ABSTRACTS OF PRESENTATIONS AT THE 24TH CONGRESS OF THE ISRAELI PHYTOPATHOLOGICAL SOCIETY

April 14, 2003

ARO, The Volcani Center, Bet Dagan, Israel

Keynote Address Dedicated to the Memory of Dr. Avi Grinstein

Soilborne Pathogen Research: Soil and Root Issues

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Studies of this group of pathogens have led to numerous achievements in Israel and worldwide. Nevertheless, many questions remain unanswered. Certain practices, such as monoculture (especially in intensive agriculture), and the worldwide spread of pathogens *via* infected propagation or other plant materials, have increased the incidence of plant diseases. In contrast, progress has been made in several areas. For example: the development of high-quality tomato cultivars resistant to Fusarium and Verticillium wilt and of cotton cultivars resistant to Fusarium wilt has rendered these diseases negligible. Other achievements include the production of pathogen-free propagation material by means of tissue culture, the development of highly sensitive and reliable diagnostic tools, and the development of certain disease-management methods, among others. Examples of issues to be addressed are: the development of decision-making tools based on reliable estimations of pathogen populations in soils before planting, the special problems involved in soil-less culture, and the issue of transgenic plants. The methyl bromide crisis demonstrated our inability to deal with unexpected situations; however, it enhanced the development of additional means of control. Control methods which are now being studied more intensively include grafting, biological control, biofumigation, the use of organic amendments and composts, new fumigants, sanitation and soil solarization. It is recognized that combining methods of control and adopting principles of integrated pest management (IPM) can serve as an alternative to the wide spectrum of control achieved by methyl bromide. Reducing pesticide usage now stands as one of the biggest challenges for researchers, extension personnel and the entire agricultural community. *(L)*

A: SOIL DISEASES

Etiology of Soilborne Diseases and Their Management Under Irrigation with Saline Water

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

Irrigation with saline water (EC >2 ds m^{-1}) can result in the appearance of new plant diseases and eruption of familiar plant diseases. The combination of intensive agriculture with soil disinfestation and irrigation with saline water provides conditions conducive to the eruption of soilborne diseases as never happened before. A good example is crown and root rot disease of tomatoes caused by *Fusarium oxysporum* f.sp. *radicis-lycopersici* (*Forl*), which can result in total loss of the crop. We studied the etiology, incidence and severity of crown and root rot disease under irrigation with saline water. Mycelium growth and development of *Forl* was not affected by water salinity in culture medium (amended with either NaCl or KCl). Irrigation of soils with saline water did not reduce the survival or viability of macroconidia and chlamydospores of *Forl* in naturally infested soils. Irrigation of tomato plants with saline water (EC ~3 ds m^{-1}) did not reduce plant development. However, irrigation with saline water before or after plant inoculation with *Forl*, enhanced crown and root rot expression and increased the incidence and severity of the disease in infected plants; fertilization of inoculated plants further enhanced the severity of crown rot under irrigation with saline water. Susceptibility of plants to *Forl* infection increased under saline water irrigation, resulting in the pathogen's rapid penetration and establishment in root tissues. In field experiments, partially effective soil disinfestation resulted in rapid establishment of *Forl* and fast disease eruption in such soils. Also, an inverse correlation existed between soil disinfestation intensity and population of *Forl* inside the root tissues. This occurred with both saline and fresh water irrigation, but was emphasized in the latter. Moreover, irrigation with saline water in the field enhanced the eruption of crown and root rot disease incidence and severity and also reduced plant yield. Effective soil disinfestation eliminated the initial inoculum density, thereby enabling disease control and yield increase. Soil disinfestation with dazomet or dazomet + solarization was the most effective in controlling the disease; soil solarization alone was only partially effective in disease control. The present results provide tools to control the diseases under intensive agriculture practices including irrigation with saline water. *(L)*

Effects of Ammonium, Nitrate, and Irrigation Water pH on Deformation Disease in *Gypsophila* Mother Plants

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In a previous study we described a new disease, named deformation disease, in *Gypsophila paniculata* L. mother plants; it reduces cutting yields by up to 50% but does not cause mortality of the mother plants. Although we were unable to prove the organisms involved, we had indirect proof of involvement of both fungi and bacteria in disease development. In other works we reported that fertigation of mother plants with compound fertilizer (20:20:20) at an N concentration of 720 mg l^{-1} mitigated the incidence of this disease as compared with 360 mg l^{-1}, the commercial fertilizer application level, and showed that the pertinent component was nitrogen. The objective of the present work was to study effects of nitrogen form (NO_3 or NH_4) and pH of irrigation water on disease incidence. Rooted cuttings of *G. paniculata* were planted in 10-*l* containers packed with either naturally infested or steamed black tuff (secoria). Three levels of either KNO_3 or $(NH_4)_2SO_4$ were tested: 180, 360 or 720 mg N l^{-1}, applied weekly; K, P and microelements were applied at constant concentrations. Plants were irrigated daily with 0.5 *l* per container of tap water – pH 7.5, or with acidified pH 5.5 or basified pH 8.6. During the experiment, disease incidence was monitored weekly. Fertilizing plants with ammonium sulfate (180 mg N l^{-1}) and irrigation with normal tap water resulted in a disease incidence of 50% of the initial number of plants. In the 360 and 720 mg N l^{-1} treatments, disease incidence was 18% and 10%, respectively. However, fertilizing plants with potassium nitrate resulted in a disease incidence of 64% of the initial number of plants at the highest

concentration (720 mg N l^{-1}), which increased to *ca* 90% at 180 or 360 mg N l^{-1}. When fertilizing with ammonium sulfate and irrigating with acidified water (pH 5.5), the disease incidence was further reduced to 10% at 180 mg N l^{-1} and fluctuated between 0 and 10% at 360 and 720 mg N l^{-1}; when irrigation water pH was 8.6, the disease incidence was similar to that in normal tap water. Fertilizing with potassium nitrate and irrigating with water at pH 8.6, reduced disease incidence further with the three N concentrations in comparison with normal tap water; 81%, 89% and 64% incidence in normal water *vs* 46%, 40% and 19% incidence in water at pH 8.6. When irrigation water pH was 5.5, disease incidence in the KNO_3 treatments was similar to that with the normal tap water. Nitrogen forms which are known to affect rhizospheric pH, as well as irrigation water pH, affect disease incidence in mother plants of *G. paniculata*. Ammonium – which reduces rhizosphere pH, coupled with acidified water, was the most efficient combination in reducing disease incidence, followed by nitrate – which increases rhizospheric pH, coupled with basified water. *(L)*

