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INVITED LECTURES

Metabolic innovations during host-virus interactions in the ocean

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Marine viruses that infect marine microorganisms are recognized as major ecological and evolutionary driving forces, shaping community structure and controlling the cycling of nutrients in the marine environment. A major challenge in our current understanding of host-virus interactions in the marine environment is to decode the wealth of genomic and metagenomic data and translate it into cellular mechanisms that mediate host susceptibility and resistance to viral infection. Nevertheless, the cellular mechanisms that govern these host-virus dynamics are largely underexplored. Recent reports highlighted a novel genomic inventory found in marine viruses which encode for auxiliary metabolic genes previously thought to be restricted to host genomes. Thus, these genes can expand the metabolic capabilities of the infected host cell and the flux of the nutrients and metabolites between the cell and its micro-environment. *Emiliania huxleyi* is a globally important coccolithophore which forms massive algal blooms in the North Atlantic Ocean, and is routinely infected and terminated by large DNA viruses (EhVs) (genus coccolithoviruses). We explore the molecular and metabolic basis of host-virus dynamics and the signal transduction pathways that mediate host-virus interactions. By combining genome-enabled technologies, analytical chemistry and advanced cell imaging approaches, we were able to identify several fundamental metabolic pathways that mediate these host-virus interactions. We revealed the role of viral-encoded sphingolipid biosynthesis, redox and DMS metabolism and their function in determining host cell fate and viral replication strategies. We are now studying host-virus interactions at single cell resolution to provide insight into the cellular mechanisms that govern the metabolic "arms race" during algal bloom dynamics in the ocean.

Nature's Gift

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Resilin is a polymeric rubber-like protein secreted by insects to specialized cuticle regions where high resilience and low stiffness are required (which

enables fleas to jump 100 times their height). Plant cell walls are durable composite structures made of cellulose, other polysaccharides and structural proteins. Plant cell wall composites exhibit extraordinary strength exemplified by their ability to carry the huge mass of some forest trees. Inspired by the remarkable mechanical properties of insect cuticle and plant cell walls we have developed novel composite materials of resilin and Crystalline Nano-Cellulose (resilin-CNC) that display remarkable mechanical properties combining strength and elasticity. We produced a novel resilin protein with an affinity for cellulose by genetically engineering a cellulose binding domain (CBD) into the resilin. CBD-Resilin interfaces at the nano-level between the resilin (the elastic component of the composite) to the cellulose (the stiff component).

Collagen is a central element of the human extracellular matrix and is intimately involved in tissue development, remodeling and repair and confers high tensile strength to tissues. Numerous medical applications, particularly wound healing, cell therapy and bone reconstruction rely on its supportive and healing qualities. The synthesis and assembly of collagen requires a multitude of genes and post-translational modifications. Historically, collagen was extracted from animal and human cadaver sources. However, because of the risk of contamination and allergenicity, animal/cadaver collagen was subjected to harsh purification resulting in irreversible modifications impeding its biofunctionality. A tobacco plant expression platform has been recruited to express human collagen, along with three modifying enzymes critical to collagen maturation. The recombinant human collagen type I extracted from plants forms thermally stable helical structures and fibrillates, and demonstrates bioactivity resembling that of native collagen. Today, in greenhouses all over Israel, farmers grow transgenic tobacco plants producing human recombinant collagen used for the production of medical implants for clinical use. Combining collagen at the nano-scale with resilin resulted in super-performing fibers with mechanical properties exceeding natural fibers.

Using barn owls as biological rodent control agents in agriculture

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Many species of rodents cause severe damage to agriculture. Conventional control methods do not provide an adequate solution and current rodenticides are dangerous to man and to the environment. The biological control of rodents using barn owls (*Tyto alba*) was suggested some decades ago, and is being used in many places around the world. The control mechanism utilizes the natural traits of the wild barn owls that nest in artificial nest boxes in agricultural areas. In Israel, the method has been used since the 1980's and the first positive results were found by Kibbutz Sde-Eliyahu. The application there was accompanied with research that analyzed the biological control method from various aspects. From these studies we found many important results: a steady, large population of barn owls can be established around these artificial nests; barn owls act as biological pest control in fields and orchards; barn owls can shift their diet according to the different fluctuations in rodent populations and therefore reduce rodent outbreaks. In financial terms the predation pressure of the barn owls increased alfalfa yield by 9.4%, thus improving farmers' profit far more than expenditure. Following its success in the Beit-Shean Valley, since 2008 the project was extended to most Israeli agricultural regions in a "National Barn Owl Project" led by The Society for the Protection of Nature, Tel-Aviv University, the Ministry of Agriculture and Rural Development and the Ministry of Environmental Protection. Nowadays the national project encompasses almost 4000 nesting boxes from northern Israel to the northern and western Negev. The nesting boxes produce thousands of chicks annually and therefore the project grows continuously. The

project's staff guides farmers as to how to best use this natural system, and constantly monitor the owls, prey and agriculture to improve the method. By enhancing the use of natural rodent enemies the use of chemical rodenticides in agriculture has significantly decreased. Many farmers have shifted to using barn owls, and have reduced the use of these dangerous pesticides or refrained from using them altogether, without an evident impact on yield. The biological pest control of rodents using barn owls protects crops, increases farmers' revenue, safeguards public health and conserves the environment.

DISEASE EPIDEMIOLOGY AND DYNAMICS

Aerial dispersal of *Fusarium proliferatum* by conidia from infected onion plants

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The soil-borne fungus *Fusarium proliferatum* causes diseases in agricultural crops such as onion, maize and date palm. In addition, the fungus can produce mycotoxins (e.g. fumonosins) which may pose a risk to human and livestock. On white onion *F. proliferatum* causes salmon blotch: pink lesions of large clusters of conidia on the external scales. The objectives of this study were to examine the potential of the fungus conidia to spread by air to new areas and hosts. We tested the detachment and transfer of the conidia from infected onion bulbs in a wind tunnel. We found that the more conidia that were on the onion scales, the more conidia were detached and trapped. There was a high correlation between wind speed and the number of trapped conidia on artificial surfaces and on onion leaves. In an experiment conducted in an onion field, we found a positive correlation between wind speed and the number of trapped conidia. This correlation, however, could not be related to the severity of salmon blotch symptoms on the onion bulbs that developed in the field. Infected onions could be a source for aerial dispersal of conidia, yet the pattern of their movement is unclear.

Spatiotemporal model of *Phytophthora infestans* dynamics at a regional scale using empirical data

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Potato late blight (causal agent *Phytophthora infestans*) has been and still is one of the most devastating diseases in cultivated crops resulting in billions of dollars of losses annually. To date, there are no quantitative data in the literature on the disease progress and dynamics and, as far as we know, there have been no studies that quantified the dispersal of *P. infestans* sporangia over regional distances. In the absence of such knowledge, growers spray potato fields with fungicide as a precaution, irrespective of the actual risk of blight. Here we developed a mathematical model for the spread of late blight on a regional scale using empirical data. The model was tested by comparing predicted to actual weather patterns to examine its accuracy. The model was then used to create risk maps showing the likelihood of future infections in the region. Such risk maps can help growers optimize late blight suppression and fungicide use. In addition, the model can be used to follow the spread of specific pathogen strains which may have different levels of aggressiveness or sensitivity to fungicides. This work offers a novel methodology to track disease spread in time and space and offers powerful information for future research about dispersal-related characteristics of the disease.

