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INVITED LECTURES

Swarming behavior – the locust model – from biology to robotics

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The spectacular phenomenon of marching locust hopper bands, when millions of individuals move in coordination for hundreds of kilometers wasting natural habitats and crops, continues to threaten agriculture and challenge science. One of the main reasons for this is our insufficient understanding of some of the fundamental principles underlying the dynamics of locust swarming and coordinated collective behavior. These include key questions regarding locust biology, as well as other more general-theoretical aspects. To tackle such problems we adopted a panoply of methodological approaches: (a) Locusts are investigated under controlled conditions in the lab. (b) Individual tracking and state of the art analysis methods provide insights into the dynamic behavior of the individual and the group. (c) Theoretical modeling facilitates understanding of the interactions among individual locusts. (d) Manipulations of the controlled environment and simulations of individual and swarm decisions allow understanding the interactions between individual locusts, the swarm and the surroundings. Finally, (e) All the above is facilitated by developing the use of locust-inspired artificial intelligence (AI) and robotics. These will provide a mean of validating our findings, testing predictions and implementing new ideas. The presentation will demonstrate our multi-facet approach by reviewing recent findings, both published and as-yet-unpublished, and by describing some collaborations and future directions.

From microfluidic devices to native soil studies: the role of constitutive and induced production and secretion of plant secondary metabolites in above and below ground interaction

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Plants produce an extraordinary number of metabolites, estimated to be as numerous as 300,000 structures, and most likely many more. While we are far from understanding the role of all the phytochemicals known to date, most of them are expected to be involved in plant defense responses. The biosynthesis of these molecules is either constitutive or induced in a highly coordinated manner upon developmental or environmental changes. Our overall objective is to understand how plants coordinate the biosynthesis of these metabolites in time and space, with main emphasis on the interaction with other organisms. Various chemical classes produced in the epidermis or accumulating in the cuticular layer are

targets for detailed investigation. We employ several experimental set-ups including microfluidics, hydroponics and native soil. In my presentation I will provide several examples from both above and below ground interactions in which we exploit various 'omics' technologies combined with genetics to understand pathways of biosynthesis, transport and regulation associated with plant defense responses. Part of this multidisciplinary strategy includes the engineering of plants that normally do not produce certain classes of metabolites. These engineered plants serve as excellent tools to examine the relevance of a defined chemical class to plant defense responses. In a recent example, we demonstrated in tobacco plants engineered for the production of betalains the importance of these pigments for plant-fungi interaction, and not merely to flower color and pollination. I will also discuss future attempts to experiment with multiple organisms simultaneously in a single experimental set-up and to extend the research outdoors, to natural settings.

Session in honour of Prof. Jaacov Katan on the control of soilborne diseases

Application of measures to manage soilborne pathogens: A bottleneck or a tool for improvement?

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Soilborne pathogens survive and spread through various mechanisms, hence generating many sources of inoculum, all of which have to be identified and managed to ensure soil health and crop productivity. Additional sources of inoculum aside from the soilborne are generated by soilborne pathogens, including infested propagation material, contaminated water, transmission by insects and animals, inoculum adhering to greenhouse structures, weeds and other hosts. Hence, effective management of soilborne pathogens should target all the potential sources. The current trend in modern intensive agriculture involves pest control, while considering environmentally safe and pesticide-free products. Integrated pest management approach incorporates all agricultural practices which are relevant to crop production and which impact pest onset or suppression. Effective approaches have to manipulate all agricultural practices to reduce optimal conditions for pest outbreaks and hence to provide better conditions for plant development. It is based upon effective application of the appropriate measures at each crop phase, interfering with all stage of pathogen life cycle. These involve use of soil fumigants and other chemically and physically based and cultural measures. Special attention is given to application methods and technology to ensure maximal effect on the targeted pest. Special attention is giving at minimizing negative attributes to the crop, environment and the consumer, by the reduced use of toxic pesticides. Thus, assembly of all components within a production system is expected result in effective management.

Technologies to improve the health of propagation materials

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The health of propagation materials is a key factor in industrialized agriculture. The use of low-quality material or that infested by pests may not only reduce crop potential and quality, but will also infect the production system, and cause additional economic loss. The nursery is an intensive agricultural system, inhabited year-round and characterized by a large diversity of cultivars and maximal plant density. The quality and health control of the raw materials for seedling production such as seeds, cuttings, tissue culture-gown plants and growth media, as well as the production processes themselves, poses a significant phytosanitary challenge. This includes the prevention of pests and epidemics in the nursery and its products. Optimized growing protocols contribute to pest control and improve seedling tolerance to biotic and abiotic stress during their establishment. Adjusted fertilization programs increase the concentration of calcium in *Capsicum* young plant tissues by 30%; these seedlings, when transplanted into soil naturally infested with *Pythium aphanidermatum*, expressed reduced disease incidence by 60% compared to the control. However, sustained heat stress reduced seedling tolerance to this pathogen. Grafting, which is becoming increasingly common in *Solanaceae* and

Cucurbitaceae, provided tolerance to several soilborne pathogens and improves the reproductive performance of different species. For example, grafting cucumber or watermelon on a susceptible rootstock TZ or on the *Cucumber green mottle mosaic virus* tolerant rootstock 'Nurit', resulted in 29% and 54% reduction in disease progress in the field, respectively. In addition, 'Nurit' rootstock improved cucumber yield by up to 30% compared to the control. Additional means, based on physical approaches, biostimulation and different application methods, are being tested and implemented in the production process to improve propagation material health and performance.

The use of soil solarization to control invasive species at natural ecosystems

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Soil solarization is an effective agricultural method for soil disinfestation eliminating various pests, pathogens and weeds. Initial experimental applications in natural ecosystems were conducted to suppress *Acacia saligna* reinvasion after tree-clearing at Palmachim Nature Reserve. Seed bank removal was highly effective and resulted in almost zero seedling emergence 2 years after the treatment. However, the high costs and complexity associated with soil irrigation prior to mulching, as well as the small size of the experimental plots cast doubt on the feasibility of the method in large-scale areas. Two experiments have been conducted recently to adjust soil solarization to natural ecosystems. In the first experiment, soil was mulched immediately after the last winter rains to trap the natural soil moisture until the summer. The experiment was repeated in different years and in various habitats. In the second experiment, soil solarization was conducted on dry soil without prior irrigation at a large 0.4 Ha plot on the eastern shore of Lake Kinneret. Both experiments resulted in almost complete eradication of the *A. saligna* seed bank, followed by elimination of seedling emergence in the experimental plots, while in the control plots, the seed bank remained large and alive, leading to thousands of seedlings per square meter. Recently, we found that the solarization process is effective against other woody invasive species such as *Acacia salicina, Leucaena leucocephala,* and *Parkinsonia aculeate*. Therefore, soil solarization may be effective against various invasive species in different habitats.

Soilborne plant pathogens: aspects of integrated pest management

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Soilborne pathogens (root pathogens) cause heavy damage to many crops. These pathogens are closely connected with the soil and therefore are influenced by its chemical, physical and biological components, as well as by the agricultural practices, e.g., irrigation. The resting structures of the pathogens in soil do not germinate (fungistasis) and are, therefore, protected, although germinate when exposed to root exudates. Then, the pathogen penetrates the below ground host parts, causes a disease and forms resting structures, which enable it to survive. Disrupting of any of the above activities can become a potential tool for disease control. The approach for control has changed from a simple tactic of pathogen control to the strategy of integrated pest management (IPM) which also considers crop management practices, environmental, economic and legislative issues. The main IPM tool combines management methods in an optimal manner to reduce pesticide usage. Developing decision making tools which consider pathogen population disease

levels i.e., level of damage to the crop, i.e., level of economic damage, enables disease management only when necessary and efficient. The use of resistant cultivars, grafting and soil disinfestation are the major tools for the management of root diseases. The use of genetically modified plants is under debate. The methyl bromide crisis taught us painful lessons. Soil solarization has been adopted in many countries and the reasons for that will be discussed. There are additional measures for disease management e.g., biological control, fungicides, cultural practices, organic amendments and induced resistance. Although the control potential of these additional measures was demonstrated, their commercial use is limited and the reasons for that will be discussed. We believe that the use of the above measures will be expanded in the future.

