

ABSTRACTS OF PRESENTATIONS AT  
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A: DIAGNOSIS AND CHARACTERIZATION OF PLANT PATHOGENS Part I

**Interception of Three Exotic Fungal Plant Diseases**

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The PPIS Diagnostic Service labs routinely test imported fruits, vegetables and propagation material for pests and pathogens (bacterial, fungal and viral). *Coniella granati* (Sacc.) Petrak & Sydow, the causal agent of pomegranate fruit rot, was identified from pomegranate (*Punica granatum*) scions imported from India. Stored twig segments developed superficial mycelium, which prompted a lab test. Diagnosis was based on the shape, size and color of its pycnidia and pycnidiospores, produced both on twigs and in culture on  $\frac{1}{4}$ -strength PDA. *Mycocentrospora acerina* (Hartig) Deighton, the causal agent of carrot licorice rot, was identified from symptomatic carrots imported from Belgium. Diagnosis was based on the characteristic conidia and chlamydospores produced by the fungus on  $\frac{1}{4}$ -strength PDA. *Phacidiopycnis pyri* (Fuckel) Weindlymayr, a causal agent of stem-end rot of apples and pears, was identified by us a number of times in pears imported from Oregon, USA, between 1998 and 2006. Until 2000, this fungus was considered as 'absent' in the USA and our identification was rejected by APHIS. A shipment of microscope slides, photomicrographs and infected fruit, sent by PPIS in 2000, convinced APHIS to acknowledge the presence of *P. pyri* in Oregon. Since 2002 this disease is mentioned in U.S. publications as "a recently acknowledged disease..". To the best of our knowledge, this is the first report mentioning the PPIS lab as responsible for the identification of *P. pyri* in the USA. All three abovementioned diseases are Quarantine Diseases, absent in Israel. In addition to *P. pyri*, we encountered another black rot in Oregon pears, caused by *Aureobasidium pullulans* (De Bary) Arnaud. This is not a quarantine pest in Israel, because it was found here associated with a minor disease of pear trees, papery bark. [L]

**English Ivy Pathogens in Israel**

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English ivy is one of the preferred ornamentals for ground cover, due to its ability to thrive in both shade and full sun. Most of the steep slopes of the Baha'i Gardens in Haifa are planted with English ivy. These slopes provided during 2002 – 2007 many samples of diseased ivy vines and leaves to the PPIS Diagnostic Lab. Most leaf spots started as small, brown, circular, growing in size, becoming darker, coalescing and becoming irregular, black and necrotic. Isolations revealed *Colletotrichum trichellum*, the causal agent of English ivy anthracnose in most samples. Samples in 2005 had also smaller, water-soaked, brown or black spots. Three such samples were found infected with *Xanthomonas hortorum* pv. *hederae*, a specific leaf-spot pathogen of English ivy. During 2005 there were patches with many dead plants and with vine dieback, diagnosed as *Phytophthora* sp. The

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L = lecture; P = poster.

three above-mentioned organisms are considered as the three main pathogens of English ivy. The first two are new reports in Israel. Three other bacterial pathogens identified were *Pseudomonas cichorii*, *P. syringae* ssp. *syringae* and *Erwinia carotovora* ssp. *carotovora*, all known as broad-range plant pathogens. In some of the samples we found additional fungi associated with leaf-spot diseases: *Lasiodiplodia theobromae*, *Alternaria alternata*, *Phoma* sp., *Phyllosticta* sp., as well as the physiological disorder edema, characterized by circular leaf blisters. Fungal identifications were performed by humid chamber incubation, isolation onto  $\frac{1}{4}$ -strength PDA and microscopic observation. Bacteria were identified by isolation and gas-chromatographic analysis of their fatty-acid profiles. [L]

### **Response of Different Cultivars of *Limonium* to *Colletotrichum gloeosporioides* and Identification of the Pathogen in Infected Plants by Quantitative Real Time PCR**

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*Limonium*, produced by tissue culture, is an important export perennial crop grown extensively in the Arava region of Israel. Two cultivars, 'Supreme' and 'Safora', were examined for their response to the pathogen *Colletotrichum gloeosporioides* that causes plant decline, by (i) determination of symptom severity and mortality and (ii) pathogen isolation on a semi-selective medium. Infected plants were examined for presence of the pathogen by quantitative real-time PCR (QRT-PCR) with specific primers for the  $\beta$ -tubulin gene (TUB1). Disease severity increased with higher concentrations of conidial suspensions. Cv. Safora was more resistant than cv. Supreme. Greenhouse plants were transplanted to Yair Station, Arava desert, one month after inoculation. Under field conditions, symptoms on the leaves disappeared and isolation of the pathogen became more difficult. No mortality was recorded in inoculated Safora plants compared with approximately 80% in inoculated Supreme plants and non-inoculated Supreme controls, suggesting transfection *via* roots and/or irrigation water. The pathogen was qualitatively identified by QRT-PCR in both cultivars during early stages of infection. Identification of the pathogen by QRT-PCR may in future become an important tool for early diagnosis of the disease in the nursery. [L]

### **Improving the Selection of Susceptible Plants out of Segregating Populations: Increasing Susceptibility to Fusarium Wilt of Melons by Irrigation with Saline Water**

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Selecting resistant plants out of segregating populations is a critical stage in the breeding process. The selection procedure is often accompanied by 'escape' plants, *i.e.*, those that are actually susceptible but do not exhibit the typical disease symptoms. These escapes pose a significant risk and delay the progress of breeding programs. The effects of type of growing medium, irrigation regime and fertilization on disease expression were usually investigated as a tool for disease suppression. The aim of the present study, however, was to find a way to increase disease incidence in order to reduce the number of escapes. Irrigating inoculated melon seedlings with saline water increased wilt incidence. Saline water (100 mM of NaCl) supplied to the seedlings from the third to the sixth day after fungal inoculation increased wilt incidence of the susceptible seedlings without interfering with their normal development. Irrigation with saline water reduced the number of escapes and enhanced wilt symptoms but did not 'break' the monogenic resistance to Fusarium wilt. This treatment is a

simple, inexpensive procedure that can significantly improve the selection of susceptible plants out of segregating populations. [L]

### **Mutagenesis of the Mating-type Locus in *Phytophthora infestans***

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Late blight caused by *Phytophthora infestans* is a devastating disease of potato and tomato. While sporangia, produced vegetatively, drive epidemics of late blight during the season, oospores – sexual, long-lasting spores – may initiate the disease at the beginning of the season. *P. infestans* is heterothallic, having A1 and A2 mating types. The first emigration of A2 from Mexico to Europe occurred in the 1980s, reaching Israel in 1983 and dominating the pathogen population until the early 1990s. In a recent study we noted the occurrence of a new A1A2 mating type in the field as well as in F1 hybrids produced between A1 and A2 isolates. The purpose of the present study was to examine the proneness of the mating type locus to mutate. Sporangia of five field isolates (408–412) having a mating type of A1 or A1A2 were treated with EMS and each isolate was inoculated singly onto tomato leaves. Oospores were formed only in leaves inoculated with EMS-treated sporangia of isolates 409, 410, 411 (all A1A2) but not of isolates 408 and 412 (both A1), suggesting the occurrence of A1 and/or A2, with which A1A2 can mate. Only isolate 409 kept its ability to produce oospores when re-inoculated singly on tomato leaves. Of 100 single sporangium isolates regenerated from isolate 409, 75 were A1, 15 were A1A2, one was A2 and nine were sterile. In the next generation 87, three and ten isolates were A1, A1A2 and sterile, respectively. The data suggest that A1A2 isolates are prone to alteration in mating type with a tendency to shift to A1. [P]