Using eco-informatics approach for studying spatio-temporal dynamics of pests in an agricultural environment

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While plants and individual fields are the focus of most epidemiological studies in agricultural systems, one of the major challenges in improving plant protection requires shifting the scale from field to the landscape. Many factors influence the spatial distribution of pest populations, including abiotic factors (e.g. temperature, humidity, etc.) and biotic factors (host sensitivity and vectors transmitting viruses). Despite advances in research, knowledge about the effects of various factors on the spatial-temporal spread of pathogens remains limited. Until recently, studies were based on small numbers of observations collected in the field or from the laboratory. Due to the complexity of biological systems, it is difficult to define the importance of the variables collected in controlled trials and to conclude reliable relationships. Agricultural research is increasingly becoming a data-intensive science, relaying on massive amount of data collected in the field. This has led to the development of an approach called Eco-informatics which allows characterizing the factors affecting pest development. The main objective of this research is to set out general principles regarding the factors influencing the spread of pests. To this end, a statistical tool that integrates spatial and monitoring data was developed. The tool shortens analysis time and produces a comprehensive picture of the spatiotemporal dynamics of various pests. The knowledge accumulated in this study will allow development of pest control principles that will aid in reducing costly and unnecessary pesticide sprays, reducing risk of the development of pesticide resistance, and thereby optimize chemical control and improve profitability.

Spatial analysis of the impact of landscape and environmental conditions on the distribution of pest and pathogen infestation in vineyards

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Agricultural areas are characterized by a heterogeneous landscape which includes a variety of agricultural crops alongside natural areas, forests, built-up areas, etc. Landscape and environment have an impact on the dynamics of populations and different ecological processes in space and time. Research on large spatial scales is receiving more attention in recent years with the understanding that landscape can affect pest occurrence in individual agricultural fields. The use of computerized tools and spatial analysis using Geographic Information Systems (GIS) to understand and quantify spatial phenomena became more common in recent years and allows better understanding of spatial phenomena. Understanding the effects of spatial heterogeneity can improve pest control management. In this study we explored the effects of the landscape and the environment in a large area on two important vineyard pests—European grapevine moth *(Lobesia botrana*) and Grape Powdery Mildew (*Uncinula necator*). For this purpose we built a database of 200 commercial vineyards in the Judean Plains region. We mapped all the plots and land cover (forests, agricultural fields, built-up areas and uncultivated land) within 2000 meters of every vineyard and we used multivariate regression to examine the relationship between the landscape variables and the extent of infection of the two pests. Preliminary findings from this study showed a link between the landscape components and the level of infestation in vineyards in different environments. This

work demonstrates the effects of landscape heterogeneity and context on pest infestation.

Epidemiology of fire blight disease caused by *Erwinia amylovora* in the Pink Lady® apple cultivar in Israel

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Fire Blight (FB) disease in rosaceous plants is caused by the bacterium *Erwinia amylovora*. The damage caused to pear and apple cultivars depends mainly on weather conditions. Decision Support Systems (DSS) can successfully predict the occurrence of FB infection in pears and identify appropriate timing for bactericide application. A local DSS named FB Control Advisory [FBCA]) was developed in Israel in the late 1990's. FBCA has been implemented successfully in pears, but was not tested in apples. The Pink Lady® (PL) apple cultivar flowers relatively early, when significant rain events occur, which enhance the chance for *E. amylovora* infection. The long-term objective of the present study was to develop a strategy for FB management in PL. The specific objectives of this report were to evaluate the accuracy of FBCA in predicting infections in PL and to record pathogen survival in apple trees. FBCA was observed in 83% and 36% of the PL orchards that were inspected in 2015 and 2016, respectively. In 2015, 90% of the infection events were predicted by FBCA. The DSS Maryblyt™7.1 predicted only 38% of the infection events. In 2016, both DSS's predicted all infection events, but Maryblyt™ issued 1.7 fold more warning events than FBCA. The disease did not progress more than 10 cm in the PL branches, and bacterial viability markedly decreased in the winter. In conclusion, FBCA can be used successfully for predicting FB in Israeli PL apple orchards, and the initial inoculum in PL orchards probably originates from nearby infected pear orchards.

The infection source of fungi pathogenic to olive fruit

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Cases where the quality of olive oil is low and the acidity values are higher than 0.8% are becoming more common in extensive agriculture. In a national survey (2010–2013), a significant correlation was found between the presence of fungal pathogens and high acidity of olive oil. The main fungi isolated from infected olives were *Alternaria* and *Cladosporium*, but the mechanisms of penetration into the fruit are still unknown. Here we hypothesized that pathogenic fungi penetrate the fruit through the flower, and set the following research objectives to: (a) characterize the variety of fungi inhabiting olive fruit in different developmental stages; (b) determine if pathogenic fungi penetrate olives through the flower. Fungi were identified by molecular methods, while their presence in various flower parts was determined on selective medium, and deep sequencing was performed on young fruits. The stigma was found to be the floral organ with the highest infection rate (93.5%) followed by the stigma (91.6%). Additionally, *Alternaria* and *Cladosporium* were most common in three developmental stages. Deciphering the routes of fungi infection will help increasing olive oil quality and, in turn, grower profitability.

Integrated disease management

Relative humidity-controlled fanning prevents downy mildew development in basil crops

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Basil Downy Mildew, caused by the oomycete *Peronospora belbahrii*, is a devastating disease of sweet basil worldwide. First appearance of the disease in Israel occurred in November 2011. Within a year the disease had spread to all growing areas in the country while the pathogen developing resistance to mefenoxam, making its sprays useless. As no resistant cultivars of sweet basil are currently available and only a very few fungicides are registered for control of the disease, we searched for alternative methods to control the disease. Previously, we showed that daily nocturnal fanning of basil crops from 8 pm to 8 am was highly effective in controlling the disease. The reason was that with fanning air relative humidity (RH) could almost never reach the near-saturation level required for infection and sporulation of *P. belbahrii*. Here we show that fanning can be applied for a limited period and yet be effective. We installed a computer-controlled RH-sensor 20 cm above the basil plants and programmed it to operate or stop the fans at any chosen RH. Seven fans, 50 cm in diameter, were installed above the middle row 2 m above soil level at an inclination of 30 degrees toward the soil, in a 45 m long net-house in which 3 rows of basil plants were grown, 18 plants per meter row. In the control house no fans were installed. In the first experiment, fans started operating at 80% RH and stopped at 70% RH. Disease control with this program was quite poor. In the next two experiments, fans were operated at 70% RH and stopped at 65% RH. Disease control with this program was excellent: 1–3% leaves were infected as against 85–95% in the control, non-fanned, adjacent net-house. The data suggest that, RH-controlled, automatic fanning, is an economical and highly efficient method for controlling downy mildew in basil.

Integrating fungicide application and agro-techniques to control Lettuce Big Vein Disease

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Lettuce Big Vein disease is caused by the *Mirafiori Lettuce Big Vein Virus*, vectored by *Olpidium* spp. fungi. This is a major disease of lettuce, especially during the winter when soil temperatures are low. Symptoms include clearing of the leaf lamina alongside the veins, thereby acquiring a typical enlarged vein appearance from which the disease name is derived. Also, plant malformation and stunting cause yield decreases or downgrade lettuce quality. Soil fumigation is frequently used in Israel, mainly with metam sodium applied through the irrigation system prior to planting, which reduces the levels of soil-borne pathogens. However, metam sodium fumigation is expensive and it negatively affects the environment, and its efficiency is inconsistent due to accelerated biodegradation. The objectives of the current study were to evaluate the disease control effect of integrated fungicide application prior to planting along with coverage of the plants and soil. Covering lettuce plants by Agril netting was effective in reducing disease symptoms. Previous work showed that increasing leaf temperature reduced the disease symptoms apparently due to an effect on virus activity. Covering the soil and combining

fungicides (Fluazinam and Carbendazim) reduced disease symptoms probably due to effects on the activity of the fungal vector.

