

Abstracts of presentations at the 31st Congress of the Israeli Phytopathological Society

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INVITED LECTURES

Climate change and its effect on agriculture and plant diseases

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Average global temperature rose 0.6°C during the 20th Century, and is projected to rise between 1.5 and 5.4°C by the year 2100. Anthropogenic greenhouse gas (GHG) emissions are widely believed to be the dominant causal factor for these increasing temperatures, which lead to other related changes in climate, including increases in sea level, melting of polar ice caps, changes in global air and oceanic circulation patterns, and changes in the hydrological cycle. Climate change is expected to affect all aspects of life for a wide range of human, animal and plant populations around the world. Annual emission of CO₂, one of the GHG, in Israel is 11 tons per person, similar to other developed countries.

Israel has been experiencing significant climate changes since the 1970s. These include increases in the frequency of extreme daily and seasonal temperatures, increases in the frequency of heat waves and drought, reduced precipitation in the eastern and southern parts of the country, and increased flood volume. Regional-scale climate models predict that by the year 2100, there will be an additional increase of

2.2–5.1°C in average temperature (and as much as 7°C on the margins of deserts), as well as a reduction of 4–27% in rain quantity. Extreme events such as heat waves, drought and floods are predicted to be more severe and frequent. These climate changes will accelerate desertification and contribute to the disappearance of climate-sensitive species and the proliferation of species better adapted to the warmer and drier climate. In addition, rising sea level will endanger water resource quality and quantity, threatening entire ecosystems, regional water management, and agriculture.

Production of agricultural produce and its quality will be affected by climate change at the agronomic level as well as at the level of international trade. Depending on the pest, plant disease severity may increase or decrease, and its appearance period may change. Consequently, the geographical distribution of pathogens and the crops they infect, the appearance of new pathogens, and a decline in the importance of others may occur. Pathogen virulence may change directly due to increases in CO₂ concentrations. Furthermore, changes in precipitation and temperature will also affect important stages of the plant life cycle such as pollination, fertilization, fruit setting and maturation, and seed production. Yield potential of extensive agriculture may be adversely affected. Such changes will require accommodation by virtue of alterations in management practices. To prepare for climate change, there is a need to develop draught-resistant crops and improved water-use efficiency, while protecting the increasingly fragile soil system. Due to an increase in demand for irrigation water and the reduction in available water resources, there may be significant

competition for water between agriculture and the domestic and industrial sectors.

Consumers of imported agricultural produce are beginning to demand an accounting of the ‘carbon footprint’ required to produce different crops. This societal pressure, while making a positive contribution towards reducing GHG emissions, may have a deleterious effect on export. To calculate carbon footprint, GHG emissions associated with all inputs used for crop production and trade are summed. Consequently, to stay competitive on foreign markets, local agricultural practice must find ways to reduce its dependence on carbon-rich inputs such as fossil fuels and chemical fertilizers and pesticides. In addition, it will need to reduce agricultural GHG emissions, which can be achieved through changes in livestock and through soil management to increase the amount of carbon sequestered in soil. Agriculture can also be used to create biofuels either from biofuel crops or from agricultural wastes. In conclusion, the need to minimize the vulnerability of agriculture to climate change and to maximize agriculture’s role in mitigation of climate change is already upon us. [L]

The Israeli Phytopathological Society— Forty years after its inception

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The Israeli Phytopathological Society (IPS) was founded in 1969. At that time several strong centers of plant pathology were active in Israel, such as in the Agricultural Research Organization, the Faculty of Agriculture of the Hebrew University, the Advisory Service in the Ministry of Agriculture, the Plant Protection Service, Tel-Aviv and Bar Ian Universities, and in the pesticide-related companies.

Phytopathology research in Israel (Palestine) was started in 1920 by Prof. I. Reichert at the Agricultural Experiment Station (presently The Volcani Center), who established the Department of Plant Pathology. In the early 1950’s, with the settling of new areas—Lachish, Negev etc.—and the introduction of new crops and of plasticulture, new pest problems became evident. New disciplines such as nematology, virology and

postharvest, were expanded and many additional plant pathologists started their careers in this field. Improved exchange of information among the experts was needed and in 1967 Dr. J. Palti organized the first national meeting of Plant Pathology and the two following ones. After that the Israeli Phytopathological Society was responsible for organizing and leading 30 annual meetings of the society, with the 31st one in 2010.

The contributions at the meetings reflect the research trends at the various institutions. In the first meetings lectures centered mainly on diseases and their chemical control and only a small number of contributions were on mechanisms and molecular biology. In the later meetings emphasis shifted from these areas to more basic subjects such as physiology of diseases, mechanisms of resistance and molecular biology. Thus, at the second conference (1969) about 65% of the lectures dealt with diseases and their (conventional) control, and about 11% with physiology and resistance; at the 11th meeting (1988) approximately 50% of the contributions centered on diseases and their control, 24% on non-conventional control, 15% on resistance and physiology and 6% on genetic engineering. At the 30th meeting (2009) *ca* 26% of the lectures were on conventional control, 17% on diseases, 17% on molecular biology, 14% on resistance, 11% on non-conventional control and 11% on physiology. It is evident that the trend in research moved from the phenology of the diseases and their chemical control towards non-conventional control, resistance, physiology and molecular biology. [L]

Implementation of environmentally friendly pre- and postharvest methods for reducing decay in agricultural fresh produce

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Severe restrictions on the use of chemicals as postharvest treatments to control decay in stored fresh produce call for the development of alternative methods which are safe for humans and the environment. The methods developed were designed to delay senescence processes, thereby increasing tolerance to pathogen attack. Increased ethylene production during

harvest and storage of many fresh vegetables, herbs, and fruits may result in accelerated senescence and decay. The ethylene inhibitor CO₂ can nullify these processes. Sealing fresh produce in a plastic bag results in CO₂ accumulation and oxygen depletion due to respiration. These gases are controlled in modified-atmosphere packaging (MAP) by micro-perforation of the film. Pre- or postharvest treatment with gibberellin (GA₃) results in retardation of senescence and decay in species of Cruciferae (celery, parsley, dill, coriander and chervil), Liliaceae (chives and green onion) and Compositae (lettuce). Postharvest GA₃ treatment prevented the development of *Botrytis*, *Sclerotinia sclerotiorum*, *Stemphylium* and bacterial soft rot of celery during prolonged storage. GA₃ treatment reduced the susceptibility of all of these leafy species to ethylene-induced senescence. Combining GA₃ treatment with MAP had an additive effect, allowing overseas transport of fresh herbs. Implementation of MAP is limited by the accumulation of condensed water in the package, increasing pathological and physiological disorders. In a joint venture with StePac Company (Tefen, Israel), we have developed a series of MA packages (Xtend®) with relatively high permeability to water vapor. The prevention of water condensation in these packages results in reduced decay and physiological disorders. [L]

Common names of plant diseases in Israel

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Plant diseases have been known in many species since their early domestication. Very few Hebrew names of plant disorders and disease descriptions are found in the early scripts such as the Bible and later scripts but only a few of them may be related to current names of plant

diseases. These names describe essentially blight, greening, rot and mold and only partly can be associated with biotic plant diseases (as described by D. Nevo, Bar-Ilan University). New Hebrew names were given to plant diseases during the early period of modern agriculture development in Israel, as mentioned in an Agriculture Dictionary published in 1939 by M. Zagorodesky. Some of these names may not be used today, like pseudo-powdery mildew, ‘spotty’ powdery mildew (for *Peronospora*), dusty powdery mildew or ‘whitey’ (both for powdery mildews), soft powdery mildew (today’s downy mildew), Jaffa disease, and gray rot (today’s gray mold). In spite of the fact that a significant portion of the diseases in Israel do carry various names, many of the diseases were not yet assigned Hebrew names. Over the last year a committee, nominated by the Israeli Phytopathological Society, has produced lists of common Hebrew names for plant diseases caused by bacteria, fungi, fungal-like organisms, nematodes, viruses, viroids and phytoplasma that prevail in Israel. The lists include names adopted from early plant pathologists in Israel, like Prof. I. Reichert, Prof. T. Rayss, Dr. G. Minz, Dr. Z. Volcani, Dr. J. Palti, Dr. F. Nitzani, and Dr. R. Barkai-Golan, as well as new names whenever necessary. Newly contributed names will describe symptoms, color, shape and other characteristics of the diseases. The committee is also under the auspices of the Hebrew Language Academy. Lists of disease names in Arabic are also prepared by an appointed committee, consisting of Dr. H. Yunis (chair), Dr. S. Droby and Prof. A. Gera.