### **Diversity of the Phytoplasmas Identified in Israel**

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Yellows diseases, suggesting infection with phytoplasmas, have been observed in Israel in various plant species since the mid 1970s. Until several years ago detection methods were based primarily on bioassays and on electron microscopy examination. It is only recently, with the advent of superior biochemical analysis and DNA sequencing, that groups of phytoplasma could be formed and readily identified. Phytoplasmas have been identified in Israel in numerous species of various botanical families, including important crop plants. Some of these crops (e.g. flowers, strawberry, carrot, grapevine) suffered significant economic losses. Northern Israel has the most diversity of phytoplasma groups followed by the northern Negev desert and Arava Valley, with the center of the country having the least. There are proportionally more infected flowers than orchards (including vineyards) or than vegetable crops. The abundance of identified phytoplasma in flowers may be due to the large export market, and the direct impact that phytoplasma infections have on flowers, necessitating constant monitoring of these crops. Of the 15 known 16Sr DNA phytoplasma groups, seven are represented in Israel. It is very likely that at least one group was imported into Israel from another area of the world. [P]

*B: HOST – PATHOGEN INTERACTIONS Part I*

### **The Type III Virulence Effector PthG from *Pantoea agglomerans* pv. *gypsophilae* Modifies the Sensitivity of the Plant to Phytohormones**

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*Pantoea agglomerans* pv. *gypsophila* (*Pag*) causes gall formation in gypsophila and the hypersensitive response (HR) in beet. Both pathogenicity and HR are dependent on type III secretion system (T3SS) and T3SS effectors. The effector PthG has a dual function: it induces HR in beet but acts as a virulence factor in gypsophila. Transgenic tobacco plants expressing PthG showed leaf deformation, stunting, and reduced fertility and light requirement for seed germination. The levels of auxin and ethylene produced by the transgenic tobacco plants were higher than in control plants. Regeneration experiments of *pthG*-transgenic tobacco lines revealed lower sensitivity to auxin and cytokinin at low concentrations (<0.5  $\mu$ M). At high auxin and cytokinin concentrations ( $\geq$ 0.5  $\mu$ M), respectively, fewer roots and shoots were formed. Compared with the transgenic control, the *pthG*-transgenic tobacco lines formed a higher percentage of calli. In the transgenic *pthG* lines regeneration rate (formation of roots and shoots) was higher in darkness than in light. These results suggest that the PthG protein affects the plant sensitivity to both phytohormones and light. Support for these results was obtained by noting the effects of various phytohormones and inhibitors on gall formation following inoculation of gypsophila cuttings with *Pag* 824-1 mutated in *pthG*. [L]

### Identification and Characterization of *Xanthomonas campestris* pv. *vesicatoria* Genes Induced during the Bacterium's Interaction with Tomato Plants

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The bacterium *Xanthomonas campestris* pv. *vesicatoria* (*Xcv*) is the causal agent of bacterial spot disease of tomato and pepper. The disease process is interactive and intricate, involving a plethora of both pathogen and host genes. In the pathogen, many genes are activated in response to the host environment to enable it to survive, adapt, evade defense responses, propagate, and cause disease. To understand the disease process it is imperative to broaden our understanding of the gene machinery that participates in it, and the most reliable way is to identify these genes *in vivo*. For this purpose we have adapted a Recombinase-based In Vivo Expression Technology (RIVET) approach to identify genes activated in *Xcv* during its interaction with tomato. RIVET revealed 61 unique *Xcv in vivo* overexpressed (*ivx*) genes/operons. To explore the role of some of these genes further, we generated knockout mutants for 13 genes and characterized their ability to grow *in planta* and to cause disease symptoms. This analysis revealed that a *citH* homolog encoding a citrate transporter is required for wild-type levels of virulence. The *citH* homolog has 61% identity to the characterized citrate transporter CitN of *Bacillus subtilis*. The *citH* mutant was unable to grow in minimal medium with citrate as the sole carbon source, suggesting the *citH* product facilitates citrate transport in *Xcv*. Analysis of the promoter activity of this gene showed that relative to its induction in rich media, it is induced to more than two orders of magnitude *in planta*. *In planta*, it was shown to be induced to up to 20 times more than the promoter of *hrpA*, a gene which is essential for pathogenicity, and is induced selectively *in vivo*. Our goal is to understand better how *citH* contributes to the virulence of *Xcv*. [L]

### Field Isolates of Tomato spotted wilt virus Overcoming Resistance in Commercial Pepper Cultivars Carrying the *Tsw* Gene

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*Tomato spotted wilt virus* (TSWV) genus *Tospovirus*, family Bunyaviridae, has a broad host range including over 900 plant species from different botanic families, and is transmitted via several thrips species in a persistent manner. The virus was first discovered in Israel in 1992, but so far has not caused significant damages to cultivated crops. In recent years an increase in disease incidence occurred in pepper (*Capsicum* spp.) crops grown in the Arava Valley and the Besor region. Resistance to the virus in pepper is conferred by a single dominant gene (*Tsw*). Plants carrying this gene usually show a hypersensitive reaction. In recent years several cases of resistance-breaking strains were reported from Spain and Italy. In the present research isolates of TSWV were collected from pepper-growing regions, and tested for their ability to break resistance obtained by the *Tsw* gene. Two resistance-breaking isolates (RB1 and RB3) were identified in the survey. These two isolates were mechanically inoculated onto 27 resistant pepper lines carrying the *Tsw* gene. RB3 isolate overcame resistance in all the cultivars tested, whereas RB1 was able to overcome resistance in only a few cultivars. All resistant cultivars were found resistant to a third wild type (WT) isolate. Cloning and sequencing of the nucleocapsid (NC) gene, the non-structural genes NSm and NSs showed no differences between the amino acid sequences of the NC protein from these isolates. On the other hand, one amino acid substitution was found between the NSm and NSs protein sequences from RB1 to the two other isolates, and three amino acid substitutions were found between the sequences of the NSs protein from RB3 and the WT isolate. [L]

### **Viral Small RNA Production by Transgene Gives Virus Resistance Depending on the Degree of Homology between the Transgene and the Challenge Virus**

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Tobacco plants (*Nicotiana tabacum* L.) were transformed with a construct containing a 'hairpin' inverted repeat of 598 nucleotides derived from the *Potato Virus Y* (PVY) N strain replicase gene. Such constructs efficiently produce dsRNA and thereby confer virus resistance by post translational gene silencing. Homozygous plants were challenged with a range of PVY strains and resistance was measured by symptom expression, ELISA titer and back-inoculation of controls with extracts from resistant plants. A transgenic tobacco line was immune to five potato-infecting PVY strains with which the transgene had homology of 99.5–88.3%. Infection with tomato and pepper PVY isolates, with a lower degree of homology with the transgene (86.8–86.3%), caused delayed symptom appearance and attenuation in transgenic tobacco compared with non-transgenic plants. To understand the correlation between resistance and the sequence homology with the transgene we analyzed the accumulation of transgene small RNA (siRNA) using a custom-designed chip for the detection of small RNA molecules. Probes of 25 bp of the transgene were printed on the microarray, covering the transgene sequence. Each probe was tiled at 1-nt intervals along the transgene so that siRNA production by the whole transgene could be mapped by 2873 probes, in both sense and anti-sense directions. Small RNA populations from transgenic and non-transgenic controls were differentially labeled and applied to the chip. siRNA peaks, generally coinciding with higher GC-content, were observed throughout the inverted repeat transgene. Resistance-breaking strains carried mutations in sequences which produced siRNA peaks. Sequence characteristics of high responding probes were considered. [L]