Management of Mal Secco disease of citrus, caused by *Phoma tracheiphila*

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Mal Secco, caused by the fungus *Phoma tracheiphila,* is devastating to citrus trees, especially lemon. Effective fungicides are not registered for the management of this disease, hence, effective measures to manage the disease are essential. The objective of this study was to screen and evaluate the efficacy of fungicides against *P. tracheiphila*. At first, the toxicity and dose-response curve was assessed in vitro for 12 different fungicides, followed by calculation of ED50 and ED90 values. The most effective fungicides were: Prochloraz, Flutiafol and a formulation consisted of a mixture of Fluopyram and Tebuconazole (FLU + TBZ); the ED50 were 1.4 ppm, 17 ppm and 0.7 ppm, respectively. A disease control assay was established with lemon transplants inoculated with *P. tracheiphila* spores in greenhouse. Application of FLU + TBZ (13 ppm), Flutriafol (53 ppm), or Prochloraz (10 ppm) two days before inoculation prevented infection and significantly reduced disease severity. Additionally, Prochloraz (10 ppm) reduced disease severity if applied two days after inoculation. Application of the fungicides 7 days after inoculation did not control the Mal Secco symptoms in infected lemon seedlings. The research is now focused on a disease management program which combines fungicides for preventing infection and disease control.

Influence of plant nutrition and biotic and abiotic induced resistance on Mal Secco in lemon

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Mal Secco is a citrus disease caused by the pathogenic fungus *Plenodomus tracheiphilus*. The disease exists in all citrus growing areas in Israel. *P. tracheiphilus* is a quarantine pathogen in most citrus growing countries. The common approach to disease management is pruning infected branches. Efficient fungicides are not available for control of conidia germination and penetration of the plant tissues. The goal of this research is to investigate the influence of nutrition and induced resistance on the disease in seedlings and young trees. The following inducers were tested: different types and concentrations of biochar and a Trichoderma preparation (Trichodex) mixed in the growing medium, copper-based fungicides (Bordeaux mixture and Kocide) and Canon in drench application or spray. Elements (calcium, magnesium, sodium, potassium, silicon, microelements (as koratin), chelates of manganese, zinc, copper and iron) were sprayed on foliage. Macroelements (nitrogen, phosphorus, potassium and different rates of ammonium and nitrate) were supplied as drenches to perlite medium. Fungal and bacterial endophytes were infiltrated to plants for disease suppression. Sprays of CuEDTA (0.01%) and magnesium sulphate (0.5%) significantly reduced disease severity in lemon seedlings. Mixing biochar EUC600 (1%) in the plant medium and drenching with Canon (0.25%) significantly reduced disease severity in

young lemon trees. Ammonium (40% of the total fertigated nitrogen of 100 ppm, and 10% of the total nitrogen concentration of 50 ppm) was also associated with reduced disease severity. It is planned to test efficient treatments in combination with other management treatments for Mal Secco suppression in lemon groves.

Involvement of bacteria in biological control

Characterization of the predation interaction between the predatory bacterium *Bdellovibrio bacteriovorus* and the cucurbit pathogen *Acidovorax citrulli*

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Acidovorax citrulli is a seed-borne and seed-transmitted bacterial phytopathogen, which causes bacterial fruit blotch (BFB) disease, a serious threat to the cucurbit industry worldwide. Although efforts have been made to develop means to cope with BFB, chemical treatments to manage the disease have limited efficiency and no BFB resistance sources are available in cucurbit germplasm. *Bdellovibrio bacteriovorus* belongs to a group of obligate predatory bacteria that prey upon gram-negative bacteria, and is found in many environments, including terrestrial, aquatic and animal gut. So far, only a few studies have assessed the potential of *B. bacteriovorus* as a biocontrol agent against plant and animal pathogenic bacteria. Moreover, many aspects of the predator/prey interactions are not well understood. In this study we characterized the interaction between *B. bacteriovorus* and *A. citrulli* and revealed two distinct phenotypes of *A. citrulli* strains: resistance or susceptibility to predation. Using a library of random mutants in the background of the *B. bacteriovorus*-resistant *A. citrulli* M6 strain, we developed a screen that allows the identification of mutants susceptible to predation. Several such mutants were identified recently, and are being characterized to identify the genes that are responsible for this phenotype.

The influence of biochar on Pythium damping off and the microbial community structure in the soil

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Biochar (the solid co-product of biomass pyrolysis), has several agronomic benefits in soil and has a great potential as a supplemental amendment in soilless media against foliar and soilborne pathogens. However, biochar positive influence is constrained when the applied concentrations exceed the optimal dose (usually >3%). We hypothesize that pre-activation of biochar will alter the microbial composition and enrich the growth media with beneficial microorganisms and diversity of microorganisms that may help to suppress disease. The research goals were to study i) the impact of pre-activated biochar on cucumber damping-off caused by *Pythium aphanidermatum* and ii) the influence of pre-activated biochar-amended soil on bacterial community composition and diversity. We found that pre-activated greenhouse waste biochar suppressed cucumber damping-off by up to 63% and improved plant growth parameters by up to 44%. Next Generation Sequencing by Illumina of the 16S rRNA gene showed substantial differences in bacterial composition between pre-activated biochar amended soils compared with non-activated biochar and control soils. Furthermore, pre-activated biochar caused a significant enrichment of bacterial abundance, increased potentially beneficial microorganisms and diversity, and shift in microbial

community structure. These changes may play an important role in the overall effects of biochar on disease suppression either through direct antagonist effect to the pathogen or indirectly via induction of systemic plant resistance.

Biological control of postharvest stem-end rot and side decay in mango, avocado and citrus fruit by endophytic bacteria

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Stem-end rot (SER) pathogens colonize the phloem of the fruit stem-end, while other pathogens penetrate the cuticle and live quiescently until fruit ripening. During ripening the harvested fruit become susceptible and the pathogenic fungi become active and develop postharvest rots. As most reported postharvest microbial antagonists are epiphytes isolated from the surfaces of fruits and vegetables, our goal was to isolate endophytic bacteria with antagonistic capabilities that could naturally colonize the inner parts of the fruit. To this end, we have isolated 150 endophytic microorganisms including fungi, yeast and bacteria from the stem-ends of mango avocado and citrus fruits, about a half of which were bacteria. All isolates were evaluated in vitro for their antagonistic activity against *Colletotrichum gloeosporioides, Lasiodiplodia theobromae, Alternaria alternata, Phomopsis mangifera, Penicillium digitatum* and *Botrytis cinerea*. Six bacterial isolates that exhibited inhibitory activity in the in vitro assays were further tested for biocontrol on the fruit against *L. theobromae* on mango and avocado, *P. digitatum* on citrus, *C. gloeosporioides* on avocado and *A. alternata* on mango fruit. These tests revealed three Bacillus spp. with promising antagonistic activity against stem-end pathogens. Various aspects related to the mechanism of action of these antagonists have been studied and preliminary results will be presented.

Characterization of genetic diversity in pathogens

Fusarium crown rot of melon: characterization of variation and genetic basis of resistance using adiverse collection and segregating populations

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Fusarium oxysporum f. sp. *radicis-cucumerinum* (FORC) is a plant pathogenic fungus causing root rot. The pathogen was identified in Israel in 2000 and has caused substantial damage and yield loss to greenhouse cucumbers since then. Recently, this pathogen was also found to affect melons in the 'Arava and other regions. Expansion of the disease to new regions and hosts is a growing threat that needs attention. We developed a methodology of effectively inoculating melon seedlings with the fungus and scoring for disease symptoms under controlled conditions. The goal of the current research is to characterize the variation in resistance level in melon and to study the genetic basis of this trait. We take two approaches: (1) genome wide association (GWAS), in which we characterize a diverse collection of melon accessions, and (2) linkage mapping, in which we use a segregating population from a cross between resistant and susceptible parents. The diverse melon collection maintained at Newe Ya'ar, composed of 180 melon accessions that represent the two melon sub-species, *agrestis* and *melo*, and encompassing 12 horticultural groups, was screened to characterize variation in resistance level using the root-dip inoculation system. Of the 180 accessions tested, 61% were highly susceptible, 29% showed an intermediate response and 10% were resistant. The collection was genotypically characterized with 24,000 SNP markers. We observed that resistant and susceptible accessions were distributed homogenously across the genetic variation and horticultural groups. In the GWAS analysis, only weak signals were observed and no major QTLs were detected. These results suggest a polygenic basis for

resistance to FORC and the presence of more than one resistance mechanism across melon diversity. In parallel, we are performing QTL mapping on a recombinant inbred line (RIL) population and bulk-segregant analysis (BSA) on an F2 population, from which we expect to be able to map QTLs that contribute to the resistance expressed by the resistant parent.