Advanced methods for the identification of disease-causing agents

Application of Nanopore sequencing technology for diagnosis of plant pathogens

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Reliable detection and identification of plant pathogens are essential for disease control strategies. Serological and molecular methods, commonly used for diagnosis of plant pathogens, have limitations in detecting several pathogens simultaneously and with symptomatic plants of unknown etiology. The development of advanced second and third generations DNA sequencing technologies has enabled determination of total nucleic acid sequence in biological samples. The aim of this study was to examine the use of the Nanopore third generation sequencing platform as a method for diagnosis of plant diseases. This technology enables sequencing of single nucleic acid molecules and sequence analysis in real time. High molecular weight DNA extracted from tomato seed and plants inoculated with *Clavibacter michiganensis* subsp. *michiganensis*, *Pseudomonas mediterranea*, *Xanthomonas euvesicatoria* and *Fusarium oxysporium f.sp lycopersici* was sequenced and analyzed by WIMP workflow of EPI2ME cloud-data analysis. All the pathogens were identified in samples of 200 seeds containing one infected seed of each pathogen. In inoculated symptomatic plants, the pathogens were identified and classified with high probability. This technology can provide a powerful diagnostic tool for laboratory and field detection of plant pathogens including those that are unculturable or unknown.

Detection of plant viruses and viroids in tomato seeds using Next Generation Sequencing (NGS)

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The recent increase in diseases caused by plant pathogenic viruses and viroids can be attributed to contaminated seed lots, which common diagnostic techniques failed to detect. Our objective is to develop new, sensitive and accurate diagnostic methods, based on Next Generation Sequencing (NGS) technology, for detection of viruses and viroids in seeds of important vegetables in Israel. We suggest that NGS technology will improve the detection of virus/viroid in low titer contaminated seed lots. Importantly, NGS technology allows simultaneous detection of various pathogens and the identification of unknown pathogens. Tomato seeds infected with *Tomato brown rugose fruit virus* (ToBRFV) and *Potato spindle tuber viroid* (PSTVd) served to calibrate the working protocol. Library constructions from both total RNA and siRNA extracted from dry or wet seeds were prepared and sequenced by Illumina platforms (MiSeq for siRNA; HiSeq for total RNA). Bioinformatic analyses of the siRNAs from the infected seeds showed reads which mapped to ToBRFV and PSTVd. In addition, the number of small RNA viral reads was significantly higher in wet seeds compared to dry seeds. To test the sensitivity of the NGS detection method, a sample of one infected seed in 50 healthy seeds was

subjected to analysis by the Hi-Seq platform. The results of total RNA allowed assembly of the complete genome of ToBRFV; viral reads from total RNA were higher in dry compared to wet seed samples. Our results suggest that NGS sequencing could be used for detection of low titer of viruses and viroids in seeds.

Development of a molecular marker for the detection of an *Alternaria alternata* isolate which causes "Black Heart" disease of pomegranate

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Pomegranate fruit rot, known as "heart rot", caused by the pathogenic fungus *Alternaria alternate* is a major disease that impacts production worldwide. Heart rot is characterized by black rot of the fruit core that spreads from the calyx area, while the outer peal retains a healthy appearance. Although 90% of the stigma are infected by *A. alternata*, only up to 50% of the fruit develop disease in the orchard. One possible explanation for the disease incidence in the orchard is the relatively low abundance of a species-specific pathogenic *A. alternata* strain (pathotype), causing heart rot of pomegranate. The disease damage causes economic loss due to yield loss. Disease management today is only by sanitation of symptomatic fruit from the orchard. Fungicides are not effective. Development of a molecular marker for detection of the pathogenic pathotype is a significant tool for future disease management. The main goal of this study is to find a molecular marker for the detection of the pathogenic *A. alternata* pathotype. Isolates of *A. alternata* from symptomatic fruit were collected along with other *A. alternata* pathotypes from persimmon, citrus and pomegranate (black spot), for isolation of DNA and apPCR amplification by 50 different primers. In addition, full genome sequencing of the different *A. alternata* pathotypes is proceeding. Differences found in the full genome of the heart rot pathotype compared to the others will be used to design specific primers to heart rot *A. alternata* pathotype.

Epidemiology and disease dynamics

Systemicity of *Peronospora belbahrii* in basil plants

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The oomycete *Peronospora belbahrii* causes severe downy mildew disease in basil plants. The disease appears in the field as chlorotic lesions on leaves, which gradually turn necrotic and abscise. Here we report that *P. belbahrii* can spread systemically in basil plants. In controlled experiments in growth chambers, potted basil plants were inoculated on their cotyledons, first, second, third, fourth or fifth pair of leaves and incubated overnight in a dew chamber to ensure infection, then at 25 °C under light to allow symptom production and a night at 100% air relative humidity to allow for sporulation. Symptoms, sporulation, microscopy and PCR were used to determine the movement of the pathogen in the plants. In all cotyledon-infected plants, the newly developed two or four true leaves sporulated heavily, and the stem displayed the specific 134 bp DNA fragment of *P. belbahrii* after PCR reaction. Such systemically infected plants stopped growing. Plants inoculated at the 2-4 leaf stage showed systemic spread in 70–90% of the plants; those inoculated at the 6–8 leaf stage showed systemic spread in about 50% of the plants and those inoculated at 10–12 leaf stage showed systemic spread in about 20% of the plants. In 6–12 leaf plants the auxiliary buds below and above the inoculated leaves became infected. Microscopic examinations and PCR assays confirmed the movement of the pathogen from the lamina of an inoculated leaf to its main vein and then to the lower and the upper stem. However, the pathogen never reaches the root. The data confirm that *P. belbahrii* can move systemically in basil plants along the stem vascular tissues to reach the apical meristem and auxiliary buds, more so in younger than in older plants.

An unprecedentedly delayed outbreak of potato late blight in Israel in autumn 2016

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Every year in the last decade late blight caused by *Phytophthora infestans* in potato crops erupted at late October to middle of November in Israel. However, in autumn 2016, no late blight was recorded in the country. It appeared as late as middle of March 2017, 5 months later than usual. To understand the reasons for this unusual delay in late blight appearance we examined the potato seed tubers used and analyzed the weather conditions that prevailed in October 2016–March 2017. Six hundred tubers were sown at the BIU farm under nets in December 2016. Five weeks after sowing six plants showed symptoms of late blight on foliage and below ground parts of the stems. Mother tubers looked healthy, but upon slicing and incubation in moist conditions all tuber slices showed sporulation of *P. infestans*, suggesting that the seed tubers used by farmers were symptomless-infected with late blight. Weather analysis indicated two major reasons that could account for the delayed infection with late blight: shortage of rain between October 2016 and February 2017 relative to previous years, and a 60% reduction in the number of potential infection events, namely periods of 6 h of >90% air relative humidity at temperatures of 10–20 °C. We concluded that an initial inoculum *of P. infestans* was present in autumn 2016, but the environmental conditions for its spread were not conducive.

Identification and characterization of nematodes associated with bark beetles (Scolytinae) in Israel

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Since the late 1990s the pine wood nematode (PWN) *Bursaphelenchus xylophilus,* the causal agent of Pine Wilt Disease, has spread in the western Mediterranean. *Bursaphelenchus* spp. are vectored by bark beetles and wood borers, while native *Bursaphelenchus* spp. are not considered a problem. The possible establishment of PWN in Israel and the absence of information about local *Bursaphelenchus* spp. were the incentives for the present study. Common nematode species associated with bark beetles are often phoretic and many feed on fungi or bacteria, also carried by the beetles; other are parasitic on the beetles. The relationships between nematodes and bark beetles has not been previously studied in the Eastern Mediterranean. The objective of the present study was to create an initial database of scolytid-associated nematodes in Israel with special emphasis on *Bursaphelenchus* spp. Eight species of bark beetles were examined for the presence of nematodes. The identification was based on morphological characteristics along with molecular analysis, using general primers for ITS, 18S and 28S rDNA regions common for members of the order Nematoda. The resulting sequences were compared with the GenBank database. Fourteen nematode species were identified of which five were *Bursaphelenchus* spp. The other species belong to the genera *Micoletzkya*, *Ditylenchus*, *Deviburs aphelenchus*, *Cryptaphelenchus* and two parasitic species of the genera *Parasitylenchus* and *Parasitorhabditis*. The greatest number of species were associated with the pine bark beetle *Orthotomicus erosus*. The identified nematodes are not considered a hazard to the tree hosts of their beetle vectors and none of the examined specimens was genetically related to PWN.