## Confocal Microscopy of Induced Resistance in Lettuce against *Bremia lactucae*

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Downy mildew in lettuce caused by *Bremia lactucae* often requires application of fungicides to protect the crops. Recent data from our lab showed that effective control of the disease in the field was also achieved with the chemical inducer BABA ( $\beta$ -amino-butyric acid). The purpose of this research was to study the interaction between *B. lactucae* and lettuce at the cellular and biochemical levels. Differential staining techniques with calcofluor and basic aniline blue showed that BABA applied before inoculation allowed frequent spore germination, appressorial formation and penetration of *B. lactucae* into the epidermal cells of lettuce. However, whereas in control leaves the pathogen, in 24 h post-inoculation, produced a mycelium that grew out of the epidermal cell into the intercellular space and produced haustoria inside the mesophyll cells, the pathogen in BABA-treated leaves produced only vesicles inside the epidermis cell and failed to produce a mycelium. The vesicles were heavily encased with callose and, as visualized by confocal microscopy, the primary mycelium attempting to grow from the secondary vesicle remained stunted inside the epidermal cell. When applied 1–4 days after inoculation, BABA caused heavy encasement of the haustoria with callose, enhanced the discoloration of the mycelia and prevented sporulation of the pathogen. The involvement of hydrogen peroxide in the resistance induced by BABA was evident from the enhanced scavenging activity of caffeic acid and chlorogenic acid peroxidases as well as from the fact that catalase compromised the activity of BABA. (L)

## Resistance of Wild Tomato Species to Late Blight Caused by *Phytophthora infestans*

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Late blight caused by *Phytophthora infestans* is a devastating disease of potato and tomato. Commercial tomato hybrids grown in Israel are all susceptible to the blight and thus require frequent fungicide applications. The wild tomato species (obtained from Ales Lebeda, Czech Republic) *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 were all found susceptible to our isolates of *P. infestans*, whereas Agrogene's inbred lines (F10) of *Lycopersicon pimpinellifolium* (*Lp*) 3707 and *Lp* 3708 were resistant. *Lp* PI 270448, PI 270449 and PI 270450 (recently obtained from R. Gardner, North Carolina, USA) were also resistant. The aim of this study was to reveal whether *Lp* 3707 and *Lp* 3708 carry allelic genes for resistance. F1 hybrids between the susceptible inbred ZH and 3707 or 3708 were partially resistant, suggesting that both carry a partially dominant gene(s) for resistance. F2 plants of 3707xZH segregated 3:6:7 resistant: partially resistant: susceptible, suggesting that two genes are responsible for resistance: R, a semi-dominant gene and E, an epistatic gene. F2 plants of 3708xZH segregated in different manners, depending on the accession of 3708. F1 plants derived from 3707x3708 were all resistant but F2 plants segregated in different ways. Some F2 families were more resistant than their parents. We concluded that some accessions of *Lp* 3708 carry the genes R and E for resistance against late blight whereas other accessions may carry a semi-dominant version of *Ph-3*. The combination of all three genes seemed to provide stronger resistance than R+E or *Ph-3*. [P]

C: DISEASE CONTROL AND PREVENTION Part I

## New Fungicides for Control of the Fungus *Pseudoperonospora cubensis* in Cucumber

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Downy mildew in cucumber is caused by the fungus *Pseudoperonospora cubensis* and instigates serious damage to the crop in open fields and in greenhouses. The disease strikes during all growth seasons and reaches three peaks: in spring and autumn in greenhouses and in open fields during the months June/July. Control of downy mildew is obtained with fungicides as a preventative treatment or as a response to the first disease symptoms in the crop. With the entry of new fungicides there was a need to test them during all cucumber growth seasons both in greenhouses and open fields. The new fungicides included Consento<sup>®</sup> and Infinito<sup>®</sup>, both designed (by Bayer CropScience) to control diseases that are caused by fungi from the Oomycetes class. Both new fungicides contain two active ingredients (a.i.): Propamocarb is shared by both products and has systemic properties. It strikes at various sites inside the pathogen's cells, which eventually results in preventing germination of the fungi spores and inhibiting mycelium growth. The second a.i. of Consento<sup>®</sup> is Fenamidon, which exhibits translaminar properties and inhibits the mitochondrial respiration process in the fungi cells. Infinito's<sup>®</sup> second a.i. is a completely new chemical compound from the new chemical class of acyl picolides: Fluopicolide. This compound has systemic properties, and thus both a.i. of Infinito<sup>®</sup> are systemic. The mode of action of Fluopicolide is not yet entirely clear, but the compound causes injury to the fungus' cell membrane. A series of three or four weekly treatments were applied in cucumbers for industrial processing and five weekly treatments in greenhouse cucumbers. The treatments maintained a very low downy mildew infection level of at most 8% disease coverage, whereas the infection in the control plots reached levels of 70–90%. The yields from the new fungicide treatments were 20–30% higher than those reached in the control plots. A series of treatments applied with Consento<sup>®</sup> at an initial downy mildew leaf coverage of 65% caused the disease to stop its expansion and later showed a steady decrease in leaf disease coverage. [L]

### Combining Control Treatments with Biological Control for Synergistic Improvement of Postharvest Disease Control

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Combining control methods can improve control efficacy, increase the spectrum of controlled pathogens and reduce the possibility of resistance development. Compatibility of control methods is mandatory for the success of the combined treatments: the first treatment should not adversely affect the succeeding one and, preferably, should improve it. In the last few years, carrot growers have begun to brush carrots before storage: this practice increases the appearance of black root rot, a postharvest disease caused by the fungus *Thielaviopsis basicola*. 'Shemer', a commercial yeast product based on a *Metschnikowia fructicola* isolate, is effective in the postharvest control of fungal pathogens that develop in postharvest fruits and vegetables. Shemer is licensed for use in the control of the pathogenic fungus *Sclerotinia sclerotiorum* in postharvest storage of carrots, but by itself does not provide sufficient control in all cases, including insufficient control of black root rot disease. In the present work a steam system, and technology for the precise application of steam and combined application with hydrogen peroxide and yeast, were tested. Both steam and hydrogen peroxide alone were highly effective at controlling most postharvest pathogens but caused phytotoxic or pseudo-phytotoxic symptoms at the effective doses. Application of a sublethal dosage of steam or hydrogen peroxide followed by yeast, before storage, improved the efficacy and disease control compared with each of the treatments alone. These experiments showed that disease-control agents can potentially be used for a short period and 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 the synergistic effects of using sublethal treatments sequentially with a biological control agent have the potential to reduce the use of chemical control. [L]

