

## Abstracts of presentations at the 30th Congress of the Israeli Phytopathological Society

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### Phytopathology in Israel as reflected in the meetings of the Israeli Phytopathological Society: A chronological review

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**(Dedicated to the memory of Dr. Avi Grinstein, scientist, colleague and friend)**

Activities in the field of phytopathology in Israel started nine decades ago, before the establishment of the state. We can define four major events in the history of this profession in Israel which shaped its present form. (i) The establishment of the first Department of Plant Pathology in the Agricultural Research Station in 1921, by Prof. I. Reichert; (ii) the establishment of the Faculty of Agriculture of The Hebrew University of Jerusalem in 1942; (iii) the first formal phytopathology meeting, organized by Dr. J. Palti in 1967; and (iv) the establishment of the Israeli Phytopathological Society (IPS) in 1970, with Prof. G. Loebenstein as its first President. Since then, IPS has organized the annual meetings and many activities, including awards to graduate students and training. During those nine decades, the Israeli plant pathologists carried out many studies on the main phytopathological issues, including: diagnostics, biology of pathogens and their genetics, host–parasite

relationships, epidemiology, evolution of pathogens and their hosts, disease management and many other topics. Tools for the transfer of knowledge were developed. The means for disease management in ancient times were also considered. [L]

### POPULATION GENETICS AND RESISTANCE

#### Resistance evaluation of tomato plants to Fusarium wilt (race 2)

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Developing resistant cultivars involves identification of resistant parents and progenies. This, however, requires reliable screening methodologies. Seedling tests under controlled conditions are relatively cheap and rapid. The selection can be further improved by the use of DNA markers. Tests for resistance of tomatoes to *Fusarium oxysporum* f. sp. *lycopersici* (FOL) race 2 were carried out using either 14-d-old seedlings or 35-d-old transplants of one susceptible and nine resistant lines, either heterozygous [ $I_2/i_2$ ] or homozygous [ $I_2I_2$ ] at the  $I_2$  locus. The dominant allele confers resistance to FOL race 2 already at the seedling stage. The root-dip inoculation method was used. Of the susceptible line with 14- and 35-d-old plants, 93–100% showed typical disease symptoms. Among the resistant lines, up to 31.6% and <7% of

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L = Lecture; P = Poster

the respective inoculated 14- and 35-d-old seedlings showed disease symptoms, and thus some of those lines were categorized as susceptible. The ten lines were also evaluated in two field tests, and the results were compared with those of the above two tests. The experimental plots were artificially infested at 2936 or 710 CFU g<sup>-1</sup> soil. Evaluation of adult plants was carried out 90–100 days after planting. Of the nine lines carrying the *I*<sub>2</sub> allele, seven showed no symptoms, and two had a few plants with slight discoloration but no wilting. Cuttings of asymptomatic (determined resistant) or symptomatic (determined susceptible) seedlings of a heterozygous line which were examined in the seedling test, were rooted and grown to fruit maturation. Seeds were extracted from the heterozygous plants and phenotypic comparisons between offspring of 14-d-old seedlings with the *I*<sub>2</sub> allele, revealed that up to 28% and 22% of the offspring of the asymptomatic and symptomatic plants, respectively, developed disease symptoms. It is concluded that discoloration and some wilting symptoms in *I*<sub>2</sub> plants do not necessarily indicate susceptibility; that the 14-d-old seedling test is rather severe; and that the 35-d-old seedling test reflects better the response under realistic conditions in infested fields. Seedling tests can be routinely used, but transplant tests should also be used for final determination of resistance, when necessary. [L]

#### Quantitative trait loci conferring powdery mildew resistance in a durum wheat × wild emmer wheat RIL population

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Durum wheat (*Triticum turgidum* ssp. *durum* (Desf.) MacKey.) is an important crop for the human diet (e.g.

pasta, couscous, bread, etc.), particularly in the Mediterranean Basin, where ~75% of the world's durum grain is produced. Powdery mildew caused by the biotrophic pathogen *Blumeria graminis* (DC.) E.O. Speer f. sp. *tritici* Em. Marchal (*Bgt* hereafter), is a foliar wheat disease resulting in severe yield losses worldwide. Of numerous studies reported on *Pm* genes, however, only a single *Pm* allele (*Pm3h*) is known to have originated from a *T. durum* background. In the present study, the isolate *Bgt#66* (collected from the wild progenitor of wheats, *T. turgidum* ssp. *dicoccoides*, in Ammiad, Israel) showed high disease severity in the wild germplasm and reduced aggressiveness on domesticated cultivars. The genetic basis of this reaction to *Bgt#66* was further dissected in a population of 152 recombinant inbred lines (RILs), derived from a cross between durum wheat (cv. Langdon) and wild emmer (acc# G18-16). Quantitative trait loci (QTL) analysis of the reaction to *Bgt#66* revealed a total of five significant QTLs with a LOD score range of 4.5–15.4, explaining totally 53.6% of the variance. A major QTL was detected on chromosome 1A with a LOD of 15.4, explaining 22.7% of the variance of disease severity. In addition, four minor QTLs were found on chromosomes 1B, 2B, 3A and 7A. In all QTLs the higher resistance to the *Bgt#66* was conferred by the domesticated allele (LDN). The identification of new resistance alleles from *T. durum* could contribute to wheat breeding for *Bgt* resistance by precise exploitation of the available and well studied LDN genetic platform. [L]

#### VAT - new software for consistent analysis of plant pathogen populations and their hosts

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User-friendly software VAT (Virulence Analysis Tool; [http://www.tau.ac.il/lifesci/departments/plant\\_s/members/kosman/VAT.html](http://www.tau.ac.il/lifesci/departments/plant_s/members/kosman/VAT.html)) combines a set of analysis steps to facilitate a comprehensive, effective and logically consistent evaluation of diversity within and among populations of plant pathogens and plants with virulence and resistance data, respectively. Basic characteristics of the VAT software are that it allows for the diversity analysis of sexually and asexually reproducing populations; is the only software package that provides calculations of recently developed assignment based diversity parameters; is compatible with other major statistics packages; and is applicable to molecular markers' data.

VAT has the following features: (i) Data entry, transformation, and identification of pheno- or genotypes; (ii) Descriptive tools characterizing the distributions (of phenotypes, virulences and resistances), complexities, associations, dissimilarities between individuals, diversities within and distances between populations; and (iii) Inference-statistical procedures assessing confidence intervals and significance of population parameters by re-sampling techniques. [L]

### **Dynamics of the Israeli population of *Puccinia triticina* on wheat during the years 1993–2008**

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Leaf rust, caused by the fungus *Puccinia triticina* Eriks., is the most common rust disease of wheat that occurs in wheat production regions worldwide. Monitoring the Israeli population of wheat leaf rust has been performed consistently since 1993. A total of 831 single urediniospore isolates was analyzed during 1993–2008. The structure of the pathogen population has changed to a large extent since 1993. Clear separation of the annual collections of wheat leaf rust into two distinct groups of the 1993–1999 and 2000–2008 populations was achieved using methods of diversity analysis and clustering. This differentiation among the annual pathogen populations can be attributed mainly to the following forces: (i) possible massive migration of leaf rust uredinio-

spores from the neighboring regions in 1994 (in parallel with those of yellow rust with *Yr9* virulence first recorded in Israel in the same year); and (ii) selection pressure of new wheat cultivars resistant to yellow rust that were introduced into Israel since 1997. Diversity within the annual collections of *P. triticina* isolates was highest in 1994, when many new pathotypes and linkages between virulences were observed. Single-step mutants of the new pathotypes were naturally selected and became predominant since 2000. Significant changes of virulence frequency on a number of *Lr*-genes for resistance (e.g. *Lr2a*, *Lr15*, *Lr17*, *Lr21*, *Lr26*) were also registered during the years 2000–2008. [L]

### **Quantities of DNA in the nuclei of wheat leaf rust urediniospores, as a parameter for distinguishing between leaf rust of the bread wheat type and the durum wheat type**