B: PATHOGEN – HOST RELATIONSHIPS AND RESISTANCE

Isolation of REMI Mutants of *Colletotrichum* Exhibiting Impaired Pathogenicity on Strawberry

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Colletotrichum, the cause of strawberry anthracnose, is one of the most important fungal pathogens of this crop worldwide, affecting all plant parts. The primary goal of this study was to obtain by insertional mutagenesis *Colletotrichum* mutants that are impaired in virulence on strawberry, for a better understanding of the mechanisms and processes involved in pathogenicity. A preliminary screening bioassay for a large population of transformants on strawberry seedlings was developed. A rapid disease response was observed in wild-type compared with healthy seedlings, 4 days after exposure to a nonpathogenic isolate. Six hundred REMI (Restriction Enzyme Mediated Integration) mutant transformants, which were generated by electroporation of germinating conidia, were screened for pathogenicity. Transformants that did not cause disease were subjected to a second round of screening and those that consistently exhibited reduced virulence were subjected to further analyses. Initial characterization of potential mutants was performed by comparing growth rates, development processes and extracellular enzyme production of the mutants with those of the wild-type isolate. Among these mutants, g-233 exhibited a high germination rate and less appressorial production, while Ca-5 produced significantly more appressoria than the wild type. The pattern of plasmid integration was studied and the genomic flanking region was sequenced by IPCR amplification of sequences flanking the plasmid. Further research using these mutants may provide additional information toward a better understanding of host–pathogen interactions. *(L)*

Characterization of a Non-Pathogenic UV-Induced Mutant of *Fusarium oxysporum* f.sp. *melonis*

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The soilborne fungal plant pathogen *Fusarium oxysporum* f.sp. *melonis* causes vascular wilt disease of melon. In order to identify fungal pathogenicity factors we studied a non-pathogenic UV-induced mutant. Vegetative growth, germination, biomass accumulation and dynamics of extracellular enzyme secretion of the fungal isolates were studied. Different carbon sources were used in the comparative analysis: pectin, galacturonic acid (GA), sodium polypectate, carbomethylcellulose (CMC) and melon plant tissue. In all cases the mutant showed a delay in development and enzyme secretion, when compared with the wild type. The two predominant extracellular proteins secreted when the strains were cultured in the presence of CMC or GA were purified and identified (on the basis of amino acid sequences) as exocellobiohydralase and endopolygalacturonase, respectively. The activity of both enzymes in the mutant culture medium was lower than that of the wild type, yet adding 1% yeast extract resulted in increased extracellular enzyme activity. Based on partial gene sequencing, *Fom* exopolygalacturonase and endopolygalacturonase resemble most the homologous genes in *F. oxysporum* f.sp. *radicis-lycopersici* and *F. oxysporum* f.sp. *lycopersici* (97% and 95% identity, respectively). We conclude that the FOM UV-induced mutant exhibits a general fitness deficiency that can be overcome by a yeast extract supplement. However, adding yeast extract in plant inoculation experiments did not overcome the defect in pathogenicity. *(L)*

Engineered Resistance to *Tomato Yellow Leaf Curl Virus (TYLCV)*

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TYLCV disease caused by *Tomato yellow leaf curl begomovirus* is a dominant limiting factor for the growth of tomato crops worldwide. The disease was first described in Israel, where it is impossible to grow tomatoes in the open field during the summer and autumn due to heavy outbreaks which result in total lost of the yield. A wide range of strategies has been used to develop transgenic resistance against geminiviruses: antisense RNA, coat protein genes, intact and incomplete replication proteins, defective interfering DNA and viral activated antiviral proteins. In this study we report on an engineered resistance against TYLCV in tomatoes using a truncated Rep gene from the mild strain of TYLCV-Is. A truncated replicase gene construct was introduced into VF36 tomato plants by agrobacterium-mediated transformation. Sixteen independent tomato lines were regenerated from cotyledon explants. PCR analysis of the transgenic plants using a 35S-promoter forward primer and a replicase-derived reverse primer yielded a 500 bp product (the expected size) in all plants tested, indicating the presence of the truncated C1 transgene. Resistance studies were carried out with self-pollinated R1 plants (lines 5,10,11) which originated from three independent R0 lines. All plants at this stage had a normal phenotype. Two groups of 15 tomato plants of each transgenic and non-transgenic plant were inoculated by the mild and severe strains of TYLCV *via* a whitefly-mediated inoculation. Virus symptoms were noted visually and recorded, and the presence of virus was monitored by dot-hybridization. All the transgenic plants were found to be immune to the mild strain of TYLCV on the basis of their symptomless phenotype and the inability to detect viral nucleic acid in the inoculated plants. The non-transgenic control plants, on the other hand, were all highly susceptible to the virus, evincing strong symptoms and containing high levels of virus, as measured by a strong hybridization signal. However, most of the transgenic plants (90%) inoculated with the severe isolate of TYLCV were found to be sensitive, showing strong virus symptoms and containing high levels of virus similar to the non-transgenic control plants. These results demonstrate that resistance conferred by the truncated replicase of the mild TYLCV is strain-specific and requires sequence identity of the transgene and the virus used for the challenge inoculation. The resistant state of the plants was maintained throughout the entire 3-month period of testing. *(L)*