Seed coating and drip protection against *Harpophora maydis* in the field

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Late wilt is a severe maize disease characterized by relatively rapid wilting of plants at the tasseling stage. The disease's causal agent, *Harpophora maydis*, is currently controlled using resistant maize cultivars. Earlier we showed that Azoxystrobin (AS), injected into a drip irrigation line assigned for each row, prevents the disease symptoms in the field. Here, we examine an economically practical treatment using AS fungicide in a mixture with Difenoconazole (DC), or other new fungicide mixtures, in a combined treatment of seed coating and drip protection for two coupled rows (row spacing was 50 cm instead of 96 cm). Real Time PCR tests revealed that AS-DC seed coating alone managed to delay the pathogen spread in the maize tissues up to the age of 50 days (near the appearance of the first symptoms and the fertilization at days 55–57), but was not sufficient to prevent the disease outbreak later. Drip protection with AS-DC was the most successful treatment and in the double-line cultivation reduced the fungal DNA in the host tissues to near zero levels. This treatment inhibited the development of wilt symptoms by 41% and recovered cob yield by 20%. Moreover, the A class yield (cob weight of more than 250 gr) increased from 27 to 63% with this treatment. No residues of this fungicide were identified in the maize cobs. This economically successful treatment to prevent maize late wilt disease in infested fields can now be applied in vast areas to protect sensitive maize cultivars against the pathogen.

Plant-pathogen interactions

New directions on the regulation of patulin biosynthesis during colonization of deciduous fruits by *Penicillium expansum*

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Penicillium expansum, the causal agent of blue mold rot, is a critical health concern because of the production of the mycotoxin patulin in colonized apple fruit tissue. Although patulin is produced by many *Penicillium* species, the factor(s) activating its biosynthesis are not clear. LaeA was found to regulate several secondary metabolite genes, including the patulin gene cluster and concomitant patulin synthesis *in vitro*. Sucrose, a key sugar component of apple fruit, was found to modulate patulin accumulation in a dose-responsive pattern. An increase in sucrose culture amendment from 15 to 175 mM decreased both patulin accumulation and expression of the global regulator laeA, whilst increasing expression of the carbon catabolite repressor creA. Patulin synthesis was greatly decreased in Δ*creA* mutants and metabolic profiling of these mutants reveals that CreA loss impacts the production of patulin. Δ*creA* mutants were reduced in fungal growth and delayed in spore formation and germination compared to control strains. Furthermore, Δ*creA* mutants were nearly avirulent and did not produce patulin in apples. The results suggest the importance of LaeA and CreA on the regulation of patulin accumulation.

Characterization of *Acidovorax citrulli* strains isolated from solanaceous plants

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Bacterial fruit blotch disease, caused by *Acidovorax citrulli*, is an economically important threat to the cucurbit industry worldwide. Two genetically distinct groups of *Acidovorax citrulli* are known: strains of group I infect a range of cucurbit species, whereas strains of group II are mainly isolated from watermelon. In recent years, strains that reacted positively in PCR

with *Acidovorax*-specific primers were isolated from tomato and eggplant plants showing dark-brown lesions on the foliage. The aim of this study was to characterize solanaceous strains in comparison with those isolated from melon and watermelon. Analyses with 16S rRNA sequencing, rep-PCR with BOX and ERIC primers, pulsed-field gel electrophoresis and PCR with primers based on the putative type III secretion effector gene, *Aave_2166*, revealed resemblance of the strains isolated from *Solanaceae* to *Acidovorax citrulli* group II strains. Nanopore sequencing and genome assembly of the two strains further support this similarity. Pathogenicity assays by puncture-inoculation of stems of watermelon, melon, tomato and eggplant seedlings showed that the solanaceous strains were more virulent on tomato, eggplant and watermelon than on melon.

The Root-Knot Nematode and tomato root: a glance into a sophisticated dialog

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Root-Knot Nematodes (RKN), *Meloidogyne spp*., are among the most devastating pathogens, threatening a wide range of crops' production worldwide, causing major annual economic loss. Our main hypothesis is that RKN effectors interact with genes implicated in lipid-signaling pathways to modulate plant defense responses. To reveal the function of lipid signaling involved in plant defense response against RKN infection, we explored oxylipins (fatty acids derivatives) changes following parasitic interaction. Therefore, a full oxylipin profile was determined in tomato roots inoculated with *M. javanica* by LC/MS-MS. Many oxylipins were found to fluctuate throughout different stages of infection, indicating their involvement. A transcriptome analysis was performed after exposing *M. javanica* to two oxylipins (9-HOT, 13-KOD) and tomato protoplasts which induce many potential effectors. Candidate effectors that might promote parasitism were selected and determined as potential effectors by Fluorescent In Situ Hybridization (FISH). Going forward, the target compartment of effectors will be identified *in planta* and functionally identified. This study will shed light on critical components which regulate the parasitic interaction and will provide novel insight for environmentally-friendly nematode management.

Mango fruit anthocyanin and the resistance to biotic and abiotic stress

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Mango fruit of eighty-three cultivars from all over the world were inoculated with *Colletotrichum gloeosporioides* or stored for 2 weeks at a sub-optimal temperature. Interestingly, red cultivars that accumulate high amounts of anthocyanin in their cuticle were overall more resistant to both biotic (anthracnose) and abiotic (chilling) stress. To validate that anthocyanin and red color peel of mango fruit are correlated to biotic and abiotic tolerance, red and green 'Shelly' mango fruit from the same trees were evaluated. Mango fruits developing at the exterior of tree canopy are exposed to sunlight and acquires a red peel color on the sun-exposed side compared to the green peel fruit that develop within the canopy. Measurements of the red mango peel showed a significant increase in anthocyanin, flavonoids and antioxidant accumulation, while the ripening parameters of both red and green mango fruit were similar. However, after 3 weeks of suboptimal cold storage 'green fruit' developed significantly more chilling injury symptoms than 'red fruit'. Furthermore, 'red fruit' were found to be more resistant to an inoculation with *C. gloeosporioides* and showed a reduction in general decay incidence both at the red and green side of the fruit. Organic extraction of red fruit peel showed more antifungal activity and inhibition of spore germination when compared to green fruit. Thus, red mango fruit that accumulate large amounts of anthocyanin showed increase resistance to chilling and pathogens. The

results point to new agro-technological approaches to induce red color in the peel of mango fruit to extend fruit quality and shelf life.

Search for the *Fusarium oxysporum* f.sp. *melonis* Avr and its protein interactors

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Fusarium wilt is an important disease in melons that infects the roots and spreads through the xylem vessels. In the xylem it produces and secretes small effector proteins, also called Avr factors, that play a role in infection. NBL (nucleotide binding site–leucine rich repeat) encoding genes are the prevalent class of resistance genes. Their products recognize *Avr* factors and initiate a defence response. In a previous proteomic study we identified in the xylem sap a candidate Avr^2 for the melon resistance gene *Fom-2*. In a yeast-two-hybrid test Avr^2 did not bind Fom-2, however they interacted in *Nicotiana benthamiana* leaves to elicit a hypersensitive response. We constructed a yeast-expressed cDNA library from Fusarium-infected melon roots and screened it with an Avr^2 bait. Several putative plant protein interactors were identified. These serve as a starting point to study Fusarium-melon recognition at the protein level.