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Severe leaf rust epidemics on durum wheat have been described annually for the last 20 years in some parts of wheat-growing areas in the world such as Ethiopia, North Africa, Mexico, South America and lately in Europe. The durum type belongs to the same species as the bread wheat type, *Puccinia triticina*. It forms its sexual stage on the same alternate host, *Thalictrum*, and is crossable with the bread wheat type. Except for a few differences in virulence patterns, there are no parameters to distinguish between these two types. A method for evaluating the quantity of DNA (weight in pg) in rust urediniospore nuclei was developed in the Institute for Cereal Crops Improvement. This method enables discerning between the bread and durum types and other rust species. It was applied by us for the identification of leaf rusts attacking wheat and wheat-related species, as shown in the following examples. (i) In Morocco, leaf rust attacking the durum wheat has a native alternate host, *Anchusa italica* (Boraginaceae). Its DNA content differs from *P. triticina* which causes epidemics on wheat all over the world. This rust is different morphologically too,

and belongs to a different species, *Puccinia recondita*. (ii) Leaf rust attacking *Aegilops speltoides* in Israel was defined as a different *forma specialis* of wheat leaf rust according to its different range of host genera; as both rusts are alike in their alternate host and in their morphology: teliospore size, shape of the substomatal vesicle and quantity of DNA in their urediniospore nuclei. (iii) A leaf rust was found on *Triticum dicoccoides* in the Golan Heights of Israel. This leaf rust differs in its teliospore size from the known wheat leaf rusts in Israel. Evaluation of its DNA content will contribute to the study of its systematics. [L]

## PLANT – PATHOGEN INTERACTIONS: PROKARYOTES AND VIRUSES

### Parasitic weed *Orobanche aegyptiaca* can acquire viruses from its hosts

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*Orobanche* spp. (broomrape) is a parasitic plant which subsists on roots of a wide range of host crops, thereby causing severe losses in yield quality and quantity. *Orobanche* physically connects to its hosts through haustorial structures which link the parasite and the host vascular systems (xylem and phloem). In this study we investigated the movement of host viruses from infected tobacco and tomato plants to the *Orobanche* parasite. Our results demonstrate for the first time that *Orobanche* can be thus infected by viruses belonging to different groups: *Tobacco mosaic virus* (TMV), *Cucumber mosaic virus* (CMV), *Potato virus Y* and *Tomato yellow leaf curl virus* (TYLCV). Sap-extract from *Orobanche* tissue grown on RNA virus-infected hosts was infective on test plants. We detected accumulation of both plus and minus RNA strands of CMV and TMV in *Orobanche* stems grown on infected tomato or tobacco. In addition, CMV particles and CMV-siRNAs accumulated to a high level in *Orobanche* stems grown on tobacco plants.

TYLCV DNA was detected in *Orobanche* stems grown on tomato infected with the virus. These data indicate that CMV replicates in *Orobanche* tissues. Accordingly, we hypothesize that parasitic weeds such as *Orobanche* spp., like many plant species, are hosts for viruses. [L]

### Infection of grapevine with infectious clones of *Vitivirus* and their use in functional genomics

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Rugose wood is a disease which has spread to most of the grape vineyards throughout the world. *Grapevine virus A* (GVA) and *Grapevine virus B* (GVB) from the *Vitivirus* group, Flexiviridae family, are associated with this disease. Understanding the etiology of the disease requires developing infectious clones of GVA and of GVB and developing an efficient means to infect grapevine plants. The infection with infectious cDNA clones of woody plants, especially of grapevine, represents a great research challenge. In this work, an *Agrobacterium*-mediated method for infecting *in vitro* grapevine (*Vitis vinifera*) explants with GVA and GVB infectious clones has been developed. In addition, using the *Agrobacterium*-mediated method, we utilized the GVA-based vector to silence the grapevine phytoene desaturase (PDS) gene, which is involved in the chlorophyll synthesis pathway. Roots of *in vitro* explants were incubated in *Agrobacterium* cultures containing infectious clones of either GVA-based vector (pGVA118), GVA-PDS or GVB (pGVB-33-6). Explants that were incubated in pGVA-PDS Agro-solution showed symptoms of photo bleaching 14–20 dpi, suggesting GVA infection. In addition, the infection was confirmed by means of semi-quantitative RT-PCR. In grapevine plantlets, which were inoculated with GVA-118 or GVB-33-6, the presence of the virus was confirmed by means of RT-PCR. In the future, this method is expected to allow deeper understanding of the rugose wood etiology and to be utilized in functional genomics assays of grapevine. [L]

## Identification of bacterial genes induced during the *Xanthomonas*–tomato interaction

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The establishment of bacterial pathogens in their host depends on a complex process that requires the coordinated activity of many genes, whose identity and mode of action are largely unknown. Several bacterial genes involved in pathogenicity have been identified using *in vitro* systems. However, these approaches are limited in their capability to mimic the pathogen–host interaction. Therefore, *in vivo* approaches are desirable to further our understanding of pathogenesis. We used a ‘Recombinase In Vivo Expression Technology’ (RIVET) approach to identify *in vivo*-overexpressed (*ivx*) genes of *Xanthomonas campestris* pv. *vesicatoria* (*Xcv*), the causal agent of bacterial spot disease of tomato. This screen revealed genes belonging to different categories. Knockout mutants were generated for some of these genes in an attempt to characterize them, and some were shown to contribute to bacterial virulence. One of these genes is *citH*, encoding a citrate transporter highly similar to the characterized CitN of *Bacillus subtilis*. An *Xcv citH* deletion mutant was significantly impaired in its ability to grow *in planta* and to cause symptoms compared with the wild type. This mutant was also unable to grow in minimal medium with citrate as the sole carbon source. Another gene was *lipA*, annotated as a putative secreted lipase. The *lipA* mutant grew like the wild type strain in rich medium, but its growth was significantly compromised in a medium with olive oil as the single carbon source. Also, the lipolytic activity of the extracellular fraction of the *lipA* mutant was significantly reduced relative to that of the wild type strain, thus supporting that *lipA* indeed encodes a secreted lipase. Leaf syringe infiltrations revealed that the *lipA* mutant induced disease symptoms that were less severe than those induced by the wild type strain, thus supporting the belief that *lipA* has a role in pathogenicity of *Xcv*. [L]

## Sequential expression of putative virulence and host-defense genes during infection of tomato plants by *Clavibacter michiganensis* subsp. *michiganensis*

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The plant-pathogenic bacterium *Clavibacter michiganensis* subsp. *michiganensis* (*Cmm*) is the causal agent of bacterial wilt and canker of tomato, an economically important disease. The molecular interactions between *Cmm* and the tomato plant were studied by following the expression of virulence genes on the one hand and of host-defense genes on the other hand, during early stages of infection. The following *Cmm* genes were investigated: *celA* encoding endoglucanase and *pat-1* encoding serine protease, which reside on the plasmids pCM1 and pCM2, respectively; genes residing on the chromosomal pathogenicity island, *chpC* and *ppaA*, encoding serine proteases; and *pelA*, encoding pectate lyase. Other chromosomal cell-wall-degrading enzymes that were tested included endocellulase (*celB*) and xylanases (*xysA* and *xysB*). The expression of the genes was measured in *Cmm382* (wild type), *Cmm100* (plasmids-free strain) and *Cmm27* (lacking the pathogenicity island) under *in planta* conditions by qRT-PCR. Results demonstrated that expression of *celA* and *pat-1* were significantly induced in *Cmm382* during early stages of infection (12 to 72 h). In contrast, the expression of *chpC* and *ppaA* was induced only after 96 h. The expression of *celA* and *pat-1* was substantially reduced in *Cmm27* compared with the wild type, whereas the expression of *chpC* and *ppaA* was reduced in *Cmm100*. *celB*, *xysA* and *xysB* were expressed at early stages of infection. The foregoing results indicate that *celA* and *pat-1* may elicit signals, via oligosaccharides or small peptides, triggering the expression of genes located on the pathogenicity island. The host-defense genes chitinase and a PG

inhibitor were expressed at early stages of infection with the highest level of chitinase after infection with *Cmm27*. PR1a and PR-5-isoform were expressed after 96 h and showed similar levels after infection with *Cmm382*, *Cmm100* and *Cmm27*. The results indicate that genes residing on the pathogenicity island may be involved in suppression of the host defense system. [L]