Phenotypes and genotypes of field isolates of *Phytophthora infestans* in Israel in 2015–16

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A total of 344 isolates of *Phytophthora infestans* were collected during 2015–6 from late blight-infected potato crops in Israel, mainly from the Western Negev region. Sporangia were collected from samples and tested at Bar Ilan University for sensitivity to mefenoxam (MFX), virulence factors and mating type. DNA was extracted from sporangia and analysed for SSRs in The James Hutton Institute, Scotland. MFX sensitivity data were obtained for 258 isolates: 114, 80 and 64 isolates were resistant (R), intermediately resistant (I), and sensitive (S) to MFX, respectively. Mating type assays were done in detached tomato leaves with defined tester A1 and A2 isolates. Mating type data were obtained for 84 isolates: 70 isolates were of the A1 mating type and 14 isolates were of the A2 mating type. Virulence race structure was determined for 248 isolates: 39 isolates belonged to the most frequent race 1 3 4 7 9; 90 isolates carried 1–4 virulence factors, 5 isolates had 5 factors other than 1 3 4 7 9; 76 isolates carried 6–8 factors, and 38 had 9–11 factors. Genotypic data were obtained for 148 isolates: 47 isolates were 13_A2, 80 were 23_A1, and 21 were of a lineage similar to US-7. Interestingly, nine 13_A2 isolates were A1. Six 13_A2 isolates were I and eleven isolates were S. All 23_A1 isolates were A1; 22 were I and 21 were R. A comparison between 2015 and 2016 showed a major shift from 23_A1 with mainly 6–8 virulence factors in 2015 to 13_A2 with mainly 9–11 virulence factors in 2016. The reasons for this shift in the population structure are not known. The data show that the population of *P. infestans* in Israel is highly variable probably due to import from Europe and/or oospore infection. In previous seasons A1 and A2 isolates occurred in the same fields and sometimes in the same leaf.

Host range of *Peronospora belbahrii*, the causal agent of basil downy mildew

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Downy mildew in basil (BDM) was first recorded in Israel in late 2011. Since then the disease has spread over the country causing devastating damage to basil crops. A similar rapid spread was reported in the USA. Currently, the disease occurs in Europe, the Middle East, Far East and the Americas. The reasons for this rapid spread are not known. Seed transmission and seedling trade were raised as possible reasons. Here we report that other members of the *Labiaceae* family besides *Ocimum* spp. may serve as hosts of the causal oomycete agent of BDM, *Peronospora belbahrii.* Endemic, ornamental and agricultural species of *Labiaceae* were obtained from USDA, Hishtil, Genesis Seeds Co. and the Dept. of Archeological Botany of Bar Ilan University. Altogether, we tested 102 entries belonging to 53 species and 21 genera, including mainly *Salvia*, *Rosemarinus, Mentha*, *Plectranthus, Ocimum*, *Lavandula* and *Origanum*. Potted plants were spray-inoculated with spore suspension of isolate Knafo 3 of *P. belbahrii* collected from basil plants, incubated in a dew chamber at 18 °C in the dark for 20 h, and then at 25 °C under continuous illumination. At 8–10 days post inoculation plants were re-introduced to the dew chamber to allow for pathogen sporulation. Sporulation was observed in four species: *Salvia fruticosa, Rosemarinus officinalis, Nepeta curviflora* and *Micromeria fruticosa.* Another six entries (of five genera) showed symptoms but no sporulation. To complete the Koch postulates, spores were collected from the four sporulating host species and re-inoculated onto basil plants. Such basil plants became infected and

sporulation occurred abundantly. Finally, spores were collected from the basil plants and re-inoculated onto all *Labiaceae* species. Again, only *S. fruticosa, R. officinalis, N. curviflora* and *M. fruticosa* became infected and were the site of sporulation. The plant population of the four susceptible species was heterogeneous, with only few plants of each species subject to abundant pathogen sporulation. PCR analysis, with markers specific to *P. belbahrii*, of the DNA extracted from the spores produced on the four susceptible *Labiaceae* host species resulted in a 134 bp band, typical of *P. belbahrii*. The data suggest that *P. belbahrii* is pathogenic to other species of *Labiaceae*. The role of these species in the epidemiology of BDM has yet to be determined.

Disease outbreaks, old and new

Soil-borne disease in cotton: from lack of knowledge to the challenges of the future

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Cotton cultivation in Israel is changing as a result of increased water availability, global market demand and profitability expectations. The cultivation of Acala-type cultivars in Israel was drastically reduced in recent years and Pima-type cultivars are on a rise. In 2006, 'Acalpi', an interspecific hybrid between Acala and Pima cottons was released by the Hazera Co. in Israel. In general, the Pima-type is more susceptible to soil-borne diseases. Indeed, the shift toward Pima-type cultivars was accompanied with an increase in the incidence and severity of plant wilting. Various fungi have been isolated from wilted plants. The most frequently isolated fungus has been *Macrophomina phaseolina*, a known pathogen of a wide range of crop plants including cotton. This increase in disease infestation has led to research activity amid at screening for the distribution and quantification of *M. phaseolina* in cotton fields in Israel, completing Koch's postulate (reproducing the disease under field conditions), and documenting the damage caused to cotton by the disease. The results indicate that the existence of the pathogen in the plant is not enough; the plants have to be under stress conditions to express disease symptoms. Future research directions will be a combination of pathological and genetic studies aimed at understanding the pathogenicity pattern, combined with screening cotton germplasm in a search for resistant lines as a basis for breeding resistant cultivars. The short term objective will be a characterization of stress factors enhancing disease expression and diminishing such stresses by agro-technological means.

Downy mildew of coleus, a new disease in Israel

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Downy mildew on ornamental coleus (*Plectranthus scutellarioides, Labiaceae)* was first seen in 2005 in Louisiana and New York. Within 10 years the disease had spread and became a serious threat to the coleus industry in the USA. Here we report on the first detection of downy mildew on potted nursery coleus plants in spring 2016. Green-yellow and green-variegated cultivars were more affected than red or green-red cultivars. On the former cultivars the disease appeared as chlorotic lesions that gradually turned necrotic, whereas on the latter cultivars symptoms were visible as beige-color or brown lesions. Sporulation occurred on the lower leaf surfaces. Spores were gray, unlike those of *Peronospora belbahrii* on basil which are dark-brown. Sporophore size and appearance were similar to those of *P. belbahrii* from basil (basil downy mildew). Spores were collected from sporulating coleus leaves and inoculated onto basil, coleus, sage and rosemary. Only coleus plants became infected and sporulated. Spores collected from basil plants which were inoculated with isolate Knafo 3 of *P. belbahrii* failed to infect coleus but succeeded to infect and sporulate on sage and rosemary. Completion of the Koch's procedure proved that coleus downy mildew (CDM) is a distinct disease of coleus. DNA extracted from spores of the CDM agent exhibited a different PCR profile compared to spores

of BDM. Using ITS6 + ITS4 primers, *P. belbahrii* from basil showed a 950 bp band whereas *Peronospora* sp. from coleus showed a major band of 1000 bp and a minor band of 1200 bp. Using ITS6 + DC4 primers, *P. belbahrii* from basil showed a 1200 bp band whereas *Peronospora* sp. from coleus showed a major band of 1250 bp and a minor band of 1400 bp. The data suggest that CDM is caused by a distinct species of Peronospora. Recent data from Europe and USA suggest that the causal agent of CDM is *Peronospora belbahrii* sensu lato.