Functional characterization of the type III-secreted effectors of the plant pathogenic bacterium *Acidovorax citrulli*

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Some plant pathogenic bacteria employ a type III secretion system (T3SS) to deliver protein effectors into the cytoplasm of the host cell. Type-III secreted effectors (T3Es) promote bacterial virulence through alteration of the host cell metabolism and/or by suppression of host defense responses. *Acidovorax citrulli* is a Gram-negative bacterium that causes bacterial fruit blotch of cucurbits, a devastating disease that threatens watermelon and melon production worldwide. Based on genetic, biochemical and host preferential association, the *A. citrulli* population is divided into two major groups. Group I includes strains that have been mainly isolated from melon and other non-watermelon cucurbits, while group II strains have been mainly isolated from watermelon. We performed a comparative analysis of T3E genes from a global population of *A. citrulli* strains. This analysis revealed that the two groups significantly differ each from the other in their T3Es' repertoire. One of the effectors we are interested in is AopW1, which is homologous of the *Pseudomonas syringae* effector HopW1-1. HopW1-1 was shown to promote *P. syringae* virulence through disruption of the actin cytoskeleton. AopW1 is conserved among group I and II strains of *A. citrulli*, except for a hypervariable region (HVR) of 45 amino acids, located at the N-terminal/central part of the protein. Heterologous expression in yeast and transient expression in *Nicotiana benthamiana* of AopW1, as well as virulence assays on melon and watermelon with *A. citrulli* mutants impaired in this effector, revealed that the group I version of AopW1, but not the group II version, possesses strong toxic activity and makes a significant contribution to virulence.

Reversion from virus infection in East African cultivated sweetpotatoes

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Viruses limit sweetpotato (*Ipomoea batatas* L.) production worldwide. Many sweetpotato landraces in East Africa are, however, largely virus-free; plants infected by the

prevalent *Sweet potato feathery mottle virus* (SPFMV) appear able to revert to virus-free status. The ability to revert from virus infection was tested for the *Potyviridae* SPFMV, *Sweet potato virus C* and *Sweet potato mild mottle virus*, the *Closterovirid Sweet potato chlorotic stunt virus* and the *Geminivirid Sweet potato leaf curl Uganda virus*, using the indicator plant *I. setosa* and PCR/RT-PCR. Reversion from these viruses was investigated in the East African cvs. New Kawogo, NASPOT 1 and NASPOT 11, and cvs. Resisto and Beauregard from the USA. The rates of reversion from infection by viruses of different families varied between the cultivars. The East African cultivars reverted at a high rate from most viruses, but the American cultivars seldom reverted. None of the tested cultivars reverted from single or double infections involving *Sweet potato chlorotic stunt virus*, but reversion was observed in co-infections involving potyviruses only. The SPFMV-reverted plants were successfully re-infected with SPFMV using graft inoculation (but not by sap inoculation) and would subsequently revert again at a much faster rate than during the first infection. Reversion generally increased with increasing temperature and was enhanced by improved nutrition. These results indicate that the natural ability of East African sweetpotato cultivars to revert from virus infection is malleable and could provide means of virus management.

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Towards recommissioning of the fungal collection of the Hebrew University of Jerusalem

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The National Collections of Natural History at the Hebrew University of Jerusalem assembles 12 collections from various fields, including the National Plant Collection. The plant collection contains a rare assortment of phytopathogenic fungi collected by Prof. Tscharna Rayss (1890–1965). The collection contains thousands of samples of plants with fungal diseases from Israel and abroad, identified and catalogued by Prof. Rayss. We show how, after decades of dormancy, the fungi collection now returns to life. The collection is now being cataloged digitally to build a database for the use of the research community. We will present two examples of samples from the 1940s that have successfully passed molecular identification and shows that the collection can serve as a source of biological material for various studies. In addition, after years of inactivity, the revival of the internationally recognized collection permits the deposit and documentation of new species, which started several months ago. Hence, the old collection is a valuable resource that will enable future research in the fields of pathology, ecology and evolution.

Epidemiology of diseases caused by viruses

The role of the *CsRDR1b* and *CsRDR1c* gene family in cucumber viral defense

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RNA-dependent RNA polymerase 1 (RDR1) plays an important role in antiviral defense and plant development by the silencing mechanism. The activity of these proteins is based on the synthesis of double strand RNA that triggers the endogenous gene silencing system. Four RDR1 (*RDR1a, b, c1, c2*) genes were identified in cucumber having different expression levels before and after infection by different viruses. The *CsRDR1a, b, c* genes have a homology of 60–58% at the amino acid level, while *CsRDR1c1* and *c2* are almost identical (97%), but have different

promoter sequences. A high expression level of *CsRDR1b* was characterized in cucumber varieties with partial resistance to viruses, while *CsRDR1c1* and *c2* were not expressed in healthy plants. Cucumber plants infected by different viruses showed a moderate expression level of *CsRDR1b* and high expression levels of *CsRDR1c1, c2*. The differences in the rates of expression between *CsRDR1b* and *CsRDR1c1, c2* in healthy and infected plants indicates differential regulation of members of the RDR1 gene family. Using the CRISPR/Cas9 system we created a variety of *rdr1b* mutants and *rdr1c1*,*c2* double mutants. Knockout of these genes increased host virus susceptibility together with virus accumulation. Plants with a homozygous *rdr1c1, c2* double mutation showed increased susceptibility to *Cucumber mosaic virus* 4–6 days post infection, followed by collapse few days later. The same mutants showed intensified symptoms with *Cucurbit vein yellowing virus* infection, whereas the susceptibility to *Zucchini yellow mosaic virus* infection was lessened. Deciphering *CsRDR1b* and *CsRDR1c1, c2* regulation will promote the understanding of the unique silencing mechanism in cucurbit plants.

Characterization of *Potato virus Y* populations in potato in Israel

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Potato is the largest crop in Israel. Potato virus Y (PVY) is the most common virus infecting potato worldwide. PVY sequences collected from all over the world revealed five non-recombinant strains and 36 PVY recombinant patterns. The genetic structure of the Israeli population was not previously characterized and no sequences of PVY from Israel were available in the databases. In our laboratory we analysed the infection rate of potato tubers that were collected from fields in the NW Negev region during 2014–2017. An initial characterization of PVY field strains was performed by multiplex PCR, where PCR product patterns could distinguish strains based on nucleotide polymorphisms around the PVY recombination junctions (Chikh-Ali *et al.* Plant Dis. 97, 1370, 2013). Five different patterns were determined suggesting 5 different recombinant types. To verify these results, each gel recombinant pattern was "deep sequenced" by Illumina MiSeq. The results show that 3 gel patterns represent the same PVY recombinant, PVY-NTNa, and the other strains being the recombinants PVY-NWi and PVY-SYRIII. The full sequences of these recombinant isolates show that the primers designed for PVY typing by Chikh-Ali *et al.* are not compatible with the Israeli strains. Therefore, a new set of primers for a multiplex PCR assay were designed and found to differentiate clearly between the 3 Israeli PVY recombinants. Analysis of the PVY population in potato in the NW Negev from 2014 to 2017 showed that the NTNa strain was the main PVY recombinant. Interestingly, the archetypal PVY-O strain was found only once. Also, the SYRIII strain (first isolated in Syria), was found in Israel 2015, but not in 2014 and 2017. PVY recombinant typing has an important role as a diagnostic tool, in understanding the origin of the PVY infection of potatoes and the viral population dynamics.

Sources of disease resistance

Macrophomina phaseolina: screening for resistance in strawberry (*Fragaria×ananassa*) and determining pathogen genetic diversity in Israel

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Macrophomina phaseolina is a typical soilborne pathogen causing wilting, root and crown rot, affecting a host range of more than 500 botanical species. In parallel to methyl bromide phase-out, *M. phaseolina* has become the most serious soilborne pathogen of strawberry in Israel. Recently, screening for resistant/tolerant

strawberry germplasm to *M. phaseolina* has commenced, based on the assumption that resistant plant material has been detected in other botanical species. Different inoculation techniques were assessed to aid in identifying resistance. The "toothpick method", which is the most common, appeared to be unselective and aggressive in comparison to inoculation using artificially produced sclerotia in a soil mix or drench, which was found to be more selective and efficient. Under artificial inoculation conditions in a growth chamber and natural infestation in the field, a high variability of susceptibility/tolerance of strawberry cultivars to the pathogen was found. To study pathogen genetic diversity arbitrarily-primed PCR was performed on DNA of *M. phaseolina* isolated from 195 strawberry plants and eight melon plants in Israel, and compared to a representative population of 8 isolates from strawberry and melon from California, USA. High variability was found between strawberry and melon, and also between populations from Israel and California. Genetically different groups will be further analyzed with SSR genotyping. Cross pathogenicity tests will be performed between strawberry and melon in order to determine host specificity.

