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ABSTRACT

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INVITED LECTURES

CRISPR/Cas9 genome editing technology is a plant breeding dream come true

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Crop improvement depends on genetic variation within a population. This variation is based on mutations in the genome's DNA occurring during evolution. The genetic variation can be increased by random mutation of the DNA, by mutagenic chemicals or physical treatment such as radiation. Artificial mutagenesis (EMS/radiation) has been widely used in conventional plant breeding for decades as deregulated (i.e. not government regulated) food products. In contrast to random mutations, genome editing technologies cause specific changes in a target gene. In genome editing exogenous nucleases break the DNA within the target sequence and an endogenous DNA repair mechanism ligates the DNA ends by nonhomologous end joining, leading to nucleotide deletions at the break site (causing gene knockout). In the last decade different nucleases (ZPN, TALEN and meganucleases) have been used with very limited success. Recently, a new technology called CRISPR/Cas9 has been developed for genome editing. The technology is based on a bacterial immune system, in which RNA transcribed from **C**lustered, **R**egularly **I**nterspaced, **S**hort **P**alindromic **R**epeats (CRISPR) in bacterial genomes associated with a nuclease (Cas9) cut bacteriophage DNA and prevent virus infection. In current practice, the DNA genome of a target organism is attacked by the Cas9 nuclease from *Streptococcus pyogenes*, in association with a synthetic guide RNA, inducing sequence-specific genomic DNA double-strand breaks. This technological revolution is highly efficient, simple to use and allows DNA editing in all organisms tested to date. The technology has been demonstrated in both dicotyledonous and monocotyledonous plants and protoplasts. Additionally, genome editing can occur without a transgene remaining in the plant, so that edited plants are equivalent to non-transgenic mutants. Thus, CRISPR/Cas9 is a promising technology to modify genes without rendering such plants classical Genetic Modified Organisms (GMOs), paving the way to its implementation in agricultural biotechnology without regulation. We have utilized this technology to develop novel crops with improved stress-resistance, including climate-tolerant tomato and virus-resistant cucumber cultivars. We believe that this novel technology has the potential for expediting development of disease resistance in many crops without the need for extensive backcrossing and genetic manipulation with wild resistance sources.

Global warming and potential effects on plants in Israel

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The predicted regional climate changes in many of the weather variables over the Eastern Mediterranean (including temperature, rainfall, humidity, aerosols, winds and particularly an increase of extreme climate events) have recently been computed employing an ensemble of regional climate models and different scenarios for emission of greenhouse gases (GHG). These climatic changes driven largely by the global warming due to GHG increases will undoubtedly affect plants. I will review our most recent 21st century climate modelling predictions for this region. I will present a new methodology for weighting high resolution model simulations to project future rainfall in the Middle East. Special focus will be given to potential changes in dew (Yiftah Ziv, M.Sc. Thesis, 2015). Dew is a meteorological phenomenon in which liquid phase water, usually during night time, appears on the ground or on nearby objects. Although the daily dew amount is on average less than 10% of the daily evapotranspiration volume, studies have found that dew has an effect on plant water budget. Dew is utilized by micro-organisms and insects as a water source, and contributes to soil moisture especially in arid and semi-arid regions, and helps in the creation of soil crusts. However, dew on leaves also enables pathogen spread. Therefore, the impact of dew can be negative or positive. In spite of being an important meteorological

phenomenon, dew is mostly absent in climate change research, mainly because it is a micro-meteorological phenomenon, which cannot be easily described by coarse climatic models. The potential dew amounts obtained both by modelling and observations exceed 50 mm per annum for most regions of Israel (except for Jerusalem and the central-southern Negev desert). These amounts are twofold and even fourfold past dew measurements in Israel.