Who are the actors of the FCR (Fruitlet Core Rot) disease of pineapple in Israel?

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Pineapple [*Ananas comosus L.*] is one of the most important cultivated tropical fruits. Originating from South America, it is grown in all tropical and subtropical regions. It was introduced in Israel 20 years ago and is gaining importance and attracting an increasing number of growers. Fruitlet core disease (FCR) is one of the main diseases that affect pineapple fruit, causing necrosis of the eyes and leading to important economic loss including in Israel. It was first described in Queensland more than a century ago, and is thought to be caused by the conjunction of two fungi entering the fruit through the flowers: *Penicillium funiculosum*, and *Fusarium moniliforme* (a member of the *Gibberella fujikuroi* species complex (GFSC)). Although *P. funiculosum* is recognized as the main causal agent of FCR in all pineapple growing regions, the exact role of the *Fusarium* is still questionable. In Israel, pineapple fruits showing symptoms of FCR have revealed the presence of *P. funiculosum* and *F. proliferatum* (of the GFSC). One of the possible scenarios of the development of FCR in the plant is the penetration of the *P. funiculosum* at the early stage of flowering, followed by the entrance of a *Fusarium* belonging to the GFSC.

Barley Net Blotch in Israel - characterization of the pathogen population and identification of host resistance

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Net Blotch (*NB*), a major disease of barley is caused by the parasitic fungus *Pyrenophora teres f. teres* (*Ptt*). Yield losses of barley *Hordeum vulgare L.* due to NB reach 40% in severe epidemics. The final goal of the research is to identify new genetic resources for NB resistance and to characterize loci in the barley genome which confer this resistance. Although Israel is a center of origin for barley and NB, a clear knowledge gap exists on this pathosystem in Israel. To address this, we have constructed a *Ptt* Israel collection that will be characterized for genetic diversity and virulence. In addition, we have established inoculation and screening platforms in control conditions and in the field. These platforms are now used for screening divers host collections for *NB* resistance. Among those resources are spring barley landraces, a HEB-NAM mapping population (for linkage mapping and QTL analysis) and a diverse collection of the barley wild relative *H. bulbosum*. The results of the phenotypic assay in controlled conditions showed significant differences between the growth rate of isolates in petri dishes and the appearance of chlorosis and necrosis on leaves. In addition, significant variability in virulence of selected *Ptt* isolates to different barley varieties was detected. In the field, as previously observed (e.g. Kent, 1960), *H. vulgare* was the most susceptible host, and *H. bulbosum* generally has shown impressive resistance to the disease. Lines which showed high resistance in the field are re-tested this season for additional screenings and are used for crossing.

Causes and consequences of leaf reddening in vineyards originating from imported plant material in Israel

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Leaf reddening in grapevines can be a result of physiological damage due to insects (especially leafhoppers), or diseases caused by trunk-residing fungi, viruses or phytoplasmas. The symptoms differ and it is usually possible to distinguish between the causes by the red pattern and hue of the leaf and leaf distribution in the canopy. In Grapevine leafroll disease, leaf veins remain green (including ca. 1 mm to each side) while interveinal areas turn purple-red. Symptomatic vines from vineyards planted before 2009 are mostly positive to GVLRaV3 using the classic primers LC1 and LC2 (Osman et al., 2007) while most suspected vines from younger vineyards are negative. Nine young Cabernet Sauvignon plots (each 1–2 hectares) were surveyed for symptoms since 2014. In 2015, five previously symptomatic and five asymptomatic vines were marked in each plot. Date of symptom appearance, yield and must data were compared. Nine of the 45 previously symptomatic vines did not show symptoms in 2015. Differences in brix level were negligible in seven plots and significant in one. Fruit color was always lower in symptomatic vines (significant in 3 plots). The classic GVLRav3 primers detected seven positive vines; however, new primers from the literature (Bester, 2014) or designed according to published sequences, revealed 13 more positives and with another 19 unknowns that were also negative to other known Leafroll or red blotch viruses. Deep sequencing of small RNAs did not yet reveal the cause for reddening but detected three viruses for the first time in Israel.

The efficiency of new disease control chemicals

Efficacy of soil fumigants on the control of the tobamoviruses CGMMV in cucumber and ToBRFV in tomato crops

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Viruses of the tobamovirus genus survive for a long time on seeds, which are transported between countries and thereby constitute a major source of initial infestation. After the first cycle of tobamovirus infestation, the disease is established in soil and becomes a possible source for secondary infection of a newly planted crop. Agricultural activities during the course of the growing season constitute the primary means for disease dispersion. The tobamovirus *Cucumber green mottle mosaic virus* (CGMMV) is commonly found in greenhouses of cucumber and melon crops, and in the last two years, a new tobamovirus *Tomato brown rugose fruit virus* (ToBRFV) was identified in tomato varieties that harbor the *Tm-2²* resistance gene. ToBRFV has spread to most of the greenhouse tomato growing regions in Israel. Previous laboratory experiments had shown that the use of commercial stabilized chlorine products in soil reduced infection caused by CGMMV in *Cucurbitaceae*. However, results of the use of chlorine products in commercial fields were not consistent. To test the efficacy of chlorine and several other products in reducing virus infectivity, experiments were conducted in walk-in tunnels at the R&D Darom station. In these experiments the efficacy of the soil treatments was tested to control infection both of naturally infected soil and soil infested by plant sap

contaminated with the tobamoviruses. In addition, to simulate normal planting conditions the effect of damaging roots before planting on virus transmission was also tested.

A novel antifungal approach to manage plant disease based on disruption of the NAD cycle

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Nicotinamide adenine dinucleotide (NAD) is an important metabolite in eukaryotic cells. NAD is crucial for proper redox balance and it is a cofactor of the sirtuins histone deacetylases. During histone deacetylation, sirtuins convert NAD to nicotinamide (NAM). It was previously shown that NAM can reduce disease severity caused by *Candida albicans* in mice due to inhibition of an ascomycete-specific sirtuin Hst4. We tested the ability of NAM to restrict the growth of fungal plant pathogens. NAM was able to inhibit both hyphal growth and conidial germination of *Fusarium oxysporum* and *Botrytis cinerea* in vitro and in tomato slices (IC50: 4 mM and 25 mM respectively). PNC1 is a fungal-specific enzyme that degrades NAM: it was shown to be inhibited in vitro by nicotinaldehyde (NA). We were able to show that NA inhibits the growth of *Fusarium* and Botrytis. Surprisingly, as determined by RNAseq, the mode of action of NAM and NA is completely different. Genes that are up-regulated by NAM act mainly in transcription, while genes that are up-regulated by NA belong to the redox-balance. These results suggested to us that while NAM inhibits chromatin biology, NA disrupts NAD biosynthesis. This is in agreement of the role of PNC1 in NAD salvage biosynthesis pathway. We were able to show that NA toxicity can be bypassed by addition of niacin (the next compound in NAD biosynthesis cycle). We propose that by developing better chemistry to inhibit PNC1 and HST4 novel classes of fungicides will be obtained.

Control of late blight and downy mildews with fungicidal mixtures of Oxathiapiprolin

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Oxathiapiprolin is a new, highly effective, anti-oomycete fungicide. Artificial mutants of *Phytophthora capsici* were shown to be resistant to the fungicide. To delay the build-up of resistant populations in nature it is recommended to mix a high-risk fungicide with a fungicide with another mode of action. Here we show that dual mixtures of oxathiapiprolin (OSTP inhibitor) with either mefenoxam (RNA polymerase inhibitor), mandipropamid (CesA 3 inhibitor) or azoxystrobin (QoI inhibitor) were highly effective in controlling late blight in tomato and potato, downy mildew in cucumber and basil. The mixtures exhibited high activity when applied preventively or curatively. The mixtures were especially effective against mefenoxam -resistant isolates in curative application. Such mixtures, which contain reduced amounts of oxathiapiprolin, are as active as oxathiapiprolin alone at a full rate.