Resistance to downy mildew in cucumber breeding lines

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Cucumber downy mildew (CDM) caused by the oomycete *Pseudoperonspora cubensis* is a devastating disease of cucumber worldwide. After the resurgence of the disease in 2002 in Israel and in 2004 in the USA, new mating types, pathotypes and races appeared. No resistant cultivars were available. Here we report on a successful multi-year project to develop cucumber lines resistant to multiple isolates/pathotypes of *P.cubensis.* Two wild, resistant, low quality accessions of *Cucumis sativus*, PI 197088 and PI 660328, which share some (but not all QTLs) for resistance, were first stabilized for resistance by self-pollination and thereafter crossed with each other to produce F1. After self-pollination for three generations, an F4 pedigree plant was crossed with an elite CDM-susceptible cucumber line. Multiple-isolate resistant, high-quality plants that were selected in the F2 were backcrossed to the elite susceptible parent. After six generations of selection for resistance and quality, we obtained stable, highly-resistant breeding lines with superior quality. We also report here on QTLs associated with resistance of PI 197088 against multiple isolates of *P. cubensis*. During 2016 and 2017 the response of a segregating a F2 family (SMR-18 x PI-197088, n = 170) to 7 CDM isolates was examined in both the laboratory and the field. NGS was performed for genotyping, and polymorphic SNPs were obtained from the same populations in both years. The specific QTLs obtained for each isolate were as follows: 23C (two on chr' 4 and two on chr' 5); Pol.1 (one on chr' 1, one on chr' 4, two on chr' 5); Pol.4 (two on chr' 7); US-506 (two on chr' 1 and one on chr' 2); 81C (two on chr' 4 and two on chr' 5; 88C- two on chr' 3 and one on chr' 6; 90C- one on chr' 1, two on chr' 4 and two on chr' 6); field isolate 2016 (two on chr' 3 and one on chr' 5); field isolate 2017 (one on chr' 4 and one on chr' 5). The data support the notion that inheritance of resistance is isolate-dependent. Previously, 11 QTL were identified for CDM resistances in a similar family accounting for more than 73.5% total phenotypic variance.

Search for a genetic source of resistance to carrot yellows disease

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Carrot yellows disease causes extensive damage to the carrot industry worldwide. The presumed causal agents of the disease were the Gram-positive obligatory pathogens – Phytoplasma and Spiroplasma. However, reports in the last decade show that a different bacterium, *Candidatus* Liberibacter solanacearum (Lso), is best correlated with disease symptoms. Lso is an obligatory, phloem dwelling bacteria, and is transmitted by carrot psyllids (*Bactericera trigonica* or *Trioza apicalis*). The main means of disease control include intensive

chemical spraying against the vector, which are largely insufficient. Different species of *Ca*. Liberibacter are known to cause diseases in several other crops, however, no genetic resistance was reported. In this study 99 carrot cultivars were evaluated for disease tolerance using small-scale and field experiments under the assumption that such evaluation would form the basis to breeding projects as well as assist in understanding Liberibacter diseases. Initially, symptoms were evaluated at the seedling stage using deliberate inoculation in insect cages, yet a notably resistant cultivar was not found. Subsequently, the 99 cultivars were transplanted into a commercial field. Psyllid population and egg laying were monitored, and symptom severity was evaluated at harvest. We found a positive correlation between egg laying and disease severity in the Asiatic carrot cultivars, also presenting less susceptibility then the Western cultivars. Overall, 25 cultivars presented a significantly reduced symptoms in relation to the leading commercial cultivar in Israel. Approximately 88% of symptomatic carrot samples were Lso positive in quantitative-PCR, which strengthens the correlation between Lso and disease symptoms. A direct infection experiment will be designed to confirm significant decreased susceptibility of the Asiatic cultivars.

Association of resistance to powdery mildew in watermelon with ploidy and earliness

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Powdery mildew (PM) caused by *Podosphaera xanthii* is a serious disease of watermelon, attacking the crop at all seasons and all locations. Current commercial cultivars, diploid or triploid, are susceptible to powdery mildew. Farmers rely mainly on fungicides to control the disease. Our inheritance studies showed that resistance against *P. xanthii* races 1W and 2W, derived from *Citrullus lanatus* var. *citroides* BIU-119, PI 189225, or PI 482312, is controlled by one partially dominant gene at the cotyledon stage but requires another additive gene in adult plants in the field. Here we show that disease progress in the field was dependent on both the presence of resistance genes and the ploidy of the entries used. Tetraploid watermelon lines (resistant, R or susceptible, S) were produced by colchicine treatments. They were crossed with diploid lines (R or S) to create four F1 triploid (3n, seedless) hybrids: 4n(R)x2n(R); 4n(R)x2n(S); 4n(S)x2n(R); and 4n(S)x2n(S). These four hybrids together with the diploid F1 hybrid 2n(R)x2n(S) and the tetraploid F1 hybrids 4n(R)x4n(S) were tested for resistance to PM in the field in four seasons during 2015–2017. The results showed that 4n(R)x4n(R) and 2n(R)x2n(R) were equally most resistant lines. The diploid hybrids 2n(R)+2n(S) and tetraploid hybrids 4n(R)x4n(S) showed both similar intermediate resistance. The seedless hybrid lines 4n(R)x2n(S) were more resistant than the reciprocal seedless hybrid lines 4n(S)x2n(R). The most susceptible lines were the hybrids of which both parents were susceptible. When hybrids and commercial lines were assessed for PM severity throughout the growing season, significant differences in area under the disease progress curve were recorded between lines, depending on the onset of female flowering. Early flowering lines were more susceptible compared with late flowering lines. In late flowering lines, disease severity sharply increased a few days after anthesis. We conclude that resistant to PM in watermelon depends on both ploidy level, number of resistance alleles, and onset of flowering.

Development of biological control agents

REGEV®: the first hybrid fungicide of STK

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Integrating biologicals into conventional spraying programs is the next logical revolution enabling food growers from all sectors and geographies to better meet market demand, increase their ecologically friendly footprint and thrive economically. STK's innovative biologic food protection products complete the conventional spray programs, at once extending the lifespan of chemical efficacy and mitigating its harmful effects. This hybrid approach is a practical path towards a more secure and sustainable agricultural ecosystem. REGEV® (Tea Tree Oil 400 g/L+ Difenoconazole 200 g/L EC) is a patented hybrid product with two

modes of action, belonging to 2 different FRAC groups (Group 7 and Group G1) in one product. REGEV® is an effective product against a wide spectrum of plant diseases, such as: Apple Scab, Powdery Mildews, Early Blight, leaf spot diseases and others, in a wide range of hosts including field, vegetable and row crops. REGEV® formulation is easy to handle, easy to use and with a good storage stability. Using REGEV® enables reduction of the chemical load and avoidance of negative environmental impacts. REGEV® is a valuable tool to be easily incorporated into resistance management programs and well suited for use in integrated pest management (IPM) programs, providing an outstanding value for money. REGEV® is available for growers in Israel, Serbia, Dominican Republic and Guatemala. During 2018 it will also become available in Argentina, Colombia, Honduras, Nicaragua, Panama and Peru. Other countries are in the registration process and will be available in the next coming years.

"Stinky endophytes–a wonderful surprise"

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Endophytes are microorganisms that spend most of their life cycle inside the plant without causing any external symptoms to the host. Most endophytes involve symbiotic relations with their host, contributing to it in a variety of ways among which are growth enhancement and protection against biotic and abiotic stresses. Some of these endophytes produce and secrete into their environment biologically active secondary metabolites, as part of their biological/chemical warfare against competitors. Most of the secreted compounds are soluble. In some cases these biologically active secondary metabolites are volatiles (Volatile Organic compounds–VOCs). Fungi are known as volatile producers; even though there is limited amount of reports describing the activity of these VOCs. As part of a project aimed at isolating and characterising endophytes from trees, for use in fruit trees disease biological control, we isolated a fungus, later identified as *Daldinia cf. concentrica.* The fungus had a very distinct fruity (strawberry) odor when grown on potato dextrose agar media. The unique odor intrigued us to test the fungus and its VOCs for biological activity against plant pathogenic fungi and pests. We found that some of these VOCs inhibit and kill a variety of fungi and pests of fruit, seeds and soil (nematodes and aphids). These results drove us to test the option of using these compounds as pesticides of natural origin in a variety of experiments. Currently, in collaboration with Luxembourg Industries Israel and Copia Agro & Food company we are in the process of developing soil disinfectant products based on these compounds.

