one of the methods that have been used successfully for detecting and identifying microorganisms, especially in food products, and the present study investigated the potential use of FTIR attenuated total reflectance (ATR) spectroscopy for phytopathogens discrimination. The study focused on five fungi (*Colletotrichum, Fusarium, Pythium, Rhizoctonia* and *Verticillium*) and a *Streptomyces* bacterium. In contrast to previous studies related to microorganisms discrimination using FTIR-ATR, the pathogen samples were not dried on the ATR crystal, which is a time-consuming operation. Rather, after removing some pathogen aliquots from the solution using tweezers, the material was placed directly on a flat ATR crystal and pressure was applied using a pressure clamp. Following water-subtraction, baseline-correction and normalization of the spectra, principal component analysis and canonical variate analysis were used for sample discrimination. A three-level hierarchical approach was adopted. The first classifier discriminated between bacterium and fungi, with success rates of 90% and 100% for the fungi and bacterium, respectively. The second classifier discriminated among the different fungi genera, with success rates ranging from 75% to 89%, and the third classifier discriminated between two *Colletotrichum* strains, with a success rate of 78%. Although the results of this preliminary study are somewhat poorer than results usually reported in the literature, this can be explained by the fact that the samples were not dried or otherwise treated prior to the measurement. After enlarging the database and refining the mathematical analysis, the proposed approach could lead to the development of a very simple, inexpensive and rapid procedure for discriminating phytopathogens, at least at the genus level. *[L]*

Morphological and Molecular Methods for Identification of the Root-knot Nematode *Meloidogyne incognita*

Evgeny Kozodoy, Emma Teverovsky and Ella Gomberg*

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

The root-knot nematode *Meloidogyne incognita* is one of three widespread nematodes of the genus *Meloidogyne* in Israel (*M. hapla* and *M. javanica* are the two others). The host range of this nematode is very broad, including numerous crop and ornamental plants. Usually, for the purpose of pest control, it is not necessary to identify this nematode up to species level. Nevertheless, in

certain cases it is important to determine the exact host / pathogen combination. Most species of paprika are susceptible only to *M. incognita* and are not infested by other *Meloidogyne* spp. In such cases specific identification before planting is very important. In addition to the importance for local agriculture, supplementary methods are significant for the exportation of products and the needs of quarantine. Misunderstandings between PPIS and plant protection organizations of foreign countries were solved by including molecular methods in the identification procedure. Each of the above-mentioned nematodes can be identified morphologically using the structure and perineum patterns of females. Isolation of nematodes and their morphological identification require high levels of experience and proficiency. We show that the use of PCR with specific primers facilitates detection of *M. incognita* as isolated females, eggs and also in the plant tissue of galls, with reliable results. [*L*]

Citrus Mal Secco: Identification and Diagnosis of the Causal Fungus by Advanced Methods

David Ezra,[1,*] Tammy Kroitor-Keren[1] and Avraham Sadowsky[2]
[1]*Dept. of Plant Pathology and Weed Research, ARO, The Volcani Center, Bet Dagan [*e-mail: dezra@volcani.agri.gov.il]; and* [2]*Extension Service, Ministry of Agriculture and Rural Development, Bet Dagan, Israel*