Possible Involvement of ABA in Resistance to TMV and to Fungi Exhibited by Transgenic Tobacco Expressing an Inhibitor of Viral Replication Protein

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Tobacco plants 'Samsun nn' transformed with the NC330 cDNA encoding an inhibitor of virus replication protein showed variable degrees of resistance to *Tobacco mosaic virus* (TMV), *Alternaria alternata* and *Botrytis cinerea.* Germination of transgenic tobacco seeds (S_3 generation) was inhibited in the dark or at high temperature. Abscisic acid (ABA) concentration of the transgenic seeds or seedlings was 2.3 to 4.4 times higher than that of susceptible non-transgenic tobacco seeds or seedlings. In addition, significantly longer roots were observed in the transgenic germinating seeds than in the non-transgenic control seeds. The involvement of ABA in conferring resistance to both virus and fungi was discussed. *(L)*

Grape Rootstocks Resistant or Tolerant to the Root-Knot Nematode Species *Meloidogyne javanica* and *M. incognita*

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The root system of grape plants could host a wide range of plant-parasitic nematodes. Means of reducing nematode populations in existing vineyards are limited and grape replants on nematode-infested soils often result in considerable economic damages to newly planted vines. The present work describes attempts to select grape rootstocks resistant or tolerant to two root-knot nematode species, *Meloidogyne javanica* and *M. incognita.* 'Cabernet Sauvignon' plants grafted on 13 rootstocks, *viz.,* Paulsen 1103, Richter 110, Ruggeri 140, Salt Creek (Ramsey), Freedom, Harmony, 161-49c, VR 039-16, SO4, 420 A, 216-3 Castle, 3309 C and 101-14 Mgt, were placed in 5-liter containers and inoculated with 30,000 nematode eggs per potted plant. Sampling was conducted at one-month intervals and nematode colonization on roots was assayed after bleaching and staining. Paulsen 1103, Salt Creek (Ramsey), Freedom and Harmony rootstocks were found resistant to both nematode species, whereas SO4 and 101-1 Mgt were resistant and VR 039-16 partially resistant to *M. incognita.* Rootstocks SO4 and 101-1 Mgt were also partially resistant to *M. javanica.* Experiments are underway to evaluate field resistance of these grapevine rootstocks in nematode-infested soils. *(L)*

Attachment of the Fungus *Trichoderma harzianum* to the Root-Knot Nematode *Meloidogyne javanica* for Improved Biocontrol Activity

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The fungus *Trichoderma harzianum* exhibits biocontrol activity against the root-knot nematode *Meloidogyne javanica.* Direct fungal parasitism is one of various possible mechanisms by which

the fungus can act against the nematode juveniles (J2) and eggs. The fungal hyphae coil around the J2 and penetrate them as well as nematode eggs. Microscopic observations showed that the fungal spores usually do not attach to the nematodes, whereas the germinating hyphae attach to and penetrate the nematodes. Antibodies raised against root-knot nematodes, which bind to the surface of *M. javanica* J2 and eggs, bound also to the fungal spores and agglutinated them. The presence of these antibodies enabled the attachment of the spores to the nematodes. This resulted in a significant enhancement of fungal parasitism on the nematodes. Moreover, the binding of antibodies to the spores enhanced their germination and thus improved the fungal parasitism on the nematode. The nature of the antibody binding to both nematodes and spores is currently under investigation. Use of antibodies or similar attachment mechanisms may lead to improved biocontrol of plant-parasitic nematodes by *Trichoderma. (L)*

Squash Leaf Curl Geminivirus – a New Illegal Immigrant from the Western Hemisphere and a Threat to Cucurbit Crops in Israel

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A severe epidemic of leaf curling was observed during autumn of 2002 in squash crops in all growing areas of Israel. The vegetative growth of affected plants was significantly retarded and the fruits in some varieties were distorted and exhibited blisters and green depressions. In most cases the incidence of affected plants was close to 100% and the phenomenon was always associated with the presence of large populations of the whitefly *Bemisia tabaci.* Transmission tests revealed that the disease is transmitted by *B. tabaci* to a wide host range in the Cucurbitaceae including squash (*Cucurbita pepo*), pumpkin (*Cucurbita maxima*), melon (*Cucumis melo*) and watermelon (*Citrullus vulgaris*). The disease could be transmitted also to beans (*Phaseolus vulgaris*) and tobacco (*Nicotiana benthamiana*). The causal agent was detected in squirting cucumber (*Ecbalium elaterium*) and in *Malva nicaensis*, which are common weeds in Israel. The viral nature of the disease was confirmed by ELISA tests showing a specific reaction with antiserum against *Squash leaf curl virus* (SLCV), a member of the genus *Begomovirus* of the *Geminiviridae*. The coat protein gene of the virus was cloned and sequenced, showing 98% identity with the published sequence of SLCV-E. This is the first report of an epidemic caused by a 'New World geminivirus' in the Western hemisphere. *(L)*

C: ETIOLOGY AND EPIDEMIOLOGY OF DISEASES

Invited Lecture

pH Stress Effect on the Regulation of Virulence of Postharvest Pathogens

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Postharvest pathogens infect fruits throughout the period of fruit growth, but remain quiescent for weeks or months while the fruit is immature. Upon harvest and fruit ripening, quiescent infections are activated, causing extensive damage to the fruit. Quiescent infection appears to be a fungal response to adverse physiological conditions imposed temporarily by the host. The quiescence of