Agro-terrorism: A threat to crop production and food security from plant pathogens

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Agricultural crops are the source of food, feed and fibers. It is, therefore, the soft belly of any nation's economy and stability. Most crops are vulnerable to attack by a wide spectrum of plant pathogens which can harm production and even destroy the entire yield. The deliberate introduction of a new plant pathogen into agricultural area could have a serious impact on yield, in addition to the cost of disease management over the short and long-term. Invasions by new pathogens can disrupt trade from a country or region if quarantined pathogens are introduced, resulting in lost markets and, in some circumstances, unavailability of certain foods. The development of anti-crop weapons based on plant pathogens has been made in parallel with the development of chemical weapons and anti-human biological weapons. Although anti-crop weapons have always been of lower priority compared with anti-human ones, and in most cases less successfully, this threat should not be overlooked. Any country with the military and scientific sophistication to successfully develop a biological weapon capability is likely capable of developing an effective anti-crop biological weapon. Since certain terrorist groups and some countries are suspected of actively developing biological weapons, there is a real risk to crops from anti-crop weapons in today's world. Managing a disease outbreak from deliberate introduction involves a number of interrelated or repeated types of control methods and surveys, testing, inspections and regulatory actions. Accomplishing eradication of an outbreak requires a concerted, skillfully executed program to advance toward the objective of eradication and stabilization of the production system.

EARLY DETECTION OF PLANT DISEASE AGENTS

Ergot disease in sorghum and detection of *C. africana* in seeds in Israel

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Ergot disease of Sorghum [*Sorghum bicolor* (L.) Moench] causes extensive damage to crops in all continents. It is caused by the fungus *Claviceps africana,* which originated from Africa and India, and known in America and Australia for the last twenty years. The disease infects the inflorescence before pollination. Windborne fungal conidia germinate on the female flower and hyphae penetrate and colonize the unfertilized ovaries, replacing the tissue plant with fungal structures (sphacelium and sclerotium) where assexual conidia are produced. At a later stage conidia are excreted in the honeydew produced by the diseased plant, giving the disease the name "sugary disease". In Israel, *C. africana* is a quarantine pest. During routine purity tests, classical morphological examination of imported forage sorghum seeds based on sclerotia identification usually leads to detection of the disease. However, last year ergot disease spread in sorghum fields in Israel. The fungus was identified by microscopic examination and molecular tests, and eradication was performed. The appearance of ergot disease in Israel indicates a lack of reliability of the classical disease detection test in seeds. A novel molecular test was developed to achieve a more robust detection assay. In the future the test will enable detection of *C. africana* in imported seeds where sclerotia cannot be found.

Use of Real Time PCR to measure the infection rate of potato tubers with *Potato virus Y* prior to storage

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Potato (*Solanum tuberosum*) is the largest crop in Israel. Production is based on the import of seed tubers from Europe for the spring planting. Imported tubers are generally free from virus infection. The most important virus in the world (and Israel) infecting potato is *Potato virus Y* (PVY), which can cause severe damage to marketable yields. Tubers from the spring harvest are stored over the summer for planting in the autumn/winter. Therefore it is important to be able to determine the infection rate of seed tuber lots prior to storage. A popular technique to measure tuber infection is to grow plants from tubers and measure virus in the leaves by ELISA (the "Growing-On test"), which takes 6-8 weeks to get results. There is a need for a faster test, such as Taqman-Real Time PCR, for direct

analysis of viral infection of tubers at harvest. To use Real Time PCR as a diagnostic tool it is necessary to determine if the two techniques give comparable results on batches of field-grown potatoes. Such a test was performed on potential seed tuber lots for three successive spring harvests. The results show that at a low PVY infection rate (suitable for seed tubers) there is a reasonable agreement between the techniques. However, at higher levels of PVY infection, which are anyway unsuitable as seed, there is not a good agreement between the techniques. Real Time PCR is appropriate for the determination of PVY infection in potential seed tubers in Israel.

EPIDEMIOLOGY AND CONTROL MEASURES

An integrated system approach for the management of root knot nematodes in greenhouse production of pepper crops

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The Israeli pepper crop is grown intensively in closed structures (plastic and net houses), and for export of high quality fruits. The plants are susceptible to root knot nematodes and pathogenic fungi which dwell in deep soil layers. Therefore, it is a challenging task to control nematodes and other soil-borne pests to ensure a productive and healthy crop. The objectives of this study were to develop a nematode control approach which is integrated with measures such as sanitation and special crop fertigation. Two field experiments were carried out in plastic- and net-houses for pepper production which were heavily infested with *Meloidogyne incognita*. Each experiment was for a period of two years. Each experiment was designed with three levels of treatments, including soil disinfestation, root destruction and crop fertigation. At the end of the previous pepper crop 1,3 dicholoropropen (1,3D) was applied to the plots to kill the roots and prevent the establishment of a new generation of nematodes. Soil disinfestation using combination of 1,3D or dimethyl disulfide (as Paladin) and solarization was applied during the summer. The combination of sanitation (root destruction) at the end of the previous crop together with soil disinfestation and specific fertigation reduced the number of nematode eggs in the soil to negligible levels. Fertigation with a phosphate/nitrogen based formula was applied periodically to improve the tolerance of the plants to nematode infection during the crop season. During the crop production we did not observe any damage from nematode infection in the treated plots. Root analysis at the end of the crop season indicated zero infection by nematodes in one experiment and a low rate of infection in the second. This study indicates that control of root knot nematodes in intensive crops requires concerted action of various measures within the production system, which benefits crop production and are detrimental to the pathogen.