Reducing stem-end rots in mango by applying fungicide treatments during florescence or after harvest

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Stem-end rots (SER) are one of the most common diseases amongst subtropical fruits. In Israel, species within Botryosphaeria family cause SER, and *Lasiodiplodia theobromae* is one of the main pathogens causing SER. Its spores might penetrate the plant tissue through inflorescences and wounds. After penetration, the fungus endophytically colonizes the fruit stem

phloem, without apparent symptoms. When ripening begins, the fungus switches to a necrophyte stage, resulting in SER. We examined two approaches to inhibit the fungus: by applying fungicides during florescence (to prevent inoculation), or a postharvest fruit dip with fungicides (to kill the fungi). Mango orchards (cvs. Shelly and Keitt) were sprayed with Scholar (Fludioxonil) or Switch (Fludioxonil + Cyprodinil). Samples from sprayed trees presented dramatic decreases of pathogenic populations found in young fruit stems and in postharvest fruits, which led to a dramatic decrease in SER incidence. Subsequently, 'Shelly' and 'Keitt' mango fruits were dipped post-harvest with Sportak (Prochloraz), (the current common practice) or Scholar fungicides, or with their combination. The experiments were conducted with or without *L. theobromae* inoculation. Interestingly, dipping Scholar significantly reduced both the incidence and severity of SER of both un-inoculated and *L. theobromae* inoculated fruits. Based on our results, many farmers started to integrate fungicide-spray against powdery mildew during flowering with the fungicide spray against SER. Additionally, based on our results, an application for approval was recently submitted for postharvest use of Scholar for mango fruit.

Plant-pathogen interactions

LaeA regulation of secondary metabolism modulates virulence in *Penicillium expansum* and is mediated by sucrose

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Penicillium expansum, the causal agent of blue mold rot, is a critical health concern due to production of the mycotoxin patulin in colonized apple fruit tissue. While patulin is produced by many *Penicillium* species, the factor(s) activating its biosynthesis are not clear. Sucrose, a key sugar component of apple fruit, was found to modulate patulin accumulation in a dose-responsive pattern. Increase in sucrose concentration (as a culture amendment) from 15 to 175 mM decreased both patulin accumulation and expression of the global regulator *laeA* by 175- and 5-fold, respectively, while increasing expression of the carbon catabolite repressor *creA*. LaeA was found to regulate several secondary metabolite genes, including the patulin gene cluster and concomitant patulin synthesis in vitro. Virulence studies of Δ*laeA* mutants of two geographically distant *P. expansum* isolates (Pe-21 from Israel and Pe-T01 from China) showed differential reduction in disease severity in freshly harvested fruit. Such changes ranged from no reduction for Ch-Pe-T01 strains to 15–25% reduction for both strains in mature fruit, with the Δ*laeA* strains of Is-Pe-21 always showing a greater loss in virulence. Abiotic factors are important in LaeA regulation of patulin and other secondary metabolites that contribute to pathogenicity.

Involvement of the Type III secretion system and induction of plant defenses in colonization of fresh vegetables by *Salmonella enterica*

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Salmonella enterica is a prevalent causal agent of human enteric diseases associated with consumption of fresh fruit and vegetables. Enteric pathogens, similarly to phytopathogenic bacteria, can reach the plant apoplast through wounds, stomata and other natural openings. Currently there are insufficient mitigation strategies to prevent or minimize the microbial

contamination of crops. Many Gram-negative pathogenic bacteria use the Type III secretion system (T3SS) to deliver Type III effectors (T3Es) directly into their host cells. In plants, T3Es play an important role in suppression of plant defenses which is a prerequisite for microbial colonization. Here we investigated the role of T3SS and the effect of induced systemic resistance on colonization of plants by *Salmonella*. We developed a translocation assay for T3Es into beet roots. *Salmonella* T3Es (SPI-1 and SPI-2) could not be translocated to host plants, although similar proteins were translocated by plant pathogens. Additionally, no difference was observed in endophyte colonization of lettuce leaves by *Salmonella* T3SS mutants compared to the wild type. The effect of systemic resistance inducers on leaf colonization by *Salmonella* was also examined. It was demonstrated that Bion and BABA significantly reduced the endophytic colonization in basil and lettuce, respectively. Results of this study suggest that translocation of *Salmonella* T3Es into plant cells does not play a role in *Salmonella* colonization of plants and that suppression of plant defenses occurs by other mechanisms. The use of systemic resistance inducers may provide a means to reduce crop contamination by human pathogens.

Colonization patterns of symbiotic fungi associated with the ambrosia beetle *Euwallacea* nr. *fornicatus* in avocado

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The ambrosia beetle *Euwallacea* nr. *fornicatus* inoculates avocado xylem with three symbiotic fungi: *Fusarium euwallaceae* (FE), *Graphium euwallaceae* (GE) and *Paracremonium pembeum* (PP). The fungi serve as feed for the beetles and their brood and are the main cause of tree damage. The objective was to reveal the relationships between the fungi and avocado and to determine the fungal colonization pattern. A week after initial attack, only FE was isolated from infected tissue. GE was detected two weeks later. In unsuccessful attacks, GE disappeared within five weeks; while FE was observed after 10 weeks. Brood development coincides with rapid increases in all three fungal populations. After beetle emergence, FE disappeared while GE and PP were still able to be isolated from dead tissue. A similar pattern to unsuccessful attack by the beetle was obtained after artificial inoculation by injection: approximately 8–9 weeks after inoculation, GE and PP were barely detected, while FE was recovered from tissue >12 months after inoculation. Beetle-associated stained xylem contained six flavonoids including putative epicatechin, naringenin, quercetin, and taxifolin. Fungi were isolated as far as 30 mm from beetle galleries and from stained xylem alone. These findings suggest that FE serves the beetle in coping with plant resistance and as feed during the early stages of colonization. GE and PP dominance is likely related to the weakened tissue and development of the larvae which mainly feed on GE. The survival of GE and PP in dead xylem may facilitate long distance spread of the beetle in woody materials.

Genetic adaptations of *Candidatus* Liberibacter solanacearum to its vector and plant hosts

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Bacterial plant pathogens belonging to the genus *Candidatus* Liberibacter represents a major threat to important agricultural crops. Ca. Liberibacter spp. are obligate parasites, vectored by psyllid insects and are nonculturable. Devastating diseases such as Huanglongbing (citrus greening) are caused by Ca. Liberibacter spp. and despite huge investments in research in recent years, efficient means to tackle it are lacking. One of the interesting features of Ca. Liberibacter spp. is the ability to adapt to and thrive in two different biological niches: the insect vector body and plant phloem cells. We hypothesize that to fulfill these adaptations Ca. Liberibacter spp.

differentially express genetic determinants in the different hosts. We further hypothesize that secreted proteins are of paramount importance for this task. To examine our hypotheses we sequenced the genome of Ca. Liberibacter solanacearum (haplotype D), which is associated with carrot yellows. We used bioinformatic tools to predict open reading frames and then detected proteins containing a conserved secretion signal. Primers for quantitative-PCR were designed for these genes and their expression level was analyzed in plant and in insect. Several putatively secreted proteins were differentially expressed in the two hosts. Some of these genes contain a nuclear localization signal which may indicate that they are targeted to the host nucleus. In vivo secretion of these genes will be tested as well as their interactions with host proteins. Identifying these crucial adaptation factors may serve as novel targets for developing new management tools for Ca. Liberibacter diseases.