### Development of Transgenic Resistance to Viruses in Cucurbits

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Cucurbits production in Israel and worldwide is affected by modern agricultural technologies that require massive investments of capital and know-how. Viral diseases of cucurbit crops result in heavy yield losses, and may even jeopardize the feasibility of these crops. The classical crop improvement for disease resistance in cucurbits is severely hampered by the lack of natural sources of durable resistance to some of the most destructive viruses. Generation of viral resistance by means of genetic engineering was successfully reported and widely implemented for several crops, including cucurbits. Our laboratories have been involved in recent years in the evaluation of several genetic constructs for the generation of transgenic resistance to RNA viruses belonging to several groups, which are known to affect cucurbit crops severely. The first construct included a fragment from the *Cucumber fruit mottle mosaic virus* (CFMMV) replicase gene. Several transgenic cucumber lines harboring these sequences showed a high degree of resistance (immunity) to the virus when challenged by different inoculation methods. The second construct included replicase fragments from three viruses belonging to different groups: *Cucumber vein yellow virus* (CVYV), *Cucurbit yellow stunting disorder virus* (CYSDV) and CFMMV. None of the transgenic cucumber lines that were screened showed resistance to the three viruses. However, lines were found that showed immunity to CFMMV and partial resistance to CVYV. The third construct was designed in a hairpin configuration and contains two identical fragments from the HcPro gene of *Zucchini yellow mosaic virus* (ZYMV) in opposite direction, resulting in a dsRNA transcript which potentially may effect virus silencing. Several transgenic cucumber lines which harbor this construct show immunity to ZYMV and resistance against *Papaya ringspot virus-W*. The successful development of transgenic resistance to cucurbit viruses certainly supports its advantageous application whenever classical improvement is unattainable. [L]

### Virus Diseases of Sweet Potato and Their Control

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Sweet potatoes were introduced into Israel around 1920 and grown at the Agricultural High School at Miqwe Yisrael. In the early 1950s the crop was grown to serve as raw material for starch and flour processing purposes. At first yields were very high (>60 t ha<sup>-1</sup>), but after about 5–6 years they declined because of virus diseases, due to farmers using cuttings from their old fields for the next year's planting. Renewed research in the 1980s led to the identification of the main viruses, preparation of virus-tested planting material from meristem cultures, and commercial nurseries starting to grow the virus-tested mother plants cuttings for growers. Since then sweet potato acreage has increased every year by 8–10% and become a profitable crop for farmers, mainly for export.

The main viruses identified in Israel are *Sweet potato feathery mottle virus*, genus *Potyvirus* (SPFMV), transmitted in a nonpersistent manner by aphids. *Sweet potato sunken vein virus*, genus *Crinivirus* (SPSVV). Possible synonym: *Sweet potato chlorotic stunt virus* (SPCSV), transmitted by



the whitefly *Bemisia tabaci* in a semi-persistent manner. The complex infection SPFMV+SPSVV causes a severe reduction in yields. *Cucumber mosaic virus* (CMV) severely infected sweet potato fields in the mid-1980s, causing stunting, chlorosis and yellowing of plants. Infection by CMV was dependent on the presence of SPSVV in the sweet potato plant. Apparently, there is a gene-silencing mechanism that inhibits CMV replication in healthy sweet potato, which is suppressed by SPSVV. Interestingly, in East Africa where SPFMV and SPCSV are endemic, no co-infection with CNV was observed. Comparing nucleic acid and protein sequences of the RNA 3 of the two isolates (SPCSV Uganda and SPSVV Israel) revealed a difference of ~17% and 20% in the nucleic and amino acid sequences, respectively. Apparently, the suppressor gene in SPCSV is not functional. Another virus found first in Israel is *Ipomea crinkle leaf curl virus* (ICLCV). Geminate particles were observed in crude sap preparations and the virus was transmitted by *B. tabaci* in a persistent manner. At present the best way to control virus diseases in sweet potato is to supply the growers with virus-indexed propagation material. [L]

### **Effects of Soil-applied Fungicides on Peanut Yield Losses Caused by *Sclerotium rolfii* in the Hula Valley, Israel**

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Peanut crops in the Hula Valley of Israel are damaged by *Sclerotium rolfii* Sacc., the pods being the main organs affected. Previously we reported that Folicur (tebuconazole 25% E.C.) sprays reduced the disease level only slightly, with no difference in the efficacy of two vs six spray applications per season. Greater disease control was achieved when a half-dose (1000 ml ha<sup>-1</sup>) was plowed into the upper soil layer and an additional half-dose was sprayed onto the plants during the growth season: 20% diseased pods as compared with 37% in the control. In the following experiments 2000 ml ha<sup>-1</sup> of Folicur was applied in the seeding strip, in the assumption that this level might provide the pods with better protection against the pathogen. In the 2007 season, the effects of six fungicides reported to be effective against *S. rolfii* were evaluated: Signum (boscalid+ pyraclostrobin), Amistar (azoxystrobin), Flint (trifloxystrobin), Punch (flusilazole), Agrostar (metconazole) and Ohio (fluazinam). The objective was to determine if any of these fungicides would enhance the disease control provided by soil-applied Folicur alone. All fungicides were applied to naturally infected peanut plots in which 2000 ml ha<sup>-1</sup> of Folicur had been applied to the seeding strip. The fungicides were applied in four, three and two sprays per season, starting 75, 89 and 103 days after seeding, respectively, with 2-week intervals between applications. The experiment was carried out in a randomized block design with five replicates per fungicide (=treatment), each replicate consisting of a 12-m-long bed with two rows. Diseased plants were counted three times during the season. At the end of the growing season, plants were removed from a 1-m bed row in the center of each plot, and the pods of these plants were rated as healthy or diseased with *S. rolfii*. The results of the overall plant disease counts and the pod disease counts both varied greatly and there were no significant differences among the treatments or between them and the Folicur-only treatment, regardless of the number of sprays or the fungicide. Although lower levels of overall diseased plants and pods were observed in some treatments, none of these differences was significant. [P]

### **Evaluation of *Sclerotium rolfii* Inoculum Levels in Hula Valley Soils**

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A wet sieving extraction procedure for extracting sclerotia of *Sclerotium rolfsii* from soil was developed in order to evaluate inoculum levels of the pathogen in soil. The procedure includes the following steps: dry sieving of a 500-cm<sup>3</sup> soil sample through a sieve with holes of 1180 $\mu$ , to remove plant parts and soil lumps, followed by filtering through a second, 500- $\mu$  sieve. The soil fraction that did not pass the second sieve and which contained any sclerotia, was transferred to a 2-l vessel together with 500 ml of water. The sample was stirred with a magnetic stirrer for 20 min in order to separate the sclerotia from soil particles. Then the aqueous fraction was filtered through the 500- $\mu$  sieve. The procedure of adding water, mixing manually (for 20 sec) with a spatula and sieving was repeated five times to ensure the maximum recovery of sclerotia from the soil fraction. The particles left on the sieve, including the sclerotia, were transferred to a paper towel to dry, and then examined under a stereomicroscope for sclerotia identification and collection. The viability of the sclerotia was determined by seeding them on potato dextrose agar. The extraction efficiency was tested in two types of soils, peat and mineral soil. Fixed numbers of sclerotia were added to 500-cm<sup>3</sup> samples of each of the two soils: 3, 6 and 50 sclerotia were added per sample, in five replicates. The sclerotia were mixed thoroughly with the soil samples and then extracted according to the procedure protocol. The extraction efficiency ranged from 90% to 100% and was not significantly affected by the number of sclerotia or the type of soil. In order to evaluate the number of sclerotia in naturally infested soils, samples were collected from infested fields. Each 10-l soil sample was comprised of 20 subsamples collected from the upper soil layer of a 10-m-diam area and mixed thoroughly. Four extractions (500 cm<sup>3</sup> soil each) were made from each 10-l sample. Great variability was found among the numbers of sclerotia detected in different extractions from the same 10-l soil sample, as well as among five soil samples collected from different sites in the same field. For example, 19, 44, 37 and 28 sclerotia were detected per extraction in the same soil sample; and in different sites in the same field, with an average of 18.4, 30, 7.8, 12.6 and 7.4 sclerotia counted per sample. The variability within the individual soil samples, even though they had been mixed thoroughly before extraction, is apparently due to the presence of clusters of sclerotia that were separated during the extraction procedure, or to non-uniform dispersion of the sclerotia in the sample. The variability among different soil samples collected from different sites in the same field is due to the dispersion pattern, in foci, typical of *S. rolfsii*. [P]