Observations regarding the primary inoculum source of *Phytophthora infestans* in potato and tomato fields and the spread of the disease in Israel

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Late blight, caused by *Phytophthora infestans*, is one of the most important diseases of potatoes and tomatoes in Israel and worldwide. Although the epidemiology and management of late blight have been studied in Israel for more than 50 years, it is still not clear what are the main sources of primary inoculum in both crops. The objectives of this study were: (i) to characterize the *P. infestans* genotypes prevailing in Israel, and (ii) to document the spatial and temporal onset of late blight symptoms around the country. The genotype of 41 *P. infestans* isolates sampled from potato and tomato crops in the 2013-14 autumn seasons, 2014 spring season and the 2014-15 autumn seasons was characterized. All isolates were categorized as belonging to the US-23 clonal linage, suggesting that infected potato seeds imported from European countries, did not serve as the source of initial inoculum in local production areas. Based on data recorded in 2014-15 we hypothesized that late blight epidemics in that year originated in the northwestern Negev (where host crops are grown year-round) from which the pathogen sequentially spread to other production areas of the country.

Control of Fusarium wilt in summer lettuce by sanitation and pre-planting soil fumigation

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Fusarium wilt caused by the soil borne fungus *Fusarium oxysporum* is one of the major diseases of lettuce, with highest incidence during summer when high soil temperatures prevail. Disease symptoms include chlorosis and wilt, brown discoloration of the vascular bundles, root rot and eventually plant death. The common practice in lettuce cultivation of incorporating plant residues into the soil causes an increase in soil borne inoculum. Therefore soil fumigation, mainly by metam sodium (MS) is frequently applied through the irrigation system prior to planting. However, MS fumigation has high costs, negatively affects the environment and its efficiency is inconsistent due to accelerated biodegradation. The objectives of the current study were to evaluate the effect of lettuce residue sanitation (chemical and

mechanical) at the end of the season and to evaluate the efficacy of pre-planting soil treatments (solarization alone or combined with MS) on the incidence of Fusarium wilt. In addition, the disease development pattern was characterized during the year at different soil temperatures. No significant effect of sanitation treatments on reducing disease incidence was observed. However, a significant reduction in disease incidence was observed due to pre-planting soil disinfection treatments. Solarization alone showed a significant effect on disease incidence with no additional effect of MS. Apparently, solarization may provide an efficient and sustainable solution for reducing Fusarium wilt during summer. However, other practices should be tested in early spring when temperatures are not high enough for solarization and conditions are suitable for disease development.

Meta-analysis of disease reduction and yield increase by soil solarization and its combinations

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Soil solarization is a non-chemical method of using solar heating for managing a wide range of soilborne pests. Numerous studies have been performed since 1976 to examine and validate its potential and efficacy. The phase-out of methyl bromide has boosted the use of solarization alone and in combination with other measures, such as soil fumigants, nematocides, biological agents or organic amendments. Meta-analysis refers to methods used for amalgamating results from different studies for identifying general trends of effects, analyzing sources of disagreement among studies, and suggesting explanations for failures. Meta-analytic approaches can also illuminate other interesting relationships in the context of multiple studies. The aim of the current study was to employ meta-analysis tools for examining if there is an advantage to the combinations of solarization with other agents in comparison to solarization as a sole means. We analyzed 74 documented experiments, and calculated the control efficacy of solarization alone or in combinations with fumigants or with organic amendments and with biological agents. These calculations were made for three groups of soilborne pathogens: various formae speciales and species of *Fusarium*, root knot nematodes and a group containing the pathogens *Sclerotium cepivorum*, *Verticillium*, *Pyrenochaeta*, *Rhizoctonia* and *Pythium*. In most cases combination with additional agents improved the control efficacy, reduced its variance, increased the probability that control efficacy was high (above 70%) and decreased the probability that control efficacy was low (under 40%). The last criterion was significantly low in the combinations (near zero) compared to solarization alone. Also, in many cases yields in the combined treatments were higher than that with solarization alone. These results demonstrate the importance and benefit of combining solarization with other control agents in managing soilborne pathogens.