Mal secco of citrus is a destructive disease, inflicting damage mainly to lemon, lime and citron etrog, but may infect also less susceptible citrus types. The causal agent, *Phoma tracheiphila,* a pathogenic fungus, infects the host plant by penetration through wounds in the leaves and branches, followed by its spread through the xylem to the rest of the tree. Infected trees usually exhibit typical symptoms such as leaf yellowing and shedding, dehydration of the branches, red pigmentation of infected xylem, the appearance of typical fruiting bodies on the dry plant material and, in severe cases, death of the tree. Coping with the disease involves mainly using disease-free propagation material and strict sanitation of the infected plants, followed by burning of the trimmed branches. Diagnosis and identification of the fungus are done by classical methods including symptoms identification in the field and fungal isolation in the laboratory. Symptoms identification in the field requires experience, because some of the symptoms might be present there due to other – unrelated – factors. Isolation and identification of the fungus in the laboratory require equipment, knowledge and time, and the isolation process in the laboratory is not always fruitful and reliable. Lately, citrus-importing countries have raised the question of whether fruit from Israel might be harboring *P. tracheiphila.* Fungal isolation from fruits is a time-consuming process during which the fruit is retained in the storehouse. All this has raised the need for a rapid, simple and reliable method that does not require identifying disease symptoms or any other specific knowledge for diagnosis. Such a method, PCR-based, was developed in our laboratory. We can successfully diagnose and identify the disease in infected, suspected plant material and in fruit. The method is simple to perform in the laboratory, rapid – taking approximately 3 h from sample reception in the laboratory, highly specific and accurate. [*L*]

Screening Imported Potatoes for Brown Rot

Emma Teverovsky, Faina Zlatsin, Rosa Gofman, Edna Levy and I.S. Ben-Ze'ev*
*Plant Protection Diagnostic Service, Plant Protection and Inspection Services (PPIS), Ministry of Agriculture and Rural Development, Bet Dagan, Israel [*e-mail: israelb@moag.gov.il]*

A country-wide survey was carried out in 1993 in all the seed potato fields in Israel for the presence of two quarantine diseases: Brown Rot caused by *Ralstonia solanacearum* and Ring Rot caused by *Clavibacter michiganense* subsp. *sepedonicum.* The results of this survey determined the status of Israel *vis-à-vis* the EU as a state "free of these two diseases, based on survey results". Since 1995, when *R. solanacearum* was reported in several European countries, all imported seed and

consumption potatoes have been screened for the presence of this bacterium. Sampling and testing are performed according to the EU protocol, which is upgraded periodically. Each sample contains 200 tubers, representing 25 tons. Bacteria are extracted from a piece of tissue taken from the hilum of each tuber and cultured on a semi-selective medium. Morphologically appropriate colonies are tested by ELISA. Positive colonies are further identified by their fatty acids (FA) profile. In addition, 10% of the ELISA-negative samples are tested by PCR with specific primers, which were changed three times during the last few years, to increase the detection reliability. The present method uses two pairs of primers: one pair specific to *R. solanacearum,* the second used as an internal control for plant material. Colonies determined positive by both ELISA and FA tests are used to infect tomato and eggplant seedlings to complete Koch's postulates. Preparation of samples and ELISA tests are performed by external laboratories recognized by PPIS. FA, PCR and Koch's postulates are carried out in PPIS laboratories. A single imported lot of consumption potatoes was found infected since 1995. The infection was further confirmed by the plant protection services of the exporting country. *[L]*

Identification of Pathogenic Fungi during Purity Test of Seeds Imported to Israel

Ronit Kohen*, Irina Arutyunyan, Orit Cohen, Galina Siden and Lea Mazor
*Official Seed Testing Laboratory, ARO, The Volcani Center, Bet Dagan, Israel [*e-mail: vpshedna@volcani.agri.gov.il]*

A purity test is carried out on seed samples aimed for propagation, in order to determine a seed lot's components and predict field contamination by noxious and weed seeds. The aims of this work were to stimulate awareness of the possibility of detecting *(a)* sclerotia that are mixed with the seeds and/or *(b)* lesioned seeds during purity tests and thus prevent damages that might occur due to their entrance into Israel. Sclerotia of *Sclerotinia sclerotiorum* were found by our laboratory from 1999 until 2006 in 45 crops belonging to 12 plant families. Sclerotia were found during these years in 272 samples, and in 204 of them viable sclerotia were found. There has not been any uniform direction of increase or decrease of viable sclerotia percentage during the years. In our laboratory, sclerotia of *S. minor* were first revealed in 2006 in a watercress seed sample; sclerotia of *Claviceps purpurea* were first revealed in ryegrass seeds in 2005 and in fescue seeds, ryegrass and rye samples in 2006; *Tilletia tritici* was first revealed in 2006 in a wheat seed sample. Detection and identification of sclerotia and infected seeds during purity tests and reporting the findings to the Plant Quarantine Service and/or to the Health Ministry, prevented field infestation with sclerotia and the entrance into Israel of quarantine fungi (*C. purpurea, T. tritici*). In addition, distribution of fungi that exist in Israel was avoided (*S. sclerotiorum, S. minor*), as well as new races of these fungi. Today most of the purity tests are being carried out on seeds that are aimed for propagation. Most of the imported shipments of grain are not being tested in the laboratory at all for pathogen identification. Therefore, it is important to expand the purity and health tests to edible grains in order to avoid damage to health and to prevent contamination of fields with leftovers of grain and waste. [*L*]

