

## MEETING



ABSTRACTS OF PRESENTATIONS AT  
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*Opening Lecture*

**Broomrape (*Orobanche* spp.) as an Angiospermous Pathogen: Biological Aspects  
and Their Impact on Potential Control**

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Broomrape is a higher plant, but nevertheless exhibits many similarities with fungal pathogens, being a holoparasite that connects to host root tissues and interacts physiologically directly with the host. In this respect broomrape differs significantly from other weeds that compete only in the environment of agricultural crops and do not interfere with any exploitation of crop photosynthates. Another similarity with fungal pathogens is that it is not detected in the field during much of its development, and is discovered only after much of its damage has already been done. Contrary to other pathogens, broomrape resembles its hosts in physiological terms, and therefore selective control is tricky. Our understanding that the broomrape serves as a strong sink may help in controlling it, by using systemic herbicides that inhibit important metabolic pathways in the pathogen. Glyphosate and ALS inhibitors are thus effective in broomrape control, but are not selective to many crops. The use of transgenic crops with target-site resistance to these herbicides enables effective control of broomrape. However, these herbicides will sooner or later meet with herbicide-resistant broomrape races. Another way to reduce broomrape damage is the use of broomrape resistant crops. Sadly there are so far only very few sources of resistance, and again, new broomrape races are found that successfully attack resistant crops, thanks to the highly polymorphic nature of the parasite. The arsenal of *Orobanche* control methods is limited. Strict sanitation and new control methods therefore are needed for effective control of the parasite, both depending on suitable diagnostic capabilities. In order to cope with broomrapes that show different host capabilities we have developed in recent years tools for the molecular identification of all broomrape species that are known in the Mediterranean region. We have also developed the means to identify a single soilborne seed using SCAR (Sequence Characterized Amplified Repeats). Additional means to challenge the broomrape problem should include the development of artificial resistance and manipulation of key metabolic and developmental processes in the parasite. For this purpose we have carefully studied different stages in the life cycle

of the parasite. For example, we found that the haustorium releases some wall-degrading enzymes during the penetration process. Penetration may be stopped once we provide the host with the ability to react to the presence of these enzymes, either by a hypersensitive reaction or by the release of a toxin. Another example is based on the understanding of seed conditioning, *i.e.*, the developmental stage that is needed in order to render the seeds sensitive to germination stimulants, and that takes place when broomrape seeds are exposed to water. We were able to prevent parasitism by inhibition of gibberellin synthesis during seed conditioning. This was used as a model that proves that interrupting metabolic activities during seed conditioning may enable control of the parasite by preventing its germination. Other developmental stages, such as germination stimulation, are also possible targets, and the mechanisms involved may be manipulated for specific control of the parasite.

#### A: FIELD AND VEGETABLE CROP DISEASES

### Population Dynamics of *Phytophthora infestans* in Israel: A 20-Year Perspective

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During the years 1983–2001, 820 isolates of *Phytophthora infestans* collected from potato crops in Israel were analyzed for mating type and sensitivity to metalaxyl and 324 isolates were analyzed for race structure. The A<sub>2</sub> mating type, first recorded in 1983, fully dominated the pathogen population from 1983 until 1991 (9 years). It was thereafter replaced by the A<sub>1</sub> mating type, which strongly dominated the population during 1993–2001 (except in 1999). Metalaxyl-resistant isolates were first recorded in 1982. During 1983–1991 the majority of the isolates were resistant. Isolates with intermediate sensitivity (I) to this fungicide were first observed in 1993, when both A<sub>1</sub> and A<sub>2</sub> mating types occurred in the population. The proportion of I isolates increased gradually, reaching 39–41% in 1997–98 and then declined to approximately 15–22% in 1999–2001. Pathogenicity to eight potato differential cultivars was determined for 80 potato isolates collected in 1983–91, to 11 potato differentials in 173 isolates collected in 1993–98 and in 71 isolates collected in 1999–2000. The first population was composed of five races with race 1,3,4,7,8,10 predominating (76%); the second population was composed of 19 races with race 1,3,4,7,8,10,11 predominating (63%); and the third population exhibited 42 (34 new) races with no single predominating race. RG-57 DNA fingerprinting and allozymes loci assays of 23 isolates revealed that isolates collected during 1984–86 belonged to the PO-57 lineage whereas those collected during 1997–99 belonged to the RFO-39 lineage. Among isolates collected during 1993–95, two unreported DNA fingerprinting patterns were found. Severe late blight epidemics occurred in tomato crops during 1998–2000. Of 35 tomato isolates, 28 were A<sub>1</sub> and only 7 were A<sub>2</sub>. Ninety-four percent of the tomato isolates were sensitive to metalaxyl. Almost every isolate had a different race structure on the 11 potato differentials. When inoculated onto three tomato differential cultivars, tomato isolates showed a more enhanced virulence than potato isolates. The data suggest that the Israeli population of *P. infestans* has passed three major genetic changes during the past 19 years: in 1983, 1993 and 1999. The most recent change included host specialization to tomato. (L)

### Boron Induces Systemic Acquired Resistance Against *Phytophthora infestans* and *Alternaria solani* in Potatoes and Tomatoes

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

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Boron is a microelement required for normal growth and development of plants. In light of the gradual increase in use of recycled water (which contains high concentrations of boron) for irrigation, a study was conducted of the effects of boron on plant pathogens. Boron did not induce any phytotoxic effects in the plants in any of the experiments. Effects of boron on *Phytophthora infestans*, the causal agent of late blight, was determined in four field experiments; and its effects on *Alternaria solani*, the causal agent of early blight, were determined in two field experiments. Foliar application of boron reduced late blight severity by 41% and increased tuber yield by 7.5 t ha<sup>-1</sup>, as compared with boron-non-treated plots; for early blight, disease severity was suppressed by 33% and yield was increased by 1.7 t ha<sup>-1</sup>. The fungicidal effect of boron on *P. infestans* was compared with that of the fungicide chlorothalonil in laboratory experiments. The ED<sub>50</sub> of boron on sporangia germination was 6400 times less effective than that of chlorothalonil. Thus, boron has a weak direct effect on *P. infestans*. In order to determine if boron affects the response of the host plants to the pathogens, the lower leaves of potted tomato plants were treated with boron and the upper leaves were inoculated with *P. infestans* sporangia. The late blight lesions which developed in plants where the lower leaves were treated with boron were significantly smaller than those which developed in non-treated plants. Our results suggest that boron induces systemic acquired resistance against *P. infestans* and *A. solani*. (L)

#### ***Verticillium dahliae* on Potato: Disease Control by Fungicides Applied to Seed Tubers and Furrow, and PCR Diagnostics**

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Potato early dying syndrome caused primarily by *Verticillium dahliae* may result in up to 50% yield loss in susceptible cultivars. Israeli growers currently rely on soil fumigation for primary disease management, as the pathogen produces microsclerotia that infest the soil. The present study tested a new approach to control of *Verticillium* wilt on potato, by treating seed tubers alone, or in combination with in-furrow applications. Field experiments were conducted during 1998 to 2001 in southern Israel. In spring 1998 (at Besor), disease incidence was reduced to zero by seed tuber treatment with 400 g ton<sup>-1</sup> Octave (prochloraz manganese). The incidence of infected dry stems was similarly affected (from 50% to 28%). Yields obtained from treated seed tubers were significantly higher than from non-treated tubers (control). In spring 2000 (at Gilat), disease incidence was significantly reduced by seed tuber treatment with 400 g ton<sup>-1</sup> Octave alone or combined with in-furrow treatment with Octave (2 kg ha<sup>-1</sup>), and by seed tuber treatment with 400 g ton<sup>-1</sup> Mirage (prochloraz zinc). In autumn 2000–01 (at Gilat), all tested treatments [seed tuber treatments with Octave (400 g ton<sup>-1</sup>), Amistar (azoxystrobin; 100 ml ton<sup>-1</sup>), and Sportak (prochloraz; 400 ml ton<sup>-1</sup>), in-furrow treatment with Octave (2 kg ha<sup>-1</sup>), and combined seed and in-furrow treatments with Octave, Mirage (2 kg ha<sup>-1</sup>) and Amistar (1 l ha<sup>-1</sup>) significantly reduced *V. dahliae* colonization levels in the stems. However, yields were significantly higher only in the Octave and Mirage treatments. A rapid method was developed to monitor *V. dahliae* in seed tubers. In ITS analysis with a specific primer Vd<sub>F-R</sub>, a clear 300 bp band was detected. Optimization of DNA extraction from tuber and plant tissues was carried out, and the same band was detected in infected or inoculated plants and tubers. (L)

### **Integrated Control of Powdery Mildew (*Leveillula taurica*) in Sweet Pepper**

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Powdery mildew (*Leveillula taurica*) severely attacks sweet pepper throughout the whole year. Yellowish spots appear on the upper side of the leaves and the powdery mildew pathogen appears on the underneath or sometimes on the upper side of the leaves. Infected leaves are shed and yield quantity and quality are decreased. The interactions of *L. taurica* and the control agents, sulfur (helio-sulfur), and the biological control agents *Trichoderma harzianum* T39 (Trichodex) and *Ampelomyces quisqualis* (AQ10), mixed or unmixed with the mineral oils JMS Stylet Oil and AddQ, respectively, were studied. The effect of the control agents on important life cycle stages (viability of conidia, conidia germination, leaf colonization by hyphae and disease severity on leaves) of *L. taurica* was studied under controlled conditions and in a greenhouse. In field experiments the aforementioned and more treatments were examined under different micro-climate conditions. The biological agents were more effective in disease control at 15° and 25° than at 20°C. Moreover, the colonization of the leaf by the mycelium of the pathogen was reduced. Conidia viability was drastically reduced only by helio-sulfur. The mineral oils and Trichodex reduced conidia germination. Disease control under commercial greenhouse conditions by the different agents and by a treatment of alternation of the control agents was more efficient under warm temperatures (> 25°C by day – achieved by curtain closure, and 19°C by night) than at lower temperatures (20°C by day and 13°C by night). A treatment of alternation of 'friendly' treatments (helio-sulfur, Trichodex, Neemgard and AQ10) in the warm greenhouse climate, resulted in effective disease control, similar to the standard alternation and mix of chemical agents. The former treatment in colder greenhouses was inferior to the chemical treatment. A combination of high temperature in the greenhouse with alternative sprays of the 'friendly' agents, effectively controls pepper powdery mildew. (*L*)