The help of Prof. M. Bar Yosef, Prof. D. Orion, Dr. M. Zeidan, Dr. Y. Kozodoy, and the many others who assist in producing the lists of common names of diseases is hereby acknowledged. [L]

IDENTIFICATION AND CHARACTERIZATION OF CAUSAL AGENTS OF PLANT DISEASES

Black spot disease of pomegranate

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In 2004 a new disease was observed in pomegranate (*Punica granatum*) orchards in Israel. Black spot symptoms were noted on leaves and fruits, from a single spot to more than 50% of the fruit surface; the leaves became chlorotic and abscised. Spots, which appeared at the start of the summer on all cultivars, were small (1–3 mm) and round on fruit and round to irregular (1–4 mm) on leaves. Each spot consisted of a green-yellow halo around a necrotic lesion. Damage to fruit was limited to the peel surface; the edible tissue was not damaged.

Symptomatic fruits and leaves were collected during the summer of 2007 and were used for isolation of the causal agent. Fungal colonies emerging from symptomatic tissue had morphology and conidia typical of *Alternaria* spp. Sequence analysis of the rDNA ITS region and β -tubulin gene of four different pathogenic isolates from different cultivars were found to be identical (i.e., GenBank accessions GQ240306, GQ240308), exhibiting 100% identity to *A. alternata*. PCR (specific primers, apPCR based) revealed differences between *A. alternata* isolated from pomegranates affected by ‘black spot’ and *A. alternata* isolated from ‘black rot’ disease or other fruits.

Laboratory pathogenicity was assessed by detached leaf and fruit inoculation. Spore suspensions, from different isolates, were inoculated on leaves, fruit and flowers, and placed in a moist chamber (25°C). Symptoms appeared 48–72 h later and were similar to those observed in the field. Koch’s postulates were completed *via* inoculation of greenhouse plants. [L]

Grapevine cercosporiosis in Israel

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Grapevine cercosporiosis was recorded in Israel in 1935 and 1943. Since then and until the late 1990’s it was absent in Israeli records of plant diseases. Lately, cercosporiosis leaf spots reappeared, mostly in Carignan vineyards after ripening, without apparent damage. Since 2006 the symptoms increased in severity, causing leaf blight and premature shedding.

Chemical control experiments were carried out in 2007–08 with fungicides from different functional groups, varying timing and spray intervals. The formulations ‘Flint’, ‘Signum’ and ‘Score’ were found efficient in controlling this disease. The PPIS Diagnostic Service in 1997 identified a case of grapevine leaf-spots in var. ‘Dabuki’ as *Phaeoramularia dissiliens*. Taxonomic considerations changed *Phaeoramularia* into a synonym of *Passalora* in 2001. During 2006–2008 increasing numbers of samples of Carignan from different locations were diagnosed with *Passalora dissiliens*. Cercosporiosis observed in varieties ‘Colombard’ and ‘Petite Syrah’ in 2008 was diagnosed as *Pseudocercospora vitis*. Field observations revealed that in >50% of the Carignan vineyards with *P. dissiliens*, leaf-spots were partially or completely covered by a mycoparasitic *Hansfordia* sp. (probably *pulvinata*). During 2008 *P. dissiliens*, *P. vitis* and *Hansfordia* sp. were isolated as single-spore cultures in ¼-strength PDA medium. These cultures were used for identification by DNA sequence. [L]

Bacterial fruit blotch of cucurbits: screening for disease tolerance and detection of the pathogen from infected seeds

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Bacterial fruit blotch (BFB) of cucurbits is caused by *Acidovorax citrulli*. The pathogen causes seedling blight, necrotic lesions on foliage, and fruit rot. BFB gained importance in the late 1980’s following devastating outbreaks in watermelon fields in several states of the USA. Since then, BFB has spread to many parts of the world, mainly due to the seed

transmission ability of the pathogen. As there are no resistant cultivars and there is a lack of efficient seed diagnosis procedures, the disease represents a serious threat to the cucurbits industry. We screened different melon lines for disease tolerance, using various pathogenicity assays. All tested lines were susceptible to the disease to some degree, and in most cases there was no correlation in the susceptibility of a given line among the different assays. When plants were grown to fruit maturity, less than 2% of the fruits developed characteristic symptoms; however, more than 50% of them contained infected seeds. This result emphasizes the difficulties in obtaining *A. citrulli*-free seeds. We also developed primers for specific detection of *A. citrulli*. The primers were also assessed using Immunomagnetic Separation (IMS)-PCR, which also employs specific antibodies for detection of the target pathogen. This assay was highly specific and sensitive, facilitating the detection of 1/5000 infected seed. This method represents a potential alternative to the current Grow-Out assay, which is based on seed germination and is time-, labor- and money-consuming. [L]

Winter wilting of pepper plants: Identification of the causal agent and the relation between disease appearance and environmental conditions

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Wilting of mature pepper plants, which occurs between December and February, is a well known phenomenon in the cooler regions of the Arava Valley, Israel. In the past, when methyl bromide was used routinely for soil disinfestations, this phenomenon was negligible. However, in the last few years it became significant again, following the reduction in

methyl bromide application. *Pythium* sp. is the fungus most commonly isolated from wilted plant roots. Young pepper plants were artificially inoculated with *Pythium* isolated from wilted plants and maintained at average temperatures of 20, 14, 10.5 and 8.6°C. Significant wilting was observed in plants grown at 8.6°C, with symptoms starting 2 weeks after inoculation. At 10.5°C, there was much less wilting and slower symptom development. At 14 and 20°C, inoculated plants did not exhibit wilting symptoms. The relationship between low temperatures and high disease incidence can explain the high disease incidence in the Arava during the cold winters of 1999–2000, 2004–05 and 2006–07. The pathogen was identified as a new species of *Pythium* based on its unique variable sporangium shape and internal transcribed spacer (ITS) sequence. The fungicide metalaxyl-M effectively controlled the disease in pot experiments. Pepper plants treated while maintained at 20°C or 14°C did not show any disease symptoms when transferred to 8°C, but fungicide applied when plants were already at 8°C was less effective. Temperatures of 20°C and 14°C represent the average soil temperatures in early November and early December, respectively, in cooler regions of the Arava. [L]

A leaf rust on wild emmer wheat on the Golan Heights: is it a new rust species?