### Molecular analysis of MAP kinase cascades activated by tomato MAPKKKε and their involvement in plant immunity

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Resistant plants have evolved the capability to protect themselves from disease-causing organisms by activating a wide array of defense responses. In our lab, we investigate resistance of tomato (*Solanum lycopersicum*) to two different Gram-negative bacteria: *Xanthomonas campestris* pv. *vesicatoria* (*Xcv*) and *Pseudomonas syringae* pv. *tomato* (*Pst*), which are the causal agents of spot and speck diseases, respectively. These pathogens colonize the aerial parts of the plant and cause economically important losses to tomato yields worldwide. Control of speck and spot diseases by cultural practices or chemicals is not effective and genetic sources of resistance are limited to certain *Pst* and *Xcv* strains. To isolate genes involved in these resistance responses, we used a functional screen based on virus-induced gene silencing (VIGS). Silencing of a MAP kinase kinase kinase, *SIMAPKKKε*, compromised resistance to both bacterial pathogens and its overexpression elicited pathogen-independent cell death. In addition, by silencing *SIMAPKKKε* in *Nicotiana benthamiana* plants, we discovered that this gene is a key regulator of cell death mediated by the specific Pto and AvrPto interaction. Moreover, we found that this gene is specifically involved in the development of HR activated by pairs of resistance genes and avirulence genes from tomato and the leaf mold fungus *Cladosporium fulvum*, respectively. We also tested the

hypothesis that *SIMAPKKKε* might play a role in disease susceptibility. Using VIGS, we showed that *SIMAPKKKε* kinase did not affect the bacterial growth in susceptible plants and is not essential for disease susceptibility. These results reveal a role for the *SIMAPKKKε* gene in plant disease resistance and in the elicitation of cell death. [L]

### IDENTIFICATION AND CHARACTERIZATION OF CAUSAL AGENTS OF PLANT DISEASES

#### *Pelargonium zonate spot virus* – A new viral disease of tomato in Israel

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Toward the end of 2007, symptoms were observed in open-field tomato (*Solanum lycopersicum*) in a single plot in the Lachish region of Israel. The symptoms included a light mosaic, leaf malformation and severe stunting of the plants. The causal agent was mechanically transmitted to several indicator species. Using electron microscopy, spherical particles 25–30 nm in diameter were observed. Viral particles were purified from infected *Nicotiana benthamiana* plants and cDNA was synthesized from RNA isolated from the particles. Cloning and sequencing of the cDNA showed 94% homology to the open reading frames coding for the capsid and movement proteins of *Pelargonium zonate spot virus* (PZSV). The virus belongs to the Bromoviridae family, and has a genome composed of three RNA segments. PZSV was first isolated from tomato in southern Italy in 1982 and later was also reported from Spain in 2000 and France in 2002; recently, it was reported from California, USA. The virus is transmitted mechanically to a wide host range, which in addition to tomato plants includes pepper (*Capsicum annuum*), melon (*Cucumis melo*), cucumber (*Cucurbita pepo*), *Nicotiana benthamiana*, *N. glutinosa*, *N. tabacum* and others. To the best of our

knowledge, this is the first report of the occurrence of PZSV in Israel. [L]

### Characterization of two viruses infecting eggplants in Israel

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Eggplant (*Solanum melongena*) is grown worldwide as a vegetable crop. In Israel eggplants are grown during summer in open fields in the coastal area and during autumn and winter mostly in the Jordan and Arava valleys. There has been an outbreak of two new plant viruses infecting eggplants in Israel. The priority was to identify the viral causal agents and the related vectors, or the mode of transmission. The first eggplant disease was found during autumn 2003 when unfamiliar symptoms were observed in eggplants growing in the Jordan Valley. The disease symptoms consisted of mild mottling on leaves with different degrees of fruit distortion. Viral particles morphology showed similarity to *Carlaviruses*. The molecular weight of the coat protein (CP) was determined as 36 kDa while the RNA genome consisted of a major fraction of 9.5 kb and two additional small RNA fractions, 2.6 and 1.3 kb. Viral RNA served as a template for the RT-PCR amplification of the conserved region in *Carlaviruses*. BLASTn analysis has shown 87% nucleotide sequence identity to *Carlavirus*. The aphid *Myzus persicae* failed to transmit the virus; however, it was successfully transmitted by the whitefly *Bemisia tabaci*. The second eggplant disease was observed during summer 2004 at Na'an, resulting in significant damage due to plant stunting, leaf mottling and narrowing accompanied by fruit malformation. In this case isometric particles could be isolated from infected plants. Characterization of the virus CP and sequencing part of its genome indicated that the causal agent is a strain of *Eggplant mottled crinkle virus* (EMCV), which is known to belong to the *Tombusvirus* genus. The virus is readily transmitted by mechanical inoculation. [L]

### Identification and characterization of the interactions between Squash leaf curl virus and Watermelon chlorotic stunt virus

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In the summer of 2003, two new Begomoviruses were discovered in cucurbit fields in Israel. The viruses were identified as *Squash leaf curl virus* (SLCV) and *Watermelon chlorotic stunt virus* (WmCSV). Soon after, a new disease was found in melon fields. Due to their overlapping host range, it was suggested that SLCV and WmCSV might have synergistic interactions. By analyzing dual infection on melon plants, we found that infection of melon plants with SLCV hardly affects the plants, whereas infection with WmCSV caused disease symptoms and yield loss of up to 30%. However, dual infection with both viruses induced the most dramatic effects. The plants displayed severe disease symptoms coupled with strong stunting, produced small and low quality fruits and suffered a significant yield loss. Following viral DNA accumulation level, it was found that in plants infected with both viruses, SLCV DNA accumulation strongly increased compared with plants infected with only SLCV, whereas WmCSV DNA accumulation did not change regardless of whether the virus was inoculated with or without SLCV. It was also found that growth season has an impact on the interaction between SLCV and melon plants: in the summer, plants infected with SLCV showed low viral DNA accumulation, as opposed to melon plants infected in the spring. In this work we identified and characterized a synergetic interaction between SLCV and WmCSV. This is the first demonstration that mixed infection of two begomoviruses under field conditions has an impact on the yield of an agriculturally significant plant such as melon. [L]

### Postharvest dark spots in potato tubers harvested before skin-set

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At least 40,000 tons of potatoes (*Solanum tuberosum* L.) grown in the western Negev of Israel for export to Europe are harvested before skin set. These tubers are packed in ‘Big-Bags’ mixed with peat in order to maintain conditions of high humidity that preserve their firmness. In recent years, some of the tubers have reached their destination, after shipping, with dark, necrotic irregular spots on their skin. *Rhizoctonia* spp. were isolated from most of these spots, which had developed during storage and transportation. Since attempts to conduct Koch postulates by artificial inoculation with *Rhizoctonia* mycelia were not successful in all cases, we assumed that atmospheric conditions caused by the unique package may induce expression of disease symptoms. Analysis of the temperature fluctuations in a commercial package stored immediately after harvest at 6°C showed that the bulk temperature rose during the first 4 days and equilibrated with room temperature after more than 30 days. The CO<sub>2</sub> level, representing the tubers’ respiration rate, reached its peak after 1 week and returned to the baseline after 3 weeks of storage. The peat in the pile lost 20–40% of its water capacity and after 1 month of storage a gradient in the peat water content, from 41% in the upper part of the bag to 17% at the bottom, was detected. Simulating the conditions observed in the bag contributed to the success of artificial inoculation, which resulted in typical superficial symptoms and re-isolation of the fungus. Thus, completing Koch’s postulates supported the assumption that the causal agent of the dark spots was indeed *Rhizoctonia* spp. The sclerotia phenotype of the pathogenic fungus on a petri dish is different from sclerotia of *R. solani*. The characterization of the isolate will be conducted in future work. Histological observation of wound-inoculated potatoes showed formation of a few layers of cells detected in UV-light, indicating over-suberization response, whereas only one layer of cells developed in non-inoculated tissue. To the best of our knowledge, this is the first

report on a postharvest disease caused by the soilborne pathogen *Rhizoctonia* spp. [L]