### ***Phytophthora infestans* Produces Oospores in Fruits and Seeds of Tomato**

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Tomato fruits at the mature green stage co-inoculated with A1+A2 sporangia of *Phytophthora infestans*, the late blight causal fungus, showed abundant oospores in the vascular tissues, pericarp, columella and placenta. Their density may reach as many as 160 oospores per 1 mm<sup>2</sup> of fruit tissue, similar to or even higher than their number in leaves of potato or tomato floating on water, and much higher than potato leaves in the field. Abundant oospores were formed at 12–17°C and a few at 23°C. Green fruits enabled faster blight development and oospore production than mature red fruits. Isolates of *P. infestans* which originated from tomato were more fertile than those which originated from potato. Oospores were also formed on the surface of infected fruits when kept in a moisture-saturated atmosphere. Occasionally, oospores were enclosed between epidermal hairs of the seed coat. In a few seeds, oospores were detected inside the embryo. The data suggest that blighted tomato fruits may carry a large number of oospores, thus making them a threatening source of blight inoculum. Such fruits may also release airborne oospore inoculum which may introduce recombinant genotypes within a growing season. Although *P. infestans* is known to be seedborne in tomato, this

is the first report on the occurrence of oospores in tomato seeds. Current experiments are aimed at elucidating whether such tomato fruits or seeds produce blighted seedlings in the following season. (L)

### **Host–Parasite Relations in a Population of Wild Emmer in Eastern Galilee**

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In the years 1983 to 1993 populations of wild emmer growing on pasture land near Kibbutz Ammiad in eastern Galilee were subjected to a study of fungal diseases. In their natural environment in a rainfall and temperature range suitable for the pathogens studied, the plants were practically free of disease. They showed very low incidence of infection by the following foliage fungi: leaf rust, stem rust, stripe rust, powdery mildew, Septoria leaf blotch and Septoria glume blotch and by the take-all root disease. However, over a 9-year period, during which self progenies of some 4000 plants (400 each year) from different habitats on the site were grown in 20 field- or nethouse-nurseries, heavy spontaneous infection with the three common wheat rusts and powdery mildew and moderate infection with Septoria leaf blotch were observed. Artificial inoculation of a field planting with Septoria glume blotch also resulted in heavy infection. Seedlings of some of the sets of progenies derived from disease-free plants were inoculated in controlled environments with different isolates of seven fungal species. In these tests, almost all accessions showed susceptibility to all or some of the isolates, including races of the three rust diseases and powdery mildew, which had been isolated from the wild Ammiad populations. The strategies that suppress expression of disease in susceptible wild-growing plants in their natural habitat are not well understood, and the need to study them is stressed. (L)

### **Stripe Rust in Wheat in Israel during 1994 – 2001**

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After several years of absence, wheat stripe rust reappeared in Israel in 1994. It was found in the Hula Valley on cv. 'Dariel'. Stripe rust has since appeared on wheat every year up to 2001. The disease has caused severe damage on wheat fields, necessitating chemical treatment as an immediate solution and variety change, since cv. Dariel grown on most wheat acreage has proved very susceptible to the new cultures that appeared in 1994.

During 1994–97, 119 cultures were studied on cultivars that possess the 1B/1R translocation from rye: 'Veery', 'Thatcher-Lr26' and 'Kavkaz'; on G-25 *Triticum dicoccoides* with gene Yr15; *Triticum spelta album* with gene Yr5; and on 15 Israeli cultivars. During 1998–2001, 191 cultures were studied additionally on a European set of differentials bearing 16 varieties. Five cultures were added from 1997. The extent of virulence of each differential variety and virulence patterns were studied. Variability within the rust population and distances between years were calculated using the Kosman indices. Due to the reaction of Israeli varieties and cultivars with the IB/IR translocation to cultures sampled before 1994 and to all cultures from 1994 to 2001, it was found that cultures from 1994 on were virulent on Yr9. A study using the European set of differentials has shown 46 virulence patterns. High virulence was found on the genes Yr2, Yr6 and Yr7 and no virulence was found on the genes Yr1, Yr3, Yr4, Yr5, Yr10 and Yr15. Variability estimates for the populations

of 1998–2001 have shown lower variability for stripe rust compared to leaf rust populations of the same years. Maximal variability was found for the 2000 population. Distances between populations of this rust in the given years also were lower than distances between leaf rust populations; thus, it is concluded that more stability exists in the stripe rust population. (L)

### Populations of Wheat Leaf Rust during 1993–2001

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Wheat leaf rust isolates were collected in fields and in wild emmer in natural habitats during the years 1993–2001: 464 isolates were isolated and increased on the susceptible cultivar ‘Lebanon’. The isolates were tested on the worldwide-used differential set of 17 nearly isogenic lines of cv. ‘Thatcher’ with a single resistance gene. The extent of virulence on each gene was assessed annually. Variability was measured within annual populations and distances between populations with the Kosman indices that take into account both differences of virulence incidence on single genes and the shift in pathotypes. Shifts were found in level of virulence on single genes during the studied years. The most interesting changes were the increase of virulence on Lr26 and Lr2a to 50% in 1999 and their decline in 2000 and 2001. On the other hand, virulence on Lr15 and Lr17 had a low frequency until 1999 and markedly increased in 2000 and 2001. Genes Lr9 and Lr24 were resistant to all studied cultures and genes Lr10, Lr18 and Lr23 were susceptible to most cultures. Maximal variability in genetic variation was found in 1994 and genetic distances were largest between 2000, 2001 and other years. The genetic distances between populations could be explained due to changes that took place in frequency of Lr26, Lr2a, Lr15 and Lr17. (L)

### Systemic Fungicides Enhance Oospore Formation of *Phytophthora infestans* in Host Tissues

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The effect of systemic fungicides and SAR (systemic acquired resistance) plant activators on sexual sporulation of *Phytophthora infestans*, the causal agent of potato and tomato late blight, was studied in the field, greenhouse and under laboratory conditions. Field and greenhouse studies demonstrated that preventive administration of systemic fungicides and SAR plant activators (metalaxyl, Canon, cymoxamyl, dimethomorph and  $\beta$ -aminobutyric acid [BABA]) to potato plants enhanced the ability of leaf or tuber tissues to support sexual reproduction of *P. infestans*. The enhancing effect in potato tuber tissue was restricted to fungicides possessing the ability of phloem translocation. Laboratory studies demonstrated that post-infectious administration of fungicides (metalaxyl, Canon, cymoxamyl, dimethomorph) or SAR plant activators (BABA, Bion, salicylic acid) to petri dishes containing potato tuber or tomato leaf discs, inoculated with A1+A2 sporangial mixture, enhanced oospore production. The enhancing effect was dependent on the concentration of the chemical and its time of application. No enhancement of sexual sporulation was observed when protective fungicides, herbicides or systemic fungicides, which possess no activity against *P. infestans*, were used. Metalaxyl enhanced oospore formation only when one or both isolates, comprising the A1+A2 sporangial mixture, were sensitive to metalaxyl, but not when both isolates were resistant. The data suggest that systemic fungicides or plant SAR activators which possess

activity against late blight may enhance sexual sporulation of *P. infestans*. The epidemiological significance of this finding was discussed. (P)

### **Vegetative Compatibility Groups in *Colletotrichum coccodes*, the Causal Agent of Black Dot on Potato**

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Black dot of potato, caused by *Colletotrichum coccodes*, is a disease of increasing economic importance. It is characterized by the development of small black sclerotia on senescent and dead plant tissues, on decaying roots and stems, on stolons and on daughter tubers, reducing tuber quality and yield. *C. coccodes*, which is a soil-, seed- and airborne pathogen, is classified to Deuteromycotina, where vegetative compatibility may be a means of genetic exchange among isolates. However, the degree of genetic diversity and pathogenic differentiation among *C. coccodes* isolates is unknown. Using Nit mutants, vegetative compatibility groups (VCG) diversity for *C. coccodes* was characterized for 110 isolates originating from Israel, the Netherlands and France. *nit1* and NitM mutant classes were recovered at frequencies of 38.5% and 7.2%, respectively, and 12 isolates were selected as tester isolates. Using these testers, we defined four multi-member VCG groups at 7.3%, 35.5%, 20.0% and 10.0% frequency in this sample. Thirty isolates (27.3% of all tested isolates) could not be assigned to any of the major groups, and showed only self-compatibility. The frequency of recovery of Nit mutant sectors was highest in isolates from VCG4, with 50.9% and 13.6% recovery for *nit1* and NitM, respectively. However, no differences were detected in the frequency of mutant classes among the three countries of origin. In pathogenicity tests, isolates from VCG3 were the most aggressive to potato, as expressed by high stem colonization levels and sclerotia density on root and crown. These results suggest that there is significant VCG diversity in this species and that this diversity may be correlated with pathogenic characteristics/specialization. (P)

### **Collection and Evaluation of *Aegilops* spp. of the Sitopsis Section in Israel**

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Sitopsis section of the genus *Aegilops* includes five diploid species growing in Israel. This section possesses the S genome from which the B genome of cultivated wheats evolved. These species possess a very important genepool for future wheat breeding. The Lieberman genebank at the Institute for Cereal Crops Improvement has one of the world's largest collections of this group, which was collected and evaluated for disease resistance during the last 20 years. A thorough study was undertaken in the Institute dealing with leaf rusts of *Aegilops* and wheats, to clarify the interrelation between different rust types, their host and alternate host ranges. The species in the genebank are: (i) *Ae. speltoides*, which grows over a vast area of the fertile crescent and in Israel, in primary habitats of Mediterranean batha, on terra-rosa, and on deep clay soils of secondary habitats in the coastal plain. *Ae. speltoides* is highly resistant to numerous rust diseases – leaf rust, stripe and stem rust as well as powdery mildew and septoria. A rust disease specially adapted to *Ae. speltoides* was discovered in native populations, studied at the Institute; it is closely related to leaf rust of wheat. (ii) *Ae. longissima*, which grows on lime soils in desert areas and sandy red loam in the coastal plain. Resistance to rust diseases, powdery mildew and septoria was found in this species. (iii) *Ae. searsii*, a species with limited area of dispersal, grows on primary habitats – marginal batha and secondary

habitats in Mediterranean margins on terra-rosa and basalt. It shows little disease resistance. (iv) *Ae. sharonensis*, an endemic species of Israel's coastal plain and southern Lebanon, grows on sandy soils only. It is revealing resistance to leaf and stripe rust of wheat. (v) *Ae. bicornis* inhabits a limited growing area in southern Israel, Egypt and Libya. It grows on loess sand soils and desert sands. Resistance to nematodes has been found in this species. Powdery mildew resistance was recently found on it at the Institute for Cereal Crops Improvement. Additional collections, evaluation and increase of new *Aegilops* accessions are undertaken annually. (P)