Use of resistance sources to withstand disease

Sources of resistance against downy mildew in cucumber

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Downy mildew caused by the oomycete *Pseudoperonospora cubensis* is a devastating disease of cucurbits worldwide. Cucumber cultivars resistant to the disease prior to 2004 became susceptible due to the appearance of new pathotypes and pathogen mating types, probably transmitted by infected seeds. Here we report on the genetic resistance of wild cucumbers to the disease and on their possible use in breeding programs. Of the nine USDA accessions tested, PI-197088 and PI-330628 were most resistant to multiple isolates. Crosses were made between each PI and the susceptible SMR-18. Plants were exposed to natural infection in the field in 2013 and 2016. Plants were examined for disease symptoms once a week using the following visual scale: 0 = no disease; 1 = 1–10% of leaf area infected; 2 = 11–25%; 3 = 26–50%; 4 = 51–75 and 5 = 76–100% of total leaf area infected. Data shown represent the final disease score at fruit set. In both years, the PI parents were highly resistant (0–1% of leaf area infected) whereas the F1 plants were partially resistant (scored 2–3). In summer 2013 the F2 population (75 plants) segregated into 1:14:1 resistant (scored 0), partially resistant (scored 1–4) and susceptible (scored 5), respectively. In summer 2016, two populations were tested (105 plants and 156 plants) and both segregated at a ratio of 1:14:1. BC plants in 2016 were all susceptible (scored 4–5) while BCr plants were all partially resistant (scored 1–2). The data suggest that several partially-dominant loci are responsible for downy mildew resistance. Some molecular markers segregated with resistance. To enhance resistance, the two resistant PIs were crossed and their F2 population examined for disease resistance. Fifty-eight plants were resistant (scored 0) and 4 plants were partially resistant (scored 1–3) under field conditions, suggesting that PI-197088 and PI-330628 share some, but not all, loci for resistance to downy mildew prevailing in Israel.

The involvement of the VPS4 gene in virus resistance in cucumber

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The vacuolar protein sorting-associated protein 4 (VPS4) is translated from a conserved gene and is part of the endosomal sorting complexes required for transport (ESCRT) system. Recently we mapped two amino acid substitutions in cucumber involved in *Zucchini yellow mosaic virus* (ZYMV) immunity (Amano et al., Theor. Appl. Genet. 2013). In addition, ZYMV resistance was found to be recessive, suggesting a VPS4 interaction with virus proteins. Sequence analysis revealed that all tested resistant Israeli cultivars (‘Samara’, ‘Kfir’ and others) have the same mutations. Similar VPS4 gene expression levels were observed in both susceptible and resistant

cultivars. Moreover ZYMV infection of susceptible cultivars did not alter their VPS4-like expression level. Therefore our findings support that ZYMV resistance is associated with affecting structure or function in the ESCRT complex by a mutated protein, rather than at the transcription level. To characterize resistant cultivars, we developed a diagnostic system to identify VPS4 gene changes using primers specific to the mutations. It should be mentioned that low virus accumulation of five additional viruses was identified in cucumber cultivars carrying mutations. In addition we showed that silencing of VPS4 gene expression by VIGS leads to plant death and indicating the importance of the gene for the plant. Results indicate that using genomic editing for creating mutations in VPS4 gene in different crops might offer a new solution for fostering broad virus resistance.

Characterization of a resistance-breaking tobamovirus in tomatoes in Israel

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An outbreak of a new disease infecting tomatoes occurred in October–November 2014 in Ohad village in the Bsor area in southern Israel. Symptomatic tomato plants showed a leaf mosaic pattern accompanied occasionally by leaf narrowing and yellow spotted fruit. The disease spread mechanically and rapidly, reminiscent of tobamovirus infection. Epidemiological studies showed the spread of the disease in various growing areas, in the south and towards the southeast and northern parts of Israel within a year, and within two years spread across all the tomato growing area in Israel. Transmission electron microscope (TEM) analysis showed a single rod-like form characteristic of the *Tobamovirus* genus. We confirmed Koch's postulates for the disease in tomato plants followed by partial host range analysis and observed that tomato cultivars bearing the *Tm-2*^{2} resistance gene were susceptible to the new viral disease. Next Generation Sequencing (NGS) of total small RNA or transcriptome sequencing (RNASeq) were performed on leaves of two cultivars grown in two different locations, and on tomato seeds collected from infected tomato plants/fruits. We found a single virus caused the disease in samples collected from commercial cultivars across Israel. The new Israeli tobamovirus showed high sequence identity to the Jordanian *Tomato brown rugose fruit virus*.

Identifying tomato Tm-2 resistance-breaking mutations in a new and emerging tobamovirus

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Recently, a new tobamovirus was discovered in Israel able to break 55 years of durable *Tm-2*-gene-mediated resistance in tomato. Following isolation and sequencing the virus was found to be *Tomato brown rugose fruit virus*, a new tobamovirus recently identified in Jordan. Previous studies on mutations causing resistance-breaking, including *Tm-2*-mediated resistance, demonstrated that the resistance-breaking phenotype was mediated by only a few mutations. Identification of such residues in resistance-breaking isolates is hindered by significant background resulting from 9 to 15% differences in virus genomic sequences compared to known species. We have utilized a comprehensive genomic comparison of tobamovirus species to identify five potential resistance-breaking mutations in the viral movement protein (MP), the primary target of the *Tm-2* resistance, and two in its helicase. Future work

should include similar unbiased computational analysis of all the viral proteins followed by reverse genetic-based validation of the identified potential resistance-breaking mutations.

Posters

A selective medium to isolate Esca-related spores from spore traps in the vineyard

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"Esca proper" disease causes heavy losses in wine and table grapes around the world. It is associated with a complex of xylem-residing fungi in the vine trunk. The main pathogens are *Fomitiporia mediteraneae, Phaeoacremonium aleophylum* (*PAL*) and *Phaeomoniella chlamidospora* (*PCH*). Infection occurs usually through pruning wounds, by spores which are air-dispersed following rain events and high humidity. Spores can be detected by a spore-trap. However, slow growth of esca is occasionally masked by faster growing fungi—mainly *Penicillium*. Therefore, a selective medium to inhibit the development of fast growing spores is required. The aim of this study was to develop a selective medium to isolate and identify esca spores in spore traps. The effect of different fungicides on the development of *Penicillium*, *PCH* and *PAL* spores in vitro was examined. Esca spore viability was tested by transferring them to a new fungicide-free medium 10 dpi. Signum, Scholar, Switch and a combination of Tachigaren + Rizolex were added in various concentrations (modified from Serra et al. 2008) to PDA plates supplemented with chloramphenicol 250 mg/l. Signum and Scholar (10 mg/l) were highly effective in inhibiting *Penicillium* growth without affecting the viability of esca spores, and therefore can be used in a selective medium. An effective method for isolating esca spores in the vineyard will enable to assess the potential of infection and can serve as a basic tool towards the development of agro-technical methods to reduce infection rates in the vineyard.

qPCR-based method for detecting and monitoring *Harpophora maydis* inside host tissues

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Late wilt is a severe vascular disease of maize characterized by relatively rapid wilting of maize plants after fertilization. The disease is caused by the fungus *Harpophora maydis* and is currently controlled using resistant varieties. Earlier, several fungicides were tested against the disease, one of which, Azoxystrobin, applied using irrigation lines, inhibited the development of wilt symptoms in the field and recovered cob yield by 100%. Nevertheless, this treatment is not economical. The current work aimed at developing a Real-Time PCR (qPCR)-based method for detecting and monitoring *H. maydis* DNA inside the host tissues. This method was applied to evaluate the efficiency of seed coating with Azoxystrobin against late wilt. The chemical treatment completely reduced the pathogen DNA appearance in seeds in vitro, and minimized damage to plant biomass and development. In sprouts (up to 40 days, four leaves), the seed dressing treatment produced similar results. These results were supported by the new qPCR-based detection method, which proved to be much more sensitive than the traditional PCR method. In a field experiment (summer 2016) with Azoxystrobin-coated seeds, the qPCR method enabled the detection of the pathogen 20 days after sowing, a month before the first detection using the PCR method. The chemical coating caused a reduction in fungal DNA in the plants on most days, but did not prevent the disease symptoms or yield loss. This work encourages further examination of other fungicides using the qPCR detection method to evaluate their influence.