Colletotrichum gloeosporioides in unripe fruits and vegetables has been attributed to the presence of high concentrations of preformed antifungal compounds or inducible antifungal compounds. It was recently reported, however, that pathogens may activate processes improving their virulence during fruit ripening. This suggests the importance of the pathogen for activation of the quiescent infections. Local pH modulation of the tissue was reported as a factor enhancing the expression of virulence factors. This regulation differs between pathogens and according to the virulence factors that are activated to cause symptoms of decay. A general understanding of the fungal mechanism modulating the expression of virulence factors is of primary importance for the manipulation of resistance and can lead to creating the basis for novel crop protection strategies. *(L)*

Factors Governing the Severity of Powdery Mildew in Vineyards in Israel

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Powdery mildew caused by *Uncinula necator* is the most important disease in vineyards in Israel and elsewhere. The severity of the disease and the resultant damage vary from year to year and from location to location and are governed by numerous factors. The purpose of this work was to quantify the relative role of the factors involved in the development of the disease in Israel, and the damage it causes. The first factor involved is the source of primary inoculum, which directly influences the severity of the disease on leaves at the beginning of the season. The disease may be initiated from flag shoots (shoots that carry dormant pathogen hyphae in buds infected during the previous season) or cleistothecia (which survive within the bark of the plant or in soil). It was previously assumed that flag shoots are the only source of primary inoculum in Israel. However, in research conducted recently it was found that essential cleistothecia are formed throughout the entire vine-growing areas in Israel, and that these cleistothecia are capable of releasing ascospores in the spring. Moreover, it was found that cleistothecia play a major role in determining powdery mildew severity on leaves only when spring rainfall coincides with vine sprouting. The damage imposed by the disease to the grape bunches relates to the severity of the disease on the leaves, but it is affected by the environmental conditions as well. The fungus may develop over a wide range of temperatures and relative humidites, but it is restricted under hot and dry episodes (*i.e.*, temperature >32°C and relative humidity <25%). Powdery mildew epidemics in untreated plots recorded during the years 1996–2002 were used to demonstrate the role of spring rain in the initial development of the disease on the leaves and the influence of hot and dry episodes on the damage occurring on grape bunches. This information is being used to determine the need for spraying vineyards with fungicides. *(L)*

Shifts in Wheat Leaf Rust Population during 1993–2002: Dynamics of Virulence Gene Association

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Wheat leaf rust samples were collected and evaluated for 10 years, 1993–2002, and a total of 534 isolates were studied. During the study period several population shifts took place, as was reported last year. Population shifts were accompanied by changes in virulence on varieties in cultivation. Due to virulence frequencies we can point out shifts between 1993 and 1994, 1994 and 1995, stability from 1995 through 1999, another shift in 2000, and stability during 2000–2002. These shifts were confirmed by variability within populations (Kosman's diversity index, *KW*), distances

between populations (Kosman's distance, *KB*), and also in linkage disequilibrium between genes for virulence. *(L)*

Current Status of Corn Wilt Diseases

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Corn wilting in the northern parts of Israel and especially in the Hula Valley has been observed during the last 15–20 years. The severity of the wilting problem has increased during recent years. The objective of the current study was to identify and characterize the causes of the wilting phenomenon. Previous studies suggested that *Fusarium verticillioides* might be the primary pathogen causing corn wilting. We produced GFP-transgenic *F. verticillioides* isolates and used them to characterize the *Fusarium* – corn interaction. In addition, we carried out numerous inoculation experiments. Despite a variety of conditions and a large number of repetitions, we were unable to reproduce the symptoms by artificial inoculation with *F. verticillioides*. In the summer of 2001 we sampled a large number of plants from diseased plots. In addition to *Fusarium* we consistently recovered another fungus from the diseased plants. It was identified as *Cephalosporium maydis*, the cause of late wilt of corn. This pathogen causes severe damages in Egypt but its presence in Israel has been unknown. The full symptoms were reproduced in artificial experiments. Thus, *C. maydis* and not *F. verticillioides* is the main cause of corn wilting in Israel. *(L)*

Transmission of *Phytophthora infestans via* Seeds or Fruits of Tomato

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Tomato fruits at the green mature stage were inoculated with mixed sporangial suspension of various A_1 (sensitive to metalaxyl) and A_2 (resistant to metalaxyl) isolates of *Phytophthora infestans* and oospores of the pathogen were allowed to produce inside the fruits. Seeds were extracted from the blighted fruits carrying oospores and sown in soil or on agar media, after surface disinfection, to verify the transmission of late blight to the emerging seedlings. Only 23 (0.1%) of approximately 23,000 seedlings developed the blight. In contrast, up to 13% blighted seedlings developed when whole blighted tomato fruits carrying oospores were cut and planted at 24 days post-inoculation in potted soil. The emergence of diseased plants from cut whole blighted tomato fruits declined to 0.1% and 0% when fruits were planted at 50 and 102 days post-inoculation, respectively. Some isolates of *P. infestans* recovered from the emerging blighted seedlings were probably of oosporic origin, as they differed phenotypically (mating type, virulence, sensitivity to metalaxyl) from the parent isolates used to inoculate the fruits. Thus, some isolates were A_1 resistant to metalaxyl, some were A_2 sensitive to metalaxyl and some were A_1A_2 resistant to metalaxyl. Many isolates showed virulence race structure different from their parents. The results suggest a low probability of transmission of *P. infestans via* seeds but a high probability of transmission *via* fruits. Transmission was often accompanied by altered phenotype of the pathogen. The data also suggest that the pathogen may not survive prolonged desiccation in tomato fruits. *(L)*

Diversity of Plant Pathogen Populations. How to Measure?