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The wheat leaf rust (*Puccinia triticina*) attacks wild and cultivated wheats. Another rust occurs in Israel on *Aegilops speltoides* only, and is a forma specialis of *P. triticina*. Leaf rusts belonging to *P. recondita* are pathogens of rye and *Aegilops* spp.; in Morocco, there exists a form which attacks mainly durum wheat. Their alternate hosts belong to the Boraginaceae family. In the last decade, a new leaf rust was found annually in Israel on wild emmer wheat (*Triticum dicoccoides*) in a limited area, on the Golan Heights, characterized by its much larger telia compared with

the wheat leaf rust. The new rust is different from *P. triticina* in a number of parameters: The teliospores and the urediniospores are 30–40% larger. The basidiospores are double the size. The nuclear DNA content in the urediniospores is 35% greater. There are substantial differences in the host range of the two rusts. The shape of the uredinial substomatal vesicle of the new rust resembles wheat leaf rust. By comparing the new rust to *P. recondita*, a similarity exists in the teliospore dimensions and in the DNA content. The genetic distance, according to the DNA sequence in the ITS region, is being examined. No alternate host of the new rust was found in nature; thus it is unclear how the pathogen survives from one season to the next in the small area in which it exists. Efforts to inoculate *Thalictrum* and different species of the Boraginaceae failed. With the lack of the alternate host we are still unable to determine the form and species of the new rust. [L]

EPIDEMIOLOGY AND CONTROL OF CAUSAL AGENTS OF PLANT DISEASES

Studying the epidemiology of tarragon rust as the basis for developing a rational disease management strategy

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Puccinia dracunculina, the causal agent of tarragon rust, develops on tarragon leaves and reduces the quantity and quality of both fresh and dried products. Knowledge of the biology of the pathogen and the factors governing its development is limited and precludes development of rational means for adequate disease suppression. We studied the life cycle of the pathogen and quantified the role of environmental

parameters on disease development. The uredinial stage is the primary form involved in dispersal and infection of the tarragon crop. The pathogen may overwinter by survival of asexual urediniospores or by completing the sexual stage. In early winter, black, submerged telia may develop on leaves and stems. The teliospores survive until the next spring, when they germinate and produce basidiospores which infect the newly emerged leaves. Next, pycnia are produced and following the infection of the pycniospores, uredinial sori are formed. In controlled-environment experiments it was found that >80% of the urediniospores germinate at temperatures of 15° to 25°C when moisture lasted 10 h or longer. Information about the pathogen's life cycle and the environmental parameters that promote epidemic development were used to develop a rational strategy for disease management. The strategy is based on reduction of the amount of initial inoculum and decreasing the rate of urediniospore proliferation by application of fungicides. The strategy was tested experimentally in 2009 with appreciable success. [L]

Disinfection of cold rooms and agricultural produce by electrolysis of brine and absorption of microorganisms as a method to prevent the development of molds after harvest

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Molds which are present on fruit and vegetables or in the air of the storage room are central factors in the development of postharvest diseases and their control is a continuous challenge. The products of electrolysis of brine solutions are very effective disinfectants. An instrument developed in Israel implements this principle and delivers the electrolysis products to the air phase in a very efficient manner and high throughput. Independently of the active release of disinfectants, the instrument can absorb microorganisms which are present in the air. In a series of experiments it was proven that the technology can perform complete disinfection of the air in closed spaces and kill conidia of *Botrytis cinerea* which were placed on

solid medium. In addition it was found that the technology can prevent the development of molds on a variety of agricultural produce after harvest and during storage. It was also shown that this system can be scaled up to a commercial level. Current research is aimed at establishing and expanding the findings and in testing the various implications of the technology. [L]

Source-sink interactions affect the development of *Leveillula taurica*, the causal agent of powdery mildew of pepper

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Powdery mildew (caused by *Leveillula taurica*) is the most devastating foliar disease of pepper in Israel. Growers suppress the disease by frequent application of fungicides; in most cases the disease is adequately suppressed but unnecessary sprays are often applied. It was observed recently that the disease develops rapidly at the vegetative stage and that the rate of disease development decelerates around the time of harvesting. Based on this observation it was hypothesized that the source-sink relationships within the plants influence the rate of disease progression. In order to test this hypothesis, two experiments were carried out during 2008 and 2009 at the Be'sor experimental farm located in the western Negev. The experiments included two treatments: in the first, the sink was manipulated by removing all blossoms soon after they were formed; in the other treatment the blossoms were kept and the fruits developed. Until the time of first harvest, the rate of disease development did not differ significantly between the treatments. However, when fruits were harvested in the former treatment, the rate of disease progression had slowed down. In plants without fruits, the disease continued to develop at the same rate and a change was observed only when the vegetative growth was halted. These results suggest that high rates of disease

development are expected at times when the demand of the sink tissues (vegetative and/or reproductive) is high and *vice-versa*. Understanding the factors affecting disease development is fundamental for developing rational strategies for disease management. [L]

Chemical control of the sudden wilt disease in melons caused by *Monosporascus cannonballus*

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Melon is an important crop in the Arava Valley of southern Israel. The sudden wilt disease, caused by the fungus *Monosporascus cannonballus*, is the main threat to melon cultivation in the Arava and in other regions in southern Israel. This disease is managed by pre-plant methyl bromide fumigation. Currently, for the spring melon growing season, there is no chemical alternative to methyl bromide for pre-planting soil disinfestations. The efficacy of various fungicides, applied during the growing season, to control the sudden wilt disease was studied. First, the efficacy of various fungicides in inhibiting fungal vegetative growth was evaluated in the laboratory. Then, between 2006 and 2009, selected fungicides were evaluated under field conditions during the spring and autumn cropping seasons. Application timing, frequency, and fungicide doses were studied. Four fungicides effectively controlled the disease and prevented melon plant wilting. Three of them are strobilorins (Amistar; azoxystrobin) or strobilorins combined with other fungicides (Signum: pyraclostrobin + boscalid and Comodor: azoxystrobin + chlorothalonil). The fourth effective fungicide (Sportak: prochloraz) belongs to the imidazoles. Some fungicides have already been registered against the sudden wilt disease; others are currently undergoing the registration process. The knowledge obtained so far is already being used successfully by melon growers in the Arava Valley and other areas and has contributed significantly to a reduction in sudden wilt damages, allowing yield enhancement. [L]

Adapting grafted melon cultivation in the Arava

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The use of grafted melons for managing soilborne diseases is increasing rapidly in Israel. In the Arava valley of southeastern Israel, growers prefer to trellis melon plants in the spring season because trellising allows lengthening of the growing season, resulting in higher yields. Melon plants grafted onto commercial rootstocks and grown trellised often collapsed, even in disease-free soils, when they were loaded with fruits and exposed to high temperatures. In order to find better rootstocks enabling growing trellised melons, we employed a two-step selection process. First, experimental rootstocks were evaluated for resistance to *Monosporascus cannonballus* in the autumn season and, second, the resistant rootstocks were evaluated in the subsequent spring season for adaptation to trellised cultivation. Nineteen of the experimental interspecific F₁ *Cucurbita* rootstocks out of the 122 evaluated were resistant to *M. cannonballus* and five of the 19 resistant rootstocks did not collapse during the spring season, that is, they evinced adaptation to trellised cultivation. The implications of these results for breeding rootstocks and adapting their usage for specific demands and needs of growers in certain areas and conditions were discussed. [L]

Biochar induces systemic resistance to disease in plants

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Biochar (charcoal) is a product of biomass pyrolysis (thermal decomposition in the absence of oxygen). The carbon in biochar is permanently sequestered and hence biochar's use as a soil amendment has been proposed for reducing greenhouse gas emissions. Biochar in soil improves soil tilth, nutrient retention and crop productivity. Based on the chemical, microbial and physical improvements reported for biochar-amended soil, we tested whether biochar also had a positive impact on plant diseases. Biochar amendments to soil and potting mix at 1–5% suppressed the foliar fungal pathogens *Botrytis cinerea* (gray mold) and *Leveillula taurica* (powdery mildew) in pepper and tomato. Since the site of infection (leaves) was spatially separate from that of the amendment (roots), it is highly logical that the biochar induced systemic resistance. Resistance was observed in leaves of different ages for as long as 105 days following inoculation of pepper by *L. taurica*. Disease suppression ranged from 33% to 92% for gray mold and 72% to 92% for powdery mildew in both crops. This result was not due to trivial differences in nutrition, since plants were constantly fertigated and leaf nutrients' concentrations did not differ between treatments and controls. Additionally, plant growth and productivity were both enhanced by biochar amendments. Biochar was found to have a strong influence on culturable microbial populations of *Pseudomonas*, *Bacillus* and *Trichoderma* spp., all of which are known to potentiate plant systemic resistance. Induced resistance may also have been elicited by biochar-borne chemicals, including resorcinol and various fatty acids. Studies designed to clarify the important systemic resistance metabolic pathways and its elicitors are underway. [L]

Secondary spread of *Clavibacter michiganensis* subsp. *michiganensis*, the causal agent of bacterial canker of tomatoes, in time and space