Classification of *Rhizoctonia* spp. to Anastomosis Groups *via* ITS Sequence Analysis

Michal Sharon,[1,*] Shiro Kuninaga[2] and Baruch Sneh[1]
[1]*Dept. of Plant Sciences, The George S. Wise Faculty of Life Sciences, Tel-Aviv University, Ramat Aviv, Israel [*e-mail: ms_me100@yahoo.com]; and* [2]*Dept. of Integrated Human Sciences, School of Dentistry, Health Sciences University of Hokkaido, Hokkaido, Japan*

Rhizoctonia spp. isolates are divided according to the number of nuclei in their young hyphal cells, into uninucleate (UNR), binucleate (BNR) and multinucleate (MNR). The classical method divides them subsequently according to the ability of the isolates' hyphal tips to fuse with the hyphae of representative isolates of designated anastomosis groups (AGs). This method is time-

and labor-consuming and requires meticulous experience to determine the hyphal fusion accurately. In addition, some isolates do not anastomose with certain isolates of their own AG, or even self-anastomose. Recently, molecular methods which are based on genetic relatedness have provided more accurate tools for classification. rDNA-ITS sequence analysis was found to be the current most appropriate available method for classification of different fungi, including *Rhizoctonia* spp. The present study attempted to provide a comprehensive classification of *Rhizoctonia* spp. *via* multiple alignment of rDNA-ITS sequences from the GenBank and cluster analysis. Generally, the isolates were located within clusters of AGs and subgroups in neighbor joining and maximum parsimonious trees, supporting the genetic basis of the classical anastomosis grouping method. These analyses were complemented with percent sequence similarity ranges of isolates within, as well as among, the AGs and subgroups. A threshold of percent sequence similarity range could not be determined for isolates of the same AG or subgroup because of an overlap between the percent similarity range within an AG or a subgroup and that among the AGs or subgroups. The present study represents a first attempt to provide a comprehensive classification of UNR, BNR and MNR *Rhizoctonia* spp. based on genetic relatedness according to the rDNA-ITS sequences available in GenBank. The multiple alignment and percent sequence analyses may provide a relatively handy tool for the identification and classification of new or unknown *Rhizoctonia* spp. isolates. [*P*]

Invited Lecture

Wild Emmer Wheat as a Source for Disease Resistance Genes: From Genetic Diversity to Positional Cloning

Tzion Fahima

Institute of Evolution, University of Haifa, Mount Carmel, Haifa, Israel [e-mail: fahima@research.haifa.ac.il]

Wild emmer wheat, *Triticum turgidum* ssp. *dicoccoides* (genome AABB), the progenitor of domesticated wheat, was discovered by A. Aaronsohn in 1906 in northern Israel. Aaronsohn had the pioneering vision that the progenitor of wheat would become a source of genes for wheat improvement. Nevertheless, traditional approaches for utilization of wild alleles are usually very slow. For example, transferring disease resistance genes from exotic sources is sometimes slower than the emergence of new pathogenic races in the field. The advanced genomic technology available today may help to increase the efficiency of utilization of wild germplasm for crop improvement.