Integrated management of downy mildew caused by *Peronospora belbahrii* with focus on cultural techniques

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Basil downy mildew (BDM) caused by *Peronospora belbahrii* is a severe disease in sweet basil (*Ocimum basilicum*). Since 2012 BDM has spread to all growing regions in Israel. High relative humidity and moderate temperatures are required for the pathogen to cause severe damage. The main means of control of BDM is the application of fungicides. Unfortunately, there are only a limited number fungicides available, as well as issues of food safety, environmental contamination and the development of pathogen fungicide resistance. Therefore, control methods are required to manage the disease. Experiments were conducted to identify potential climate-management techniques in greenhouse and tunnels at Eden Experimental Station, Emek Hama'ayanot and in Zohar Experimental Station, Kikar Sedom. The methods tested included polyethylene soil mulch, passive heating of the tunnels by closing the aeration openings, air circulation and reduction in plant density. Polyethylene soil mulch reduced the disease severity compared to uncovered soil. Air circulation reduced the disease severity compared to structures with no air circulation. Plant density of 15 plugs/m^2 reduced the BDM severity compared to a higher density of 30 plugs/m^2. Passive heating of the tunnels significantly reduced BDM severity. Polyethylene soil mulch and a plant density of 15 plugs/m^2 reduced the disease severity in east-west aerated tunnels. Heating the root zone (to 32°C under controlled conditions) before canopy inoculation with BDM reduced the disease compared to non-heated plants. We conclude that heat exposure of plants induced resistance to BDM. Cultural methods therefore have the potential to suppress the disease.

Fanning suppresses downy mildew in sweet basil

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Downy mildew, *Peronospora belbahrii*, is currently the most serious disease of sweet basil (*Ocimum basilicum*) in the world. Previously we demonstrated that effective control of the disease in nature was achieved by nocturnal illumination which suppressed pathogen sporulation on leaf surfaces. We also showed that the spores and the mycelium of *P. belbahrii* are sensitive to heat and that day-time solar heating, achieved by covering basil crops in net

houses with polyethylene sheets, was highly effective in suppressing disease development and increasing yield. We now report on another physical means to prevent the pathogen from attacking basil: fanning the crops at night. We show that nightly nocturnal fanning (wind speed of 1-1.5 m/s) from 8 p.m. to 8 a.m. applied to basil crops growing in net houses dramatically suppressed downy mildew development. In three experiments conducted during 2015, leaves in control (non-fanned) net-houses reached, within a month, a level of 90-95% infection as against 0.5-1.7% infection in adjacent fanned net houses. Nocturnal fanning avoided water saturation of air and consequently reduced infection and sporulation of the pathogen. The data suggest that nocturnal fanning is highly effective in suppressing downy mildew epidemics in sweet basil.

PLANT INTRODUCTION × PATHOGEN

Plant hormones regulate the development of *Harpophora maydis*, the cause of late wilt in maize

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Late wilt, a severe vascular disease of maize caused by the fungus *Harpophora maydis*, is characterized by rapid wilting of maize plants before tasseling. The late phenological plant stage at which the disease appears suggests that plant hormones may be involved in the pathogenesis. Our work revealed that plant growth hormones, auxin (indole-3-acetic acid) and cytokinin (kinetin), suppress *H. maydis* in culture media and in a detached root assay. Kinetin, and even more auxin, caused significant suppression of fungal spore germination. Gibberellic acid did not alter colony growth rate but had a signal suppressive effect on pathogen spore germination. In comparison, plant senescence and defense response regulators ethylene and jasmonic acid had minor effects on colony growth and spore germination rate. Their associated hormone, salicylic acid, had a moderately suppressive effect on spore germination and colony growth rate, and a strong influence when combined with auxin. Despite the anti-fungal auxin success *in vitro*, field experiments with dimethylamine salt of 2,4-dichlorophenoxyacetic acid (that mimics auxin) failed to suppress late wilt. The evidence presented here is important to encourage further and more in depth examinations of this intriguing hormonal complex regulation and its role in the maize-*H. maydis* interactions.