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Bacterial canker and wilt disease caused by *Clavibacter michiganensis* subsp. *michiganensis* (*Cmm*) is an important disease of tomato, causing substantial economic losses in Israel. Disease development in time and space was recorded in commercial tomato-growing facilities in 2009. The disease spread from initially infected plants only within rows and not among or even adjacent rows. The resultant foci vary in size from a few to more than 30 plants. Accordingly, it was hypothesized that the disease spread spatially during the routine agrotechnical procedures employed by the farm workers. This hypothesis was examined in two experiments which showed that indeed, the workers were responsible for the spread of the diseases within rows, but the distance of dispersal was limited to two to four plants. Observations of tomato plants showed that under some climatic conditions guttation droplets were formed on the leaves. The involvement of these droplets in the spatial spread of the pathogen was studied in two greenhouse experiments. Tomato plants, inoculated by dipping their roots in *Cmm* suspension, were planted at the beginning of a non-inoculated row of plants. After one week, guttation was induced and the plants were touched gently starting from the inoculated plants. A week later, typical disease symptoms were observed on the non-inoculated plants reaching the end of the rows (four and 15 plants, respectively, in the two experiments). After 45 days, most of these plants were severely infected. These results may suggest that guttation plays a major role in the spatial spread of *Cmm* in commercial production. [L]

Are Ascochyta blight pathogens from wild peas a source of primary inoculum for domesticated pea crops?

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Wild pea (*Pisum fulvum*) is common from the northern Negev to the Galilee and the Golan regions

of Israel. Domesticated pea (*P. sativum*) is grown for processing in the north, close to wild pea populations. The main disease affecting wild and domesticated pea is Ascochyta blight. The primary inoculum sources of the pathogen are sexual and asexual spores that develop on pea straw, and soilborne chlamydospores. There is no information on whether *Mycosphaerella pinodes* (the primary Ascochyta pathogen) from wild peas can serve as a source of primary inoculum for domesticated peas. Accordingly, we quantified the ecological requirements of isolates sampled from wild and domesticated peas and studied their aggressiveness against domesticated pea. Controlled-environment experiments revealed that there were no significant differences in the response to temperature and in moisture requirements between isolates sampled from wild and domesticated pea, nor were aggressiveness differences between the isolates against domesticated pea. The variation in the studied parameters between isolates was high, but not related to the original hosts. From the lack of host specialization we conclude that spore exchange between wild and domesticated pea is possible, and that it is likely to occur in early season when optimum conditions for disease development prevail. The high level of aggressiveness of isolates from wild pea populations may threaten pea crops no less than other inoculum sources. These findings are important for understanding the causes of epidemics in fields and for planning effective strategies for disease management. [L]

Late wilt of maize: characterization of the pathogenesis and identifying means of control

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Late wilt of maize, caused by the fungus *Harpophora maydis*, is one of the most important fungal diseases in Egypt. It has been reported also from India and Hungary. Common disease symptoms have been documented for 20 years in the Upper Galilee (northern Israel). Recently, its prevalence has increased. The

pathogen is currently controlled by the use of reduced-sensitivity maize varieties, but these varieties are threatened by a pathogenic variant of the fungus known in Egypt. In earlier work we modified a molecular method for use as a diagnostic tool to evaluate the disease progress in field-infected plants. The assay identifies the pathogen 50 days after seeding, before the emergence of disease symptoms, in both susceptible and resistant host plants. Surprisingly, the pathogen also spreads in non-symptomatic resistant plants, and therefore their seeds may become vectors for disease spread. Two plant hormones, auxin (IAA) and cytokinin (kinetin), and several fungicides suppressed *H. maydis* *in vitro*. One of these fungicides, azoxystrobin, at the rate of 1125 g ha⁻¹, inhibited the development of disease symptoms in a field experiment, and brought about a 100% increase in ear yield, in comparison with untreated plots. [P]

Influence of microclimate on pathogen-biocontrol agent interactions in the tomato powdery mildew (*Oidium neolycopersici*) pathosystem

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Oidium neolycopersici is the causal agent of tomato powdery mildew, causing powdery white lesions on the leaf surface, petioles and calyx. Severe infections lead to a marked reduction in fruit size and quality and to leaf wilt. Several applications of chemical fungicides are currently used for its control, and therefore alternative methods, such as biocontrol, are required. Beneficial microorganisms can provide sufficient control, but they are sensitive to environmental conditions. A better understanding of the influence of environmental factors on pathogen-biocontrol agent interaction can help in improving their efficacy. The effect of microclimate on the development and survival of two powdery mildew antagonists was evaluated. When applied on detached leaves and incubated for 7–14 days at various

temperatures, relative humidities, and disease levels, the bacterium (*Pseudomonas* sp.) survival was poorer at high temperatures and low r.h. compared with the yeast (*Rhodotorula* sp.). The microorganisms survived well at 10–15°C and high r.h. Tomato plants were grown in a net house with climate regimes of high and low r.h. and two disease levels were induced. Plants were sprayed weekly with the two microorganisms. The bacterium survived better under high r.h. as compared with lower r.h. and on leaves with powdery mildew as compared with symptomless leaves. The yeast was less affected by microclimate conditions, and survived well for 14 days even with a single application. Similar results were obtained in the grape powdery mildew system. It was concluded that the yeast survives better than the bacterium under various microclimate conditions. [P]

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Influence of climate change on plant-pathogen-BCAs interaction in high humidity-promoted diseases on tomato

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Climate change refers to changes in mean and/or variability of climate properties. Plant pathogens have a range of environmental conditions which allows them to survive and cause disease. Environmental changes might cause alterations in distribution, survival and plant-pathogen interactions, while these changes can increase / decrease epidemic events. The efficiency of beneficial organisms is affected by environmental conditions. Late blight (*Phytophthora infestans*) is a polycyclic, rapidly developing and destructive disease in tomato and potato plants. A critical factor for disease epidemic occurrence is 6–8 h of wetness, which enables penetration into the plant tissue. Gray mold (*Botrytis cinerea*) infects many crops including tomato and proliferates at high

humidity. A few hours of wetness are usually a prerequisite for its infection. Tomato plants inoculated with *P. infestans* with wetness duration of 4, 6, 8, 12 and 24 h showed a decrease in disease severity under 8 and 12 h wetness as compared with 24 h. With *B. cinerea*, infection severity was different between 4 and 10 h wetness duration. Spraying the plants with two yeasts and three bacteria isolates and *Trichoderma*, and inoculation with *P. infestans* under wetness durations of 8 and 24 h, revealed that two of the BCAs reduced disease significantly as compared with control treatment at the 8 h wetness duration, whereas at 24 h wetness duration no disease control was observed. It seems that environmental conditions affect both the disease intensity and the efficacy of the introduced microorganisms. [P]

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Monitoring for resistance to polyoxin in *Botrytis cinerea*, a foliar pathogen of basil

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Sweet basil is highly susceptible to gray mold (*Botrytis cinerea*), which also develops at postharvest. The chitin synthase inhibitor polyoxin AL is commonly used on basil in Israel. Resistance to polyoxin has never been studied in Israel, although it is known from abroad. A total of 260 *B. cinerea* isolates collected from diseased sweet basil plants and from the air in seven sweet basil greenhouses situated at five sites in the Jordan Valley, were tested for sensitivity to polyoxin AL using a mycelial growth test. First, 50 isolates were tested on a range of polyoxin AL concentrations, and concentrations causing 50% inhibition of mycelial growth (EC_{50}) and a discriminatory concentration which allows distinguishing between sensitive and resistant isolates (DD) were defined. The isolates did not show any significant growth retardation on Czapek Dox agar amended with 0.001, 0.01 and 0.1 $\mu\text{g ml}^{-1}$ of polyoxin AL; a concentration of 1 $\mu\text{g ml}^{-1}$ caused 10% to 90% growth retardation, and this concentration was regarded as DD. A

concentration of 10 $\mu\text{g ml}^{-1}$ caused 80% to 100% growth retardation, and no isolates grew on media amended with 100 $\mu\text{g ml}^{-1}$. Isolates showing less than 55% relative mycelial growth (RG) on DD were regarded as sensitive (mean $EC_{50}=0.8 \mu\text{g ml}^{-1}$); isolates showing >55% RG on DD were regarded as less sensitive (mean $EC_{50}=5.3 \mu\text{g ml}^{-1}$). An additional 210 isolates were tested on DD. Greenhouses differed in frequency of isolates less sensitive to polyoxin AL. Minimal frequency of 23% was found in a greenhouse in Gilgal and maximal frequency of approximately 80% in greenhouses in Havat Eden and Neot HaKikar. Greenhouses in Mehola and Rehov had a frequency of approximately 60% isolates less sensitive to polyoxin AL. [P]