#### B: BIOLOGICAL CONTROL

##### **Combining Biocontrol Agents with Several Different Mechanisms to Improve Biological Control**

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Two biocontrol agents, a yeast (*Pichia guilliermondii*) and a bacterium (*Bacillus mycooides*), were tested separately and together for suppression of *Botrytis cinerea* on strawberry leaves and plants. We reported recently that the combination of these two biocontrol agents reduced variability and improved efficacy of *B. cinerea* suppression. Scanning electron microscopy revealed significant inhibition of *B. cinerea* conidial germination in the presence of *P. guilliermondii*, whereas *B. mycooides* caused breakage and destruction of conidia. When both biocontrol agents were applied in a mixture, conidial inhibition and destruction were more severe. The modes of action of each of the biocontrol agents were elucidated and the relative quantitative contribution of each mechanism to suppression of *B. cinerea* was estimated using multiple regression with dummy variables. The improvement in control efficacy achieved by introducing one (or more) mechanism(s) at a time was calculated. *P. guilliermondii* competed with *B. cinerea* for glucose, sucrose, adenine, histidine and folic acid. Viability of the yeast cells played a crucial role in suppression of *B. cinerea* and these cells secreted an inhibitory compound(s) that had an acropetal effect and was not volatile. *B. mycooides* did not compete for any of the sugars, amino acids or vitamins examined. Viable cells and the compound(s) secreted by them contributed similarly to *B. cinerea* suppression. The bacteria secreted volatile and non-volatile inhibitory compounds and activated the defense systems of the host. The non-volatile compound(s) had both acropetal and basipetal effects. A mixture of *P. guilliermondii* and *B. mycooides* resulted in additive activity, as compared to their application separately. The combined activity was due to the sum total of biocontrol mechanisms of both agents. This work provides a theoretical explanation for our previous findings of reduced variability in disease control with a mixture of *P. guilliermondii* and *B. mycooides*. (L)

##### **Isolation of Nonpathogenic Mutants of *Fusarium oxysporum* f.sp. *melonis* for Potential Biocontrol of Fusarium Wilt in Cucurbits**

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Two nonpathogenic mutant isolates – 4/4 and 15/15 – of *Fusarium oxysporum* f.sp. *melonis* (race 1,2) were isolated by a continuous dip-inoculation technique following UV-mutagenesis of the virulent wild-type FOM1.2 isolate. No disease symptoms or detrimental effects were observed following inoculation of muskmelon seedlings by isolate 4/4. In contrast, isolate 5/15 caused



mortality of susceptible cultivars although to a lesser extent than the wild-type isolate. Isolate 4/4 colonized a variety of muskmelon and watermelon cultivars. In muskmelon cv. 'En Dor', seedlings were dipped in a conidial suspension of isolate 4/4 and planted in medium amended with the mutant to achieve 100% colonization of roots and between 30% and 70% of the lower stem tissues, 7 days after planting. Similar percent colonization of watermelon seedlings by isolate 4/4 was recorded. In cross-protection experiments with muskmelon cultivars, a significant reduction in seedling mortality was observed between 4/4-colonized FOM1.2-challenged plants compared with that of wild-type-challenged plants alone. Similarly, isolate 4/4 was able to reduce significantly mortality of watermelon seedlings caused by *F.o.* f.sp. *niveum* race 2. This novel approach of 'creating' nonpathogenic mutants for biocontrol in *Fusarium* and other fungal pathogens from virulent wild-type strains may be beneficial for control, since the mutant strains, lacking only in pathogenicity, may compete more efficiently than other biocontrol organisms against the pathogen of origin. (L)

### **'Serenade' – A New Biofungicide**

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'Serenade' is a new biological fungicide with broad-spectrum activity, manufactured by Agra-Quest, USA. This biofungicide in WG formulation is a mixture of a microbial strain of *Bacillus subtilis* (QST-713) and its toxins. In its natural environment, *B. subtilis* releases cell contents during growth, which inhibits development of competitor organisms, including plants pathogens. Serenade can prevent plant pathogen spores germination, disrupt the germ tubes and mycelial growth, and inhibit attachment of the plant pathogen to the leaf. This biofungicide is suitable for preventative treatments and is active on fungi from the sub-divisions Imperfecti, Oomycetes, Ascomycetes and bacterial pathogens, including some fungi and bacterial strains which are resistant to chemicals. Serenade is registered in the USA and South America for use on vineyards, fruits, vegetables and field crops in controlling foliage diseases (powdery mildew), rot diseases (*Botrytis*, *Rhizopus*) and bacterial diseases (fire blight). As a biological fungicide Serenade is suitable for organic farming and its low toxicity enables application until the day of harvest. In field trials conducted in Israel, Serenade was tested at concentrations of 0.5% and 1%. In the trials during the last 2 years, the efficacy of Serenade 0.5% controlling fire blight in pears was 70–80%, in comparison with 90% of the standard treatment. The efficacy of Serenade 0.5% in controlling powdery mildew in vineyards was 90%, vs. 95% efficacy of the standard. Applying Serenade for the control of *Oidiopsis taurica* in pepper gave an efficacy of 90%, which did not differ statistically from the standard chemical treatments. Serenade is a biological and environmentally friendly fungicide suitable for organic and conventional agriculture. Similar to *B.t.* products, Serenade's activity is due mainly to the toxins, which are released during the fermentation process, and thus its activity is consistent and it is not highly dependent on environmental conditions. (L)

### **Control of Powdery Mildew on Organic Pepper**

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Powdery mildew of pepper, caused by *Leveillula taurica*, is a disease affecting most of the varieties. Disease symptoms include white powdery mycelial growth on the underside of the leaves, and chlorotic spots on the upper surface of the leaves, which turn to be necrotic. Affected leaves

tend to drop off the plant, causing direct damage to the fruits due to exposure to sunlight. In organic management sulfur-containing agents are used efficiently to control the disease. However, since sulfur agents may harm beneficial insects in the greenhouse which help to control pests, our main objective in this study was to search for alternative control means. Two experiments were carried out in protected tunnels at Yair station in 1999–2000 using cvs. 'Fiesta' and '107' and in 2000–01 with cvs. 'Nibla' and 'Parker'. In the first experiment, sulfur agents either sprayed on the foliage or fumigated in the greenhouse were, as expected, very efficient in controlling the disease. They were better than the other tested treatments: aqueous extract of cattle manure compost, Kaligrin (calcium bicarbonate) and Rifol (fish oil). Also in the second year, all treatments including Neemgard (97% neem oil), water extract of grape marc compost, AQ10 (*Ampelomyces quisqualis*), Kaligrin and Rifol, significantly reduced disease incidence compared with the nontreated control; however, disease incidence in plots treated with sulfur was the lowest. Higher yields were obtained in all treatments (especially with Neemgard) only in cv. Nibla. (P)

C: VIROLOGY

**Transformation of Tobacco with cDNA that Codes for the Inhibitor of Virus Replication (IVR) Causes Partial Resistance to TMV and to Some Fungi: Possible Action Through the ABA Biosynthesis Pathway**

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An expression cDNA library was prepared from induced-resistant *Nicotiana tabacum* cv. Samsun NN and screened with polyclonal antibody raised against the IVR protein. A 1016 bp clone (named NC 330) was isolated. The predicted NC330 protein had 199 amino acid, with a molecular weight of 21.6 kDa. This protein contained leucine-rich repeats, nucleotide binding site motifs, as well as six phosphorylation, two myristilation, and one glycosylation site, all of which are found in the R-gene family. The IVR-like gene was present in the genome of both resistant and susceptible tobacco, at least with one copy, but differed in its polymorphism in agarose gel. NC330 transcripts were observed in induced-resistant tobacco tissue but not in un-induced or susceptible tobacco. The NC330 protein over-expressed in *Escherichia coli* reduced TMV replication in tobacco Samsun nn leaf disk assay. The NC330 cDNA was inserted into the binary vector pBJFLO and used to transform susceptible tobacco Samsun nn. Seventy-four regenerated transgenic plants were obtained; two of them were highly resistant to TMV. At the S<sub>1</sub> generation, 5 of the 92 transgenic plants were resistant; at the S<sub>2</sub> generation, 7 of the 117 plants showed high levels of resistance to TMV in leaf disk assay. When the whole plant was inoculated with TMV, a delay of 12–14 days in symptom appearance was observed. At the S<sub>3</sub> generation, PCR test for presence of NPTII and NC330 revealed that less than one third of the plants were transgenic, the majority of which showed high resistance to TMV. NC330 transcripts were detected in all the transgenic plants, including those that did not show TMV resistance. Compared to non-transgenic susceptible plants, germination of transgenic Samsun nn seeds in the dark or at high temperatures was inhibited. Abscisic acid (ABA) concentration in transgenic tobacco seeds or seedlings was higher than in non-transgenic controls. In addition, roots of transgenic tobacco seedlings were significantly longer than those of the non-transgenic controls. Seeds of the transgenic plants were darker than those of the control plants. Transgenic seedlings expressing the NC330 cDNA were resistant to *Alternaria alternata* and *Botrytis cinerea*. The involvement of ABA in resistance to viruses or fungi, and the possibility of post transcription silencing, were discussed. (L)

## Development of a Genetic Transformation Protocol for Cucumber and Its Use in the Generation of Transgenic Resistance to the CFMMV Virus