Proteomic approach to the molecular interaction between *Fusarium oxysporum* and melon

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Plant immune response against pathogens is based on recognition between pathogen secreted effectors (Avr) and plant resistance proteins. *Fusarium oxysporum* causes vascular wilt by invading the plant's xylem vessels. The recognition between *Fusarium* and its host is unique, occurring in the vessels and the surrounding cells. In this project we adopted a proteomic approach to identify proteins participating in the melon-*Fusarium* interaction. We compared protein profiles of xylem sap from susceptible and resistant melon genotypes inoculated with *Fusarium*. Proteomic analysis identified xylem proteins from fungal and host sources, and produced relative quantification for each protein. A total of 522 different proteins were identified: nine fungal proteins and 513 melon proteins. Many proteins, including defense related proteins, demonstrated differential expression following inoculation. To identify the fungal proteins evoking the plant resistance reaction (Avr factors), we looked for fungal gene distribution among *Fusarium* races. One of the fungal genes whose product was found in the proteomic experiment was present in the genomes of *Fusarium* races 0 and 1, and absent from the genomes of races 1.2 and 2, as predicted by the "gene-for-gene" theory. The fungal gene was also identified by another research group as the *Fom-2* avirulence gene. Agro-infiltration of *Fom-2* resistance gene together with Avr^{Fom-2} avirulence gene in *Nicotiana benthamiana* triggered a hypersensitive reaction, suggesting a molecular interaction between the gene products.

Effect of mineral nutrition on sweet basil susceptibility to *Peronospora belbahrii* downy mildew

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Sweet Basil Downy Mildew (BDM), caused by *Peronospora belbarhii*, has become a major disease in the basil (*Ocimum basilicum*) crop. Under conditions of high relative humidity and mild temperatures, the pathogen spreads rapidly, and sometimes damages the entire crop. Currently, the main means of control is chemical fungicides. Since the use of fungicides is problematic, it is necessary to develop alternative control methods. The present research focuses on mineral nutrition and its ability to reduce host susceptibility to BDM. The aim of this work is to test the effect on BDM disease severity of the composition and concentration of mineral nutrients in the irrigation water and by foliar nutrient spraying. To this end, we treated plants that grew on perlite medium with various nutrient applications. Disease severity was highest at higher N concentrations. In addition, disease severity decreased at higher ammonium/nitrate proportion. An increase in potassium concentrations in the irrigation water increased disease severity. On the contrary, foliar spraying of potassium significantly reduced disease severity. Moreover, a decrease in disease severity occurs also upon increased concentrations of calcium and magnesium in the irrigation water. In an experiment under commercial-like conditions, calcium fertilization (total concentration of 240 ppm) in water with magnesium (40

ppm) reduced disease severity most effectively. Magnesium addition (total concentration 80 ppm) also reduced disease severity. This research is still ongoing, but it is already clear that an appropriate fertilization regime lessens BDM severity, and that combination with other agrotechnical techniques may result in a better disease control.

Oxylipines – lipid signals regulating plant defense response to root knot nematode infection

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The root knot nematode *Meloidogyne* spp. is a very important soil pathogen around the globe. The nematode maintains sophisticated interactions with root cells based on ongoing signal exchanges. The role of oxylipins, oxidation products of fatty acids including jasmonic acid, is still unknown in this signal interchange. Different methods have been used but without any success. In this study, by combining genetic and biochemical tools, we have investigated the oxylipin involvement in the interactions between the nematode, *M. javanica*, and the host, *Arabidopsis*, while focusing on deciphering those signals. To understand the involvement of jasmonic acid biosynthesis, the roles of Allene Oxide Synthase (*AOS*) and Allene Oxide Cyclase (*AOC*) host gene families have been characterized. In addition, the effect of oxylipins on juveniles and hatching processes has been studied. The accumulated results indicate that the expression of host *AOS*, *AOC3* and *AOC4* genes is strongly induced by nematode infection. Furthermore, *aoc3* and *aoc4* mutants showed increased susceptibility. When exposed to certain oxylipins, a rise in nematode mortality and hatching delay has been observed, while products of the studied cascade showed no effect on nematodes. It is possible that other oxylipins may indirectly be active through regulation of other plant pathways. Continuing the research may lead to a better understanding of factors regulating the outcome of this parasitic interaction.