The influence of fertilizer element concentration on sweet basil morbidity of *Sclerotinia sclerotiorum* and *Botrytis cinerea*

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Sweet basil (*Ocimum basilicum*) is infected during winter by gray mold (*Botrytis cinerea*). *B. cinerea* causes infection on stems during growth and branch rot at postharvest. *Sclerotinia sclerotiorum* infects the basil stem base and other canopy parts. Within the framework of the 'Hosen Basil' project we studied the effect of fertilization on plant susceptibility. In one field experiment (at Eden Station) two commercial fertilizers were used: one with high ammonium concentration (30%) and no added calcium, and the other with 10% ammonium concentration enriched with calcium; the frequency of gray mold was higher in the high ammonium treatment. The type of fertilizer did not affect *S. sclerotiorum* plant mortality. Nitrogen nutrition and $\text{Ca}(\text{NO}_3)_2$ spray were tested at the Tzvi Station. Treatments included three ammonium concentrations – 6%, 27% and 41% and also 6%

ammonium with the addition of calcium. Gray mold was reduced to a certain extent by the low ammonium concentration. Intensity of conidiation was reduced by the low ammonium + calcium treatment. Calcium spray had no significant effect on the severity and frequency of gray mold. A significantly lower yield was obtained in the low ammonium treatment. Experiments with specific nutrients were conducted in perlite pots using nitrogen (0.4–14.1 mM), phosphorus (0.01–0.62 mM) and potassium (0.25–5.33 mM). A high concentration of nitrogen increased the intensity of *S. sclerotiorum* on basil stem bases and reduced the intensity of gray mold in harvested branches. A high concentration of phosphorus reduced *S. sclerotiorum* and increased gray mold severity. A high concentration of potassium reduced the severity of both diseases. Similar effects were obtained at the end of a storage and shipment simulation period. [P]

Developing a spatial decision support system for plot allocation with potatoes to minimize the occurrence of *Verticillium* wilt

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The soilborne and seedborne fungus *Verticillium dahliae* is the primary causal agent of potato early dying (PED), resulting in yield reduction. The incidence of PED in potato fields is affected by numerous factors such as previous infestation, crop and variety susceptibility, source of reproduction material, soil characteristics, growing season and chemical treatments. The objective of this study was to reduce the risk of *V. dahliae* infestation in potato by developing a knowledge-based prediction model of infestation and a spatial decision support system (SDSS) for crop allocation according to predicted disease intensity. The development included the formation, by experts, of an agreed set of factors

including treatments and abiotic and biotic causes affecting the manifestation of *V. dahliae*. These factors were ranked and weighted using pairwise comparison and aggregated into a ranking matrix to calculate the weighted linear combination of the SDSS. The model was used in time series to simulate and illustrate the effects of crop rotation practices and cultivar sensitivity on multiyear disease incidence. Plot allocation pattern was examined by constructing 3-year spatial scenarios. The inter-rater agreement for simulations compared to 47 commercial and study plots dataset was high (weighted kappa=0.9). The temporal scenarios indicated that the disease severity in plots with a tolerant cultivar would remain low over 10 years without fungicide application with 2-, 3- and 4-year rotations. However, in monoculture, fumigation would be necessary every 5 years. The spatial scenarios indicated that during the 3 years the estimated infestation was low for all the plots. [P]

Bio-Film—a spreader-sticker additive to improve the efficacy of fungicide treatments

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Bio-Film is a spreader-sticker wetting agent produced by Kalo Laboratories Inc., USA. Bio-Film improves the dispersion, wetting and sticking of spray droplets on foliage. The objectives of this trial were to quantify the contribution of Bio-Film to fungicide application, to examine its ability to retain deposits on the plant foliage and to enhance fungicide activity. As a model, the following factors were set: potato and tomato foliage, fungus, Manzidan fungicide (mancozeb 75% WG); and the adjuvants: the sticker 'Dabak' or the spreader-sticker 'Bio-Film'. The trial was carried out in Eshel Hanassi, on greenhouse tomato (cv. 449) and on potato (cv. 'Draga'). Treatments were applied to foliage by knapsack sprayer. When the fungicide was adsorbed, the plants were irrigated by sprinklers, and leaf samples from the plots were taken to the lab for inoculation with *Phytophthora infestans* spore solution. The inoculated leaves were

incubated at room temperature in trays covered with polyethylene sheet to maintain humidity. Seven days later, leaves were uncovered and leaflets infected by late blight were evaluated. The implemented method of ongoing inoculation in a humid atmosphere allows the reliable testing of the efficacy of different treated products. With this method, the inoculation rate of untreated potato foliage was 100%, and the inoculation rate of the untreated tomato foliage was 68%. The results indicate that mancozeb deposits to foliage can be retained if wetting agents and stickers are added to the tank mix. Extending the effect of mancozeb on potato and tomato foliage resulted in better late blight prevention. Manzidan + 0.1% Bio-Film was more efficient than Manzidan + Dabak 0.5% in preventing late blight infection on the two tested crops. This result can be attributed to both the wetting agent and spreader-sticker features of Bio-Film, whereas Dabak is only a sticker. Bio-Film at a lower rate (0.05%) acted mainly as a wetting agent, and was not effective enough as a sticker. Similar results were obtained in two additional trials, carried out on potato at two sites, Or Haner and Eshel Hanassi. [P]

Carial MZ—a new fungicide for late blight control in potato and tomato

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Carial MZ is a new fungicide, developed and produced by Syngenta, Switzerland. Carial MZ in WG formulation is composed of two active ingredients:

Mandipropamid, at the rate of 5%, is the first representative of a new chemical family—Mandelamides, which is very active in preventing and inhibiting zoospores germination and mycelia growth, of foliar Oomycete diseases. Mandipropamid has been tested in Israel, and was found to be highly effective against metalaxyl-resistant strains of *Phytophthora infestans*. No strains resistant to the product have been detected so far. The excellent rainfastness and translaminar activity can explain the long lasting and effective consistent disease control under field conditions.

Mancozeb, at the rate of 60%, is a broad-spectrum protectant fungicide of the dithiocarbamate family.

In trials carried out during 2007–2009, Carial MZ at rates of 2.0–2.5 kg ha⁻¹ was found effective against late blight in potato and tomato. This product is applicable either as a preventive or translaminar curative tool, when applied soon after first symptoms are observed, in repeated consecutive treatments at 7–10-day intervals.

The tank mixture of Carial MZ at 2.0 kg ha⁻¹ with Curzate (Cymoxanil 60%) at 0.6 kg ha⁻¹, resulted in rapid and effective late blight control in potato and tomato, and quite successful in preventing the fungus from spreading to healthy plants or new growth. The contribution of the tank-mixed treatments to the qualitative and quantitative yield parameters was positively correlated to the efficacy level of disease control. Carial MZ, as well as the tank mixture with Curzate at the recommended rates and at double rates, were well tolerated by potato and tomato grown in the open field and under cover.