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Cucumber fruit mottle mosaic virus (CFMMV) was identified in the early 1990s in limited areas of Israeli greenhouse cucumber production. CFMMV causes severe mosaic symptoms with vein banding and yellow mottling on leaves. Moreover, fruits are not suitable for marketing, due to deformations, bright mottling or mosaic. In some cases fully developed plants show severe wilting symptoms leading to plant collapse. Recently we cloned the full genome of CFMMV and classified it as a new cucurbit-infecting tobamovirus (Antignus *et al.*, *Phytopathology* 91:565-571 [2001]). No genetic resistance was detected against this aggressive virus by screening numerous cucumber genotypes, and we therefore attempted to generate resistant lines by using the Pathogen-Derived Resistance (PDR) approach. An efficient technique for transformation of cucumber was established, based on the protocol published by Tabei *et al.* (*Plant Cell Rep.* 17:159 [1998]). *Agrobacterium*-mediated transformation of cotyledon explants with the GUS reporter gene was optimized by adjusting parameters related to media composition, level of acetosyringone, age of seedlings, concentration of *agrobacteria*, and selection schemes. Representative transformation frequencies reached a level of 25%. The genes coding for the coat protein or the putative 54 kDa replicase component were cloned into binary vectors under the SVBV (strawberry vein banding virus) promoter (Wang *et al.*, *Virus Gene* 20:11-17 [2000]). *Agrobacterium*-mediated transformation was performed as described above aided by the NPT II selectable gene. Kanamycin-resistant segregants from R1 progenies were screened for virus resistance after mechanical inoculation with purified virus (100 µg/ml), by symptomatology and ELISA assays. From a total of 14 replicase-containing lines, eight exhibited full resistance, while three resistant lines were found among a total of nine CP-containing lines. Further work on highly resistant lines indicated an immune response when challenged by mechanical inoculation, or by root infection, as performed by planting in CFMMV-contaminated soil. Furthermore, transgenic scions grafted on infected non-transgenic rootstocks remained virus-free throughout the growing period. (*L*)

## Characterization of the Fig Mosaic Virus: A Widely Spread but Little Known Virus

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Fig (*Ficus carica*) growing in Israel is undergoing many changes to adapt it to intensive cropping, such as the introduction of new cultivars and novel cultivation methods. Screen cover of some plantations is one of the methods to prevent damage by the fig fly and the fruit fly without using toxic insecticides. These expensive cultivation procedures require the use of the best propagation material in order to produce the best yield. Figs are propagated from cuttings, and therefore the new trees carry all the infested disease of the pre-existing trees. A small survey conducted in cultivar introduction plots and some commercial plots in the northern part of the country revealed mosaic symptoms of various degrees in all surveyed trees. In order to associate the mosaic symptoms with a virus

infection, leaves were extracted and the sap was examined by electron microscope (EM). Elongated virus particles similar to a potyvirus were observed in the sap. Since some serological cross-reaction occurred among various potyviruses, we analyzed antisera against potyviruses found in our lab. Of the antibodies examined, only the one against leek yellow stripe virus (LYSV) reacted with fig leaves sap. For the isolation characterization and antibody production against the fig mosaic virus (FMV), it should be propagated in a herbaceous test plant under controlled conditions. Sap transmission failed in all plants except cucumber seedlings, similar to a 1972 report from Turkey. Mechanical inoculation of cucumber cotyledons resulted in necrotic local lesions appearing in the first true leaf from 6 days post-infection. Uncommon symptoms were mosaic at the edge of the leaves and downward bending of the edges of symptomatic leaves. The sap of symptomatic cucumber leaves was used to inoculate cucumber seedlings further. Utilizing two different procedures it was possible to isolate FMV from fig and cucumber, although still mixed with some plant proteins. Inspecting such preparations in the EM, elongated particles similar to the Potyviridae were observed. PAGE-SDS analysis of the FMV preparations revealed a polypeptide of ~32000 Da. This polypeptide reacted in western blotting procedure against the LYSV antibody and the antibody against FMV raised in cucumber. Sections in mosaic-diseased fig leaves from cv. 'Brazilian' inspected by EM revealed double membrane particles typical for the Rymoviruses, which are mite-transmitted. Preliminary examination indicates the existence of an additional virus in some of the newly imported cultivars. The production of a specific antibody and the isolation of the coat protein will enable the partial sequencing of its amino acids, which will be translated into specific DNA primers for PCR analysis. The sensitive serological and molecular FMV-detection methods will be applied to tip-culture-propagated fig in order to develop virus free trees. (L)

### **Involvement of the Helper Component Protein in the Transmission Specificity of Potyviruses by Different Aphid Species**

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Aphids transmit potyviruses in a non-persistent manner. Transmission by aphids requires the involvement of two viral proteins, the coat protein and the helper component (HC). It is generally accepted that non-persistent viruses have a low specificity for aphid vectors. However, there are a few reports on specificity between potyviruses and aphid vectors. In order to elucidate the phenomenon in this virus group, we selected six species of aphids (*Myzus persicae*, *Aphis gossypii*, *Lipaphis erysimi*, *Brevicoryne brassicae*, *Rhopalosiphum padi*, *Rhopalosiphum maidis*) colonizing plants of different botanical families (cereals, Cruciferae, and cucurbits) and we examined the capability of each of these aphids to transmit the cruciferous turnip mosaic virus (TuMV) and the cucurbit zucchini yellow mosaic virus (ZYMV). Plant-to-plant transmission tests have shown that all six species were able to transmit TuMV, but only *M. persicae* and *A. gossypii* could transmit ZYMV. In order to exclude a possible host effect on aphid behavior, transmission tests were also done with aphids that acquired the virus (in mixture with helper) from stretched Parafilm membrane. Transmission results obtained for membrane-acquired virus corroborated those acquired from plants. Specificity may derive from an affinity either between the aphid and the HC, or between the HC and the virion. The availability of purified virions and HCs of the two viruses allowed examining these possibilities by acquiring each virus with a respective homologous or heterologous HC. The results have shown that *M. persicae* and *A. gossypii* were capable of transmitting TuMV virions with the ZYMV-HC but were not capable of transmitting ZYMV virions with TuMV-HC. These findings demonstrate that aphid species differ in their recognition of HC-virus systems. They also suggest that specificity may be due to the affinity between the HC and virions. The significance of these specificities in transmission of potyviral mixtures in plants was discussed. (L)

## Induction of Chitinase and $\beta$ -1,3-Glucanase Activities in Potato Leaves During Viral Infection

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Induction of defense enzymes is an important part of plant protective mechanisms. Our research was focused on chitinase and  $\beta$ -1,3-glucanase which have been investigated in fungal and bacterial infections, but their role in virus infection is not clear. The influence of potato virus X (PVX) on activity of chitinase and  $\beta$ -1,3-glucanase in resistant 'Sante' potato and susceptible 'Tamasha' potato was investigated. Significant differences were found in the level of chitinase and  $\beta$ -1,3-glucanase activities as compared with the uninfected potato plants. The resistant variety Sante had a considerably higher specific activity of soluble and cell-wall-bound enzymes in comparison with the susceptible Tamasha. Inoculation of the resistant plants with the PVX for 10 days did not show any changes in the activity of the soluble and the bound chitinase and in  $\beta$ -1,3-glucanase in comparison with the control. Strong induction of the soluble activity occurred in the susceptible Tamasha potato, whereas soluble  $\beta$ -1,3-glucanase did not change at all. Soluble chitinase reached maximum activity 72 h after virus inoculation and its activity was found to be ten times greater than in the control. Activities of both cell-wall-bound enzymes were induced as early as 1 h after virus inoculation and exceeded a level of approximately three times that of the control. We assume that the significant growth of enzyme activity in the susceptible potato leads to partial breakdown of the plant cell wall and allows the virus to penetrate into the plant tissue. (P)

D: SOILBORNE DISEASES

*Invited Lecture*

### Ethylene and Reactive Oxygen Species in a Plant – Pathogen System

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The reaction of plants to infection by pathogens is complex and variable. A detailed research study was conducted in recent years to investigate the involvement of reactive oxygen species (ROS) and the plant hormone ethylene in the pathogenesis of *Botrytis cinerea*. Host plants were French beans (*Phaseolus vulgaris*), tomato (*Lycopersicon esculentum*) and Arabidopsis (*Arabidopsis thaliana*). As a result of infection, the level of ethylene is increased and ROS and antioxidant enzymes (mainly catalase) and lipid peroxidation are enhanced in the infected tissue and around the rot. As a result of lower leaves' infection, it is possible to detect a systemic reaction in upper leaves that previously were not infected. A selection process resulted in oversensitive and resistant Arabidopsis lines. Infected resistant plants produce less ethylene and ROS as compared with sensitive plants. The induction of increased tissue ROS levels results in elevated ethylene and severe disease. The above mentioned plant reactions to infection can be related to the contact with *B. cinerea* cells, since application of dead conidia induced ROS and ethylene production. The effect was also obtained by leaf injury, H<sub>2</sub>O<sub>2</sub>, pH reduction, oxalic acid and botrydial (both are produced by the pathogen). Ethylene added to the system or produced internally promotes the disease development, whereas inhibition of ethylene production by the plant suppresses the disease. Interestingly, ethylene also promotes conidia germination and subsequent penetration to the host tissue. Ethylene-signaling mutants of Arabidopsis showed extremely high susceptibility to *B. cinerea*; it is possible that the

pathway that leads to susceptibility is independent of other ethylene signal transduction pathways. *B. cinerea* has the potential to produce ethylene, and thus it is possible that the ethylene that plays a role in the system originates not only from the plant. Treatment with antioxidants reduced ethylene production and disease severity. In conclusion, infection induces both ROS increase and ethylene production. Ethylene induces auto-catalytic production of the same hormone in exposed tissue. Ethylene promotes the deterioration of the infected tissue and the ROS development and *vice versa*. Consequently, antioxidant levels are changed in the rot area or in symptomless leaf tissues surrounding the rot. The reaction can be negated by inhibition of ethylene or external application of antioxidants. (*L*)