Previous screening of wild emmer wheat collections revealed high genetic diversity at the DNA level and for many agronomic traits, including disease resistance, drought tolerance and grain quality. Our current studies are focused on genetic mapping and positional cloning of several wild emmer wheat genes. The availability of advanced cereal genomic tools, such as physical maps, EST database, wheat BAC libraries, and the complete rice genome sequence, enabled us to initiate positional cloning of disease resistance and high grain protein genes derived from wild emmer wheat. These genes include *Yr15*, conferring resistance to stripe rust, and *PmG3M*, a novel powdery mildew resistance gene. The high grain protein gene, *Gpc-B1*, was cloned by the positional cloning approach. The *T. dicoccoides* allele of *Gpc-B1* was found to confer pleiotrophic effects that include earlier senescence and increased protein and grain mineral concentrations. These studies demonstrate the impact of modern genomic technology on the exploration and utilization of the rich allelic variation residing in wild wheat germplasm. [*L*]

E: PATHOGEN CHARACTERIZATION AND PROCESSES IN PATHOGENICITY

Climatic Conditions that Affect the Progress and Control of Tomato Powdery Mildew (*Oidium neolycopersici*)

Dana Jacob,[1,2,*] D. Rav-David,[1] A. Sztejnberg,[2] Y. Messika,[3] G. Reshef,[3] H. Yehezkel,[4] L. Ganot,[4] D. Shmuel[4] and Y. Elad[1]

[1]*Dept. of Plant Pathology and Weed Research, ARO, The Volcani Center, Bet Dagan [*e-mail: danasjacob@hotmail.com]; [2]Dept. of Plant Pathology and Microbiology, The Hebrew University of Jerusalem, Faculty of Agricultural, Food and Environmental Quality Sciences, Rehovot; [3]Extension Service, Bet Dagan; and [4]R&D South, Besor Experiment Station, M.P. haNegev, Israel*

Tomato powdery mildew is caused by the obligate pathogenic fungus *Oidium neolycopersici*. Symptoms on tomato include powdery white lesions on the upper surface of infected leaves. These lesions also appear on all other aerial parts of the plant, except for the fruit. Severe damage to the foliage results in a reduction in photosynthesis, which can lead to the appearance of necrotic spots on the leaves and eventual defoliation and plant death. The incidence of this disease has increased in Israel in recent years. The objectives of this study were to examine the effects of various biotic and abiotic factors on different components of the disease cycle (germination, appressoria formation, conidiation and survival) in tomato plants and to determine which environmental conditions may limit the progress of an epidemic. The highest levels of conidial germination and the formation of appressoria were observed at 25°C and high relative humidity. High light intensity reduced germination and enhanced appressoria formation. The highest levels of conidiation were observed at 22°C and 70–85% r.h., under high light intensity. Low levels of disease severity were observed at 28°C. During the autumn of 2005 and the spring of 2006, we examined disease development in walk-in greenhouses with various microclimatic conditions at the Besor Experiment Station. A positive correlation was observed between disease severity and the length of time that environmental conditions ranged between 15° and 25°C and 60% and 90% r.h. A negative correlation was observed between higher temperatures and lower r.h. levels. Most of the commercial control products employed effectively suppressed disease. However, the efficacy of the different products varied with differences in the application time relative to the time of infection, and with the frequency of the sprays. The results of this project are being applied to the development of an integrated system for the control of powdery mildew in tomato. [*L*]

Isolation of Reduced-pathogenicity Mutants of *Fusarium oxysporum* Using *Agrobacterium tumefaciens*-mediated Transformation

Youlia Denisov,[1,2,*] O. Yarden[1] and S. Freeman[2]