### **Mutagenesis of *Bremia lactucae* for Resistance against CAA Fungicides**

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Downy mildew caused by *Bremia lactucae* is a serious disease of lettuce which often requires application of fungicides due to the failure of genes for disease resistance to protect the crops adequately. The Carboxylic Amide Acid (CAA) fungicides dimethomorph, iprovalicarb, benthialavalcab and mandipropamid were recently reported to protect lettuce effectively against downy mildew. Our objective in the present study was to predict the proneness of *B. lactucae* to mutate for resistance against CAAs. Our recent data showed that resistance to CAAs did not develop in *Phytophthora infestans* whereas it is known to occur naturally in *Plasmopara viticola*. Spores of six isolates of *B. lactucae* were treated with 0.2% EMS (ethyl methane sulphonate) or UV light (254 nm, 1000 mJ cm<sup>-2</sup>) for 5 min and inoculated onto CAA-treated leaves of lettuce. Both treatments yielded slow-growing mutant isolates that could resist 10 $\mu$ g ml<sup>-1</sup> DMM or MPD and 50  $\mu$ g ml<sup>-1</sup> BENT or IPRO. Wild-type isolates of *B. lactucae* could tolerate no more than 0.01  $\mu$ g ml<sup>-1</sup> of the first three fungicides listed above. The stability and fitness of these mutant isolates are currently being examined. If resistance is found to be stable, its inheritance will be studied. [P]

### **Resistance to Six Fungicides among *Botrytis cinerea* Isolates from Vineyards in Israel**

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*Botrytis cinerea* is a ubiquitous plant pathogenic fungus, which is responsible for gray mold in grapevine, inducing quantitative and qualitative losses in wine production. Chemical control remains the main way to suppress gray mold, although the use of fungicides can enable the selection of resistant populations of *B. cinerea*. We monitored the resistance to six widely applied fungicides in two vineyards (Sha'al and Ortal) in the Golan Heights where different plots were treated with benomyl (benzimidazole fungicide), fenhexamid (hydroxyanilide), fluazinam (phenylpyridinamine), fludioxonil (phenylpyrrole), iprodione (dicarboximide) and pyrimethanil (anilopyrimidine) during two growing seasons. About 300 isolates were sampled from diseased plants and from the air, and characterized for resistance to the above-mentioned fungicides using a mycelium growth test. Thirty different phenotypes showing resistance or less sensitivity to one or more fungicides were recovered. In general, across all phenotypes, alleles of strong resistance to the older fungicides benomyl (BenR) and iprodione (DicR) were the most frequent, with BenR 5.7% in Ortal and 15.6% in Sha'al, and DicR 10.6% and 19.5% in the two locations, respectively. High resistance to pyrimethanil was found with a frequency of 2.4% in both vineyards. A few isolates (less than 1%) were resistant to the relatively newer fungicides fenhexamid or fludioxonil. No high resistance to fluazinam was detected. Additionally, 4% to 7% of the isolates showed reduced sensitivity to pyrimethanil, iprodione, fenhexamid and fluazinam, frequently combining several traits in one isolate, which may possibly be explained by the existence of multi-drug resistance. No good correlation was found between the plot treatment and the *B. cinerea* phenotypes recovered. Compared with the newer fungicides, the efficacy of benzimidazole and dicarboximide fungicides treatments was low, which correlates with the relatively high frequency of resistance to these fungicides in the tested population of *B. cinerea*. [P]

### **Volatile Antimicrobial Compounds from Isolate OB-RB1A, a Novel Endophytic Fungus Isolated from an Olive Tree**

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Biological control of plant diseases has gained increasing attention in recent years, due to public concern regarding environmental and health implications of the use of chemicals for the protection of crops from pests and diseases. Many microorganisms capable of inhibiting plant pathogens have been discovered and tested. Several commercial biological control products are even available for use in agriculture. These products have had limited success, mainly in the lab and greenhouse setting. It is not infrequent that difficulties prevent the successful application of many such products in the field and orchard setting. To overcome these limitations, it is possible that stronger, different microorganisms are required. We screen for such microorganisms, particularly endophytes (microorganisms inhabiting the inner parts of the plant tissues) which are characterized by extraordinary biological antimicrobial activities. During the screening process, we have discovered a unique fungus, isolate OB-RB1A. OB-RB1A is an endophytic fungus isolated from an olive tree and belonging to the Xylariaceae family. This fungus effectively inhibits and even kills a number of plant pathogenic fungi *in vitro*, via a mixture of volatile organic compounds produced by it. These compounds underwent chemical analysis by gas chromatography and mass spectrometry (GC-MS). OB-RB1A's activity is similar to that displayed by the endophytic fungus *Muscodora albus*, which also belongs to the Xylariaceae, although the profile of volatiles produced by it is different. This biological activity hints at OB-RB1A's potential as an agent of plant disease biocontrol. The first

step in testing its potential *in vivo*, is to introduce OB-RB1A into a model plant. This model plant will then be used to test OB-RB1A's ability to prevent infection and disease development caused by selected pathogens. The biological activity of OB-RB1A *in vitro* and the chemical profile of volatile compounds produced by it are reported. [P]

#### D: ETIOLOGY AND EPIDEMIOLOGY

### **Epidemiological Aspects of Mango Malformation Disease Caused by *Fusarium mangiferae***

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Mango malformation caused by *Fusarium mangiferae* is one of the most destructive diseases of this crop, occurring in most mango production regions worldwide. Very little is known about the epidemiology of the disease, conidial dispersal pattern, location of penetration site, penetration process, and colonization in the tree. Experiments on seedlings and observations in a diseased orchard were performed in order to determine several epidemiological aspects of this disease. The objectives of the study were to: (i) locate and quantify the source of primary inoculum in the orchard; (ii) study the pattern and timing of conidial dispersal; (iii) locate the infection site in the tree; and (iv) determine the duration of primary infections in the orchard. Infected inflorescences from the Volcani orchard were sampled from April through June over a 3-year period. Significantly more conidia per gram panicle were found in May and June than in April in all 3 years, and also the number of malformed inflorescences per tree was higher during these months. Airborne conidia were successfully trapped by two methods: (i) Burkard volumetric spore trap and (ii) exposure of plates with selective media in the orchard. Dispersal of conidia was detected during two consecutive years in which the pattern revealed a peak of airborne conidia during May and June. Inoculation experiments of four plant organs of mango seedlings were performed in order to identify the penetration sites. Mango buds, primarily apical, were found to be the exclusive penetration sites in the tree. Young branches were sampled from the Volcani orchard in every growth flush during two consecutive years. Primary airborne infections of the buds occurred mostly in May and June, a period that corresponds with the time of maturation and dispersal of inoculum in the orchard. Results from this study indicate the times of infections in the orchard, which may assist in developing an improved control program for mango malformation disease. [L]

### **Aspects in the Pathogenicity of *Colletotrichum coccodes*, the Causal Agent of Black Dot in Potato and of Anthracnose in Tomato**