### Molecular diagnostic for *Harpophora maydis*, the cause of maize late wilt disease in Israel

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Late wilt disease causes the wilt of maize, and is well documented in Egypt, where it causes severe damage, as well as in India and Hungary. The pathogen can survive in the soil for long periods, and at present the most effective means of contending with it is the use of reduced-sensitivity maize strains; however, a pathogenic virulent strain of the fungus is known, which may be a threat to relatively resistant maize strains. For the last 20 years, common symptoms for this disease were documented in the Upper Galilee area of northern Israel, particularly in the Hula Valley. Recently, the prevalence of the disease has increased. Here we report for the first time that the direct and primary cause of the disease, in Israel, is the fungus *Harpophora maydis*, of which there has been no previous record in Israel. We used a molecular method and identified the pathogen 50 days after seeding, before the emergence of disease symptoms, both in the susceptible and resistant strains. *H. maydis* DNA measurements fit the previous description of the disease progress in a susceptible maize strain. However, the results revealed that the pathogen spread in resistant plant organs as well, although no disease symptoms were observed. Furthermore, we have shown that seeds from apparently healthy resistant strains may become infected and therefore serve as vectors that spread the disease. The molecular assay is now used for testing the health of export seeds before shipment. [P]

## PREVENTION AND CONTROL OF PLANT DISEASES – A

### Endophytes as plant disease biocontrol agents

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Many micro-organisms capable of inhibiting plant pathogens and providing biological control are known, yet the commercial success of this approach has been limited so far. Nevertheless, this field has gained much attention in recent years. To overcome the limitations of many of the existing biological fungicides, there might be a need for micro-organisms with a higher activity than those applied to date. Endophytes, micro-organisms inhabiting plant tissues without causing disease symptoms, compete with pathogens on the same ecological niche. This fact, and the ability of some endophytes to secrete antimicrobial compounds and to induce resistance in plants, makes them good candidates for biocontrol. We have screened and tested 1,023 endophytic bacteria, fungi and yeasts, isolated from 46 different plants residing in nature reserves, abandoned orchards, ornamental gardens and botanical gardens. The activity of all isolates was tested against eight plant pathogenic fungi in petri dishes. Isolates demonstrating a particularly strong or unusual activity were examined further. Selected isolates were tested *in planta* in rough lemon seedlings against the mal secco disease, caused by the pathogenic fungus *Phoma tracheiphila*. The screening resulted in the discovery of 102 bacterial isolates and 18 fungal isolates which inhibited all pathogens in over 95% of the cases, on average. In addition, a unique and new fungal species (*Daldinia* sp.) was discovered. This isolate secretes volatile antimicrobial compounds capable of inhibiting and even killing various fungal pathogens in the laboratory and in fruits postharvest. Another fungal isolate, which was identified as a member of the genus *Penicillium*, according to a morphological and molecular analysis, was found to

secrete compounds capable of inhibiting late blight and bacterial rot in tomatoes, and these diseases' respective pathogens. These findings and their potential applications in agriculture are discussed. [L]

### Effect of microclimate on powdery mildew (*Oidium neolycopersici*) in tomato

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*Oidium neolycopersici* causes severe powdery mildew of tomato. Symptoms of the disease caused by this ectoparasite include powdery white lesions on leaf blades and, in severe epidemics, similar lesions may also be observed on other organs, e.g. petioles, stems and sepals, but not on fruits. The disease can eventually cause the death of the entire plant canopy. The objective of the present work was to study the interaction between the host plant, the pathogen and the microbial population on plant surfaces under changing microclimate conditions. The parameters (microclimate, growth conditions and agro-technology) that affect various stages of the disease cycle as well as populations of beneficial microorganisms were characterized. Conditions that restrict disease development and cultural methods that suppress the disease were found and integrated control was carried out. A high disease level occurred under field and controlled conditions at high relative humidity (60–90%) and moderate temperatures (15–25°C), whereas a slight change towards higher temperatures resulted in significantly lower disease severity. Therefore, daytime heat treatment by closing the side walls of the greenhouse was applied under commercial-like conditions. The daytime warming was found in two

experiments to be very effective in disease suppression and it was superior to a spray program that was used and to genetic resistance. The combination of increased day temperatures and spray treatments was found to be very effective. In addition to the applicable result, the research emphasizes the ability of global climate change to affect pathogens and the antagonistic microorganisms and to affect their interaction with plants. [L]

### Combining human- and environment-friendly methods to improve postharvest disease control synergistically

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Management of postharvest diseases is a prerequisite for a stable and profitable food supply. Combining different control methods can improve efficacy, increase the spectrum of controlled pathogens and reduce the possibility of resistance development. During the last few years, carrot growers have begun to brush carrots before storage to remove the outer peel of the root. In the present study we showed that this practice enhances the appearance of black root rot during storage, a postharvest disease caused by the fungus *Thielaviopsis basicola*. The chemical fungicide iprodione is usually applied before storage to reduce the possibility of development of this disease. In the present study, we evaluated the efficacy of combining physical, low-residue chemical and biological control agents as an alternative to the conventional chemical control approach. A technology for the precise application of steam and combined application with stabilized hydrogen peroxide (Tsunami<sup>TM</sup>) and a commercial yeast product (Shemer<sup>TM</sup>) were tested. Both the steam and Tsunami<sup>TM</sup> alone were highly effective at reducing disease decay but were phytotoxic to the roots. Application of combined treatments of sublethal steam followed by a sublethal dose of Tsunami<sup>TM</sup> or the recommended dose of Shemer<sup>TM</sup>, improved efficacy and disease control synergistically compared with each of the treatments alone. The same pattern was observed by application

of a non-compatible combination of Tsunami<sup>TM</sup> first, washing off with water, and then Shemer<sup>TM</sup>. These experiments showed that disease-control agents can potentially be used for a short period, then washed off—if necessary—and efficiently followed by application of a biological control agent. The biological pathway and mode of action are still under investigation but to the best of our knowledge this is the first study using the synergistic effects of sublethal treatments applied sequentially to control postharvest disease as a potential method to reduce the use of chemicals in fruits and vegetables. [L]

### Cross resistance to CAA fungicides in artificial mutants of *Bremia lactucae*

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Carboxylic acid amide (CAA) fungicides such as dimethomorph (DMM), benthialvalicarb (BENT) and mandipropamid (MPD) are highly effective in controlling plant pathogens belonging to the *Peronosporales* (except *Pythium* spp.). While resistance to CAAs occurs in natural populations of *Plasmopara viticola*, no natural resistance was reported in other *Peronosporales*. Artificial mutagenesis failed to yield resistant mutants in *Phytophthora infestans* but produced less sensitive mutants in *Bremia lactucae*. Our aim here was to study the cross resistance to CAAs among mutant isolates of *B. lactucae*, namely, to learn if a mutant selected for resistance to one CAA, is resistant to the other two CAAs. For this purpose, five different mutants, produced by exposure to a mixture of 0.15% or 0.66% EMS (ethyl methane sulfonate) and 5 mg  $\Gamma^{-1}$  CAA, were propagated for four asexual generations on CAA-free lettuce plants (7 days old, cotyledon stage, cv. ‘Noga’) and thereafter, in the fifth generation, on CAA-treated plants. Results showed that the minimal inhibitory concentration (MIC) for all five mutants tested, regardless of whether originally selected for resistance to DMM, BENT or MPD, was higher (12.5–50 mg  $\Gamma^{-1}$ , technical grade) for DMM and BENT than for MPD (1.56–3.12 mg  $\Gamma^{-1}$ ).