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Most conclusions from studies of populations of plant pathogens are based on comparisons between populations using various indices of diversity as well as clustering procedures. Unfortunately, many studies do not explain or justify why the specific analysis was chosen for that specific data set. The methods are rather formally applied without consideration of the options available. For example, an inappropriate choice of measure of similarity between isolates can lead to misleading conclusions. Cluster analyses start with a matrix of calculated dissimilarities between isolates. Three different similarity/dissimilarity measures (Jaccard, Dice, and simple mismatch coefficients) are generally used. The structure of clusters based on these different measures will not necessarily be the same for a given data set. Therefore, the choice of which coefficient to use should be justified by the type of data obtained. Meanwhile, one can find applications of all spectra of dissimilarity measures for data of the same type. Similar potential problems arise in comparison between populations using different measures of distances (Nei, Rogers, and Kosman indices). Diversity within populations measured by various indices (Nei, Shannon, Simpson, and Kosman diversities) may result in qualitatively different relationships between the populations. Logical consistency in the application of different measures and methods in population studies is also of great importance for the validity of conclusions. *(L)*

Factors Affecting Germination of *Sphaerotheca macularis*, the Causal Agent of Strawberry Powdery Mildew

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Powdery mildew of strawberry caused by the obligate pathogenic fungus *Sphaerotheca macularis* f.sp. *fragariae,* affects leaves, flowers and fruits of this crop worldwide. The long-term aim of the research is to study the biology of the pathogen and develop management tools for the limitation of chemical sprays. Laboratory experiments were conducted to examine the effect of different environmental factors on germination and average conidial germ tube length of the pathogen. A range of temperatures between 5 and 35°C was examined; optimal germination percentage on leaves occurred between 15 and 25°C. Relative humidity (r.h.) ranging between 5% and 97% was examined. Highest germination occurred above 95% r.h. In a double factorial experiment, which combined both temperature and r.h., the previous results were confirmed: optimal germination percentage occurred at 20°C and 97% r.h. Disease severity and maximal conidia production occurred at 25°C and 85% r.h. Germination percentage and average germ tube length decreased with inoculum age (the larger the lesion, the older the inoculum). Germination rate was higher on mature and young leaves than on medium-aged leaves. The following treatments significantly reduced germination and elongation of germ tubes on detached strawberry leaves: Milsana (extract of Giant Knotweed) and Neemgard (neem oil 97%); chemical sprays with Clipman (mancozeb 62.5% + famoxadon 6.25%), Polar (polyoxin AL 50%), Signum (pyraclostrobin 6.7% + nicobifen 26.7%), Anvil 5 (hexaconazole 50 g l^{-1}); and other chemical sprays containing sulfur: Gofrithar (sulfur 825 g l^{-1}), Heliosulfre (sulfur 700 g l^{-1}) alone and in combinations: Domark combi WP (tetraconazole 100 g l^{-1}) + Gofrithar, Sequestrine + Signum, Sequestrine + Heliosulfre. *(P)*

Detection of *Xanthomonas campestris* pv. *campestris* in Crucifer Seeds by a PCR-based Method

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Black rot caused by *Xanthomonas campestris* pv. *campestris* (*Xcc*) is a serious disease of crucifers which occurs worldwide and causes economic losses in Kazakhstan and Israel. The bacterium is seedborne, and seeds are considered the most important source of primary inoculum. A joint project between Israeli and Kazak scientists was aimed at developing a specific and sensitive PCR-based method for detection of *Xcc* in cabbage and other crucifer seeds. A collection of *Xcc* strains isolated from infected crucifer plants or seeds from Israel and different regions of Kazakhstan was established. Isolations were made on four semi-selective media and the isolates were subjected to biochemical and pathogenicity tests on cabbage seedlings. Random amplified polymorphic DNA (RAPD) analysis was carried out with DNA isolated from 106 strains using 77 different arbitrary primers. Two primers were found to reveal diagnostic polymorphisms between *Xcc* and other *X. campestris* strains. An amplified fragment of 0.46 kb, obtained with one of the primers, was cloned, sequenced and provided a source for generation of two specific primers. PCR carried out with these primers amplified the same fragment only with DNA of *Xcc* and not with other strains of *X. campestris* or with non-pathogenic strains. No homology to known genes could be found in the GenBank. Recently, the whole genome of *Xcc* was sequenced and the cloned sequence was found to be homologous to a hypothetical protein from *Xcc*. The sensitivity threshold of the PCR procedure, determined by adding different concentrations of *Xcc* to an extract of cabbage seeds, was between 2 and 20 bacterial cells in the reaction mixture. The threshold level of the detection procedure was determined with artificially inoculated cabbage seeds. The minimum threshold of infested seeds that can be detected by Bio-PCR is 6–7 cfu ml^{-1} or 90–110 cfu per 1000 seeds, and by direct PCR it is 9–10 cfu per 1000 seeds. *(P)*

Seedborne Diseases in Imported and Domestic Potato Seed Tubers (1999–2002)

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Monitoring of seedborne diseases in potato seed tubers continued during 1999–2002. Brown rot caused by *Ralstonia solanacearum* was not observed in any of the imported lots. Common scab was detected in most of the imported lots; 63% of the imported lots were contaminated at moderate and high levels, whereas only 5% of the domestic seed lots were contaminated at these levels. Black scurf was detected in most of the imported lots; on average, 44%, 38% and 1% of the lots were contaminated at low, moderate and high levels, respectively, and 18% were disease-free. In contrast, most of the domestic lots were either disease-free (69%) or had a low disease incidence (13%). Only 16% and 1% of the lots were moderately or highly contaminated, respectively. Silver scurf was observed in most of the imported lots during all years of the survey, with no difference between the producing countries. On average, 10%, 48% and 35% of the lots were contaminated at low, moderate and high levels, respectively. Half of the domestic lots were disease-free, and an average of 11%, 36%