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*Colletotrichum coccodes* (Wallr.) S. J. Hughes, the causal agent of black dot in potato and of anthracnose in tomato, is a major pathogen on these hosts worldwide. The objectives of the present study were to understand the disease mechanism and to isolate pathogenicity-related genes. Identification of reduced- or impaired-virulence mutants depends on a rapid, reliable, large-scale screening method. The current bioassays for assessing *C. coccodes* aggressiveness use potato plants or tissue culture plantlets. Such bioassays are labor-intensive and time-consuming. A bioassay using ripe tomato fruit puncture was found to be unsuitable for classifying the aggressiveness of

*C. coccodes* isolates. We have developed an alternative bioassay, in which the conidial suspension is injected into the stem scar of a mature green tomato fruit. Seven days later, the fruit is sliced and the lesion behind the stem scar is measured. This bioassay has demonstrated its reliability in determining isolate aggressiveness, it is faster and less costly than currently used bioassays and it can easily be applied to large-scale screening. The assessed aggressiveness of different *C. coccodes* isolates using tomato and potato bioassays was highly correlated. This newly developed bioassay enabled us to differentiate among wild-type isolates, based on aggressiveness. The most aggressive isolates secreted pectate-lyase (PL) 6 h post-induction at pH 6.0, whereas less aggressive isolates secreted the enzyme 12 h post-induction (conducted with anti-PL from *C. gloeosporioides* kindly provided by Prof. D. Prusky). The *C. coccodes* PL sequence was cloned by PCR using degenerate primers to the conserved areas of the enzyme and the whole sequence was isolated through DNA walking. A recombinant DNA cassette containing partial PL sequence and a hygromycine-resistance gene was used to transform germinating conidia of an aggressive isolate. Transformants were selected by hygromycine resistance. Two hygromycine-resistant lines were found to undergo gene disruption via homologous recombination. The gene disruption decreased pathogenicity to tomato fruit by 20%.

It was demonstrated that *C. coccodes* may act as a seedborne pathogen in tomato. Seeds collected from inoculated fruit (14 days post-inoculation) appeared necrotic but no sclerotia were visible under a light microscope. Nevertheless, when these seeds were placed on selective medium, typical colonies of *C. coccodes* developed around several seeds, and typical sclerotia including setae were observed on the seed coat. Seeds that were heavily covered with fungal structures failed to germinate; others that were less colonized deteriorated soon after germination, and some were affected at later seedling stages. Treatment with fludioxonil reduced the incidence of infected seeds but did not abolish it completely. [L]

### The Israeli Solution for the 'Uganda Virulence'

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Stem rust epidemics have been a real obstacle to wheat growing for many centuries. The wheat stem rust problem was 'solved' with worldwide use of the *Sr2a* gene and its complex and later by the *Sr31* gene (derived from rye). In 1999 a new stem rust race, termed Ug99, was found in Uganda and overcame *Sr31* and most of the other known resistance genes. This race spread into Kenya and Ethiopia and lately 'jumped' across the Red Sea to Yemen. In 2005 a global initiative (GRI) was founded to combat the new threat for worldwide wheat cultivation, led by CIMMYT, ICARDA, FAO and USDA. Israel (TAU – ICCI) has joined the efforts to track the spread of Ug99 and to search for genetic sources of resistance to this race. Trap nurseries composed of relevant genes were set up at three locations in Israel: Bet She'an Valley, Bet Dagan and Mivhor. In addition, the virulence of stem rust secured from wheat fields and wild relatives is being studied annually. In our work with wild wheat relatives, much emphasis is given to the Israeli endemic species *Aegilops sharonensis*. This species possesses the *S*<sup>1</sup> genome, closely related to the B genome of cultivated wheat. In this species we located resistance genes to several diseases: leaf rust, yellow rust and stem rust, including Ug99 resistance! Crosses aimed at transferring this resistance to bread wheat have been done lately. Due to only partial homology between the *S*<sup>1</sup> genome and the bread wheat genome, the use of cytogenetic techniques is required. [L]

*E: DISEASE CONTROL AND PREVENTION Part II*

### Melon Wilt in the Yizre'el Valley: Causal Agents and Management Measures

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Plant wilting just prior to fruit ripening and harvest is a common affliction of Ananas-type melons grown during the summer in the Yizre'el Valley (northern Israel). Disease incidence is dependent on cultivar and on prevailing environmental conditions throughout the growing season. The fungi *Macrophomina phaseolina* and *Fusarium* spp. are commonly isolated from the roots of wilted plants and are suspected as being causal agents of wilting. Results of experiments conducted over the past 3 years have shown that grafted melons grown in infested fields did not wilt even though non-grafted ones wilted severely. However, the expense of grafted transplants motivated us to check the economic feasibility of using grafted melons in this area by evaluating the yield as related to the stand in the field. Two melon cultivars, '6405' and 'Eyal', were grafted on the *Cucurbita* rootstock TZ 148 and were transplanted according to common commercial practice, that is, 60 cm between plants within the row. For comparison, the plants were transplanted at two additional spacings, 90 and 120 cm within the row. The two cultivars responded differently to the spacing. Reduction of plant density of Eyal resulted, as expected, in lower yields from both grafted and non-grafted plots. However, the yield of 6405 did not differ significantly among the three plant densities. It seems that there is a difference in the compensation ability between these two melon cultivars. In addition, cv. 6405 responded better to the grafting process and the percentage of yield increase was higher than that of Eyal. This finding suggests a way to lower expenses of the grower by helping him choose melon cultivars that will be more suitable for grafting. [L]

### Suppression of Soilborne Pathogens by Ammonia: Mode of Action

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The fungicidal action of ammonia on various genera of fungi is well known. In order to understand its mode of action, laboratory experiments were conducted in which two fungi, *Fusarium oxysporum* f.sp. *lycopersici* [Fol] and *Sclerotium rolfsii*, were exposed to different ammonia concentrations (exposure time 20 min) and the following parameters were measured: leakage of glucose, potassium and organic nitrogen from mycelium cells (membrane functioning), activity of ATPase on the plasma and mitochondrial membranes of *S. rolfsii* mycelium only (membrane functioning), activity of GDH in the mycelium cells (nitrogen metabolism), and the content of ATP in the mycelium cells (general metabolism). The leakage pattern in *S. rolfsii* was similar for potassium, glucose and organic nitrogen, increasing up to 1 mg NH<sub>3</sub> l<sup>-1</sup>; below this ammonia level, the leakage of the three components decreased. *Fol* exhibited a similar pattern of potassium leakage, although the leakage ratio was similar for the entire range of ammonia concentrations. The glucose leakage decreased at the lower ammonia concentration (below control), and increased in the medium-high ammonia concentration. There was no leakage of organic nitrogen, with a reduction tendency as the ammonia concentration increased. The activity of NADPH-GDH in *S. rolfsii* increased up to 1 mg NH<sub>3</sub> l<sup>-1</sup>, after which there was a slight reduction in the NADPH-GDH activity (very similar to the leakage behavior). The activity of NADPH-GDH in *Fol* increased as the ammonia concentration rose but NADPH-GDH activity remained stable over the entire ammonia concentration range. Measurement of ATP content in the mycelium cells of *S. rolfsii* indicated that in the lower ammonia concentration, the ATP quantity decreased and in the medium-high ammonia concentration the ATP quantity increased; the ATP content in *Fol* decreased as ammonia concentration increased. The activity of ATPase in the plasma membrane and mitochondria of *S. rolfsii* displayed the same tendency: increased activity until 1 ml l<sup>-1</sup> ammonia, followed by a reduction in activity. The

plasma ATPase activity was higher than the mitochondria ATPase and their activity patterns were very similar to the activity of NADPH-GDH and the leakage measurement. In summary: ammonia can (i) alter the permeability of the cell membrane; (ii) increase the activity of NADPH-GDH (increase the production of amino acid); (iii) affect the activity of the TCA cycle by using carbon intermediates ( $\alpha$ -ketoglutarate and oxaloacetate) for biosynthesis of amino acids; (iv) increase the activity of ATPase (pH regulation); and (v) lead to higher consumption of ATP and carbon skeletons for amino-acid biosynthesis, and pH regulation. [L]