In a mini-epidemic experiment conducted under closed plastic with mutant CH-Bent/5 at generation 7, a mean of 68 lesions/plant were counted on CAA-free inoculated plants at 15 dpi as against 9 lesions, 17 lesions, and one lesion/plant ( $n=16$ ) in plants treated with 25 mg  $\Gamma^{-1}$  (formulated grade) DMM, BENT and MPD, respectively. The data suggest that artificial mutants of *B. lactucae* can tolerate relatively higher doses of DMM or BENT than MPD. [L]

### Management of gray mold in lisianthus

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Gray mold (*Botrytis cinerea*), severely affects the stem base and cut stems left after flowers are harvested in lisianthus (*Eustoma grandiflorum*) crops. The disease commonly causes the death of plants. Chemical fungicides frequently fail to control the disease. Various levels of pathogen resistance towards botryticides were recorded by us. The objectives of the present work were to examine factors that influence *B. cinerea* infection of lisianthus stems under non-heated greenhouse conditions and to establish cultural methods in commercial greenhouses that will be combined for management of the disease. Although the lower nodes of lisianthus stems are typically infected, they were found to be less susceptible than higher nodes. On the contrary, the development of gray mold along lower leaves that are adjacent to the soil toward the stem was more rapid than along higher leaves. Leaf sensitivity and dense canopy that are associated with disease-promotive microclimate, promote infection and its development along the leaf towards the stem base. Viability of pathogen sclerotia or plant debris containing the

pathogen was 64–73% after 3 months in soil. Soil treatments totally killed the sclerotia in soil but not the *B. cinerea* inoculum in plant debris. Under commercial greenhouse conditions, supplemental calcium [ $\text{Ca}(\text{NO}_3)_2$ ] applied in fertigation or as a spray led to moderate yet significant reduction in disease severity. Cultural methods such as polyethylene soil cover, the use of buried drip irrigation instead of surface drip irrigation, reducing planting density and in-bed air movement suppressed gray mold significantly. Combining cultural methods and chemical botryticides resulted in control of lisianthus gray mold in non-heated greenhouses. [P]

### RAPD, mating type, virulence and resistance to metalaxyl in field isolates of *Phytophthora infestans* in Israel

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One thousand and forty-one isolates of *Phytophthora infestans* were collected from potato and tomato crops in Israel during 1983–2008 and some of their characters were analyzed. During 9 years, from 1983 to 1991, all 574 isolates collected belonged to the A2 mating type; in the 7 next years, from 1992 to 1998, the proportion of A2 among 173 isolates decreased to 16%; and during 1999–2005, among 251 isolates it decreased further, to 3.7%. In the last 3 years, 2006–2008, no A2 isolates were found among the 70 isolates collected; of these isolates, 63 were A1 and seven belonged to the unusual mating type A1A2, which can make no oospores in pure culture but can make them when mated with either A1 or A2. During 1983–1991, one third of the A2 isolates were sensitive to metalaxyl and two thirds were resistant, with no intermediate isolates detected. Isolates with intermediate resistance to metalaxyl emerged in 1993 parallel to the appearance of A1 isolates. The proportion of intermediate isolates increased from approximately 5% during 1993–1996 to approximately 50% during 1997–2008. They survived in the population in the last 3 years in spite of the absence of A2 isolates. RAPD analysis of 42 isolates collected in 2007–2008 showed a large variability in DNA fingerprinting, with almost every isolate exhibiting a

different banding pattern. Virulence race structure analysis confirmed the presence of all virulence genes in the population. Virulence factors 1, 3, 4, 7, 9 were most frequent (present in ~90% of the isolates), the frequency of 2, 8, 10, 11 was intermediate (12–28% of the isolates) and that of 5, 6 was lowest (~5% of the isolates). Complexity of the isolates increased with time: during 1983–1991 isolates carried 3–6 virulence factors; during 1993–1998, 4–7 factors; during 1999–2006, 4–9 factors; and in 2007–2008, 4–10 factors. No association was found between virulence, mating type, and/or response to metalaxyl. The data show that the population of *P. infestans* in Israel, which was already highly variable in the past, continues to change. Sexual reproduction might be responsible for some of these changes. The most dramatic changes in the last few years were: the disappearance of isolates with A2 mating type (because of unknown reasons, as they suffer no reduced fitness); the appearance of isolates with A1A2 mating type; the increase in virulence complexity; and the decrease in frequency of metalaxyl-resistant isolates that have probably derived from reduced applications of metalaxyl. [L]

#### PLANT – PARASITE INTERACTIONS: FUNGI AND OOMYCETES

##### Ammonium secretion by *Colletotrichum coccodes* activates host NADPH oxidase activity enhancing host cell death and fungal virulence in tomato fruits

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*Colletotrichum* pathogens of fruits and leaves are known ammonium secretors. It was found that *C. coccodes* virulence, as measured by tomato (*Solanum lycopersicum* cv. Motelle) fruit tissue necrosis, correlates with the amount of ammonium secreted. Ammonium application to fruit tissue induced hydrogen peroxide accumulation. To examine whether the tomato NADPH oxidase, LeRBOH, is a source for

the ammonium-induced H<sub>2</sub>O<sub>2</sub>, wild-type and anti-sense lines abrogated for LeRBOH were examined. Wild-type lines produced 7.5-fold more reactive oxygen species when exposed to exogenous ammonium than did LeRBOH<sup>-</sup> lines. *C. coccodes* colonization of wild-type tomato lines resulted in higher H<sub>2</sub>O<sub>2</sub> production and faster fungal growth rate compared with colonization in the LeRBOH<sup>-</sup> mutant, although the amount of ammonium secreted by the fungi was similar in both cases. Enhanced ion leakage and cell death of fruit tissue were correlated with H<sub>2</sub>O<sub>2</sub> accumulation, and treatment with the reactive oxygen scavenger N-acetyl-L-cysteine decreased H<sub>2</sub>O<sub>2</sub> production, ion leakage and cell death. The production of H<sub>2</sub>O<sub>2</sub> could be reduced by the addition of calcium chelators and calcium-channel blockers, consistent with a role for calcium in the control of LeRBOH activity. Importantly, the activation of reactive oxygen species production by ammonium was positively affected by an extracellular pH increase from 4 to 9, implying that ammonium exerts its control *via* membrane penetration. Our results show that *C. coccodes* activates host reactive oxygen species and H<sub>2</sub>O<sub>2</sub> production through ammonium secretion. The resultant enhancement in host tissue decay is an important step in the activation of the necrotrophic process needed for colonization. [L]

##### Role of the putative anti-apoptotic gene BcBIR1 in pathogenicity of the grey mold disease *Botrytis cinerea*

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*Botrytis cinerea*, also known as the gray mold disease, is a wide host-range necrotrophic plant pathogen that attacks over 200 plant species. It causes soft rot of all aerial plant parts in the field as well as postharvest damages to vegetables, fruits and flowers. Previous works have suggested that fungus-induced programmed cell death (PCD) of the host plant facilitates disease development. Recent evidence suggests that PCD might be similarly induced in the fungus by plant metabolites. Accordingly, we propose that induced fungal PCD might serve as a defense mechanism of

plants. In order to test this hypothesis we have been investigating the role of candidate apoptotic genes in development and pathogenicity of *B. cinerea*. Here we report on our results with BcBIR1, a homolog of the human anti-apoptotic gene survivin. Survivin belongs to the IAP family of proteins, which includes eight members in humans. A single homolog was found in *B. cinerea*. The gene (BcBIR1) was isolated and knockout and over-expression transgenic strains were generated. We have been unable to obtain  $\Delta$ bcbir homokaryones suggesting that BcBIR1 is an essential gene. The BcBIR1 over-expression strains were more stress resistant and exhibited reduced apoptosis under PCD-inducing conditions. These strains were also hypervirulent on beans. Thus, the cell death-protected strains also had higher virulence. Although circumstantial, these results support the hypothesis that induced fungal cell death might reduce disease spread. [L]

#### **Involvement of protein tyrosine phosphatase 1 (*ptp1*) in the development and pathogenesis of *Sclerotinia sclerotiorum***

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*Sclerotinia sclerotiorum* is a sclerotium-producing, phytopathogenic, filamentous ascomycete, with worldwide distribution. The sclerotium is a pigmented, multicellular, firm resting structure composed of condensed vegetative cells and is capable of surviving years in soil. Protein tyrosine phosphatases (PTPs) are a group of enzymes that remove phosphate groups from phosphorylated tyrosine residues on various proteins. Among the four PTPs in *S. sclerotiorum*, we found that *ptp1* exhibited a marked (30-fold) increase in expression levels, during the white, premature, sclerotium development phase. We partially impaired *ptp1* expression by inserting an inducible *ptp1* antisense cassette into *S. sclerotiorum*. Antisense expression resulted in complete cessation of the development of sclerotial initials and impaired pathogenesis (as determined by a detached tomato leaf bioassay). Reactive oxygen species (ROS) have

been shown to be involved in developmental and pathogenicity-related signaling in *S. sclerotiorum*. Reduced levels of *ptp1* expression conferred a reduction in NADPH oxidase, but not in superoxide dismutase, activity levels, suggesting a functional link between PTP1 and ROS formation by NADPH oxidases in *S. sclerotiorum*. [L]