PLANT – PARASITE INTERACTIONS

The influence of cuticle content and composition on plant defense responses

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During their life cycle, plants are exposed to a variety of biotic and abiotic stresses. To overcome those stresses, plants have developed a range of constitutive and inducible defense mechanisms. The cuticle protects plants from mechanical injury and pathogens. In addition to its role as a physical barrier against pathogens, there is growing evidence that cuticle components, such as the cutin monomer (CM), activate pathogenesis signals in the invading pathogens, and trigger the plant's defense responses as well. The *SHN1* gene encodes the transcription factor AP2/EREBP, which has been suggested as being involved in lipid biosynthesis. *Arabidopsis thaliana*

plants that overexpress *SHNI* (*SHNI^{OXp}*) are greener, shinier and have a tenfold thicker cuticle than the wild type (WT). Following infection with *Botrytis cinerea*, we found a significant difference in the yellowness and intensity of *SHNI^{OXp}* leaves compared with the WT; in addition, we found accelerated plant cell death and reactive oxygen species (ROS) accumulation in *SHNI^{OXp}* vs WT plants. Microarray analysis of *B. cinerea*-inoculated plants revealed that genes associated with senescence, oxidation stress and defense responses are up-regulated in *SHNI^{OXp}* to a much greater extent than in WT plants. CM extracted from *SHNI^{OXp}* and WT plants showed no influence on *B. cinerea* spore germination or development *in vitro*. However, when WT leaves were inoculated with *B. cinerea* spores that were supplemented with *SHNI^{OXp}* CM, symptom inhibition was observed. Based on our results, we hypothesize that the change in CM content and composition in *SHNI^{OXp}* plants triggers excessively strong defense responses resulting in plant sensitivity and death. However, adding a moderate amount of CM from *SHNI^{OXp}* leaves triggers a plant defense response that results in plant resistance. [L]

Postharvest dark skin spots in potato tubers are caused by over-suberization response to *Rhizoctonia solani*

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Israeli farmers export 250,000 tons of potato tubers (*Solanum tuberosum* L.) a year, while about 40,000 tons are harvested before skin set. In recent years there has been an increase in the prevalence of dark skin spots on potato tubers (cv. 'Nicola'), harvested in

the early stage and packed in large bags containing peat to retain moisture. The irregular necrotic spots form in commercial packages during storage and overseas transport. Characterization of the conditions required for symptom development, indicates that target temperature (8°C) is obtained only 25 days after harvest. Isolates from typical symptoms were identified as *Rhizoctonia solani* and by artificial inoculation performed at 13–14°C, Koch postulates were completed. The isolate was assigned to anastomosis group 3 (AG-3) of *R. solani*, using phylogenetic analysis of the internal transcribed spacer sequences (ITS1 and ITS2) of ribosomal DNA (rDNA) genes. Artificial inoculation of wounded tubers with *R. solani* mycelia resulted in an over-suberization response and development of a thick periderm layer. The expression of genes *StKCS6* and *CYP86A33* increased 6.4- and 3.4-fold, respectively, 24 h after inoculation, and this was followed by a 1.5-fold increase in *POP_A* gene expression 48 h after inoculation. We suggest that postharvest dark spots disease is a tuber over-suberization response to *R. solani* inoculation before skin set. [L]

The role of ambient pH modulation in patulin biosynthesis and pathogenesis of *Penicillium expansum*

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Penicillium expansum, the causal agent of the blue mold rot in apples and other deciduous fruits, is a major concern for human health because of the production of a toxic secondary metabolite (SM), patulin (PAT), during pathogenesis and the subsequent consumption of this mycotoxin in contaminated fruit products. Notably, PAT has been shown to be produced in large quantities over a small range of pH, viz. 3.2–4.0. Our preliminary results indicate that the necrotrophic process of *P. expansum* is accompanied by host tissue acidification. *P. expansum* causes a pH decrease from values ranging from 3.95 to 4.31 in healthy mesocarp, to values ranging from 3.64 to 3.88

in decaying tissue. Recent findings demonstrated that gluconic acid (GA) is the primary organic acid secreted by *P. expansum* during tissue acidification, and that pH values of 3.5–4.0 are optimal for endopolygalacturonase expression, a pathogenicity factor in *P. expansum*. Two genes encoding for glucose oxidase (GOX) were identified in *P. expansum* genome, *gox1* and *gox2*. To address the role of each *gox* gene in *P. expansum* development and pathogenicity, mutant strains carrying the invert repeat transgene (IRT) of *gox1* and *gox2* were generated. Results demonstrated that reduction of an individual *gox* gene has an effect on host colonization, gluconic acid formation and PAT accumulation. These results led us to study the association between gluconic acid and PAT formation during fungal growth. It was found that GA and PAT production are alkaline-induced processes which are strongly induced at pH 6–7 as compared with pH 4–5. Our results indicate that the highest induction of GA and PAT accumulation is actually at pH 7.0 compared with pH 3.0. The association between both GA and PAT formation was further illustrated by glucose and sucrose induction compared with complex carbon sources that inhibit both processes. In addition, induction of both processes by controlled atmosphere conditions *in vitro* and *in vivo* of stored apple fruits at high oxygen concentration compared with low oxygen (6%) further emphasizes the link between GA and PAT. These new findings indicate that GA and PAT share similar pathway cascades that control GA and PAT production. Understanding the mechanism governing their production will aid in developing strategies to prevent PAT contamination and maintaining the safety of deciduous fruits. [L]

Acidification of fruit environment by gluconic acid during decay development caused by *Phomopsis mangiferae*

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Colonization of mango and other deciduous and tropical fruits by *Phomopsis mangiferae* was affected

by local acidification of the host tissue. The fungus acidified the host tissue in mango and grape from pH 5.1 and 4.1, respectively, to 3.8 and 2.5, respectively. Analysis of the acidification process in colonized fruits showed that gluconic acid was the main organic acid that accumulated at the infection court. In culture conditions the relative induction of transcripts of *pmGOX1*, encoding for glucose oxidase (GOX), was 8–12 times greater at pH 7.0 and 8.0, than at pH 4.0. In infected fruits the detection of high levels of transcripts of *pmGOX1*, accumulation of gluconic acid and H₂O₂ in the decayed tissue, suggested that glucose oxidase contributed to the acidification of the tissue. At the same time, transcripts encoding the endopolygalacturonase gene *pmPG1* were enhanced under acidic culture conditions, suggesting the importance of the acidification mechanism as a factor for enhanced pathogenicity of *Phomopsis*. Our results indicate that ambient pH is a regulatory cue for processes linked to pathogenicity of postharvest pathogens, and that specific genes are expressed as a result of the environmental pH created by the pathogen. [L]

Inhibition of gall formation by *Pantoea agglomerans* in gypsophila and beet plants through the application of quorum sensing signal molecule

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Pantoea agglomerans has evolved from an epiphytic bacterium into *hrp*-dependent tumorigenic pathogens: *Pantoea agglomerans* pv. *gypsophilae* (*Pag*) and *Pantoea agglomerans* pv. *betae* (*Pab*) that cause galls on gypsophila and beet, respectively. Pathogenicity of both pathogens was shown to be affected by molecular interactions among the type III system, phytohormones and the quorum-sensing (QS) regulatory system. The major QS signal was identified as N-butanoyl-L-homoserine lactone (C₄-HSL) and the genes encoding for C₄-HSL biosynthesis (*pagI*) and

its transcriptional regulator (*pagR*) were characterized in both pathovars. Disruption of *pagR*, *pagI* or both genes reduced gall size in gypsophila cuttings by 50% to 55% when plants were inoculated with 10^6 cells ml^{-1} . Moreover, significant reductions in gall size (70% to 90%) were achieved by overexpression of *pagI* or addition of C₄-HSL. The aim of the present study was to investigate the possible application of C₄-HSL for controlling gall formation in gypsophila and beet plants. Results indicated that addition of a concentration higher than 50 μM C₄-HSL inhibited the formation of galls in gypsophila cuttings and beet cubes taken from infected plants. Because the QS signal was more effective in preventing gall development at low bacterial concentration (10^6 cells ml^{-1}), we tested also the effect of copper hydroxide (Kocide at 0.15%) on reduction of bacterial population. Results showed that addition of the QS signal combined with Kocide caused greater reductions in gall formation than their single application. [L]

Involvement of the NADPH oxidase complex in pathogenicity of *Colletotrichum acutatum* on strawberry