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

The soilborne fungal plant pathogen *Fusarium oxysporum* f.sp. *melonis* (*Fom*) causes vascular wilt disease of muskmelon. This study was aimed at identifying and characterizing pathogenicity-related genes of *Fom*. Using an *Agrobacterium tumefaciens*-mediated transformation, we created a collection of 2000 tagged mutants. Five reduced-pathogenicity isolates were identified following a screen of muskmelon seedlings. To determine sequences flanking the inserted T-DNA, we employed a TAIL-PCR approach. A reduced pathogenicity mutant, D122, caused a reduction of approximately 80% in plant mortality, when compared with the wild-type isolate. In addition, D122 is characterized by excessive mycelial branching. Mutant D122 was found defective in the gene encoding a Snt2-like transcription factor of *Schizosaccharomyces pombe*, which regulates activity of stress-related genes in yeast. [*L*]

The Species *Fusarium proliferatum* in Israel

I.S. Ben-Ze'ev,* Genya Elkind, Emma Teverovsky, M. Zeidan and Edna Levy

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

The phytopathogenic species *Fusarium proliferatum* was described three decades ago. During most of this period it was confused with other species in section Liseola and mentioned under the name *F. moniliforme.* Records of *F. proliferatum* in Israel are very scarce, a fact that we aim to correct here. *F. proliferatum* resembles *F. verticillioides* and *F. fujikuroi,* both morphologically and pathologically, in causing similar maize and rice diseases, respectively. Forty-nine plant hosts have been recorded for this fungus in Israel at the PPIS phytopathology lab since 1991. Assuming a certain degree of correlation between the frequency of identifying a disease in the lab and its relative occurrence in the agricultural environment, these are the main diseases caused by *F. proliferatum* in Israel: corm and root rot of asparagus; leaf blight of onion and chives; bulb rot of onion and garlic; retardation and death of gypsophila; leaf rot of date palm and other palms; crown rot of capsicum; various rots of carnation; leaf spots, corm and root rot of curcuma; bulb rot of lilium; stalk and cob rot of maize; tuber rot of potato. The diseases of maize, asparagus, onion and date palm have been mentioned abundantly in the literature. Some of those mentioned here could be more suited to the local conditions. In many of our identifications *F. proliferatum* was not the only fungus and possibly not the main pathogen. Certain isolates of this species were considered for use as biological control agents. Considering the broad host range and the pathogenicity of this species, such a use seems to be highly questionable. Molecular identification was performed by amplifying and comparing sequences of the *tub-2* and *tef-1* genes with homologous ones found at GenBank. [*L*]

Involvement of Type 2A Ser/Thr Phosphatase in the Morphogenesis and Pathogenesis of *Sclerotinia sclerotiorum*

A. Erental,* A. Harel 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: ariel_erental@hotmail.com]*

Sclerotinia sclerotiorum is a sclerotia-producing, broad-range phytopathogenic fungus. The sclerotium is a pigmented, multicellular, firm resting structure composed of condensed vegetative cells and is capable of surviving for years in soil. Plant infection depends on the formation of melanin-rich infection cushions, secretion of hydrolytic enzymes and oxalic acid. Type 2A Ser/Thr phosphatases (PP2As) are involved in several cellular signal transduction pathways. PP2As are comprised of a catalytic subunit, an anchoring A subunit and a variable, regulatory, B subunit. The catalytic subunit-encoding gene (*pph1*) was isolated and part of it was cloned in an antisense orientation. When antisense expression was induced, inhibition in fungal growth could be observed, indicative of a crucial role for PP2A in fungal growth. A construct containing the B regulatory subunit-encoding gene, *rgb1*, designed for RNAi-based gene silencing, was produced. In isolates in which *rgb1* RNA levels were decreased, inhibition in melanin production and reduced pathogenesis were observed, apparently due to impaired infection cushion production. We examined the relative expression of *rgb2* (encoding an additional B subunit) during sclerotial development. Expression of *rgb2* in mature sclerotia was threefold higher than in hyphae, implying that *rgb2* is involved in sclerotial development. When attempting to produce mutants containing an antisense *rgb2* construct, none of the transformants produced sclerotia, confirming our hypothesis for the involvement of *rgb2* in sclerotial development. As sclerotial morphogenesis has been shown to be induced by phosphatases and as protein phosphatases are known to be sensitive to reactive oxygen species, we determined the relative expression and the activity of catalase, superoxide dismutase, and superoxide-NADPH oxidase during sclerotial development. We demonstrated that the expression, as well as the activity, of the genes/enzymes changes throughout the development process in partial correlation with the changes that were observed in the activity of protein phosphatases. [*L*]