### Suppression of Soilborne Pathogens by Ammonia: Dose and Incubation Time Effects

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Pathogen control efficiency of diverse soilborne fungi differed under the same conditions (soil type, ammonia concentration). The assumption was that different soilborne fungi have different sensitivity to ammonia. We examined the influence of the ammonia concentration (0–30 mg  $l^{-1}$ ) and exposure time (5–30 min) on the soilborne fungi mycelium of *Sclerotium rolfsii*, *Verticillium dahliae*, *Rhizoctonia solani* and *Fusarium oxysporum* f.sp. *lycopersici* in laboratory experiments. It was found that: (i) Effective control (disinfection) depends upon the product of the ammonia concentration and the exposure time, each reduction in one component necessitating an increase in the other component. (ii) Some of the fungi can tolerate a low concentration of ammonia for a short time (nitrogen metabolism), meaning that we must apply an ammonia concentration below a certain threshold that can be specified for each fungus in order to obtain the depression/mortality of the fungi. (iii) Grading the fungi according to their ammonia sensitivity from high to low, listing ammonia concentration and exposure time in order to obtain 100% control efficacy: *S. rolfsii* was the most sensitive (15 min exposure, 1.2 mg  $l^{-1}$  ammonia conc.), *F.o.* f.sp. *lycopersici* (30 min, 8 mg  $l^{-1}$  ammonia), *R. solani* (20 min, 22 mg  $l^{-1}$  ammonia), and *V. dahliae* was the most resistant (30 min, 16 mg  $l^{-1}$ ). These results were obtained from direct contact between ammonia and the fungal mycelium. However, under normal conditions, soilborne fungi are mixed with the soil particles, and this arrangement can interrupt the direct influence of the ammonia. In laboratory experiments we examined the influence of the following parameters: soil type (sand, loamy sand, sandy loam, clay and sandy clay loam), soil moisture content, pH of the soil solution and different injected ammonia concentration on the ability of the soil ammonia to disinfect the soil (exposure time of 4 h). The conclusions from these trials were: (i) The ammonia retention of the soil increases along with the clay content and the cation exchange capacity of the soil, and therefore less ammonia is available for disinfection. (ii) As the water content of the soil increases there is less ammonia available for disinfection. (iii) The pH of the soil solution affects to a certain extent the concentration of ammonia available for disinfection. (iv) Efficient suppression/mortality of soilborne fungi by ammonia can be achieved in soil types like sand, loamy sand, sandy loam. [L]

### Development of a Plant Virus as a Unique Platform for Oral Vaccination of Poultry

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Oral vaccines offer the hope of more convenient immunization strategies in livestock and poultry. As plant viruses accumulate to high levels and are harmless to animals, they could be used as carriers

for immunogenic epitopes by fusing them to the exposed coat protein. Such recombinant virions display numerous copies of the epitope on their surface, thus enhancing their immunogenicity, providing a potentially effective, inexpensive and safe vaccine. We developed a novel attenuated *Zucchini yellow mosaic potyvirus* (ZYMV-AGII) as a non-pathogenic virus-vector in cucurbits and have demonstrated that AGII can express foreign epitopes on its viral surface without impairing squash development or crop yield. The optimal epitope integration site on AGII coat protein was determined by comparing the effects of different fusion sites on epitope exposure and viral accumulation. To test the immunogenic potential of this vector we created a Human cMyc expressing recombinant virus, AGII-cMyc. Chicks were either fed with purified virus, or voluntarily consumed fresh or desiccated infected zucchini leaves expressing this virus. Specific anti-cMyc antibody responses were determined and compared with those induced following intramuscular injection of recombinant virus in adjuvant. Our observations show AGII-cMyc to be immunogenic in all instances. After the model with AGII-cMyc was established, we extended our vaccine system to include epitopes of a well known avian pathogen: epitopes HN and F of the Newcastle disease virus (NDV). An epitope expressing AGII-NDV-HN and AGII-NDV-F was successfully constructed and the ability of these AGII chimeric particles to induce anti-epitope specific antibody responses following injection or voluntary intake of fresh and dry infected zucchini leaves was demonstrated. Our findings demonstrated the ability of AGII chimeric particles to induce an immune response against a foreign epitope both upon their injection into chicks and upon their being ingested on fresh and desiccated infected squash leaves. [L]

#### F: HOST – PATHOGEN INTERACTIONS Part II

##### **Interactions between Soilborne Fungi and *Rhizoglyphus robini* in Liliaceae**

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The soil bulb mite *Rhizoglyphus robin* (Acaridae: Astigmata) is frequently found on various species of Liliaceae in Israel. Severe damage to underground plant parts is associated with high populations of the mite and fungal pathogens. In a series of laboratory experiments with onion seedlings, we attempted to characterize the major fungal pathogens, examined the influence of the fungi on the mites, and studied the interactions between the mites and the fungi. Different levels of pathogenicity were detected between fungal species and between isolates of the same fungus. Mites were attracted to most tested fungal species and strains. The degree of attraction was not related to pathogenicity. The combination of *Fusarium oxysporum* and mites was more severe on onion seedlings than either factor alone. Currently, *R. robini* management is based on highly toxic insecticides. Our results suggest that these treatments could be avoided and that fungal control could be a more effective and environmentally friendly method to prevent the damage inflicted by these two causal agents. [L]

##### **Effect of Plant Age at Inoculation on Expression of Genetic Resistance to *Tomato yellow leaf curl virus***

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In order to determine the effects of plant age at inoculation on the expression of genetic resistance to *Tomato yellow leaf curl virus* (TYLCV), six TYLCV-resistant and two susceptible tomato varieties



were inoculated at 14, 28 or 45 days after sowing (DAS). Inoculation at 14 and 28 DAS was performed in the greenhouse, and the plants were transplanted to the field at 30 DAS. Inoculation at 45 DAS was performed in the field, by covering the target plants with polypropylene ('Agril', Agriferma S.A.) sheets and releasing viruliferous whiteflies under them. Resistance was assayed mainly by comparing yield components of inoculated plants with those of control, non-inoculated plants of the same variety. Symptom severity and plant height were also followed. Plant age at inoculation had no effect on disease-severity scores of the susceptible varieties, and little or no effect on those of the resistant varieties. In contrast, plant age at inoculation had a significant effect on the yield of all varieties tested. All varieties suffered a significant yield reduction due to inoculation with TYLCV. However, the older the plant was at inoculation, the smaller the TYLCV-induced yield loss. Our results clearly demonstrate the occurrence of age-related (or mature-plant) resistance in tomato plants to TYLCV. By assaying TYLCV accumulation level in susceptible plants, it was found that the age-related resistance is manifested by reducing viral accumulation levels. [L]