and 4% of the lots were contaminated at low, moderate and high levels, respectively. Black dot was observed in a considerable portion of the shipments from Holland during all years of the survey, and an average of 55% of the lots were infected. Shipments from France, Germany and Scotland were infected at levels of 28%, 19% and 6%, respectively. In the domestic lots, black dot was detected only in 2000, and at a low incidence (5%). Latent infections with *C. coccodes* were detected in 70% of the examined imported lots, and in only 3% of the domestic lots. Powdery scab was detected at high levels only in shipments from Scotland (72%), whereas only 6% and 3% of lots from France and Holland, respectively, were infected. The incidence of *V. dahliae*-free domestic seed tubers lots was 56–62%. In 22–35% of the lots the level of infection was <5%, and in 8–17% of the lots the level was >5%. We checked 84 lots of imported seed tubers and *V. dahliae* was detected in only 10% of the lots, and at low levels. The survey findings demonstrate transmission of seedborne pathogens, most of which can become established in the soil and eventually cause severe outbreaks of disease in potatoes grown in Israel. *(P)*

Mycelial Interaction among *Botrytis cinerea* Strains Tested by Heterokaryon Formation or Barrage Phenomenon

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A total of 21 *B. cinerea* strains originating from five host plants at ten sites throughout Israel, were tested for mycelial interaction. Complementation between auxotrophic selenate-resistant (*sul*) mutants, and antagonism (barrage) between wild-type strains served as criteria for compatibility: Complementation indicates that the parent strains are compatible, whereas barrage indicates that the strains are incompatible. *sul* mutants resistant or sensitive to chromate were paired on the *Botrytis* Minimal Medium. Wild-type growth of complementary heterokaryons was always observed between different mutant phenotypes derived from the same parent, as well as in some inter-strain pairings. Most isolates were placed into the same group based either on positive complementation reaction between their mutants or, more often, on positive reaction with mutants of 'bridging' strains, compatible with two or more isolates that were not compatible directly. Barrage between wild-type isolates, in the form of a zone of sparse mycelium with or without dark pigmentation of the agar along the line of confrontation, was observed in ~35% of pairings. Although isolates were often incompatible with each other, all of them were eventually placed into one group based on their compatibility with a common third strain ('bridging'), confirming the data of heterokaryon compatibility by *sul* mutants. *sul* mutants were pathogenic to bean and strawberry and could be used as marked strains in epidemiological studies. *(P)*

Dissemination of Pathogens in Recycled Water in Strawberry in Soilless Medium and Its Prevention

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A study was conducted of dissemination of pathogens and microorganisms in a recycled water system and ways of its prevention. Fungi and bacteria were found in the recycled water, but no *Erwinia* or *Pythium*. *Phytophthora* in recycled water was examined using three methods: fruit baiting,

isolation from plant crowns, and filtering through membranes. It was found that *Phytophthora* can be spread *via* water. Water salinity (E.C.) had the greatest effect on number of zoospores released and on their mobility. As E.C. rises above 0.6 dS m^{-1}, zoospore viability is adversely affected. The chemical composition of the solution had a great influence on viability of zoospores. In the experimental system tested, the dissemination of pathogens in water did not cause apparent disease symptoms in plants in the same season and did not affect yield. The lack of disease symptoms will be studied further. Water disinfection using slow filtration through a column containing tuff was found to be effective in the prevention of pathogen dissemination. It appears that there are potential ways to minimize the risk of dissemination of pathogens *via* recycled water. *(P)*

D: BIOLOGICAL AND CHEMICAL CONTROL

Improved Application and Reduced Pesticide Residues in Herb Crops

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A significant increase in herb crop production for export, in both field and greenhouse, has occurred in Israel. It has been accompanied by an increase in the occurrence of known soilborne and foliar pathogens as well as with some new diseases and new forms of existing diseases. In order to maintain a continuous and quality supply of fresh herbs, production has been expanded to new areas where climatic conditions are better suited for year-round production. Chemical pest management in herb crops is problematic, because herbs contain a significant fraction of essential oils in plant tissues and special glands and thus retain pesticide residues for an extended period of time. Avoiding or minimizing the use of pesticides is especially important with these crops due to the restriction on pesticide residues on the marketable produce. Efficient pesticide application technologies which will enable marketing of a pesticide-free product, are essential. Previously we showed the effectiveness of aerosol generators for pesticide application in herb crops, the advantages being effective pest control and the potential to reduce pesticide dosage. Cold foggers enabled rapid dissipation of pesticides from plant tissues; reducing pesticide concentration further enhanced pesticide dissipation from plant tissue. In the present study we developed a new backpack sprayer which produces fogging size droplets in a low-volume spray, with the assistance of fast air-jets. The new sprayer consists of a twin fluid nozzle (similar to that of cold foggers) and air blower, with special adjustments to modify air velocity and droplet size as needed. With this sprayer we achieved high coverage of both upper and under sides of leaves, and spray penetration to hidden parts of the plants. Droplet density on the target surface was very high (over 3000 cm^{-2} of tissue surface). Pest control experiments were established in greenhouses and net-houses. Pesticide application with the new sprayer resulted in effective control of whitefly, powdery mildew, and downy mildew in various herb crops, while using 20% of the spray volume and pesticide dosage. Such low dosage enabled also fast dissipation of pesticide residues from the herb tissues. The research is focused now on improving the performance of the sprayer. The application approach developed in this study will enable effective pest control with low pesticide dosage, rapid pesticide dissipation, and pesticide-free herbs on the market. *(L)*