Molecular Tools Development for Efficient Homologous Recombination and Direct Hyphal Transformation in *Sclerotinia sclerotiorum*

Maggie Levy,* A. Erental 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: vpshedna@volcani.agri.gov.il]*

Harnessing homologous recombination can provide powerful and efficient possibilities for studying gene function *via* disruption, replacement or other manipulations. The KU80 protein (ATP-dependent DNA helicase II 80 kDa subunit) is part of the nonhomologous end-joining DNA repair mechanism. We identified and disrupted the homologous gene in *Sclerotinia sclerotiorum* and generated *sku80*-deficient mutants. These mutants show almost no phenotypic defects when compared with the wild type; sclerotial formation as well as pathogenicity on tomato fruits were normal. The frequencies of homologous recombination in these strains were markedly higher than those of the wild type when transformed with a *cna1* (calcineurin) replacement construct. This indicates that *sku80* disruption strains can be used as efficient recipients for gene-targeted procedures. In addition, we increased the ease of *S. sclerotiorum* transformation by adapting a direct Bim-lab apparatus-mediated transformation procedure which uses compressed gas to assist the aerosol of transforming DNA to penetrate fungal hyphae and sclerotia. This procedure is efficient, reproducible and does not reduce fungal fitness. Combining the use of *sku80* strains with Bim-lab-assisted transformation significantly enhances our capabilities to manipulate this phytopathogen genetically. [*L*]

Seedling Disease and Growth Retardation of Onion and Corn in Israel

A. Gamliel,[1,*] Y. Kapulnik,[2] Orna Ucko[3] and S. Dobrinin[3]
[1]*Inst. of Agricultural Engineering and* [2]*Inst. of Crop Sciences, ARO, The Volcani Center, Bet Dagan [*e-mail: agamliel@agri.huji.ac.il]; and* [3]*Extension Service, Ministry of Agricuture and Rural Development, Be'er Sheva, Israel*

A new disease was detected recently in onion and corn crops in the southern part of the Arava Valley in Israel. The area is characterized by a hot summer and mild winter, which allows crop planting in the early autumn for winter production. The disease symptoms in onion plants are damping off and poor plant stand. The growth of the entire crop is retarded and yield decline is significant. The symptoms in corn plants are evident during flowering and are exhibited by plant stunting, poor root system and root rot. The disease is spread in the fields in foci which are typical of a soilborne disease pattern. The objectives of the study were to identify the causal organism and study its behavior and eruption in the region. Isolation of the pathogen was conducted using a selective agar medium which consists of Czapek salts amended with onion root extract. A heat-tolerant fungus was isolated from diseased onion and corn roots. The fungus colonizes the roots intensively during the early stage of seedling development. The pathogenicity of the isolated fungus to corn and onion was verified following artificial inoculation in pot experiments. Disease symptoms and the damage to onion seedlings in the field were greater following soil solarization compared with in non-treated soil. On the other hand, fumigation with methyl bromide, dazomet or metam sodium effectively reduced damping off symptoms in onion and improved seedling emergence, and plant growth. Yield of onion bulbs was significantly increased in the fumigated plots (10 kg m^{-2} in the fumigated plots compared with 3–4 kg m^{-2} in the non-treated or in the solarized plots). The curative effect of fumigation on disease control and improved yield was evident even following three consecutive onion and corn crops. Continuous efforts to further identify and characterize the pathogen, its biology and epidemiology are currently underway. [*L*]

First Report of *Neonectria radicicola* on Avocado in Israel

Edna Levy,[1,*] Miriam Zilberstaine,[2] Genia Elkind,[1] M. Zeidan,[1] Emma Teverovsky[1] and I.S. Ben Ze'ev[1]