Nobactra: a biological pesticide registered for the control of bacterial diseases in plants

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Bacterial diseases are common in all crops and cause severe economic damage. Current control methods focus on prevention including use of clean propagation materials, phytosanitary methods, and creating a non-conducive environment for the pathogens. Copper compounds, and in exceptional cases, antibiotics are also used. The weakest link are pesticides, which have regulatory limitations, can be phytotoxic, show resistance and have low efficacy rates. Nobactra's solution is biological, safe, environmentally friendly and leaves no residues. It consists of two groups of active ingredients which when combined create a synergistic effect. The first component is a cocktail of antagonistic bacteria and the second component is a special powder formulation that contains essential oil. This combination is novel as it uses of antagonistic bacteria in a cocktail. Nobactra comprises 10 bacteria isolated from agricultural land which have not been genetically modified. The advantages of the cocktail are: efficiency in different growing seasons, consistency in different environmental conditions (due to the different requirements of each bacterium), multiple modes of action, low probability of developing resistance against the mixture, and a wide spectrum of activity. Additionally, the oil powder formulation of Nobactra has advantages including a significant reduction of phytotoxicity, ease of transport, and

the addition of a unique carbon source for the bacteria. Nobactra is registered in Israel for use in nurseries and for the treatment of salmonella in hatchery eggs. The label is being expanded to include further diseases which this formulation controls in nursery and farming conditions: bacterial canker in tomatoes (*Clavibacter*), bacterial scab in potato tubers (*Streptomyces* spp.) and black rot in crucifers (*Xanthomonas* spp.). We are working to develop a solution for Citrus Greening caused by the bacterium *Candidatus liberbacter spp*. Trials are underway in Florida.

Synthetic volatile mixture, from a fungal origin, controls *Sclerotium rolfsii* both *in vitro* and in soil

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Sclerotium rolfsii (also called *Athelia rolfsii*) is a phytopathogenic fungus and is the causal agent of "southern blight" disease in economically important crops. The elimination of this fungus is problematic due to its extremely wide host range and its capacity to form sclerotia. Recently we have shown that the endophytic fungus *Daldinia cf. concentrica* emits biologically active volatile organic compounds (VOCs). We also demonstrated that a synthetic volatile mixture (SVM), comprising 4-heptanone and trans-2-octenal in volumetric ratio of 1:1, was the most effective against various phytopathogenic fungi. The objective of this work was to evaluate the potential of this SVM to control hyphae and sclerotia of *S. rolfsii*, both *in vitro* and in soil. We found that the SVM fully controlled hyphae and sclerotia in Petri plates. We used a controlled laboratory system to simulate soil conditions, and found that the SVM affected the viability of both hyphae and sclerotia of *S. rolfsii*. We also examined the ability of the SVM to affect the fungus viability in different soils. Loam soil enabled the most efficient control of the fungus, followed by sandy soil. In contrast, planting mixture was less suitable for this purpose with nearly no effect of the SVM on *S. rolfsii* hyphae and sclerotia. In addition, application of SVM at a concentration of 0.5 ml/kg soil had no effect on the growth of tomato seedlings. Based on our result, we suggest the use of our SMV as an alternative approach for the control of *S. rolfsii*.

Random peptide mixtures inhibit different phytopathogenic bacteria and reduces symptoms of plant diseases caused by *Xanthomonas*

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Plant pathogenic bacteria are among the most important causal agents of plant diseases with almost all major crops being severely affected by one or more important bacterial diseases. The chemical control of bacterial plant diseases in the field greatly relies on copper-based bactericides, yet with limited efficacy. Moreover, the occurrence of copper resistant strains has been reported. Antimicrobial peptides have been studied as potential crop protection agents. In the present study we explored the potential of two random peptide mixture (RPM) models as novel crop protection agents. These unique peptide mixtures consist of random combination of L-phenylalanine and L/D-lysine (FK and FdK, respectively) along a 20-mer peptide chain. Both RPMs displayed powerful bacteriostatic and bactericidal activities towards strains belonging to several plant pathogenic bacterial genera including *Xanthomonas*, *Clavibacter* and *Pseudomonas*. Greenhouse studies revealed that RPMs significantly reduced disease severity of tomato and kohlrabi plants infected with *Xanthomonas perforans* and *Xanthomonas campestris* pv. *campestris*, respectively. Moreover, RPM effects on reduction of disease severity were similar to those exerted by the commercial copper-based bactericide Kocide 2000, applied at 12-fold concentration to the active compound relative to the RPM

treatments. Importantly, the two tested RPM compounds had no toxic effect on survival of bees and Caco-2 mammalian cells. The present study demonstrates the potential of these innovative RPMs to serve as crop protection agents against crop diseases caused by *Xanthomonas* species and other phytopathogenic bacteria.

Isolation and characterization of a new *Cripavirus*, study of its pathogenesis against aphids, and its integration in future biological control of aphids

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Aphids are important agricultural pest insects, and are vectors of plant viruses that cause significant economic loss due to crop damage to quantity and quality in various species. Currently, management of plant viruses is based on control of insect vectors through chemical pesticides, which are environmental contaminants and have a negative impact on human health. Aphid pathogenic viruses may be integrated into the future chemical or biological control arsenal. The genus *Cripavirus* of the *Dicistroviridae* family, contains several viruses that are pathogenic to aphids and other insects e.g., *Aphid lethal paralysis virus* (ALPV) and *Rhopalosiphum padi virus* (RhPV). In this study, five isolates of a new *Cripavirus* were identified in *Aphis gossypii* that grew on *Hibiscus rosa-sinensis* plants. The viral RNA was first sequenced by Next Generation Sequencing followed by RT-PCR and Sanger sequencing validation. The obtained sequences allowed assembly of the complete genome sequence (10,200 nts). Icosahedral particle morphology (30 nm diameter) were observed by electron microscopy. SDS-PAGE followed by Coomassie brilliant blue staining allowed visualization of three viral structural proteins (~26, ~27 and ~30 kDa). Comparison of the genome sequence with the GenBank database revealed 71% nt sequence identity with the *Cripavirus Rhopalosiphum padi virus*; 88% and 87% with *Big Sioux River Virus* and *Wuhan insect virus 33* respectively, which are suggested to belong to *Cripavirus*. The new virus was putatively named *Aphis gossypii virus* (AgoV). Currently the study is focused on the pathogenicity of the virus to selected aphid species.

Posters

Development of CRISPR/Cas9 genome editing system *via Agrobacterium rhizogenes*-induced hairy roots

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With the advent of the CRISPR/Cas9 technology, *Agrobacterium tumifecians*-mediated plant transformation plays an essential role in developing new crop varieties. However, it is a costly and time-consuming procedure to observe the efficiency of the CRISPR/Cas9-sgRNA construct. Alternatively, *A. rhizogenes*-mediated plant transformation could be used as cheaper and timelier validation of CRISPR/Cas-sgRNA constructs in *in vitro*-grown hairy roots. Mobilized CRISPR/Cas-sgRNA plasmid constructs to *A. rhizogenes* strains ATCC15834 and K599 were used to infect explants of tomato and potato, or melon and cucumber, respectively. Following *in vitro* selection of transgenic roots, DNA was extracted and analyzed by PCR and sequencing of cloned PCR fragments. We observed deletions of 4 or 38 bp and insertion of a nucleotide in 3 different melon roots. In cucumber roots we found deletions of 1, 32 and 69 bp and insertion of 53 bp at the sgRNA target region. In potato and tomato roots deletions that range from 4 to 9 bp and insertions or substitutions of 1 or 5 bp were detected. In melon roots, where two sgRNAs were designed to target two different sites, substitutions of single nucleotides at the

sgRNA1 site and deletions of 4 nts in the sgRNA2 target site were observed. In one incident, where calluses developed on melon cotyledons infected with *A. rhizogenes,* deletions of 288 and 303 bps in the DNA strand lying between the two-sgRNA target sites were observed. The results show that *A. rhizogenes* induced hairy roots could be utilized for validating CRISPR/Cas-sgRNA constructs.