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The fungus *Colletotrichum acutatum* J.H. Simmonds is the major causal agent of strawberry anthracnose in Israel. Previously it was shown that reactive oxygen species (ROS) and the ROS scavenger machinery are associated with pathogenicity and development of the fungus. Since ROS, produced by NADPH oxidase (NOX), is a major factor involved in pathogenicity, the aim of this study was to assess the effect of two different NOX genes (*nox1*, *nox2*) on disease and development of the pathogen by reverse genetics. Full genomic sequences of *nox1* and *nox2* were determined and used as a basis for preparation of disruption cassettes for gene replacement experiments. Mutant

strains Ca1.5 and Ca2.3 were generated for *nox1* and *nox2*, respectively. Ca1.5 grew more rapidly than the wild-type isolate, whereas less conidia were produced by this mutant. No differences were evident between strain Ca2.3 and the wild-type isolate regarding growth rate and conidia production. Mutant strain Ca1.5 caused reduced symptom development on detached strawberry leaves, whereas strain Ca2.3 was completely nonpathogenic. Future research will concentrate on additional developmental characteristics such as formation of appressoria, colonization and infection processes for the Δnox1 and Δnox2 mutant strains. [L]

Global transcriptome analysis of *Botrytis cinerea* at low temperature

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The fungus *Botrytis cinerea* is responsible for the 'gray mold' disease in more than 200 plant species. It attacks mainly pectin-rich soft fruits, such as table grapes, tomato, and strawberry, but also vegetative tissues in humid greenhouses. *B. cinerea* is a major postharvest pathogen, mainly due to its exceptional ability to develop at low temperature, which is the major postharvest tool to maintain the quality of fresh produce. The aim of this research is to understand the whole complex of physiological and genetic factors enabling *B. cinerea* to develop under low temperature. The major hypothesis is that genes which are up-regulated at low temperature may be involved in the mechanisms of cold tolerance. A first series of genes which are unregulated at low temperature was previously identified by cDNA subtraction and verified by quantitative PCR. Another group of genes that was selected by a search in the literature did not appear to be up-regulated at low temperature. The genome sequencing of two strains of the fungus facilitated global transcriptome analysis by microarray with 21,000 elements. Gene expression was compared for growth at 2.5°C and 22°C after 1, 4, 10 and 24 h. Many of the genes which changed significantly ($P \leq 0.01$) do not have any assigned function. Of the genes with assigned functions many

are not known to be involved in cold response. Diverse cellular functions are activated with notable increase in transporters as well as genes involved in translation and transcription and other functions. These results reinforce the hypothesis that cold response demands drastic changes in various cell activities. [L]

One-step construction of *Agrobacterium* recombination-ready plasmids (OSCAR)—an efficient and robust tool for gene deletion construction in fungi, and its validation in *Verticillium dahliae*

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Many fungi including *Verticillium dahliae* are amenable to *Agrobacterium tumefaciens*-mediated transformation (ATMT). OSCAR was developed by us for rapid generation of gene deletion constructs for fungal ATMT. We designed a marker vector that contains a hygromycin B resistance gene driven by the *Aspergillus nidulans trpC* promoter, flanked by the *attP1r* and *attP4* recombination sites. We also modified a binary vector (pPZP-RCSII, a gift from V. Citovsky, SUNY) to contain the *ccdB* gene flanked by *attP2r* and *attP3* recombination sites. Generally, two PCR amplifications generate 5' and 3' gene flank products with ends possessing the *attB* recombination sites *attB2r*, *attB1r* and *attB4* and *attB3*, respectively. A single BP clonase reaction containing both the above PCR products and vectors results in a deletion plasmid containing the resistance cassette flanked by the upstream and downstream PCR products between the T-DNA borders. *V. dahliae* is a fungal pathogen and the primary causal agent of Verticillium wilt on a wide variety of crop species. The recent genome sequencing of *V. dahliae* (<http://www.broad.mit.edu/>) provides an important tool for functional analysis and we tested OSCAR in this species. Deletion constructs for six genes, predicted to affect *V. dahliae*

pathogenicity, were made to evaluate the efficiency of the OSCAR method. In all cases correct deletion constructs were obtained at high frequency. One OSCAR construct (VDAG_02161) was tested in fungal ATMT and resulted in a complete gene deletion. In summary, OSCAR methodology combines PCR and Gateway technology to enable rapid and robustly generated precise deletion constructs for ATMT for homologous gene replacement. [L]

A secreted lipase contributes to the virulence of *Xanthomonas campestris* pv. *vesicatoria*

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Xanthomonas campestris pv. *vesicatoria* (*Xcv*) is a Gram-negative bacterium which causes bacterial spot disease in tomato and pepper. The disease is distributed worldwide and has high economic significance, especially in areas characterized by high temperatures and relative humidity. In addition, *Xcv* is a model bacterium in research of plant–pathogen interactions. A recombinase-based *in vivo* expression technology (RIVET) approach was developed to identify *Xcv* genes specifically induced during its interaction with tomato. One of the identified genes was *Xcv0536*, which putatively encodes a secreted lipase (*lipA*). Several studies showed lipases have a role in virulence of some fungal pathogens. In contrast, little is known about their role in bacterial pathogenicity. We characterized the expression of *Xcv0536*, and examined its possible contribution to virulence. By homologous recombination we generated a mutant strain impaired in this gene. The mutant had reduced ability to grow in a medium containing olive oil as the sole carbon source, relative to the wild type. In addition, the culture supernatant (from minimal media) of the mutant showed a significantly reduced lipolytic activity relative to that of the wild type. Inoculation experiments with tomato revealed that disease symptoms induced by the *lipA* mutant progressed more slowly and were clearly less severe

than those induced by the wild type. Also, a consistent trend of a slight reduction of growth *in planta* of the *lipA* mutant relative to the wild type was observed. Altogether, these findings indicate that Xcv0536 indeed encodes an extracellular lipase and contributes to *Xcv* virulence. [P]

RESISTANCE AND DEFENSE MECHANISMS

Utilization of comparative genomic tools for genetic mapping of *PmG3M*, a novel powdery mildew resistance gene derived from wild emmer wheat, *Triticum dicoccoides*

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Wild emmer wheat (*Triticum turgidum* ssp. *dicoccoides* (Körn.) Thell.), the tetraploid progenitor of cultivated wheat, is a valuable source for disease resistance. The *PmG3M* gene derived from wild emmer wheat is a novel major dominant gene conferring resistance to powdery mildew, located on chromosome arm 6BL. Developing EST-based markers through the exploitation of wheat-rice-*Brachypodium* colinearity is a promising tool in wheat genomic research. In the current study we have used this strategy to develop new EST-based markers based on homology of wheat ESTs with rice and/or *Brachypodium* genes. A genetic map of chromosome 6BL was constructed using seven SSR, seven EST-based markers and the *PmG3M* gene. The EST-based markers served as anchors to the physical map of rice chromosome 2 and *Brachypodium* Bd3. The markers flanking *PmG3M* gene, Xuhw229 (proximal) and Xedml49 (distal) are delimiting the colinear rice and *Brachypodium* sequences to

640 Kb (seven BAC clones) and 3.8 Mb, respectively. *PmG3M* gene, which confers an impressive resistance to all 59 isolates tested so far, is an excellent example for exploiting the genetic resources of wild emmer wheat. Moreover, the high-density genetic mapping of the *PmG3M* region and the high colinearity with rice and *Brachypodium* genomes provide a solid base towards positional cloning of this gene. [L]

Additive effects of dsRNAs expressed in *Arabidopsis* on root knot nematode development

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Ectopically expressed dsRNAs have recently been shown to suppress parasitic success of *Meloidogyne* spp. in plants. We have targeted two genes from the root-knot nematode *Meloidogyne incognita*: a dual oxidase gene implicated in the tyrosine cross-linking of the developing cuticle, and a subunit of signal peptidase—a protein complex required for the processing of secreted proteins. While these genes are involved in different aspects of nematode development, the phenotypic consequences of RNAi in *Arabidopsis* were similar, with ≥50% reduction in nematode numbers in the roots and retardation of development to the egg-producing saccate females. Expression of processed dsRNA was observed, but no evidence of detectable levels of small interfering RNAs (siRNAs) was found in the transgenic plants. We show for the first time that combining expression of these dsRNAs by crossing appropriate *Arabidopsis thaliana* lines resulted in an additive effect that further reduced nematode numbers and developmental

capacity. Combining RNAi target genes has the potential to enhance the efficacy of RNAi and may allow control of different nematode species or genera in the crop of interest. [L]