[1]*Plant Protection and Inspection Services (PPIS) [*e-mail: ednal@moag.gov.il] and* [2]*Extension Service, Ministry of Agriculture and Rural Development, Bet Dagan, Israel*

Blighting and wilting of young avocado (*Persea americana* Mill.) trees accompanied by root rot were observed during the last 3 years in a few locations in Israel. Isolations revealed a *Cylindrocarpon* sp., after which a survey was initiated in avocado nurseries for fungi and nematodes. *Cylindrocarpon* sp. was isolated from the roots of 10–100% of the seedlings in all the surveyed nurseries. The sale of seedlings was prohibited and phytosanitary measures were undertaken immediately. Perithecia of the fungus were observed in the lab, first on roots and later on PDA medium. Single-conidium and single-ascospore isolates were obtained for morphological identification of this *Cylindrocarpon / Neonectria* species. Molecular methods were employed for further identification. DNA was extracted from mycelium grown in shake culture. Conventional PCR was performed using three universal primers for fungi: (i) small subunit mitochondrial rDNA, (ii) β-tubulin and (iii) a sequence of the ITS region. Sequencing of these three products revealed homologies of 99.8%, 100%, and 94%, respectively, with *Neonectria radicicola,* the telemorph of *Cylindrocarpon destructans. N. radicicola / C. destructans* is a known agent of root rot disease in nurseries of raspberry, grapevine, ginseng and forest trees. Due to discrepancies in morphological descriptions of this species by different authors, it is now commonly accepted that *N. radicicola* is a complex of species. Our avocado isolate has morphological features which may warrant its segregation from the complex, as a self-standing species, after more data are analyzed from a number of isolates from different locations. This is the first report of *Neonectria radicicola* in *Persea americana. [L]*

Ecological and Epidemiological Characteristics of *Didymella rabiei* Isolates Originating from Wild and Cultivated Chickpea

O. Frenkel,[1,2,*] A. Sherman,[2] D. Shtienberg,[3] T. Peever,[4] M. Chilvers[4] and S. Abbo[1]
[1]*Inst. of Plant Science and Genetics, The Hebrew University of Jerusalem, Faculty of Agricultural, Food and Environmental Quality Sciences, Rehovot, Israel [*e-mail: omerfre@yahoo.com];* [2]*Dept. of Genomics and* [3]*Dept. of Plant Pathology and Weed Research, ARO, The Volcani Center, Bet Dagan, Israel; and* [4]*Dept. of Plant Pathology, Washington State University, Pullman, WA, USA.*

Ascochyta blight, caused by *Didymella rabiei*, is one of the most significant diseases of domesticated chickpea. Recently, the pathogen was isolated from natural populations of wild *Cicer* species (*C. judaicum*). As epidemic intensity in the wild seems to be less severe than in the domesticated crop, we quantified the ecological requirements and the epidemiological characteristics of isolates from wild and domesticated origins. Hyphal growth rates of isolates from both origins were measured at 15° and 25°C and the ratio between them was used to determine the adaptation to high temperatures. The results showed that the growth rate of isolates from domesticated origin was significantly higher ($P<0.001$) at the high temperature than the growth rate of isolates from wild origin. This finding is in line with the fact that the wild chickpea is a winter annual, whereas domesticated chickpea has a long evolutionary history as a late spring – summer crop. Analyzing the disease components of the isolates on domesticated chickpea revealed that isolates from domesticated fields were significantly more aggressive (shorter incubation period, higher disease rate and disease severity) than isolates from wild origin. On wild *Cicer* lines, the wild isolates were more aggressive in at least some of the disease components. The results suggest that isolates from each origin are better adapted to their relevant environmental conditions and to their main host. Therefore, it is unlikely that wild *D. rabiei* populations serve as a source of inoculum to domesticated fields and *vice versa*. A successful crossing between isolates from wild and cultivated origins may help us to develop a useful tool for studying the evolution, ecology and genetics of this pathogen and its relationships with its hosts. [*L*]