### **Colonization of Melons Grafted onto Squash Plants by the Fungus *Fusarium oxysporum* f.sp. *melonis***

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Colonization of non-grafted melon plants, squash plants used as rootstocks and melon (*Cucumis melo*) plants grafted onto squash (*Cucurbita* spp.), by the fungus *Fusarium oxysporum* f.sp. *melonis* (*FOM*), was observed in greenhouse experiments conducted in order to understand better the interaction between the pathogen and the grafted host. Two methods for evaluating the fungus level inside the plants were compared: counting the colony-forming units (CFU) and evaluating the percentage of colonization in the plants' organ segments. High correlation was found between the two methods and thus the segments method – that was easier to use – was employed in all experiments. Colonization rate of resistant, susceptible and partially resistant melon plants by *FOM* was studied in the first stage of the research. High colonization of the roots and the stem (95–100%) of the susceptible melon cv. 'En Dor was observed as compared to lower colonization of roots (40–60%) and stems (0–20%) of the resistant cv. 'Hemed'. Colonization percentages of the partially resistant cv. 'Noy Yizre'el' were intermediate. Root colonization of the squash rootstocks 'Brava' and 'TZ 148' were as high as in roots of the susceptible melon. The colonization of the lower part of squash stems was similar to that of the partially resistant melons. Colonization of the upper stem by the pathogen was similar to that of resistant melons. The grafting procedure did not affect plant colonization significantly. Colonization pattern was similar in non-grafted resistant or susceptible melons and in self-grafted genotypes. Roots of TZ 148 were highly colonized (65–80%) when they served as rootstocks to resistant and to susceptible melon plants, whereas stems of both resistant and susceptible melons grafted on this rootstock were colonized in only 0–45% of cases. A similar colonization pattern was evident in grafted melons grown in a field naturally infested with *FOM*. *Fusarium*-resistant melons used as rootstocks were more effective than squash rootstocks in suppressing both scion colonization and disease incidence. (*L*)

### **Effect of N-Viro Soil (Ecosoil) and NH<sub>4</sub> on Controlling Soilborne Pathogens**

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In a previous study we reported on the fungicidal activity of N-Viro soil (Ecosoil) applied together with a nitrogen ammonia source. In the present work we studied the effect of pH values on fungicidal activity of Ecosoil on *Fusarium oxysporum* f.sp. *dianthi* (*F.o.dianthi*) in naturally infested soil in laboratory and in field experiments. The effect of four treatments was studied: (i) Ecosoil 40 mg g<sup>-1</sup> soil; ammonium sulfate 1 mg g<sup>-1</sup> soil; combined treatment - Ecosoil + ammonium sulfate (i + ii); and (iv) control. The soil samples were irrigated to field capacity and the count of *F.o.dianthi* was determined at various time intervals following incubation at 25°C. The pH values of the soil samples treated with Ecosoil alone or Ecosoil + ammonium sulfate were the same. In the combined treatment, control of *F.o.dianthi* was observed at a pH range between 10 and 11. The highest control activity was achieved at a pH range of 10.5–11, 24 h after irrigation. At this pH range Ecosoil alone reduced fungal count between 50% and 70%. The pH of the soil samples decreased gradually and reached the original value 16 days after the irrigation. At pH values slightly below 10, 90% of the *F.o.dianthi* population was controlled, and at pH below 9 there was no fungicidal activity. Ammonium sulfate had no fungicidal activity and was similar to the control. In two field experiments, one in sandy soil and the other in medium loessial soil, we studied the effect of the four treatments on potato wilt caused by *Verticillium dahliae*. Following the incorporation of each material into the top 20-cm soil layer, soil samples with *F.o.dianthi* and *V. dahliae* were inserted at various depths down to 30 cm. In addition, soil samples from each replicate were collected from two soil layers, 0–20 and 20–30 cm, to determine the number of *Fusarium* spp. and *Pythium* spp. propagules. The pH values in the combined treatment were 11.0 and 9.6 in sandy soil and medium loessial soil, respectively. In the combined treatment, 99% control of *F.o.dianthi* and *V. dahliae* as well as of *Fusarium* spp. and *Pythium* spp. was observed in the top 20-cm layer in the sandy soil field. In the medium loessial soil field, however, the combined treatment was less effective than in the sandy soil in controlling these fungi. (*L*)

### Control of *Fusarium* and Root-Knot Nematodes in Carnations Using Steam or Chemicals

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The most destructive soilborne pathogens of carnation (*Dianthus caryophyllus* L.) are *Fusarium oxysporum* f.sp. *dianthi* (*F.o.dianthi*) and root-knot nematodes, *Meloidogyne javanica*. A comparison between methyl bromide and soil steaming or metham sodium (Edigan, Vapam) in controlling the two pathogens was carried out in a naturally infested field. The field was divided into ten blocks, of which five were fumigated with the liquid nematicide 1,3-dichloropropene (Condor), 25 ml m<sup>-2</sup>. Then, each of the ten blocks was treated with the following four treatments: (i) control – irrigation with water; (ii) Edigan – 180 ml m<sup>-2</sup>; (iii) soil steaming for 5 h using a negative pressure method; and (iv) methyl bromide 70 g m<sup>-2</sup>. The efficiency of *Fusarium* control was determined by counting *F.o.dianthi* propagules in soil collected from three depth layers of each replicate: 0–20, 20–40 and 40–60 cm. The *M. javanica* population was estimated qualitatively on tomato roots planted in soil collected from the 0–20-cm layer. Two carnation varieties: Lior - susceptible to the two pathogens, and Galit – moderately resistant to *F.o.dianthi* and highly susceptible to *M. javanica*, were planted at the end of June. Incidence of the two diseases was estimated at 14-day intervals from 90 to 180 days

after planting. Propagule counts of *F.o.dianthi* treated with Edigan, steam or methyl bromide were very low in the three soil layers tested. In control plots not treated with Condor, disease incidence caused by *F.o.dianthi* reached the maximal level faster and growth inhibition caused by *M. javanica* was greater, than in control plots treated with Condor. Disease incidence by the two pathogens in methyl bromide plots with or without Condor was similar and very low. Steam or Edigan treatments were similar to methyl bromide in reducing disease incidence caused by *F.o.dianthi* but had only a partial effect on disease caused by *M. javanica*. Addition of Condor fumigation reduced further nematode disease in both treatments and was similar to the effectiveness of methyl bromide. (L)

### **Toxicity of Formaldehyde to *Pythium* Following Repeated Applications**

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A formulation of formaldehyde (Fordor 37) has been found to be effective in controlling peanut pod rot disease caused by two *Pythium* species, *P. myriotylum* and *P. aphanidermatum*. In this work the effect of repeated application of Fordor 37 on its toxicity to these two *Pythium* species was studied. The same sample of sandy loam soil collected from a field was used for all experiments, with a one-month interval between one experiment and the following. In the first experiment, Fordor 37 at 300 ppm controlled completely both *Pythium* species. In the second and third experiments a higher concentration of the chemical was necessary to control *P. aphanidermatum* (600 ppm) but not *P. myriotylum* (400 ppm). In the following experiment control of *P. aphanidermatum* in 'old' soil (already treated three times with Fordor 37) was compared with that in a 'new' soil sample collected from the same field. In the 'new' soil, *P. aphanidermatum* was 100% controlled at 300 ppm, whereas in the 'old' soil (fourth application) control of 93% was achieved at 600 ppm. In the last experiment we compared control of *P. aphanidermatum* in sterilized vs nonsterilized 'new' and 'old' soils. With 300 ppm Fordor, 100% control of the fungus was achieved in both sterilized and nonsterilized 'new' soil and also in the sterilized 'old' soil. In the nonsterilized 'old' soil, however, 600 ppm Fordor controlled only 56% of the fungal population. In the present work we demonstrated that repeated applications of Fordor 37 reduce its efficiency in controlling both *P. myriotylum* and *P. aphanidermatum*, due to biodegradation of Fordor 37 in the soil. Although repeated application at this frequency is not the normal practice in the field, the results indicate a problem that could occur if growers intensify the use of this chemical, following the phasing out of methyl bromide. (L)

### **Vine Decline and Yield Reduction in Watermelon: The Causal Agents and Evaluating Chemical Control Efficacy**

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The fungus *Macrophomina phaseolina* causes vine decline and yield losses in cucurbits. The wide distribution of *M. phaseolina* constitutes a serious threat to watermelon cultivation in its major production areas of Israel. Currently, control of the disease in Israel is possible by soil disinfestation treatment. The goal of this study was to find fungicides from different chemical groups that will inhibit *M. phaseolina*, as a first step towards establishing a disease control approach using fungicide application during the growing season. Of the 15 fungicides tested *in vitro*, the benzimidazole fungicides carbendazim, thiophanate methyl and benomyl totally inhibited the



growth of *M. phaseolina* in culture at a concentration of 10 µg a.i. ml<sup>-1</sup>. Also the experimental fungicide cyflufenamid (NF-149), fluazinam and cyprodinil + fludioxonil (Switch) exhibited the same efficacy. The fungicide efficacy in suppressing the pathogen inside watermelon roots was tested in pot experiments. The fungicides were applied to the soil one month after sowing when roots were already colonized by the pathogen. Carbendazim had a curative effect, being capable of controlling the fungus inside the roots and of maintaining a low colonization level up to 5 weeks after application. Azoxystrobin was effective for the whole experiment period only when applied at the low concentration of 0.025% to the sandy soil. Cyprodinil + fludioxonil (Switch) acted differently in the different soils and concentrations; Switch was highly effective throughout 5 weeks when applied to the heavy soil in the high concentration (0.1%) and to the sandy soil in the low concentration. When applied to the sandy soil at the high concentration, it suppressed *M. phaseolina* completely in roots sampled after one week but enhanced root colonization by *Fusarium solani*. (L)

### **Combined Resistance to Fusarium Wilt and Root-Knot Nematodes in Carnation**

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Carnation response to Fusarium wilt caused by *Fusarium oxysporum* f.sp. *dianthi* (*F.o.dianthi*) and/or to root knots caused by the nematode *Meloidogyne javanica*, was studied during two successive growth seasons in the field and in containers, in naturally infested soil. Carnation response to both pathogens was recorded from 90 to 180 days after planting, at 2-week intervals. The proportion of plants which wilted due to *F.o.dianthi* was classified into six groups: 75.1–100%, very susceptible or susceptible cultivars; 50.1–75%, low resistance; 25.1–50%, moderate resistance; 10.1–25%, high resistance; 0.1–10%, very high resistance; and 0%, possibly complete resistance. Cultivar response to *M. javanica* was determined by scoring growth inhibition of plants. Carnation response to Fusarium wilt resembled durable resistance as the cultivars tested were distributed among the six classification groups. The response to *M. javanica*, on the other hand, resembled monogenic resistance response, as the cultivars were either resistant or susceptible. Resistance to nematodes was found in the six groups of resistance to *F.o.dianthi*. In some carnation cultivars resistant to both pathogens, presence of the nematodes reduced slightly the resistance to *F.o.dianthi*. In all carnation cultivars susceptible to nematodes, however, the presence of the nematodes reduced considerably the resistance to *F.o.dianthi*. In the second growth season we tested wilt and response to nematodes of six cultivars from the first season together with new cultivars. The response of the six cultivars was similar to that observed in the first season. Distribution of wilt or growth inhibition was similar to the first season. In previous work we reported on the existence of complete resistance to *F.o.dianthi*. In the present work we reported on the existence of resistance to *M. javanica* and to both nematodes and Fusarium, in commercial cultivars and lines of carnations. (P)