### **The Genetic Base of Temperature Adaptation in the Chickpea Pathogen *Didymella rabiei***

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In the Levant, domesticated chickpea (*Cicer arietinum*) has been grown sympatrically with several wild *Cicer* species since the dawn of agriculture. For more than 10,000 years chickpea was sown in late spring while the wild species kept their autumnal germination. This 4-month shift in the cultigens' life cycle is a means of avoiding the potential damage of *Ascochyta* blight, which is an important disease of chickpea especially under mild and rainy winter conditions. Recently, the pathogen was isolated from different populations of wild *Cicer* species (*C. judaicum*). These isolates are less adapted to high temperatures than those from domesticated chickpea and are better adapted to *C. judaicum* than to *C. arietinum*. The research objective was to study the genetic base of the temperature adaptation of the pathogen. The phenotypic distribution of progeny populations of a cross between isolates from wild and cultivated origins to temperature was studied *in vitro*. The distribution was continuous, departed from normality, and some progenies had better adaptation to higher temperature than their parents (transgressive segregation). *In vivo* experiments did not support the hypothesis of close linkage between temperature adaptation and aggressiveness. In addition, aggressiveness of the parents under favorable conditions (on their original host and in typical temperatures) was higher than their progeny. The results indicate that there is a potential for adaptation to higher temperatures, but the chances for formation of hybrids that are capable of attacking both hosts in a broad temperature range are slim. [L]

### **Characterization of Ammonia Secretion and pH Modulation during Pathogenicity of *Colletotrichum coccodes* on Tomato Fruit**

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*Colletotrichum coccodes* alkalinizes the decaying tissue of tomato fruits by the accumulation and secretion of ammonia. Alkalinization dynamics caused by ammonia secretion from growing hyphae

was examined on the microscopic level using the pH-sensitive fluorescent dye BCECF (2',7'-bis (carboxyethyl)-5(6)-carboxyfluorescein). A pH of 4.2 was detected in healthy tissue whereas in infected tissue a sharp gradient from pH 7.9 to pH 6.0 was observed in the first 10 mm from the growing hyphal tips. Ammonium accumulation at the infection site was found to be dependent on the initial environmental pH and the type and availability of nitrogen source. Treatments with low (4.0) and high (7.0) pH buffer at the site of infection site resulted in high and low levels of ammonia secretion, respectively. In the former case, a higher rate of infection was observed, indicating that ammonia accumulation is important for pathogenicity. In order to examine further the necessity for ammonia secretion, *nit<sup>-</sup>* and *AreA<sup>-</sup>* mutants with reduced ammonia accumulation were isolated and characterized. In both cases, the mutant lines showed reduced decay development. The reduced infection rate of *nit<sup>-</sup>* mutants could be complemented by the addition of glutamine at the infection site. Our findings demonstrate that ammonia accumulation is a critical factor contributing to pathogenicity of *C. coccodes* on tomato fruits. Furthermore, the acidic pH of fruits is conducive to ammonia secretion and subsequent alkalization of the infection site and stimulating fungal transformation from the quiescent-biotrophic stage to the active necrotrophic state. [L]

#### G: DIAGNOSIS AND CHARACTERIZATION OF PLANT PATHOGENS Part II

##### Phytoplasma Diseases in Ornamental Crops

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Phytoplasmas are plant pathogens associated with yellows diseases in several hundred plant species of many important food, vegetable, ornamental and fruit crops. The pathogens, which belong to the class Mollicutes, are cell wall-less bacteria and populate phloem sieve elements. The phytoplasmas are transmitted mainly by sap-sucking insect vectors belonging to the Cicadelloidea (leafhoppers). They have not been cultivated *in vitro*. Traditionally, phytoplasmas have been identified and characterized based on their biological properties. Recently, the introduction of molecular biology-based analyses advanced the art of phytoplasmal disease diagnostics, and made it possible to detect a wide array of phytoplasmas associated with diseases in hundreds of plant species. Phytoplasma diseases have been identified, in Israel, in cultivated ornamentals, vegetables, field crops, fruit trees and in wild crops. In the summer of 1999, the disease was identified in *Anemone*, *Cellusia* and *Cosmos* spp. In these cases the disease was sporadic with no serious economic losses. However, in grapevines and carrots the disease is causing losses of great economic significance. During 2000–2007, the disease was identified in *Limonium* spp., *Eustoma* and *Gypsophila* spp. in the Arava Valley. [L]

##### Sweat Boxes and Selective Media for the Detection of *Acidovorax avenae* subsp. *citrulli* in Melon and Watermelon Seeds

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Bacterial fruit blotch (BFB) is caused by *Acidovorax avenae* subsp. *citrulli*, a bacterium that is introduced into watermelon or melon fields mainly by infected seeds and transplants. The bacterium can be a surface contaminant of seed harvested from infected watermelon. The pathogen can be

asymptomatic on transplants and can lead to high numbers of infected transplants entering field plantings. The most important management strategy for BFB is exclusion of *A. avenae* subsp. *citrulli* by using pathogen-free seeds and seedlings. It is impossible to guarantee pathogen-free seed, but seed health testing reduces the risk of outbreaks. Currently, the standard seed test for BFB is the seedling grow-out assay in which 10,000 – 30,000 seeds per lot are planted under conditions conducive for BFB development. Seedlings are visually inspected after 18 days for BFB symptoms. Our method combines the grow-out assay, but in sweat boxes with isolation and partial identification of the suspected seedlings using a new selective and partial diagnostic medium (Kritzman AAFBD medium). Special care is taken to eliminate from the sweat boxes other pathogens that can mask the symptoms of the BFB or that produce unclear symptoms. The tested seeds are sown into a sterile environment supplemented with fungicide. After 15 days of incubation, seedlings that evince suspected symptoms are taken to the lab for isolation of the pathogen on our selective medium. Suspected colonies are inoculated into melon or watermelon seedlings for a pathogenicity test, and further identified by mean of serology, PCR or fatty acid contents. This method enables us to detect one naturally infected seed out of 10,000 melon or watermelon seeds, easily and in a manner safe for the environment. [L]

### **Characterization of *Pyrenochaeta lycopersici* Isolates, the Causal Agent of Corky Root in Tomato, Using PCR-based and VCG Assays**

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Corky root, caused by *Pyrenochaeta lycopersici*, is an important soilborne disease and may cause 30–70% yield reduction in the tomato crop. The symptoms of the disease are stunting, wilt and typical necrotic brown lesions on the roots that become corky, with swollen cracked bark surface. This disease became rare in Israel during the years that methyl bromide was used, but the phase-out of this fumigant has led to increasing disease incidence. The existence of two types of the pathogen that differ in several physiological features was detected recently. Specific PCR markers of 147 and 209 bp were obtained for isolates 1 and 2, respectively, and enable easy identification. Another method, which is genetic-based classification, is the Vegetative Compatibility Group (VCG) test, which involves biological processes in cell functioning. With this method, chlorate-resistant mutants which reflect mutations at the nitrate reductase structural locus are formed. These mutants are unable to utilize nitrate as a sole nitrogen source (*nit* mutants). Only closely related isolates can produce heterokaryons using this assay. The objective of this research was to study (a) whether the VCG test can serve as an additional tool for indicating genetic relatedness between different types of the pathogen *P. lycopersici*, and (b) the agreement between these two tests. We compared several isolates originating from Israel and Italy using PCR markers and VCG tests. With the PCR markers, all Israeli isolates were identified as type 1, and those from Italy were from both types. The VCG test revealed that the Israeli isolates belong to the same VCG group, but those of type 1 that originated from Italy do not belong to this VCG group. Preliminary studies show that type 1 and type 2 isolates (all originated from Italy) do not belong to the same VCG group. This is the first report regarding VCG tests carried out for *P. lycopersici*. Additional isolates will be tested in the future in order to broaden the knowledge regarding the genetic variation that exists among isolates of *P. lycopersici* that originate from different sources. [L]