#### **Involvement of the *snt2* gene in pathogenesis and development of *Fusarium oxysporum* f. sp. *melonis***

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The soilborne fungal plant pathogen *Fusarium oxysporum* f. sp. *melonis* (FOM) causes vascular wilt disease of muskmelon. In order to identify pathogenicity-related genes of FOM, we utilized a tagged-mutagenesis approach. Following *Agrobacterium tumefaciens*-mediated transformation we isolated a mutant (D122) that exhibits a reduction of approximately 80% in plant mortality, when compared with the wild type isolate. The defect in D122 was attributed to an insertion in the gene encoding a *snt2*-like transcription factor (TF), which harbors three PHD (plant homeo domain) fingers and a BAH (bromo-adjacent homology) domain. Suppression Subtractive Hybridization was used to compare expression profiles between the wild type and D122 isolates. One of the differentially expressed genes encodes a putative basic leucine zipper (b-ZIP) TF. Its *Podospira anserina* homolog (*idi-4*) has been shown to be involved in execution of autophagy-associated programmed cell death. Using Real Time PCR we determined that *idi-4* expression was 16- and 30-fold higher in D122 and in a  $\Delta$ *snt2* strain, respectively, when compared with the wild type, suggesting that *snt2* may be a novel negative regulator of *idi-4*. Both D122 and  $\Delta$ *snt2* showed reduced radial growth and a significant reduction in conidial production when compared with the wild type, demonstrating that the

*snt2* is also involved in fungal development. In addition, deregulation of SOD expression was observed in both *snt2* mutant strains. Elucidating the links between *snt2* function and other regulatory pathways may provide a better understanding of fungal pathogenicity and development. [L]

### Effect of plant hormones on the development of the maize late wilt agent, *Harpophora maydis*

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The ascomycete fungus *Harpophora maydis* is the cause of one of the most severe corn diseases: late wilt. Its main characteristic is the dehydration of mature maize plants, 60–80 days after sowing. The disease was first described in Egypt, in 1960, and it can appear in 70% of plants and can cause 40% losses of maize seeds. Since its discovery the disease has spread rapidly to most areas of Egypt in which maize is grown. Late wilt of maize was also reported in India, in Hungary and, in 2002, in Israel. The pathogen attacks roots, reproduces asexually by conidia and produces sclerotia-like bodies that can remain dormant in the soil for long periods. Today, the most effective way to control late wilt is by the use of resistant maize strains, but a virulent strain of the fungus is known, which may be a threat even to resistant plants. Here we examine the effect of plant hormones on the pathogen's development. While the addition of gibberellin to the fungal culture had no apparent effect on the pathogen, auxin and kinetin caused a significant decrease in colony growth. These findings may explain the appearance of the disease in mature plants and in plants that are under dehydration stress. On the other hand, the rising ethylene concentrations in mature plants may cause the pathogen's aggressive invasion into the host tissues. When an ethylene-releasing compound (Ethral) was applied, we identified a concentration-dependent influence on colony growth and sporulation. A root pathogenicity

assay followed by fungal DNA measurements, showed a complete inhibition of the root infection, by *H. maydis*, in the presence of auxin. Kinetin caused a delay in the root penetration while ethylene had no apparent effect. [L]

### Proteins associated with resistance against *Phytophthora infestans* in wild tomato

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The wild tomato species *Solanum (Lycopersicon) pimpinellifolium* was reported to carry race-specific genes against late blight (*Phytophthora infestans*). The gene *Ph-1* was transferred to the cultivated tomato (*Solanum esculentum*) cv. 'New Yorker', the gene *Ph-2* to the cultivars 'West Virginia', 'Pierline', and 'Macline', and the gene *Ph-3* from *S. pimpinellifolium* L3708 was incorporated into several Taiwanese and US cultivars. All these resistant cultivars were reported susceptible within a few years of growth in the field due to the appearance of virulent isolates of *P. infestans*. Another source of resistance was reported by Irzhanski and Cohen in *S. pimpinellifolium* L3707. After several cycles of self-pollination and selection they developed a resistant breeding line 3707 which carries race-non-specific resistance against late blight. In the present study we compared the profiles of soluble neutral proteins of our resistant breeding line *S. pimpinellifolium* L3707 with that of the susceptible *S. pimpinellifolium* 14377. Using two-dimensional gel electrophoresis we identified a 41 Kd protein which appears in the resistant but not in the susceptible line. Amino acid sequencing revealed that this protein shares 60% homology with Rubisco activase and 63% homology with malate dehydrogenase. Decreasing mitochondrial malate dehydrogenase activity in transgenic tomato was reported to enhance photosynthesis. To verify whether or not the resistance in our breeding line is related to sugar supply by the host to the pathogen, we floated inoculated leaf discs of 3707 on increasing doses of sucrose and monitored sporangial production with the aid of an epi-fluorescent microscope. While the

number of sporulating discs floating on water was zero ( $n=24$ ), the proportion reached 70% in leaf discs floating on sucrose of 10–50 mM. Further studies are required to support the hypothesis that over-expression of malate dehydrogenase in 3707 is related to resistance against late blight. [L]

### Age-dependent resistance against late blight (*Phytophthora infestans*) in wild tomato

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Late blight caused by *Phytophthora infestans* (Mont.) de Bary is a most devastating disease of tomato and potato worldwide that requires frequent fungicide applications for disease control. No commercial cultivars resistant to late blight are available in Israel. In previous work we found that the wild tomato *L. pimpinellifolium* accession L3707 carries two genes for race-non-specific resistance, E and R. Studies from Taiwan showed that *L. pimpinellifolium* accession L3708 possesses the dominant *Ph3* resistance gene. In the present work we show that the resistance to late blight in *L. pimpinellifolium* accession L3707 and accession L3708 is age-related. In order to assess this hypothesis, the resistant lines (3707 and 3708) and the susceptible lines (*Lycopersicon esculentum* ZH and *Lycopersicon pimpinellifolium* 14377) were sown weekly and inoculated when they reached the age of 2 weeks to 9 weeks post-sowing. Intact plants were inoculated by spray with sporangia ( $2000 \text{ ml}^{-1}$ ) of the local isolate 411 (A1, nine virulence factors, sensitive to metalaxyl). Disease records were taken at 7 dpi and sporulation records at 8 dpi. All lines, including the resistant genotypes 3707 and 3708, were susceptible (100% blighted leaf area with approximately  $250 \times 10^3$  sporangia per gram fresh weight tissue) at the juvenile phase (3 weeks post-sowing). Similar responses were recorded in plants inoculated at 4 and 5 weeks after sowing. However, at 6–9 weeks post-sowing, inoculated plants belonging to the resistant genotypes showed 0–12% blighted leaf area and nearly zero sporangia per gram fresh weight tissue. Our data suggest that late blight resistance genes are probably expressed at  $\geq 6$  weeks

post-sowing. Breeders should consider this fact when breeding for late blight resistance in tomato. [L]

### Resistance to carboxylic acid amide fungicides in *Pseudoperonospora cubensis*

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The carboxylic acid amide (CAA) fungicides dime-thomorph (DMM), benthiavalicarb (BENT) and mandipropamid (MPD) are highly effective in controlling plant pathogens belonging to the *Peronosporales* (except *Pythium* spp.). Resistance to CAAs occurs in natural populations of *Plasmopara viticola*, but no natural resistance has been reported in other *Peronosporales*. Our objective in this study was to monitor the sensitivity of some field isolates of *Pseudoperonospora cubensis* to CAA fungicides. Isolate B was collected from melon plants infected with downy mildew in summer 2006 in Bilu, in the southern coastal plain of Israel. Isolate N was collected from cucumber plants in autumn 2006 at Achituv, in the central coastal plain. Sporangia were harvested in water and inoculated onto potted melon plants previously spray-treated with CAAs. ED<sub>90</sub> values (concentration, in  $\text{mg l}^{-1}$ , required to control 90% of the disease) for MPD, DMM, IPRO and BENT with isolate N were  $<0.39$ , 1.56, 25 and 6.25, respectively. With isolate B, they were 25  $\text{mg l}^{-1}$  for MPD and 400  $\text{mg l}^{-1}$  (the highest dose tested) for the other CAAs. This implies that isolate B expressed a resistance factor of 64–256 relative to isolate N. After three cycles of propagation on CAA-treated plants, both isolates became resistant to 400  $\text{mg l}^{-1}$  of either CAA, suggesting that the original isolates carried both sensitive and resistant sporangia. Another ten isolates were collected in the central coastal plain in winter 2008, one isolate per plastic house. Sporangia were mixed with CAAs and inoculated onto detached cucumber leaves in petri dishes. Nine isolates were sensitive and one was resistant. The competitive fitness of one pair of isolates was studied. Sporangia of isolates #4 (sensitive) and #7 (resistant) were

mixed at a 1:1 ratio and inoculated onto CAA-free cucumber plants growing in a plastic greenhouse in winter 2009. At 38 dpi (approximately four disease cycles), sporangia were removed separately from single lesions and their resistance was tested. About half of the lesions were sensitive and half were resistant. The data show that resistance to CAA occurs in a natural population of *P. cubensis* in Israel. The resistant and the sensitive isolates tested seemed to have equal fitness under the conditions used. MPD was more effective than the other CAAs tested. [L]