Biological Control of Strawberry Fruit Rot by 'Shemer' - a Yeast-Based Pesticide

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The fungal pathogens *Botrytis cinerea* and *Rhizopus stolonifer* cause significant losses of marketable fruit and shorten shelf life of strawberries. Several chemical pesticides are currently used for *Botrytis* control, but none is both effective and registered for *Rhizopus* control. A biological product ('Shemer') based on the yeast *Metschnikowia fructicola* was found effective for the control of different fungal rots in a variety of fruits. The efficacy of the product in controlling fruit rot in strawberries was demonstrated over a period of several years, in the field and during postharvest storage. Weekly applications of Shemer decreased fruit rot in the field by 40–60% for *Botrytis* and by 30–60% for *Rhizopus*. Rot reduction was observed in fruit from the treated plots also during postharvest storage: 20–70% reduction in *Botrytis* and 40–70% reduction in *Rhizopus*. The efficacy of Shemer in the control of fruit rot was comparable to that of standard chemicals (Fenhexamide and cyprodinil/fludioxonil). Thus, the use of Shemer offers a biological alternative which increases the yield of marketable fruit, while reducing the input of toxic chemicals. *(L)*

The Use of Several Phytopathogenic Fungi to Control Broomrape

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Broomrapes (*Orobanche* spp.) are obligate, chlorophyll-lacking root parasites, which parasitize many dicotyledonous species and cause severe damage to vegetables and field crops worldwide. In Israel sunflower broomrape (*Orobanche cumana* Wallr.) parasitizes sunflower (*Helianthus annuus* L.), causing severe damage to the crop; and Egyptian broomrape (*O. agyptiaca*) is the main constraint in growing processing tomatoes in northern Israel. In recent years we established a collection of fungi and bacteria isolated from diseased Egyptian broomrape inflorescences. *Fusarium solani,* one of the fungi in the collection, demonstrated high potential to control Egyptian broomrape without causing any disease symptoms to the corps that were tested. In order to enhance the efficacy of the fungus, a combination of *F. solani* together with *Fusarium oxysporum* f.sp. *orthoceras,* specific pathogen of *O. cumana*, was tested. The results of the experiment conducted in the summer indicated that soil inoculation with both fungi caused severe disease symptoms to sunflower broomrape and prevented completely the appearance of broomrape inflorescences above soil level. When the same experiment was conducted in the winter, *F. solani* and *F.o.* f.sp. *orthoceras* caused 60% and 83% of sunflower broomrape mortality, respectively. Inoculation with the two fungi together resulted in 90% mortality. *F. solani* demonstrated a high level of control of Egyptian broomrape in the summer and only moderate activity in the winter. Addition of *F.o.* f.sp. *orthoceras* to the formulation reduced the control efficacy of *F. solani*, both in the winter and in the summer. Inoculation with *F.o.* f.sp. *orthoceras* did not cause any damage to Egyptian broomrape. *(L)*

Clipman – A Broad Spectrum Novel Fungicide

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Famoxadone (produced by du Pont de Nemours) is a novel fungicide of the novel chemical class Oxazolidinedione, whose principal mode of action is the inhibition of mitochondrial electron transfer at complex III. Famoxadone acts mainly as a protectant fungicide, being active mainly on the initial phase of the fungus's life cycle. It remains mostly on the surface of treated plants. Most of the deposits are strongly associated to the epicuticular waxes and are protected from wash-off by rain or sprinkle irrigation. Famoxadone controls a broad spectrum of fungal diseases belonging to the

Oomycetes, Ascomycetes and Basidiomycetes. With a view to minimizing the risk of resistance and in order to enhance its activity, Famoxadone is commercialized only in mixture with other appropriate companion fungicides, having a different mode of action. Such ready mixtures have recently been approved in France, Switzerland and Italy for fungi control in tomatoes, potatoes, cucurbits and grapes. Clipman is a ready-mixture of du Pont de Nemours which contains 6.25% Famoxadone and 62.5% mancozeb in dispersible granules formulation. In field trials carried out in Israel over the years 1998–2002 on various crops, it was found to be particularly effective against the following diseases:

***Didymella rabiei* in chickpeas:** Clipman at 1.0–1.5 kg ha^{-1} exhibits good preventive efficacy with persistence of ~17 days. Its curative effect is rather poor, and therefore Clipman is recommended only for preventive treatments, when rainfall is expected. ***Alternaria macrospora* in cotton:** Clipman was tested on the susceptible var. Pima and effectively prevented Alternaria damage. Preventive treatments with 1.0–1.5 kg ha^{-1} at intervals of 10-14 days, controlled Alternaria effectively, and maintained a low infestation rate up to harvest. Treatments were applied by sleeve boom tractor blower sprayer, at a spray volume of 200 *l* ha^{-1}. ***Foliage diseases* in tomato:** Preventive treatments of Clipman at 1.0–1.5 kg ha^{-1} protected foliage effectively from *Phytophtora infestans, Erysiphe* spp. and *Leveillula taurica.* In trials on trellising tomatoes in covered houses, Clipman was applied by blower knapsack sprayer at a spray volume of 500–1000 *l* ha^{-1}. ***Alternaria citri*:** In field trials carried out in the Sharon and Hevel Ma'on regions, Clipman was effective against the disease on Mineola foliage and fruits. It was applied at 0.15% concentration at 14-day intervals, from fruit setting until the end of July (4 or 5 treatments). Similar results were obtained in autumn applications (two treatments) for preventing late season inoculation. Clipman exhibits high selectivity to the easy peelers Mineola, Nova, Murcott and Michal varieties. Applied by tractor blower mist sprayer at a spray volume of 1500–3000 *l* ha^{-1}, Clipman was tested and successful in the recommended anti-resistance regime treatments for Alternaria contol in citrus groves. *(L)*