Characterization of a toxin-antitoxin system of the phytopathogenic bacterium *Acidovorax citrulli*

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Toxin–antitoxin (TA) systems are commonly found in plasmids and chromosomes of bacteria and archaea. These systems appear as biscystronic genes encoding a stable toxin and a labile antitoxin, which protects the cells from the toxin's activity. Under specific conditions such as stress or initial host infection, the unstable antitoxin is degraded, the toxin becomes active and growth is arrested. Using genome analysis we identified a locus encoding a putative TA system in the genome of the plant pathogen *Acidovorax citrulli*, the causal agent of bacterial fruit blotch disease of cucurbits. Phylogenetic analyses suggested that this locus, *vapBC*, is unique to group II *A. citrulli* strains and several xanthomonads, and that it is not found in other sequenced *Acidovorax* species. Using biochemical and molecular analyses we show that the *A. citrulli* VapBC module is a *bona fide* TA module in which VapC is a toxin with ribonuclease activity that can be counteracted by its cognate VapB antitoxin. We further show that transcription of the *vapBC* locus is induced by amino acid starvation, chloramphenicol and during plant infection. Due to the possible role of TA systems in both virulence and dormancy of human pathogenic bacteria, studies of TA systems are gaining a lot of attention. Conversely, studies of TA systems in plant pathogenic bacteria are lacking. The study presented validates a role for the VapB/VapC proteins in *A. citrulli* growth regulation, and suggests an involvement in host-pathogen interactions. To the best of our knowledge this study is the first systematic analysis of a chromosomally encoded *vapBC* locus of a plant pathogenic bacterium.

USE OF RESISTANCE SOURCES TO WITHSTAND DISEASE

Reducing damage caused by *Sclerotium rolfsii* in peanut cultivars in the Hula Valley by cultivar testing and chemical control

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Stem rot caused by *Sclerotium rolfsii* causes severe losses in Hula Valley peanut fields. In the U.S. damage reduction is due to the use of tolerant cultivars and efficient fungicide usage. In Israel, preliminary observations show that cv. Hanoch is more tolerant to *S. rolfsii* than 'Harari', and 50% Tebuconazole + 25% Trifloxytrobin (Nativo) is less efficient in Hula Valley heavy (peat and mineral) soil than in U.S. sandy soil. The research aims here were to examine the mobility of Nativo in soil columns and screen the crossing of 'Hanoch' and 'Harari', together with fungicide treatment. Application of 1 kg/ha Nativo on the soil column resulted in significantly more efficient inhibition of sclerotium germination in sand, with 93.5% and 56% inhibition on the soil surface and at a depth of 30 mm, respectively, and only 56% and 50% inhibition on the surface of peat and mineral soils, respectively. At the 30 mm depth, inhibition decreased to 21.4% and 11.5% in peat and mineral soil, respectively. In a field trial in peat, 'Harari' was more sensitive to *S. rolfsii* than 'Hanoch'. Line B65 was the most tolerant and line B77 was the most sensitive. Four applications of 2 kg/ha Nativo, but not 1 kg/ha, were significantly more efficient only in line B77. 'Hanoch' yields were 10% higher than 'Harari', contrary to a nearby healthy field, where the 'Harari' yield was 9% higher than 'Hanoch'. Nativo activity on Hula soils was less efficient than on sand and reduced disease only in line B77. Line B65 was the most tolerant compared to the commercial cultivars, but not significantly.

Variation in the response of melons and watermelons to *Macrophomina phaseolina*

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Macrophomina phaseolina is the causal agent of the charcoal rot disease attacking many plant species, including plants belonging to the *Cucurbitaceae*. The fungus is isolated frequently from wilted melon and watermelon plants in Israel. Nevertheless, the role of *M. phaseolina* in disease progress and wilting induction or yield reduction is not clear and not similar in melon and watermelon. The objective of the current study was therefore to investigate the disease progress and document its damage in watermelon and melon. Watermelon and melon were seeded in a field naturally infested with *M. phaseolina* and in 10 L pots containing dune sand in a greenhouse. Thirty-five-day-old plants were inoculated using two methods: drenching macerated fungus around the crown area or stabbing the lower stem with an *M. phaseolina*-infested toothpick. Disease progress was documented in the field and in the greenhouse. Plant colonization and symptom development were more pronounced in melon than in watermelon. Large lesions and significant internal-rotting developed in melon plants inoculated by the two methods in both field and greenhouse. Yet, in watermelon, no disease symptoms were observed and the internal rotting was limited. Charcoal rot was observed only in the field a short time before harvest, and was expressed as partial wilting and significant loss of foliage and fruit fresh weight. No wilting was observed in the greenhouse experiment.