Transcription profiling of the infection process of *Botrytis cinerea* on whole tomato plant leaves

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Botrytis cinerea is a foliar necrotrophic fungal-pathogen which is capable of infecting over 1,400 plant species, and was ranked second worldwide for its scientific and economic importance. In spite of the importance of this pathogen, a transcription profiling of its infection process on a whole plant (as opposed to detached tissue) of a widely used crop (except for cucumber and lettuce) was not yet studied. We analyzed the transcriptome of *B. cinerea* (strain B05.10) infection on tomato (*Solanum lycopersicum*, cv. M82), an important vegetable crop. We sampled infected leaf tissues at 0, 16, 23, 40, and 48 h post infection. Approximately 35% of *B. cineria* and 45% of *S. lycopersicum* genes were differentially expressed during pathogenicity, respectively, demonstrating the global effect of this process. Preliminary KEGG enrichment analysis of the *B. cineria* transcriptome illustrated expression of genes involved in regulation of transcription, translation, DNA repair and recombination, in early stages of the infection. Genes involved in interaction with the environment and plant secondary metabolite synthesis pathways (e.g., phenylpropanoid and isoquinoline alkaloid biosynthesis) were upregulated in the later part of the infection (i.e., establishment). The latter could illustrate how *B. cineria* manipulates plant growth/defense systems to accomplish establishment. Altogether, our analysis and its future validation, may increase our understanding of plant-fungal interactions essential for successful infection.
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Disruption of the NAD cycle as a potential approach to manage fungal plant pathogens

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Plant pathogenic fungi are a major threat to food security and impose severe economic burdens. Therefore, there is a continuous need to develop new strategies to manage fungal plant pathogens. We suggest the NAD pathway as a target for pesticide development. NAD oxidation status determines the metabolic fate of the cell. NAD is also consumed by sirtuins which are histone deacetylases. These proteins remove acetyl groups from histone and thus regulate gene expression and chromatin accessibility. Sirtuins convert NAD to nicotinamide; the latter was shown to inhibit sirtuins. We were able to show that nicotinamide is fungistatic to plant pathogens albeit in high concentrations *in vitro*, on tomato slices and on cherry tomato berries. Nicotinamide inhibits hyphal growth much more than conidial germination. We thought to inhibit an enzyme that further metabolize nicotinamide, to increase the efficiency of application. Nicotinaldehyde inhibits *in vitro* pnc1, a nicotinamidase, part of the NAD salvage pathway. Nicotinaldehyde is fungistatic and inhibits germination much more than hyphal growth. The cellular response to nicotinaldehyde was overexpression of genes related to redox potential. However, we also found a decrease in the NAD+ level and deviation from the redox ratio upon exposure of cells to nicotinaldehyde. Niacin, a substrate for the salvage pathway, was found to slightly increase the rate of germination of cells

exposed to nicotinaldehyde. Currently, we are studying the mode of action of nicotinamide and nicotinaldehyde to develop a novel strategy to manage fungal diseases.

Identifying genes induced by ferulic acid and dependent on transcription factor ChAP1 in *Cochliobolus heterostrophus*

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Ferulic acid (FA) belongs to a group of phenolics widespread in plants, which are perceived by the necrotrophic maize pathogen *C. heterostrophus* as a stress signal. The mechanisms of signaling and toxicity of FA are unknown. We used RNAseq to follow the transcriptome in response to short (30 min) exposure to 0.5–2 mM FA. Since FA promotes nuclear retention of the redox-sensitive transcription factor ChAP1 without up-regulating genes for oxidant tolerance, we are identifying specific ChAP1-dependent FA targets. Comparing the transcriptome of Δ*chap1* and WT showed 819 genes that were significantly differentially expressed, at a $p < 0.05$ threshold. These genes are clustered mainly in two groups. At low FA, transcripts in both clusters are regulated in the mutant in correlation with the wild type pattern. At high FA concentration the regulation in both clusters shows a striking mirror-image of the WT pattern. Interestingly, the annotation of some of these genes suggests that they might participate in the regulation of programmed cell death (PCD), like ankyrin domain containing protein and a NACHT nucleoside triphosphatase domain protein. ChAP1 apparently suppresses the expression of this class. In contrast, other genes, like a major facilitator superfamily member, are positively regulated by ChAP1. In response to FA the fungus apparently operates two mechanisms to cope with this stress. One is a defense pathway in which the fungal cell can overcome the stress by removing and metabolizing FA, and the other is a PCD pathway. The transcription factor ChAP1 has an important role in determining the balance between defense and PCD.

Resistance of *Peronospora belbahrii* the causal agent of basil downy mildew to multiple fungicides

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The first attack of basil with downy mildew occurred in Israel in late 2011. Farmers immediately employed mefenoxam to cope with the sudden appearance of this new disease. Within a year, isolates of the pathogen showing resistance to mefenoxam (rRNA polymerase I inhibitor) appeared in Northern Israel, which thereafter spread to all other growing regions in the country. In 2013, isolates insensitive also to dimethomorph and mandipropamid (*Ces 3A* inhibitors) appeared in several locations. During 2013 and 2014, isolates with triple resistances were maintained in the fungal population. The first isolates resistant to azoxystrobin (QoI inhibitor) showed up in 2016. During 2016–2017 isolates which carrying resistance to all four fungicides were frequently collected. No isolate resistant to the new fungicide oxathiapiprolin (XSBP inhibitor) was detected. This is the first report on resistance of *P. belbahrii* to *Ces 3A* and QoI fungicides. The data explain the great difficulty of farmers to cope with basil downy mildew with commercial fungicides.

Powdery mildew on Oriental Plane (*Platanus orientalis*) in its natural environment in Israel

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Oriental Plane (*Platanus orientalis*) is an impressive indigenous tree, occurring in nature mainly in the Galilee and the Golan and planted as a shade-tree in Israel. The wild population is small, and under extinction threat. Since 1948, the species population in northern Israel has decreased by 80%. Powdery mildew on *P. orientalis* is caused by the fungus *Erysiphe platani* attacking leaves and fruits. In this study, we examined the association of the disease with the tree crown health. The study focused on riparian stands along the banks of Snir, Banias, Samach, Kziv and Jordan streams. Isolates were collected from powdery mildew colonies on leaves in all five sites. Morphological and genetic identification of the fungus was performed, together with detached leaves inoculation fulfilling Koch's postulates. Powdery mildew infection and three crown health parameters (dieback, density and foliar transparency), were examined on 60 trees. Disease incidence on offshoot leaves was 80%, compared to 43% on the crown leaves. Disease incidence and severity increased as crown distance from the ground decreased. No significant correlation was found between the three crown health parameters to powdery mildew infection. Disease was found on leaves and fruits of wild and ornamental hybrid trees. The main threats to *P. orientalis* in Israel includes: water overexploitation, the beetle *Euwallacea nr. fornicatus*, hybridization with other exotic sycamores, and fungi such as *Ceratocystis platani*. It is possible that powdery mildew serves as a secondary factor weakening the trees, thus contributing to the decline of the *P. orientalis* native population in Israel.

Rad21 paralogs, the cohesin complex proteins have a role in cell cycle regulation

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Fusarium oxysporum is a soilborne fungal pathogen having great genetic plasticity. Its dynamic genome provides an ability to develop resistance against fungicides. To understand the mechanism for genetic diversity we studied cohesin subunit proteins. Cohesin complex ensures proper segregation of the sister chromatid during cell division and also participates in DNA repair, transcription and maintenance of chromosome structure. Rad21, a cohesin complex protein, has three paralogs in *F. oxysporum*: conserved rad21, non-conserved rad21 (rad21nc) and meiotic Rec8. The rad21nc gene is found only in one family of molds and *Fusarium solani* and was probably integrated into the *F. oxysporum* genus via horizontal gene transfer. All rad21 paralogs are expressed in *F. oxysporum*, although the canonical paralog is expressed much more than the others. It is rather surprising that the rec8 paralog, which is known to be meiosis-specific, is expressed in *F. oxysporum* that has no sexual life cycle. We have created deletion mutants of rad21nc and rec8 in *F. oxysporum*. The deletion mutants of rad21nc showed irregular conidiation and were defective in germination under mitotic stress. We hypothesize a role for the rad21nc paralog in a checkpoint-like response during fungal cell division or sporulation. The possible role of such a checkpoint protein in fungal pathogenesis will be further discussed.