BioSafe for the Control of Root-Knot Nematodes in Organically Grown Basil

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The basil crop is extremely sensitive to root-knot nematodes, which may cause significant yield loss. Basil varieties resistant to nematodes are not yet available, and thermal disinfestation (mainly soil solarization) is currently the only treatment used in organic farming for the control of nematodes. BioSafe is a bionematicide, based on the bacterium *Bacillus firmus,* which is registered in Israel for the control of root-knot nematode in organically grown vegetables. Field trials conducted through 1998–2000 demonstrated the efficacy of stand-alone BioSafe applications in achieving a significant reduction in the nematode population and damage in basil. To study the incorporation of BioSafe in IPM programs, and the effect of repeated treatments on nematodes, a series of trials was conducted in a greenhouse that was treated with BioSafe following soil solarization. In the first crop cycle, BioSafe was applied immediately following solarization. Nematode control by solarization alone was insufficient. BioSafe reduced the nematode population significantly and increased the yield by 83%, when compared with solarization alone. In a less infested area, BioSafe increased the yield by 6%. Before the second crop cycle (the following spring), BioSafe was applied to previously treated plots and to nontreated plots and compared with soil solarization (before the first crop), which served as a control. During the second crop cycle the nematode population increased, leading to significant root infestation already 4 weeks following planting. The yield of plots which received a single BioSafe application (before the second cycle only) increased by 30% compared with solarization. Two consecutive BioSafe treatments increased the yield by 73%. These results demonstrate clearly the efficacy of BioSafe for nematode control and for damage reduction to organically grown basil.

They also point up the advantage of preventive BioSafe treatment in each crop cycle, including immediately after soil solarization. *(P)*

Synergistic Interaction between BABA and Mancozeb in Controlling *Phytophthora infestans* in Potato and Tomato and *Pseudoperonospora cubensis* in Cucumber

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Spray mixtures consisting of the plant activator BABA (DL-β-aminobutyric acid) and the protectant fungicide mancozeb were significantly more effective than BABA or mancozeb alone in controlling late blight (*Phytophthora infestans*) in potato and tomato and downy mildew (*Pseudoperonospora cubensis*) in cucumber. A mixture composed of 5 parts BABA and 1 part mancozeb (w/w, a.i.) exhibited a higher synergy factor than the 1:1 or the 1:5 (BABA : mancozeb) mixtures. No synergistic interaction was measured between BABA plus mancozeb in controlling sporangial or cystospore germination, nor mycelial growth of *P. infestans in vitro*. The results showed enhanced effect of mancozeb in BABA-induced plants, suggesting, therefore, that lower dosages of this fungicide may be required to control late blight or downy mildew under field conditions. *(P)*

Efficacy of Dry Mycelium of *Penicillium chrysogenum* in Controlling Wilt Diseases of Cotton in Field Miniplots

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Dry mycelium (DM) of *Penicillium chrysogenum* is a waste product of the pharmaceutical industry. Earlier studies showed that when DM or its water extract (DME) were supplemented to cotton seedlings in the greenhouse, plants became partially resistant to challenge inoculation by either *Fusarium oxysporum* f.sp. *vasinfectum* (*Fov*) or *Verticillium dahliae* (*Vd*). In the present study, DM was applied to miniplots in the field which were thereafter inoculated with *Vd*, whereas DME was applied to miniplots after being inoculated with either *Fov* or *Vd*. 'Acala' and 'Pima' cotton were grown in the *Vd*- or *Fov*-infested miniplots, respectively, and plant growth and wilt development were monitored for 100 days. Results showed that both DM and DME significantly increased, in a dose-dependent manner, plant growth and raw-cotton yield; and that DM significantly suppressed wilt development in both uninoculated and inoculated miniplots. Yield increments in DM-treated Acala reached 127% or 88%, in miniplots inoculated with a mild strain or a severe strain of *Vd*, respectively. These increments were accompanied by 66% and 47% protection against the mild and severe strains of *Vd*, respectively. Application of DME to Acala plants enhanced production of raw cotton by 55% and provided 67% protection against *Vd*. Similarly, DME applied to Pima plants increased yield by 116% while providing 64% protection against *Fov*. The data suggest that DM (powder or water extract) incorporated into *Vd*-infested or *Fov*-infested soils may protect cotton plants against their respective wilts and facilitate cotton production. The implications of these findings may be of special significance in organic farming, where usage of pesticides or mineral fertilizers is unacceptable. *(P)*

BABA Induces Resistance in Sunflower Against *Puccinia helianthi*

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BABA (DL-β-aminobutyric acid) induces resistance in sunflower plants against the rust disease caused by the fungus *Puccinia helianthi*. BABA was effective when applied as a soil drench or foliar spray. Soil drench at the rate of 25 mg BABA kg^{-1} soil, applied 3 days before inoculation, provided 90% protection against the disease. Foliar spray at a dose of 2000 $\mu g\ ml^{-1}$, applied 2 days before inoculation, controlled the disease completely. BABA was effective also when applied post-infection. Plants treated with BABA as a foliar spray of 2000 $\mu g\ ml^{-1}$, 1 or 2 days after inoculation, showed 80% and 70% protection, respectively. BABA was effective against the rust also under field conditions. Thus, plants inoculated one week after spraying with 2000 $\mu g\ ml^{-1}$ of BABA, showed 60% protection (evaluated 21 days after inoculation). Leaf disc bioassays showed that BABA and its enantiomers were effective in controlling the disease, whereas the isomer AABA (DL-α-aminobutyric acid) had much lower activity, and the isomer GABA (DL-γ-aminobutyric acid) was ineffective. Of the two (*R* and *S*) enantiomers of BABA, the *R* form was more active in inducing resistance. Thus, 100% protection was achieved with 25, 12.5 and 50 $\mu g\ ml^{-1}$ of BABA, R-BABA and S-BABA, respectively. BABA had no direct effect on uredospore germination of *P. helianthi in vivo* or *in vitro*. Microscopic examinations of sunflower leaf tissue revealed no accumulation of callose or lignin as a result of treatment with BABA and inoculation. (*P*)