### Resistance of wild watermelon genotypes (*Citrullus* spp.) to powdery mildew

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Powdery mildew, *Podosphaera xanthii* (syn. *Sphaerotheca fuliginea*), affects many cucurbit crops worldwide, limiting yields and increasing the need for fungicide applications. In Israel, all commercial watermelon cultivars are susceptible to powdery mildew. Our aim in this study was to identify genotypes of watermelon with resistance to race 1 of powdery mildew. (A similar study was conducted in 2007 by Davis et al. in the US.) For our purpose, 253 genotypes of *Citrullus* spp. which were collected from Asia, Africa, America, Europe and Israel (obtained from the Introduction Center of The Volcani Center, ARO, at Bet Dagan), were examined for resistance to race 1 of PM. Plants were raised from seeds in Speedling trays and inoculated at the cotyledon developmental stage by dusting spores of *P. xanthii*. The appearance of fungal colonies was monitored on the cotyledons, hypocotyl and leaf 1 until 14 dpi. Of the 253 genotypes, 211 germinated, of which only four genotypes (one *C. coloncythis*, two *C. lanatus*, one *C. mucosospermus*) were fully resistant (no fungal colonies developed), eight genotypes demonstrated partial resistance, and 23 genotypes segregated for resistance, with some individuals showing full resistance. All the other 176 genotypes were susceptible, showing fungal colony development on the cotyledons and/or the hypocotyls and/or leaf 1. Four resistant individuals (all *C. lanatus*, with white or yellow flesh, low sugar content) were propagated in net-houses

during four generations by self-pollination. Current work is aimed at determining the nature of resistance by microscopy and the inheritance of resistance by crossing with the susceptible genotypes. [L]

### PREVENTION AND CONTROL OF PLANT DISEASES – B

#### Reducing peanut yield losses caused by *Sclerotium rolfsii* in the Hula Valley

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The fungus *Sclerotium rolfsii* Sacc. causes severe damage to a wide range of crops in the Hula Valley, Israel, including peanut, sunflower, potato and watermelon. Yield losses in peanut are due to pod and peg rot caused by the fungus. Foliar applications of Folicur (tebuconazole 25% a.i.) were previously reported to be effective in reducing peanut yield losses, but in the Hula soils (peat and heavy mineral soil) this treatment was ineffective, even when six Folicur sprays were applied over the growing season. In laboratory experiments conducted to examine the efficacy of Folicur soil applications, the fungicide was stable in three types of soils over a period of 7 months. In field experiments, the combined treatment of an in-furrow application with a foliar spray was most effective for reducing yield losses, as compared with the other treatments. In the 2007 season, Folicur (2000 ml ha<sup>-1</sup>) was applied in furrows and the effects of foliar applications of six commercial fungicides were evaluated. Although pod yields were higher in several fungicide treatments, they were not significantly different from those observed in the control plots, which were treated in the furrow only. In the 2008 season, three rates of Folicur were applied in furrows (1000, 2000 and 4000 ml ha<sup>-1</sup>). Two additional foliar sprays were applied to these plots during the growing season. Each plot received two

sprays of Nativo (tebuconazole 50%+trifloxystrobin 25% a.i.), Amistar (azoxystrobin 25% a.i.) or Signum (boscalid 26.7%+pyraclostrobin 6.7% a.i.) We tested two different rates of each of these chemicals (Nativo: 1000 and 2000 g ha<sup>-1</sup>; Amistar: 1000 and 2000 ml ha<sup>-1</sup>; Signum: 500 and 1000 g ha<sup>-1</sup>). The control plots were left untreated. In-furrow applications of Folicur increased pod yield by an average of 2000 kg ha<sup>-1</sup> and decreased disease incidence by 50%, as compared with the results from the control plots. There were no significant differences in efficacy among the different rates of soil-applied Folicur. Among the foliar sprays, only the higher rate of Nativo significantly increased pod yield and decreased disease incidence, as compared with the untreated control.

Rotation to non-host crops to reduce the amount of sclerotia in the soil is an important practice for the management of *S. rolfisii*. In two fields in which non-host crops had been grown, the sclerotia population decreased by 97% in 2 years in one field, and by 90% in one year in the other field. Nonetheless, it is important to note that the fungus' mycelia grow on the roots of these crops, and plowing crop residue into wet soil would accelerate mycelial growth and increase the sclerotia population. [L]

#### Evaluation of DMDS as a soil fumigant: Application, distribution and control of soilborne pests

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DMDS has been developed and evaluated in France, Italy, Spain and the USA as a viable alternative to methyl bromide for control of soilborne fungi and nematode species. The objectives of this study were to study the behavior of DMDS in the soils of Israel, and explore the technology for its application, in order to develop an effective use of this fumigant for the agricultural industry in Israel. DMDS, which is applied under plastic tarp in the field, dissipated from the soil after a period of 4–7 days depending on the

rate applied. DMDS is highly toxic to *Meloidogyne javanica*. In contrast, the toxicity of DMDS to soil fungi varied among certain pathogens. *Fusarium* species appear to be the most sensitive among the tested fungi; *Macrophomina phaseolina* was highly tolerant to DMDS. When applied through the drip irrigation system, DMDS moves mainly along the water front. The lateral movement of DMDS, however, is limited, resulting in decreased concentration away from the drip pipe. Thus, effective and uniform distribution of DMDS requires a spacing of 40 cm between the drip pipes. Similar results were obtained by inserting the fumigant to a depth of 20 cm using hand injectors. DMDS permeates rapidly through a common low-density polyethylene film. In contrast, application of DMDS under impermeable film resulted in higher amounts of the fumigant in the soil, more uniform distribution in the soil profile, and an extended retention period. The combination of solarization and DMDS yielded effective control of pathogenic fungi and nematodes at all tested soil depths. Such a combination enabled reducing the rate of DMDS and shortening the solarization period (2 weeks), while achieving the same level of pest control. [L]

#### Paladin (DMDS), a new soil disinfectant: From controlled trials out to the field

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Paladin (DMDS=dimethyl disulphide) is a new soil disinfectant, for the control of root-knot nematodes and soilborne diseases, and for weed prevention. Paladin is produced by Arkema. The product was tested in many countries; and in Israel – by the Rimi company, in cooperation with the Agricultural Research Organization, The Volcani Center. Paladin's mode of action is by inhibition of the mitochondrial activity.