Recent Outbreak of *Erwinia chrysanthemi* in Israel: Monitoring in Seed Tubers

L. Tsror (Lahkim),[1,*] O. Erlich,[1] S. Lebiush,[1] M. Aharon,[1] U. Zig,[2] S. Warshavsky[2] and J.J. van de Haar[3]

[1]*Dept. of Plant Pathology, ARO, Gilat Research Center, M.P. Negev, Israel [*e-mail: tsror@agri.gov.il]; [2]Ma'on Enterprises Ltd., M.P. Negev, Israel; and [3]HZPC Research BV, Metslawier, the Netherlands*

Erwinia chrysanthemi (*Ech*) was reported only once (in the 1980s) in Israel on potato plants originating from imported Dutch seed tubers; however, in recent years it has been occurring more frequently, causing economic damage. The disease was observed on crops of various cultivars grown from seed tubers imported from the Netherlands. In addition to typical wilting symptoms on the foliage, in cases of severe infection progeny tubers rotted in the soil. Samples were obtained from potato plants and tubers collected from commercial plots during the spring seasons of 2004 to 2006. Six bacterial isolates were characterized by biochemical, serological and molecular tests. All tests verified the isolates as *Ech*. One of the isolates was used for pathogenicity assays on potato cvs. 'Nicola' and 'Mondial'. Symptoms appeared 2 to 3 days after stem inoculation, and 7 to 10 days after soil inoculation. Seed tubers sampled from commercial lots were tested for latent *Ech* infection in spring 2006. The test was based on bio-PCR or enrichment ELISA. Of 43 lots, 41.9% were positive in both the laboratory and the field (typical symptoms were observed); 48.8% were negative in the laboratory tests and no disease was found in the field; 4.6% of the samples were positive in the laboratory but no symptoms were observed in the field; and 4.6% were negative in the laboratory test but mild symptoms were observed in the field. In conclusion, the developed protocol for detection of latent *Ech* infections in seed tubers can be used to prevent the introduction of *Ech* into Israel. [*L*]

Causal Agents of Onion Rot and Their Prevention: Summary of Field Research 2004–2006

H. Yunis,[1,*] N. Omeri,[1] E. Margalit,[1] Y. Baum,[2] B. Baloum,[3] D. Sarid,[4] A. Omeri[5] and O. Naot[5]

[1]*Extension Service, Ministry of Agriculture and Rural Development, Bet Dagan [*e-mail: yhisham@shaham.moag.gov.il]; [2]CTS Chemicals Ltd., Hod haSharon; [3]Makhteshim Ltd., Lod; [4]Agan Industries Ltd., Ashdod; and [5]Lidorr Chemicals Ltd., Ramat haSharon, Israel*

Several agents (including *Aspergillus niger* and *Fusarium oxysporum* f.sp. *cepea*) cause onion rot under field and/or storage conditions. The existence of latent fungi in the field cannot be observed *a priori*, but during storage may cause considerable damage by reducing the final yield by an order of between 25% and 50%. The fungal inoculum infects the crop during the growth season in the field. First symptoms might be observed only at harvest, but the bulk of the damage caused to the crop develops during storage. Because of this particular feature, we have attempted to answer the question whether these storage rot-diseases can be prevented using chemical means during the growth season. The research was carried out during the years 2004 to 2006. Basic treatments included canopy spray applications at 2-week intervals starting after full emergence and until leaf drop. In addition, treatments were tested during either the first half or the second half of the growth season. The harvested crop was stored at 65% r.h. and 25–26°C for at least 3 months. At the end of this period, the stored onions were visually classified according to the particular disease. During this study it was proved that it is possible to reduce significantly the level of onion rots by the above described means, and that treatments applied during the second half of the season were superior to those applied earlier. The trend of the results has been very consistent over the years. The various fungicides showed different efficacy levels. [*L*]