### **Heterokaryon Compatibility in *Verticillium dahliae* Tested by Comparing *nit* and Auxotrophic Mutants**

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Isolates of *Verticillium dahliae* defined as VCG1, VCG2A, VCG2B and VCG4B using nitrate nonutilizing (*nit*) mutants were marked by mono- or diauxotrophic mutations and by resistance

to carbendazim. Heterokaryon compatibility between marked strains belonging to the same or to different VCGs was tested using the 'hanging drops' or the 'blocks' technique. Combinations involving marked strains from the same VCG were successful (23 combinations of different auxotrophic markers), whereas most inter-VCG pairings failed (48 combinations of auxotrophs). In a few cases prototrophic growth formed by auxotrophs from different VCGs was proved to be cross-feeding using interposed cellophane membrane. Reversion to prototrophy in one parental strain was the reason for prototrophic growth in a few other cases. In some inter-VCG combinations, very slow or inconspicuous prototrophic growth was observed, which is similar to weak complementation occasionally observed at the contact zone between complementary *nit* mutants belonging to different VCGs. It was concluded that VCGs defined by *nit* and by auxotrophic mutants coincide. Parasexual cycle, tested by recovering prototrophic recombinants and diploid conidia from high-temperature-treated heterokaryons, was demonstrated between isolates belonging to the same VCG. (*P*)

*E: FRUIT CROP DISEASES*

### **Overwintering of *Unicula necator* and the Initial Stages of Grape Powdery Mildew Development in Israel**

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*Unicula necator*, the causal agent of powdery mildew, is the most devastating foliar pathogen of grapevines in Israel. The pathogen infects all the green parts of the plants but most of the damage results from bunch infections. Final disease severity and the resultant losses are influenced by the time of disease onset and by the spatial development of the disease at the beginning of the epidemic. *U. necator* may overwinter as mycelia in dormant buds, and in the form of cleistothecia that survive the winter and mature in the following spring. When infected buds flourish, the growing branch and the newly developed leaves are covered with *U. necator* mycelia and conidia. The diseased branches (flag-shoots) are assumed to be the only source of initial inoculum in Israel. In a study that was conducted in the years 1998 to 2001, the appearance of flag-shoots over time and space, and their role in disease development at the initial stages of the epidemic, were studied. Flag-shoots were found only in orchards of the cultivar 'Carignan' but not in 'Cabernet Sauvignon', 'Chardonnay' or 'Merlot'. The number of flag-shoots varied from vineyard to vineyard, but never exceeded 350 per ha. In the years 1998 to 2000, disease development in time and space was related to the appearance of flag-shoots: infections on leaves were observed only after the emergence of flag-shoots, and were located only in close vicinity to flag-shoots. In these years, disease onset was relatively late and the severities of the epidemics were mild to moderate. In 2001, on the other hand, the disease appeared earlier and its spatial distribution was not restricted to the location of the flag-shoots. This may suggest that ascospores, produced in over-wintering cleistothecia, served as another source of initial inoculum in 2001. (*L*)

### **Sensitivity of 'Red Delicious' Apple Fruit at Various Phenologic Stages, to Infection by *Alternaria alternata* in Relation to Moldy-Core Control**

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The fungus *Alternaria alternata* is considered to be the predominant fungus involved in moldy-core of 'Red Delicious' varieties of apple. The sensitivity of various phenologic stages to infection by *A. alternata*, and the efficacy of various fungicides in controlling moldy-core disease in apple orchards, are reported here. Artificial inoculations conducted in the orchard during the 1999 and 2000 seasons revealed that the beginning of bloom (10–30%) and full bloom were the most susceptible developmental stages for infection by the fungus. Natural infection with *A. alternata* in fruits was relatively high, reaching 44%, 46% and 46–50% of the fruits on control non-treated trees in 1999, 2000 and 2001, respectively. Two to four foliar applications of polyoxin B (Polar), and the sterol inhibitor difenoconazole, starting from the beginning of bloom until fruit set, reduced the number of infected fruits by 54–72% and 61–75%, respectively, compared with control non-treated trees. There were no significant differences between two, four and six foliar applications of difenoconazole, or between two and four applications of polyoxin B (Polar). In large-scale trials two or three foliar applications of each fungicide during the bloom period were effective in reducing disease incidence. *Alternaria* was isolated from the inner part of the core region of 71–88% of non-treated fruits, but less was recovered from the external part of the core region. Fruits from difenoconazole and Polar-treated trees showed reduced colonization by *A. alternata* in the core region. Results indicate that a control program based on spray applications of difenoconazole or polyoxin B (Polar) during the the bloom period, can effectively reduce *Alternaria* on Red Delicious. (L)

### **Occurrence and Management of Alternaria Brown Spot in Israel - 2002**

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*Alternaria* brown spot, caused by *Alternaria alternata* pv. *citri*, was first observed in Israel in 1988 on Minneola tangelo, in an orchard at the central coastal plain. Since then the disease had spread to most citrus-growing areas, where nights are characterized by much fog and dew. There are large variations in the disease infection and intensity between adjacent plots. *Alternaria alternata* pv. *citri* inflicts severe damage to cvs. 'Minneola', 'Murcott/Mor', 'Nova/Yanuv' and 'Michal'. Commonly grown cultivars such as Satsumas, Clementines, Ortanique, oranges, grapefruits, pommelos and lemons were found to be resistant under field conditions. Or 1, a newly developed hybrid, proved to be resistant to *Alternaria* brown spot. The disease occurs mainly on fruit on the outside of the trees, the typical symptoms being minute brown spots which become corky. Fruit infection may develop at any stage of fruit development from fruit set to harvest. In the years following the first occurrence of the disease in Israel, fruit infection occurred mainly in spring and early summer (March to June). During the last few years there has been a marked increase of fruit infection in autumn, from August to December, especially during November; atypical large, sunken and blackish lesions form, which may cause fruit abscission. In addition, light green spots occur quite frequently. In most orchards chemical disease management is practiced: fungicides are usually sprayed at 2–3-week intervals in the spring and early summer, and at 3–4 week-intervals in autumn. Registered fungicides include iprodione, dithiocarbamates, triazole, strobilurine, famoxadon, and copper compounds (the latter which are less effective). To reduce the chance of resistance development, it is recommended to alternate between fungicides with different modes of action. Some fungicides are limited to early season use only, to avoid residues at harvest. During the 2001 season, a series of sprays with potassium phosphite (Canon<sup>®</sup>) significantly reduced brown spot lesions. Likewise, treatments with imidacloprid (Confidor<sup>®</sup>), an anti-aphid insecticide, significantly decreased brown spot incidence. (L)

### **Is Pruning Always the Way to Cope with Pear Tissues Infected with Fire Blight?**

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Fire blight, caused by the bacterium *Erwinia amylovora*, is a devastating disease of pomaceous fruit trees. The bacteria penetrate the plant through the blossoms and wounds. The disease then progresses within the tree tissues, causing wilting and death of branches; if it reaches the trunk, the entire tree may die. Pruning and removal of infected tissues is one of the means to cope with the disease and growers are advised to cut infected tissues *ca* 30 cm below the symptoms. The only factor taken into account in this recommendation is the pathogen. The effects of various factors related to the host on the success of pruning were tested in a series of experiments conducted in pear orchards naturally infected by fire blight. The factors were: (i) pruning site (cutting and removing only the infected tissues or cutting 30 cm below the point of visible symptoms); (ii) growth habit of the tree (few or many new branches); (iii) time of infection (spring or autumn); and (iv) phenological stage of the trees at the time of pruning (blooming, fruit set, etc.). The success of the pruning procedures was governed, to a large extent, by the physiology and phenology of the tree. Moreover, it was found that in some cases it is preferable not to prune at all (because the bacteria remained restricted to the blossom cluster) rather than to prune (because cutting induced rapid progression of the bacteria within the branches). Based on the results, the recommendations to growers were modified and host factors are now taken into consideration as well. (L)

#### **Changes in Resistance and Susceptibility of *Erwinia amylovora* Population to Streptomycin and Oxolinic Acid**

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Fire blight caused by the bacterium *Erwinia amylovora* is a devastating disease in all pear-growing areas in Israel. Streptomycin was used for managing the disease since 1986. However, in a survey conducted during 1994–97, streptomycin-resistant strains were detected in pear, apple, loquat and quince orchards all over the country. As of 1997 streptomycin was removed from use for fire blight management and instead oxolinic acid (Starner) has been used commercially. Oxolinic acid is currently the only bactericide available for management of fire blight. Consequently, it is important to detect changes in the response of *E. amylovora* populations to oxolinic acid (appearance of resistance) and streptomycin (reappearance of susceptibility). Samples of infected flowers and shoots collected from pear, apple and quince orchards were examined on plates containing 50 g ml<sup>-1</sup> streptomycin, and on 5 and 50 g ml<sup>-1</sup> oxolinic acid. Strains resistant to oxolinic acid were not detected in 22 orchards that were sampled during 1998. In 1999, oxolinic acid-resistant strains were isolated from two pear orchards in the northern part of Israel. In a survey conducted during the spring seasons of 2000 and 2001, respectively, 48 and 55 orchards of pear, apple and quince from all over the country were sampled. Oxolinic acid-resistant strains were detected in several orchards located in two restricted areas in northern Galilee. In contrast, populations of streptomycin-susceptible strains were widespread all over the country. Resistant strains could be found only in restricted regions in western Galilee and the Golan Heights. No strains with resistance to both streptomycin and oxolinic acid were found. Results of this survey are used for directing bactericide sprays against fire blight. (L)