It is produced in two formulations: EC – for application via the drip irrigation system, and a formulation for shank application. Field trials were conducted in commercial fields, to test Paladin at various rates, application volumes, soil types, loca-

tions, and application timings. It was tested in both drip and shank application methods. In all trials, VIF (virtually impermeable film) mulch was used. Paladin was found safe for all tested crops (pepper, tomato, eggplant, melon, cucumber, cabbage, lettuce, basil, coriander, dill, asphodel, and phlox), when applied via the drip irrigation system, and to potato, strawberry, and carrot when applied by shank. It was found to be efficient in the following: control of root-knot nematodes (*Meloidogyne* sp.); control of *Fusarium* sp., which causes severe damages in cucumber in the center of Israel; control of *Pythium* sp. and *Rhizoctonia solani*; and prevention and inhibition of weed emergence. Paladin is a solution for the control of nematodes, soilborne diseases and weeds in various crops and under different environmental conditions. RIMI's Plant Protection Department is constantly seeking additional solutions for soil disinfection, for the days following methyl bromide phase-out. [L]

#### Accelerated degradation of metam-sodium in soil: Isolation and characterization of the involved microorganisms

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Accelerated degradation (AD) of methyl-isothiocyanate (MITC), the active ingredient of metam-sodium (MS), develops in soils following repeated application of MS. The present study was carried out under controlled environment conditions and in agricultural fields. AD of MITC can also be induced in soils by incorporating non-history soil (non-degrading soil) with MS history soil (degrading soil). The objectives of the present study were to isolate and to characterize the soil microorganisms which are involved in AD of MITC. We

developed a method to “extract” and concentrate the degrading organisms from the history soil. The liquid soil extraction contained microorganisms which can rapidly degrade MITC. Addition of formalin (1000 ppm) to the soil extract from history soil did not reduce AD of MITC. Two bacteria which rapidly degrade MITC were isolated from Rehovot history soil and were identified by DNA sequence and fatty acid profile analysis. Both isolates belong to the Oxalobacteraceae family and were identified as *Naxibacter* sp. Mixing bacteria culture with non-history soil resulted in AD in this soil. We also found rapid degradation of MITC in En Tamar soil (non-degrading soil) after its inoculation with one of the bacteria. By using molecular analysis, we found that the Oxalobacteraceae population was dominant in Rehovot soil, after the first application of MS. These results indicate that the signal for MITC degrading is induced in soil already after one application. However, the bacterial populations were not dominant, indicating that other populations may be also involved in degradation of MITC. [L]

#### Induced soil suppressiveness to root diseases by herb amendments and solarization

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Soil suppressiveness to soilborne pathogens exhibits reduced disease incidence and severity in a sensitive host even in the presence of a pathogen and favorable environmental conditions. Incorporation of herb residues combined with subsequent soil solarization is effective in controlling soilborne plant pathogens and in inducing soil suppressiveness. The objective of this work was to validate soil suppressiveness under field conditions with commercial cropping. Two field

experiments, both in fields infested with *Meloidogyne javanica*, were carried out with tomato and snapdragon crops. The first stage of the study included growing of *Diploaxis tenuifolia* (wild rocket) in the plots, followed by incorporation of the foliage after 6 months and applying moderate solarization, to achieve a partial control of the pathogens in the soil. Indeed, nematode viability in the tomato greenhouse was not affected by any of the treatments. However, disease index of nematodes on tomato roots (cv. 870) was reduced by 40% (severe galling index) by amending wild rocket or soil solarization, compared with the control. The yield of tomato fruits did not differ among the treatments. In the second experiment, herb amendment and solarization totally controlled *M. javanica* at a depth of 40 cm. Galling on the roots of snapdragon (cv. Photomek) by *M. javanica* was not reduced in any of the treatments, compared with non-treated plots. Severity of infection was increased by 25% in the solarized plots. No differences in yield were found among treatments. Soil suppressiveness to root diseases, which follows soil disinfection, may extend the efficacy (but not in all cases) of the disinfection under commercial conditions; however, crop sensitivity and cropping period affect the level of suppressiveness. [L]

## PREVENTION AND CONTROL OF PLANT DISEASES – C

### *Trichoderma* as a biocontrol agent against plant-parasitic nematodes

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Plant-parasitic nematodes cause great economic losses to agricultural crops and attempts are made

to develop safe measures to reduce nematode damage. We had shown that several *Trichoderma* species and isolates were effective against root-knot nematodes, *Meloidogyne* spp. (sedentary endoparasites), on vegetable crops in different soils. In this work we concentrated on the activity of two *Trichoderma* species, *T. hamatum*-382 and an isolate of *T. asperellum*, against *M. incognita* and *M. javanica*, on ornamentals, in potting mixes. *Trichoderma* peat-bran preparations (fresh or dried in several batches) were mixed with nematode-inoculated potting media, with or without colonizing the seedlings with the fungi, 1–2 weeks before planting *Begonia* or *Impatiens* seedlings in the pots. Results showed improved plant growth, reductions in root galling indices, and inhibition in nematode development that resulted in reduced egg production, after one to three nematode life-cycles. *Trichoderma* treatments usually improved growth, also without nematodes, and in poor *Meloidogyne* hosts, like *Pelargonium* and *Chrysanthemum*. *Trichoderma* was tested also against other plant-parasitic nematodes in naturally infested soils in pot experiments. Soil containing *Pratylenchus penetrans* (migratory endoparasites) and *Rotylenchus robustus* (ectoparasites) was pre-treated with each of the *Trichoderma* isolates and lettuce was planted. After 2 months, reductions in nematode populations in soil were recorded, as well as improvement in plant growth. *Pratylenchus projectus* (ectoparasites)-infested soil that was pre-treated with each of the *Trichoderma* isolates, revealed a reduction in nematode populations in each of three lettuce planting cycles (2 months each cycle). This work demonstrated the ability of *Trichoderma* isolates to control several important polyphagous nematode species which cause damage worldwide, and to improve growth of various plants. [L]

### Three-year-long monitoring for resistance to fungicides among *Botrytis cinerea* isolates from vineyards

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*Botrytis cinerea* is the important plant pathogenic fungus responsible for gray mold in grapevine. We monitored the *B. cinerea* populations of two vineyards in Ortal and Sha'al in the Golan Heights of Israel for resistance to six widely applied fungicides. Different plots in these vineyards were treated with Delsan (carbendazim, benzimidazole [Ben] fungicide), Mythos (pyrimethanil, anilinopyrimidine), Ohaio (fluazinam, phenylpyridinamine), Rovral (iprodione, dicarboximide [Dic]), Switch (mix of fludioxonil, phenylpyrrole and cyprodinil, anilinopyrimidine), and Teldor (phenhexamid, hydroxyanilide) during three growing seasons. Disease incidence differed across vineyards and years, whereas relative efficacy of the fungicide treatments was rather similar: the older fungicides Rovral and especially Delsan were ineffective or weakly effective; Ohaio was a little more effective but did not differ significantly from Rovral; the newer fungicides Mythos, Switch, and Teldor were effective, reducing disease incidence by 50–60%, although the efficacy of Mythos decreased in the third year. Approximately 500 *B. cinerea* isolates were sampled from diseased plants and from the air, and characterized for resistance to the above-mentioned fungicides using a mycelium growth test. Approximately 20 different phenotypes showing resistance to one or more fungicides were recovered. Alleles of strong resistance to benzimidazole and dicarboximide fungicides were the most frequent, counting BenR up to 10% in Ortal and 23% in Sha'al, and DicR up to 20% and 25% at the two locations, respectively. High resistance to pyrimethanil was found, with a frequency of ~3% in both vineyards in the first 2 years of observations, which increased up to 10% at Sha'al (where the disease was found) in the third year. A few isolates (less than 1%) were resistant to fenhexamid or fludioxonil. No high resistance to fluazinam was detected. The low efficacy of Delsan and Rovral treatments correlates with the relatively high frequency of resistance to these fungicides. Also, the lower efficacy of the newer fungicide Mythos in the third

year of observation correlates with increased frequency of resistance to this fungicide. [L]

### Development of a method for repeated broomrape biocontrol agent application through drip irrigation systems

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The genus *Orobanche* (broomrape) consists of obligate root parasitic plants that cause severe quality and yield reduction of many agricultural crops in Israel and worldwide. Broomrapes are extremely difficult to control and therefore may serve as a target for the development of a biological control system. One of the main obstacles in the application of biocontrol agents, especially soilborne agents, is their low effectiveness and sensitivity to environmental conditions. Efficacy of biocontrol agents could be enhanced by releasing them into favorable environmental and feeding conditions, allowing uniform distribution and multiple applications. At present, soilborne biocontrol agents are applied as granular formulations, at sowing or planting time, and repeated applications are extremely difficult or even impossible to perform. In order to test the applicability of the drip irrigation system for multiple applications of biocontrol agents, we used spores and mycelia of *Fusarium oxysporum* f. sp. *orthoceras* (*Foo*) and *F. solani*. The efficacy of *Orobanche cumana* control in greenhouse experiments was evaluated based on the (a) distribution of fungal propagules through low- and high-pressure drip irrigation systems; (b) uniformity of spores or mycelia distribution along the dripper line; (c) correlation between the amount of fungal propagules and mixture of the two agents on control efficacy; and (d) effectiveness of one, two and three applications on broomrape biocontrol. Multiple applications of *Foo* mycelia through a low-pressure irrigation system were found to be the most effective for *O. cumana* control on sunflower. [L]