Bacterium isolated from a disease vector has remarkable endophytic characteristics

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A *Dyella*-like bacterium (DLB) was isolated from the insect vector *Hyalesthes obsoletus*, which transmits yellows disease of grapevine, caused by phytoplasma. Recent studies have shown that when DLB is grown in vitro in large quantities and then sprayed on grapevine foliage in the vineyard, it can suppress yellows disease symptoms and increase overall yield. Although the mechanism of action of DLB is still unclear, fluorescent labelling of DLB coupled with confocal microscopy provide evidence that it is localized to the plant phloem tissue. Interestingly, the grapevine yellows pathogen, phytoplasma, is restricted to the phloem tissue. These findings urged us to test whether DLB can be used to suppress other phloem-restricted bacterial diseases, such as those caused by *Candidatus* Liberibacter spp. First, we tested the host range of DLB by applying it by both spraying and soil drenching to carrot, cotton, citrus, grapefruit, periwinkle, sesame and tomato. DLB occupied the tissue of all tested plants but tomato, and persisted for 30 days and more in some of the tested plants. DLB also appeared to have an astonishing systemic capability, migrating from soil to leaves in three days. Confocal microscopy of carrot sprayed with DLB revealed that DLB aggregates close to the leaf stomata, and later was found inside the leaf in close proximity to the phloem tissue. Overall, these results provide an important and promising basis to test the capability of DLB to suppress important world-wide diseases caused by phloem-restricted pathogens, such as citrus greening.

High CO_2 effect on *Leuconostoc mesenteroides* pathogenicity in stored carrots

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Carrot (*Daucus carota* L.) is an important vegetable crop in many countries worldwide, both economically and nutritionally. In Israel, the primary inoculum in the field or the packing house, combined with the volatiles environment created during long shipments, creates micro-climate conditions leading to a dramatic change of the microbial populations on the carrots' surface. The microbiome shift permits the establishment of different phytopathogens causing soft rots of the entire root. Specifically, this shift, induced by high CO_2 levels, leads to the dominance of the lactic acid bacteria *Leuconostoc mesenteroides*. It has been found that this new strain of *L. mesenteroides*, named YL48, produces liquid slime over the carrot surface, termed oozing. Oozing facilitates the development of other microorganisms, some phytopathognic, for example the mold *Mucor fragilis*. Characterization of the oozing sugar and protein components led to the identification of an abundant sugar polymer (dextran), along with several major bacterial glucosyltransferase enzymes involved in extracellular polymeric substance production. Dextran is produced by the bacterium while fermenting sucrose, the main sugar in carrots. This dextran shows favorable physiochemical properties and is a valuable compound in a variety of industries, including biomedical and food. Therefore, identifying the optimal environment leading to increased yield of dextran by *L. mesenteroides* YL48 has beneficial outcomes, both for the carrot growers and for other industries.

In cupo: an autotrophic closed plant production and manipulation technology using easily available materials

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Sweetpotato (*Ipomoea batatas*) is a major crop in Israel and worldwide, and is generally propagated vegetatively by cuttings. Sweetpotato stock plants are commonly maintained in vitro, and multiplied ex vitro for agricultural production as necessary. *In cupo* (Latin = tub or cask) is novel propagation method whereby plants can be propagated in closed vessels much larger than is usual for plant tissue culture, under photoautotrophic conditions in a temperature-controlled growth room. The vessels reported are 1 L food-grade polypropylene boxes with a large paper filter inserted into the lid. The substrate is a horticultural growing plug, a variety of which were tested. Slow release fertilizer is provided once only. Distilled water is supplied as necessary. The plants grow autotropically by photosynthesis under fluorescent lighting (100 μmol photons PAR m^{-2} s^{-1}) in these closed conditions protected from pathogens for several months, and can be subcultured when necessary, every 6 to 8 months. Such conditions are not axenic, and the boxes are opened as necessary so that the distilled water can be renewed (every 2–3 weeks). By this means we have cultured healthy sweetpotato plants continuously for years, with subculture at intervals of several months, without the possibility of infection by viruliferous aphids or whiteflies. Sweetpotato plants grown *in cupo* can be transferred directly to a greenhouse without further hardening. Besides use as a hardening and multiplication method, this technique can be used for graft-transmission of viruses from virus-infected sweetpotato scions to healthy rootstocks.

Grape downy mildew appearance in Israel as a result of a continous or single rain event

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Grape downy mildew (DM) is caused by the oomycete *Plasmopara viticola*. Infection may be reduced through timely fungicide application. The Israeli spring is characterized by random rains and hot dry spells. Farmers currently apply fungicides in spring, before every rain which threatens mainly young clusters. It has recently been observed that plots in the Golan Heights and Galilee are sometimes treated unnecessarily. The research hypothesis was that DM infection develops only following continuous rain events with more than 5 mm, on a background of wet soil. The long-term objective of the present study was to reduce fungicide application against DM. We monitored the disease in untreated plots, studied the effect of dry spells on DM sporulation and the differences in susceptibility to DM infection during water stress. DM appeared in eight of 25 plots only after a continuous rain event, with damage developing in only 3 plots. A single day of rain (>5 mm) or two days with less than 5 mm on the second day, did not cause infection (observed over 10 rain episodes). DM germinated on all leaves collected after 4 or 7 days of hot dry spells after overnight incubation. DM sporulated significantly less on mature leaves collected from water stressed (−16 pa) compared to well-watered grapevines (−8 pa). In conclusion, DM incidence in northern-Israel was low in 2013–2016. Fungicide application can be significantly reduced and applied only following continuous rain forecasts. Hot spells can delay DM development, but cannot prevent its re-sporulation. Water stress reduces grapevine sensitivity towards DM.

Sister Chromatid Cohesion in *Fusarium oxysporum*

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The soil-borne fungal plant pathogen *Fusarium oxysporum* (FO) is extremely genetically diverse, as shown by the existence of sub-species uniquely adapted to different hosts. Sister chromatid cohesion is an essential and conserved process that is mediated by the cohesin complex, which is also important for chromosome condensation, DNA repair, and transcription. Using comparative genomics, we found that sister chromatid cohesion has significant differences in FO compared to other eukaryotes. FO does not encode eco1, an essential acetyl transferase that is thought to activate cohesin. Interestingly, the target sequence for acetylation on the cohesion subunit, Smc3, is conserved. We hypothesize that differences in sister chromatid cohesion in FO contribute to its genetic diversity. Revealing how FO lives without Eco1 can be used to develop specific fungicides. To determine whether Smc3 is acetylated in FO, we will test the status of K112/113 acetylation. For this purpose, we made a construct of smc3 with tef1 promoter and 3XFLAG tag and co-transformed it with hygromycin resistance cassette to *Fusarium oxysporum* f. sp. *lycopersici* protoplasts. Twenty six clones were resistant to hygromycin, and four were found to have the construct in their genomic DNA. We plan to test the tagged Smc3 protein expression level by western blot analysis with α-FLAG antibody. We also found that FO is unique among fungi in having at least two paralogs of the mitotic cohesin subunit Rad21: one evolutionarily conserved and another that is not. Additionally, we found that FO expresses rec8, a meiosis-specific paralog of rad21, despite the fact that meiosis has never been demonstrated in FO. Currently, we are examining the expression pattern of different paralogs of rad21 in various conditions using real-time PCR.