## Epidemiology of *Verticillium dahliae* on Olive

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*Verticillium* wilt on olives caused by *Verticillium dahliae* is characterized by desiccated leaves and branches, defoliation and death of parts of the tree, which lead to severe economic losses. Only 15% of the olive orchards in Israel are irrigated. During the last 6 years new plantations have been established in the Negev (southern Israel), where saline water is used for irrigation. In 1998 *Verticillium* wilt was detected in a 3-year-old plantation, planted in a field previously cultivated with susceptible crops. Disease incidence in cv. 'Barnea' increased from 20% to 40% within one year, and to 60% after another year. However, disease severity decreased from 4.1 (on a scale of 2–10) to 2.6 and 2.0 in the second and third years, respectively. The average yields of symptomatic trees in the first and second harvests were significantly reduced (by 44% and 13%) compared with the yields of the healthy trees. Disease incidence in cv. 'Picual' increased from 22% in 1999 to 40% in 2001. Disease severity also increased, from 2.6 to 3.5. The yields of symptomatic trees in the first and second harvests were significantly reduced (by 80% and 71%, respectively) compared with the yields of the healthy trees. In cv. 'Souri', there were increases in both disease incidence (from 23% to 35%) and severity (2.4 to 3.1) within one year. The fungus was isolated from branches at the top, middle and bottom of both healthy and symptomatic trees. Positive isolations of the fungus were higher in autumn-winter (61%) than in summer (5%). Forty isolates collected from various sites were assigned to VCGs, with 87% and 13% classified to VCG4B and VCG2A, respectively. (L)

## Control of *Alternaria* Brown Spot (*Alternaria alternata* pv. *citri*) by Potassium Phosphate and Potassium Phosphite

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In an experiment conducted in the spring and autumn of 2001, two compounds which are known as acquired resistance inducers, Peak<sup>®</sup> (KH<sub>2</sub>PO<sub>4</sub>) and Canon<sup>®</sup> (K<sub>2</sub>HPO<sub>3</sub>), were tested for their ability to control *Alternaria alternata* pv. *citri* in 'Nova' tangelo. In August 2001 a significant difference was found among the four treatments tested in the percentage of fruit in class A, suitable for packing (less than five small spots of *Alternaria* per fruit). In the untreated control only 60% of the fruits were of class A. In the commercial chemical recommendation (five sprays, one every 2 weeks), 90% were class A. In the Canon treatment (four sprays, one every 4–5 weeks), 88% were class A. The Peak treatment did not differ from the control, with only 70% class A. The results at the end of autumn were similar, with only 50% class A fruits in the control and 58% in the Peak, which were significantly lower than 85% class A in the commercial and 86% in the Canon treatment. The encouraging results from the spring experiment led to three additional experiments conducted in the autumn of 2001, on 'Yanov' (Nova), 'Murcott' and 'Minneola' with only three treatments: untreated control, commercial recommendation (three sprays with dithiocarbamate, one every 4 weeks) and Canon (three sprays, one every 4 weeks). In this experiment the percentage of class A fruits in untreated control was significantly lower than in the commercial and Canon treatments: respectively, 53% in contrast to 77% and 83% in Yanov; 38% in contrast to 74% and 77% in Murcott; and 43% in contrast to 93%

and 84% in Minneola. From these results it is suggested that Canon has a fungicidal effect on *A. alternata* pv. *citri*. The mode of action is not fully known. (L)

### **Improved Pesticide Application and Pest Management in Vineyards Using Air-Assisted Spraying**

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Wine production in Israel has been a developing industry during the last two decades and much research is conducted on the control of pathogens in the vineyards. Major pests of grapes in Israel include the European vine moth (*Lobesia botrana*) and the honeydew moth (*Cryptoblabes gnidiella*), and the fungus causing powdery mildew (*Uncinula necator*). The moths are active throughout the crop season and cause substantial damage to the yield. Pest control measures include frequent pesticide applications through hydraulic, air-carried and air-placement sprayers, *inter alia*. Low-volume sprayers based on air placement have been introduced in recent years, but the performance of these sprayers has not been satisfactory. In tests it was found that the air velocity diminishes rapidly between the orifice and the vine, which results in deflection of the spray droplets around the berries. The effects of this air barrier are particularly significant during the early stage of fruit development, when there are small berries and sparse clusters. The objective of the present study was to improve the performance in vineyards of a low-volume sprayer that uses air-placement sprayers. The modified air-assisted sprayer provided better spray coverage than the commonly used sprayers, as exhibited in more effective coverage of the grape clusters, and improved pest control. The quality of spray coverage with the air-assisted sprayer depended on the phenological stage of the grapes. In the early stage, with small berries and sparse clusters, good coverage was accomplished with large-volume nozzles applying large droplets. The modified air-assisted sprayer provided better spray penetration than the conventional sprayer, as indicated by improved spray coverage and control of grape rot. The use of sprayers with nozzles that apply large volumes demonstrated the need for sufficient energy to ensure droplet deposition on round targets. Nevertheless, it is essential to reduce pesticide volume, and the ability to reduce spray volume while maintaining effective application was demonstrated in this study. Increasing the air velocity made it possible to use nozzles with small droplets and reduced volume, while maintaining good coverage of the target. This will be studied further. Optimization spray application technology, in combination with other cultivation practices such as leaf removal, could provide better pest control and might enable the frequency and volume of sprays to be reduced. (L)

#### *F: POSTHARVEST STORAGE AND TECHNOLOGY*

### **Characterization of Extracellular Lytic Enzymes Produced by the Postharvest Biocontrol Agent *Candida oleophila***

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The enhancement of biocontrol activity of biocontrol agents may be the most important factor in their success in controlling postharvest fruit diseases. In our efforts to identify genetic traits

of the yeast *Candida oleophila* and to determine their potential to enhance its biocontrol activity, we have been studying the yeast's ability to produce fungal cell-wall-degrading enzymes. This study focused on four enzymes secreted by *C. oleophila*: exo-glucanase, endo-glucanase, chitinase and protease. The influence of the type of growth and carbon sources on the production of these enzymes by the yeast antagonist was determined using calorimetric assays and plates containing the septic substrates. SDS-PAGE and activity gels of partially purified enzyme preparations were used to study the isozyme profiles. In addition, a full length gene of secreted  $\beta$ -1,3-glucanase of this yeast (CoEXG1) was cloned and analyzed. Sequencing analysis revealed that CoEXG1 carries the signature pattern of family 5-glycohydrolase, has a putative secretion leader, and has a high degree of identity to other yeast  $\beta$ -1,3-glucanase. *C. oleophila* transformants harboring two copies of the *CoEXG1* and its putative promoter region exhibited increased production (at least two-fold) of  $\beta$ -1,3-glucanase. Cloning and sequence analysis of chitinase genes from *C. oleophila* are under way. (L)

### **Characterization and Experimental Approaches for Management of *Penicillium* in Litchi Fruits**

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Litchi fruits must be treated to prevent their rapid browning and to retain their appealing red color after harvest. This is currently achieved by SO<sub>2</sub> fumigation followed by dipping the fruit in dilute hydrochloric acid containing the fungicide prochloraz. SO<sub>2</sub> blocks enzymatic activity and bleaches the fruits, whereas the acid restores the red color to the fruits and prochloraz prevents fungal infection. Alternative methods, such as hot water brushing, have been developed to eliminate the use of SO<sub>2</sub>, but while this procedure reduced the fungal population it did not reduce infection. Untreated fruits are infected with several fungal species but after dipping the fruits in HCl, *Penicillium* is the sole or major cause of infection of the peel during storage. Surface sterilization after harvest and storage in a sterile environment does not control infection, which may initiate from micro-cracks in the pericarp. Storage under non-sterile but ventilated conditions can, however, prevent or reduce decay. *In vitro* growth of a *Penicillium* isolated from litchi is restricted at pH 2.0 but not at pH 3.0. The pH of the pericarp after fumigation of the fruit with SO<sub>2</sub> and dipping it in hydrochloric acid ranges between 2.5 and 3.0. Internal transcribed spacer sequence analysis of *Penicillium* isolated from litchi revealed identity to *P. commune*-related species. Management approaches include the use of compounds which perturb pH homeostasis to control spore germination and conventional approaches for immediate relief of the problem. (L)

### **pH Regulates Endoglucanase Expression and Virulence of *Alternaria alternata* in Persimmon Fruits**

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The phytopathogenic fungus *Alternaria alternata* produces one endo-1,4-glucanase, *AaKI*, which is an important factor in disease development in persimmon fruits. During growth of *A. alternata* in media containing acidified yeast extract or cell walls from persimmon fruit, the fungus

secreted ammonia and raised the medium pH. A rise in media pH from 3.8 to 6.0, in the presence of cell walls, induced the expression of *AaK1*, whereas a glucose-induced decline in pH to 2.5, repressed transcription and enzymatic production. Treatments with buffered solutions at pH 6.0, during growth of *A. alternata* in the presence of glucose, de-repressed *AaK1* expression and endo-1,4-glucanase production and enhanced decay development on the fruit. The results suggest that conditions affecting environmental pH modulate gene expression of *AaK1* and virulence of *A. alternata* in persimmon fruit. (*L*)

### **Effect of pH on *Colletotrichum gloeosporioides* PacC and *pelB* Expression and Its Relation to Pectate Lyase Secretion by the Pathogen**

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Accumulation of ammonia during *Colletotrichum gloeosporioides* attack and its effect on pH alkalization is considered as a virulence factor for fungal attack on fruits. The fungus-induced increase in pH to 6.0, changes the pattern of secretion. Secretion of pectate lyase was detected at an initial pH not lower than 5.4 and increased up to pH 6.0. At pH 4.0, neither expression nor secretion was observed. Also nitrogen source was found to affect secretion, and this was observed also at the transcription level. Contrary to *pelB*, transcriptional activation of *pg1*, encoding the endopolygalacturonase, accumulated similarly at pH 4.0 and 6.0. Analysis of the promoter region of *pelB* showed that it contains seven PacC consensus-binding sites. In order to determine whether *pelB* is pH-regulated by a signal transduction process, we cloned a putative transcription factor-encoding gene, *pac1*. Its transcription accumulates parallel to ambient pH. Our results suggest that ambient pH is a regulatory factor for processes linked to virulence and that these processes may be under the molecular regulation of a conserved pH-dependent signaling pathway. (*L*)