Interaction of *Fusarium oxysporum* f. sp. *melonis* race 1.2 with susceptible and resistant melon plants: genetic and physiological characterization

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An Israeli melon breeding line, BIZ, that is resistant to all four races of *Fusarium oxysporum* f. sp. *melonis* (*Fom*), has been characterized regarding its response to *Fom* race 1.2. This trait is considered problematic to select for, due to the quantitative nature of resistance to this race and the need for reliable, well characterized resistance sources. We showed that resistance of BIZ is expressed as a recessive trait, controlled by two major recessive genes, when severe artificial inoculation is applied, but in the field it appeared as a dominant trait. A mapping population that segregates for *Fom*1.2 resistance was used to construct a linkage map with ~90 traits and markers, and quantitative trait locus (QTL) analysis was initiated. A major recessive QTL for *Fom*1.2 resistance was located in linkage group 2. The infection process of a *Fom*1.2 strain that expresses the GFP reporter protein was monitored *in vivo* in the infected roots and stems. This analysis indicated the time points and sites in which fungal progression differed between resistant and susceptible genotypes. The expression pattern of a few defense genes was compared between the resistant and susceptible genotypes, using real-time PCR. Transcript levels of phenylalanine ammonia lyase (PAL), chitinase (CHI) and hydroperoxide

lyase (HPL) were induced to a greater extent in BIZ, compared with susceptible ‘Ein Dor’ melon seedlings. A constitutive 2–4-fold difference in the basal levels of all three transcripts was also apparent. Both the constitutive and inducible defense responses could contribute to reduced vascular colonization of the resistant genotype. [L]

Evaluation of systemic resistance mechanisms induced by hypovirulent *Rhizoctonia* spp. in *Arabidopsis* plants

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Various isolates of *Rhizoctonia* spp. infect a wide range of plants, resulting in crop losses worldwide. On the other hand, many isolates are nonpathogenic and some even protect plants against pathogenic *Rhizoctonia* and other plant pathogens. In the past, the mode of protection was not supported by competition for carbon or nitrogen sources, antibiosis, or parasitism, but was based rather on changes in the cell wall components and increased production of resistance-associated hydrolytic enzymes in the protected plants. The present study focused on the defense responses: SAR—systemic acquired resistance, and ISR—Induced systemic resistance, induced in plants by colonization with protective hypovirulent *Rhizoctonia* isolates, especially the protection against pathogenic *Rhizoctonia*. Using *Arabidopsis* mutants in the SAR pathway showed that SAR is involved in resistance triggered by the tested hypovirulent isolates. Reduced protection of plants with constitutive expression of SAR suggested that ISR is also involved. This assumption was supported by reduced protection of plants detected in a gene which mediates SAR and ISR by inhibiting ISR. Quantitative real-time PCR performed on colonized plants showed induced gene expression *via* the SAR and ISR pathways as well as phytoalexins production in the protected plants. [L]

Tomato plants transformed with the Inhibitor of Virus Replication (IVR) gene are partially resistant to *Botrytis cinerea*

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Tomato plants transformed with a cDNA clone encoding the ‘inhibitor of virus replication’ (IVR) gene were partially resistant to *Botrytis cinerea*, the causal agent of gray mold. On leaves, the transgenic plants’ rot development was significantly reduced compared with its development on the non-transgenic controls. Resistance was much reduced when plants were kept at 32°C before inoculation with *B. cinerea*, compared with that in plants kept at 17–22°C prior to inoculation. Resistance was correlated with the presence of IVR transcripts, as detected by RT-PCR. This is one of the few cases in which a gene associated with resistance to a virus, seems to be involved also in resistance to a fungal disease. [L]

Evaluation of quantitative resistance to *Fusarium* wilt of melon

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Resistance of melons to *Fusarium* wilt, caused by *Fusarium oxysporum* f. sp. *melonis*, is based on resistant cultivars bearing the dominant genes *Fom-1* and *Fom-2*. In several melon breeding lines, quanti-

tative, or field resistance exists, and is probably governed by several quantitative trait loci (QTLs). Our research is aimed at describing phenotypic and molecular aspects of such partial resistance. We first carried out artificial inoculation of seedlings from several melon cultivars or breeding lines and recorded disease symptoms and percentage of dead plants. It was found that in some breeding lines a constant level of quantitative resistance exists, with 30–70% mortality, with the remaining plants surviving despite some disease symptoms. In the same experiments, susceptible lines suffered 100% mortality whereas fully resistant ones remained healthy. In some genotypes, quantitative resistance appeared race-specific whereas in others it was effective against multiple races. Longer term resistance was tested in a greenhouse using three quantitatively resistant lines inoculated at the 2–4-leaf stage. Stem length and plant weight were measured after 4 weeks. Quantitatively resistant lines showed heterogeneity in the response among individual plants from genetically uniform lines. Average length and weight after inoculation, relative to non-inoculated plants, were shown to be reliable parameters for evaluating partial resistance and were consistent in two consecutive experiments. Values were intermediate, compared with fully resistant and susceptible genotypes, respectively. Chitinase and phenylalanine ammonia lyase (PAL) expression (measured using Real-Time PCR) were induced at one dpi in seedlings of a fully resistant genotype, but not in a susceptible one, and not in the quantitatively resistant line. In one experiment, higher levels of chitinase and PAL expression were maintained 3 dpi in a quantitatively resistant line, compared with the resistant one. More experiments are needed to confirm this result. [L]

Use of composite melon plants to characterize a *Fusarium* resistance gene-promoter

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Composite plants are obtained by *Agrobacterium rhizogenes*-mediated transformation of seedlings whose root system has been removed. The seedlings develop an adventitious, chimeric root system where some of the roots are transformed with the gene under study. Composite plants do not require tissue culture and allow the study of a large number of transformants in a short time. To study the function of *Fusarium* resistance genes, we set up a composite plant system for melons and obtained good transformation efficiency. In preliminary experiments aiming to exclude a non-specific effect of the transformation protocol, composite plants from genetically resistant or susceptible backgrounds showed no change in their resistance response to *Fusarium oxysporum* f. sp. *melonis* race 1 (FOM1) in a hydroponic culture system. In melon, a dominant gene, *Fom-2*, conferring resistance to FOM1 was identified by genetic linkage analysis, but its function has not been functionally validated. We cloned a 1450 bp-fragment upstream the *Fom-2* ORF, and wondered whether it acts as a functional promoter. For this purpose, we fused the putative promoter with the GUS reporter gene and produced composite melon plants that express the construct. It was shown that the cloned sequence promoted the expression of the reporter gene. Specific expression was observed in tissues adjacent to the vascular system, where the defense response is likely to occur, but no further induction by the fungus was seen. The function of the entire *Fom-2* gene in conferring FOM1 resistance will be addressed in future studies. [P]

INVASIVE PESTS – PANEL DISCUSSION

Invasive pests: risks and ways to cope

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Pests, including pathogens, arthropods and weeds, which do not exist in the country, may invade it and cause heavy economic, social and environmental damage. The pests may reach the invaded country *via* various means, including trade of agricultural products. The major and most dangerous vehicle for these pests is infected propagation material, but other means, *e.g.* wind, machines and wild animals, may also introduce new pests. We can cope with the invading pests by a variety of means, including inspection, quarantine measures, immediate eradication of the pest at the early stages of invasion, and eradication and control of the pest after its establishment. Detection and diagnostic tools, which are highly sensitive and reliable, are needed for this purpose. Legislation is another important tool for coping with the threat of pest invasion. Bioterror, which aims on purpose to introduce new pests, should also be considered and thoroughly discussed.