Characterization of *Streptomyces* spp. population causing potato common scab in Israel

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Common scab, caused by several species of *Streptomyces*, is an economically important disease of potato worldwide. In recent years disease incidence in potato fields has become a severe problem, especially in the Negev area of Israel. The major inoculum source is infected potato seed tubers imported from Europe (Netherlands, Germany, France and Scotland). The aims of this study were to isolate and characterize pathogenic

Streptomyces strains prevailing in Israeli potato fields and to establish a protocol for detecting the pathogen in soil prior to planting. In total, 152 strains were isolated from potato tubers with scab lesions, peanut pods with lesions, and from soils. The strains were analyzed by PCR with primers based on genes encoding thaxtomin synthetase (*txtAB*), a necrosis-related protein (*nec1*) and tomatinase (*tomA*), sequencing PCR products of 16S rRNA and ITS rDNA, and by pathogenicity tests on potato slices, radish seedlings and symptoms formed on radish roots in inoculated soil. The main species identified were *S. scabiei, S. turgidiscabiei, S. europaeiscabiei, S. stelliscabiei* that are common in Europe. Selected strains will be further characterized by sequencing whole genomes. A protocol based on quantitative Real Time PCR, for detecting pathogenic *Streptomyces* populations in soil (or plots) previously cultivated with potato, was developed. It enables detection of 10^3 to 10^6 CFU per 2 g of soil and will be used to monitor potentially infested plots prior to planting or fumigation.

Survey of bacterial and fungal seedborne diseases in imported and domestic potato seed tubers (2013–2017)

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Potato seed tubers imported from Europe are used for the spring crop, whereas domestic seeds are used for the autumn planting. Seed tubers infected with bacterial and fungal diseases are a major source of disease development during the growing season and are also a source for soil infestation. Latent or active infections of various diseases are prevalent although only certified seeds are used. Disease monitoring was performed with a sample of 200 tubers from each lot (350 lots/year) for the period of 2013 to 2017. Black dot (*Colletotrichum coccodes*) was detected in 46%, 13% and 10% of the lots at a low, moderate and high levels, respectively. Black scurf (*Rhizoctonia solani*) was detected in 46% of lots (at a low level). Silver scurf (*Helminthosporium solani*) was observed on 42%, 25% and 20% of the lots at a low, moderate and high levels, respectively. Incidence of Fusarium dry rot, caused by *Fusarium spp.*, was low (2–5%). Powdery scab (*Spongospora subterranean*) incidence was 44% in lots from Scotland, 9% from Germany, 4% from France and only 2% from Holland. Incidence of common scab (*Streptomyces* spp.) was 66%, 15% and 5% at a low, moderate and high levels, respectively. Latent infections with *Dickeya* and *Pectobacterium* spp., the causal agents of blackleg and soft rot, were tested by molecular analysis. Incidence of *Dickeya* was 24%, 56%, 22%, 7% and 30% in 2013, 2014, 2015, 2016 and 2017, respectively. Incidence of *Pectobacterium* was 49%, 48% and 62% in 2015, 2016 and 2017, respectively. Incidence of latent infections with *Verticillium dahliae*, tested only in domestic seeds, was 39% (155 lots/year).

The physiological basis of pomegranate fruit response to *Alternaria alternata*, the causal agent of Heart rot

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Heart rot, caused by *Alternaria alternata*, is a major pomegranate disease that influences production worldwide. Most fruits in the orchards are colonized by *A. alternata*. Nevertheless, symptoms are apparent on only small portion of the colonized fruits. During our previous research, we noticed that within individual orchards the incidence of pomegranate fruits exhibiting heart rot symptoms was related to the visual appearance of the trees. Trees that appear visually frail had more diseased fruits than robust trees. The specific objectives of the present study were to (i) characterize the relationship between the visual appearance of pomegranate trees and their vulnerability to heart rot; and (ii) to study the physiological basis of pomegranate fruit response to *A. alternata*. Analysis of heart rot intensity in 4 orchards in 2014 revealed large differences in heart rot incidence among trees growing side by side in the same orchard; these differences were related to the visual appearance of the pomegranate trees. There were significant differences in the germination of *A. alternata* spores in juice prepared from asymptomatic fruits originating from

these trees and comparable differences were found in the acidity level (pH) of the juices. These differences may reflect variances in the physiological response of pomegranate trees to heart rot. Studying the relationship between the acidity (pH) of pomegranate juice and the germination of *A. alternata* spores supported the hypothesis that compound(s) prevailing in the pomegranate juice, beyond pH, regulate(s) the germination of *A. alternata* spores in the juice.

Pectinolytic bacteria affect potato yield under hot climate conditions

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Blackleg and soft rot, caused by pectinolytic bacteria, transmitted by latent infection of seed tubers, might cause severe economic losses in potato. *Dickeya solani* (Ds) and *Pectobacterium carotovorum* subsp. *brasiliense* (Pcbr) development is favored by high temperature. The objectives of this study were to evaluate the prevalence of pectinolytic bacteria in seeds imported from Europe, potato plants and progeny tubers derived from such imported seed, and to characterize the bacterial populations. In 2017, out of 79 tested seed lots, 30% were infected with Ds and 62% with Pcbr. Field surveys indicated that 53% of Ds-negative seed lots had no disease symptoms in field, 15% were Ds-positive with symptoms, 16% were false negative (-Lab/+Field) and 15% false positive (+L/-F). The survey also indicated that 33% of Pcbr-negative seed lots had no symptoms in field, 30% were Pcbr-positive with symptoms, 5% were false negative (-L/+F) and 32% false positive (+L/-F). Seed tubers of 20 lots, infected with Ds (100%) and Pcbr (85%) were planted in a field plot at Gilat. Plants were infected with Ds (65%) and Pcbr (85%) and 40% of progeny tubers were infected with both pathogens. Pathogenicity of 72 Pcbr strains from imported seeds, plants and progeny tubers was evaluated using a potato tuber maceration test. Selected strains (26) were characterized by Pulsed Field Gel Electrophoresis. Two groups were identified using the restriction enzyme I-ceuI and seven groups with AvrII, regardless of their origin. The presence of Ds and Pcbr in imported seed material affects yields of the spring and winter crops in Israel.

The correct use of ELISA for detection of viral proteins

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Enzyme-Linked Immuno-absorbent Assay (ELISA) is a routine laboratory test in medicine, agriculture, quality control and biological research, and has been in use since 1971. This technique is based on the use of a specific antibody to recognize an immunogenic substance (often a protein), with a color reaction to identify the presence of the target. ELISA tests can be performed in large numbers quite cheaply, although the sensitivity is rather less that of the more expensive Polymerase Chain Reaction. We discovered a number of common problems in the performance of ELISA tests in the course of our routine work on the detection of plant viral proteins. We will describe how to perform ELISA tests effectively, and how to fault-find when routine ELISA tests fail.

Control of basil downy mildew with oxathiapiprolin-based fungicides

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Basil downy mildew (BDM) caused by the oomycete *Peronospora belbahrii*, is currently the most threatening disease of sweet basil worldwide. Reducing the MRL (maximal residual level) by the authorities and the frequent occurrence of insensitive isolates to registered fungicides, make the combat against this disease a very hard task. Here we report on the effective control of

BDM by the new fungicide oxathiapiprolin (OXTP, an OSBP inhibitor) and four oxathiapiprolin-based fungicidal mixtures. One to two preventive sprays of OXTP applied to basil crops in net houses, or 1–2 preventive sprays with one of the ORONDIS™ mixtures by Syngenta [oxathiapiprolin mixed with either chlorothalonil (multisite), azoxystrobin (AZ, QoI inhibitor), mandipropamid (MPD, *CesA* 3 inhibitor) or mefenoxam (MFX, rRNA polymerase I inhibitor)] were highly effective in season-long control of the disease. Control efficacy was pronounced even at low doses of 12.5–25 ppm active ingredient. The data suggest that the new OXTP-based fungicides can both achieve a profound control of the disease in the field and meet the new MRL levels requested by the authorities.