G: NEMATODOLOGY

### **The Role of Root Exudates in Host Detection by Plant Parasitic Nematodes**

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Plant parasitic nematodes cause substantial economic damage worldwide. Lately many chemicals employed against nematodes have been excluded from use and it is therefore necessary to develop alternative control measures. The attraction of nematodes to their host is generally based on specific signals from the root which can be detected by the nematodes and affect their behavior. Understanding these processes may enable the development of new means to interfere with nematode parasitism. In this study *Ditylenchus dipsaci*, the stem and bulb nematode, was used as a model. This nematode attacks underground and upper parts of the plants. The nematodes are transferred mainly by infected vegetative organs and can survive in soil under dry conditions. The effect of onion root exudates on nematode behavior was studied using aseptic bioassays we developed, in plates containing water-agar or sterile sand and soil layers. In comparison with control treatments, the vast majority of the juveniles were attracted to root exudates in all bioassays. The juveniles remained in the attractant zone for several hours and the attraction decreased quantitatively in correlation



with serial dilutions of the root exudates. Onion plants are known to be better hosts than alfalfa for this nematode: when juveniles were exposed to either onion exudates or alfalfa exudates, they significantly preferred the onion exudates. Boiling did not affect the attractivity of the onion root exudates and the active fraction was of low molecular weight. (L)

### Control of Root-Knot Nematodes by Ammonia

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The nematicidal activities of ammonia-releasing and ammonium compounds were tested against the root-knot nematode *Meloidogyne javanica* in pot, microplot and field experiments. Among ten compounds tested, NH<sub>4</sub>OH, (NH<sub>4</sub>)<sub>2</sub>HPO<sub>4</sub> and NH<sub>4</sub>HCO<sub>3</sub> showed greatest nematicidal activity at concentrations of 300 mg N kg<sup>-1</sup> of NH<sub>4</sub> or NH<sub>2</sub> in pots. NH<sub>4</sub>OH was found to be the most nematicidal of these compounds. Enclosure of pots containing NH<sub>4</sub>OH-treated soils in plastic bags reduced the concentration of NH<sub>4</sub>OH needed to kill the nematodes from 200 mg N kg<sup>-1</sup> in open pots to only 70 mg N kg<sup>-1</sup>. In a microplot experiment, treatment of nematode-infested soil with NH<sub>4</sub>OH at 70 mg N kg<sup>-1</sup> reduced the root-galling index to zero. In one field experiment the nematicidal efficacy of NH<sub>4</sub>OH on tomato plants at doses of 1000 and 2000 kg N ha<sup>-1</sup> was equivalent to that of Telopic C35<sup>®</sup> or metam-sodium in combination with cadusafos. In another field experiment, NH<sub>4</sub>OH at 500 and 1000 kg N ha<sup>-1</sup> increased tomato yield and at 1000 kg N ha<sup>-1</sup> reduced the galling index, compared with untreated controls. The results suggest that NH<sub>4</sub>OH may serve as a nematicide in alkaline sandy soils. Nitrapyrin, a nitrification inhibitor, was used with NH<sub>4</sub>OH in order to maintain the ammonia concentrations for a longer period in the soil. When nitrapyrin at a concentration of 10 mg kg<sup>-1</sup> (which has no adverse effect on the nematodes) was incorporated with NH<sub>4</sub>OH, an additional reduction in galling index was observed on tomato plants. Use of nitrapyrin with organic amendments, such as chitin and cottonseed meal, also enhanced the nematicidal activity. (L)

#### H: SOIL DISINFESTATION

### Occurrence of Accelerated Degradation of Metam-Sodium and Dazomet in Israeli Soils

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Metam-sodium (MS) and dazomet (DAZ) are alternative soil fumigants to replace methyl bromide in Israel and elsewhere. When applied to moist soil, these fumigants are decomposed to methyl-isothiocyanate (MITC), which is an effective wide-spectrum soil fumigant for controlling various soilborne pests (fungi, nematodes, soil insects and weeds). In recent years we found cases in which repeated applications of MS to commercial potato fields in various locations in Israel have resulted in less effective control of potato *Verticillium* wilt, caused by *Verticillium dahliae*. In such situations, farmers usually react by applying higher dosages and the results may be an upward spiral in chemical usage and costs, without improved pest control. The objectives of the present study were to determine the possible occurrence and wide spread of the problem of accelerated degradation of MITC in various field soils in Israel, and to assess prevention strategies in order to delay and/or

control the development of the phenomenon. We studied the relationship between rate of degradation of MITC after application of the fumigants in history soils (soil with previous application of MS or DAZ) compared with non-history soils. Soil samples collected from nine field sites in Israel without (non-history soil) or with a record of previous applications of MS or DAZ (history soil) were treated with the fumigants in the laboratory. The generation and dissipation rates of MITC in these soils were measured using solid-phase micro-extraction (SPME) and gas chromatography methods. Concurrently, a quantitative bioassay with two test organisms (*Verticillium dahliae* and *Fusarium oxysporum* f.sp. *radicis-lycopersici*) was used for assessing the changes in pathogen control efficacy associated with MITC degradation in soil. The accelerated degradation in history soils was expressed as a sharp reduction in the Concentration  $\times$  Time ( $C \times T$ ) values and half-life of MITC compared with non-history soils. In parallel to accelerated MITC dissipation rates, application to history soil resulted in reduced effectiveness in pathogen control compared with application to non-history soil. The occurrence and wide spread of accelerated MITC degradation in field soils in Israel was shown in this study. The management of accelerated MITC degradation requires appropriate strategies and further emphasizes the importance of alternating fumigants. The elucidation of the mechanisms involved in accelerated degradation of MITC may well provide valuable information for the adoption of suitable management tools. The phenomenon should be studied thoroughly also for other fumigants in order to develop integrated soilborne pest management schemes to prevent the rapid loss of efficacy of one chemical. (L)

### **Soil Solarization Using Sprayable Plastic Polymers to Control Soilborne Pathogens in Field Crops**

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Applying plastic films for soil solarization requires special equipment and procedures. A sprayable polymer product, 'Ecotex', was developed together with the technology for economical application on soil for various purposes. A black polymer formulation can raise the soil temperature and retain soil moisture under summer conditions in Israel. This process allows soil solarization and can significantly reduce populations of soilborne pathogens. The effect of soil solarization using sprayable polymers for controlling soilborne diseases in potato and peanut crops was tested in several field plots. Sprayable polymer (Ecotex, Nir Oz, Israel) was applied at rates of 800–1000 kg ha<sup>-1</sup>, using the special technology and sprayer which were developed for that purpose. In specific experiments, standard plastic mulch was applied for comparison. In order to enhance pest control, solarization was combined with fumigants or organic amendments, which were applied prior to mulching the soil. Soil coating using sprayable material resulted in a membrane film which can raise soil temperature to solarization level. The soil heating process with sprayable mulch is faster than that with plastic film, but the soil temperature is also cooled down more at night. Overall, soil temperatures under sprayable mulch are inferior to those obtained under plastic film. The thickness of the sprayed coat is critical to obtaining effective heating with the mulch. Combining solarization with formalin fumigation enables effective reduction of populations of *Streptomyces* at reduced dosages. Two field studies were established to test the effect of spray mulch solarization in the crop rotation, which included a 4-year rotation of potatoes, wheat, fallow and peanuts. Ecotex was combined with fumigants (formalin and applied before either the peanut or the potato crop. The combined treatment was effective in controlling Verticillium wilt in potatoes as the first crop in exp. 1 and peanut pot wart in exp. 2. The long-term effect of the treatments was evident in the increased yield of the third crop (peanuts in exp. 1, and potatoes in exp. 2) without any additional disinfection treatment. It was

pronounced also by the control of peanut pod wart (exp. 1) and Verticillium wilt of potato (exp. 2). In a third study, conducted in a field heavily infested with soilborne pathogens, Ecotex was combined with formalin or soymeal. Wheat was grown and incorporated as green manure before the peanut and potato crops in separate experiments. Solarization with sprayable polymers had a significant effect in controlling disease incidence of *Verticillium*, *Rhizoctonia*, and tuber scab in potatoes, and pod wart in peanuts. Soil solarization using sprayable membranes when combined with fumigants or organic amendments can be an effective soil treatment in field crops. (L)

### **Permeability of Plastic Films to Soil Fumigants and Mixtures: A Laboratory Study**

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Impermeable films can enable the use of reduced dosage of fumigants and thus can lower emission without decreasing pest control effectiveness. This was shown extensively for methyl bromide in many studies. The suggested alternative fumigants may also have negative environmental attributes. Evidence has shown that also other fumigants permeate through plastic films. In this study we employed an innovative approach to test the permeability of plastic sheets to fumigants which are formulated alone or in mixtures, are in liquid form and have low vapor pressure and high boiling points. However, it is also suitable for testing permeability of films to fumigants such as methyl bromide. The sampling system with the solid-phase microextraction (SPME) method enables accurate analysis of the amount of fumigant on either side of the plastic film and determination of the permeability of the tested film. The high sensitivity of the SPME fiber enables the detection of minute amounts of the tested compound, and make separate analysis of gases in mixtures or isomers of gases (such as for Telone [1,3-dichloropropene]). Thus, the behavior of fumigants in pure formulations compared with their behavior in mixtures can be assessed. All the tested fumigants permeate through the common LDPE film. The descending order of LDPE permeability to the fumigants in this study was MITC > methyl bromide > 1,3-dichloropropene > chloropicrin. The VIF (Barromid; Plastopil, Hazorea, Israel) film was impermeable to the tested fumigants. Permeation of MITC through VIF was also reduced compared with that through LDPE, but the level of reduction was lower. The permeation of some fumigants through LDPE films is influenced by the formulation applied. Permeation of chloropicrin was increased when it was introduced in mixtures with MBr (Bromopic). The permeation of 1,3-dichloropropene through LDPE was increased by 62% with a chloropicrin mixture (Telone C-35), as compared with pure Telone. In contrast, permeation of chloropicrin did not change significantly in the C-35 formulation. Permeation of both MBr and chloropicrin was increased by 25–30% when they were introduced as Bromopic mixtures rather than as pure compounds. Permeability of VIF was not altered with mixtures of fumigants. The vapor pressure of fumigants is influenced by the presence of other fumigants in the mixture. A high correlation was established between the concentration of the vapors in the source cell and rate of LDPE permeability to the fumigants. This correlation was evident for all the fumigants tested, indicating that the increase in plastic permeability to fumigant mixtures is due to increased vapor pressure. It is assumed that increased permeation of fumigant mixtures is not due to the plastic's physical or chemical properties. The data from this study should be validated in the field under normal climate conditions and agricultural practices. Confirmation of the results under field conditions can give the farmers as well as environmental protection authorities a rational and environmentally sound tool for managing soilborne pests. (L)