Transfer of resistance against downy mildew from wild basil to sweet basil

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Downy mildew caused by the Oomycete *Peronospora belbahrii* is currently the most serious disease of basil. Farmers suffer severe losses due to this disease and expect the introduction of resistant cultivars to the market. Whereas no resistance to downy mildew was found in sweet basil, *Ocimum basilicum,* a high level of resistance was detected in wild basil *Ocimum* spp (1). Sweet basil is not crossable with wild basil due to ploidy differences. We used the embryo-rescue procedure to overcome the species incompatibility. Sweet basil was pollinated with pollen from wild basil, fertilized ovules were rescued, cultured *in vitro*, and when rooted were transferred to potting medium and grown in the green house. F1 plants were all resistant to downy mildew with some showing partial female fertility, which enabled back-crosses with sweet basil. After three back crosses BC3 plants were fully resistant and fertile. Progeny analysis suggested that resistance is controlled by a single dominant gene. Further selection resulted in resistant progeny plants with elite horticultural and aroma properties.

The role of RNA-dependent RNA polymerase 1 gene in antiviral defense in *Cucumis*

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RNA-dependent RNA polymerase (RDR) is a gene family involved in plant development and defense against plant viruses. RDR1 and RDR6 play crucial roles as defense enzymes against plant viruses, by amplification of viral-dsRNA in the gene silencing pathway. We identified a number of RDR1 genes in the *Cucurbitaceae* compared to the single RDR1 genes found in most plant species. Four RDR1 genes (RDR1a, RDR1b, RDR1c1 and RDR1c2) were identified in cucumber (*Cucumis sativus*). In healthy plants RDR1a and RDR1b transcripts are expressed at relatively high levels compared to the very low expression levels of RDR1c1 and 2. RDR1c1 and 2 are highly induced in susceptible cultivars infected with viruses, but not by infection with *Pseudomonas syringae* or Powdery mildew. In addition, silencing of RDR1c1,2 leads to increased virus accumulation which may indicate the antiviral function of those genes. We observed broad virus resistance in several cucumber cultivars and in a transgenic line associated with a high level of RDR1b expression, independent of the levels of salicylic acid, a known inducer of RDR1 expression. We assume that RDR1b gene is new virus resistance gene in cucumber. However, in melon only RDR1a is expressed, while both RDR1b and RDR1c genes are truncated. The expression levels of RDR1a, RDR2 and RDR6 did not increase following virus infection. Understanding regulation of RDR1 genes and their antiviral functions may help with the development and breeding of plants with broad virus resistance.

Developing means to decrease *Cucumber green mottle mosaic virus* (CGMMV) infection in trellised cucurbit crops

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The disease caused by *Cucumber green mottle mosaic virus* (CGMMV) genus *Tobamovirus* results in significant yield losses in cucurbit crops in Israel and worldwide. Tobamoviral particles are capable of preserving infectious in soil, plant debris and seeds. Tobamoviruses can be transmitted efficiently by mechanical means: workers' hands, tools etc. and have no insect vector. Developing means to reduce CGMMV infection may help dealing with virus spread, decreasing the primary contamination sources and postpone the plant infection. Grafting susceptible plants on pumpkin rootstock enables increasing the amount of high quality

crop and extension of the growth period. Yet, those plants are exposed to virus infection in nurseries, once grafting takes place. The project goals were twofold (1) to screen for resistance to CGMMV within potential pumpkin rootstocks, and (2) to examine the efficiency of various disinfectants for grafting knifes in nurseries. Identification of the virus was carried out using a serological assay (ELISA) and a molecular assay (Reverse transcription-polymerase chain reaction). Firstly, two sequential inoculations were performed to examine the resistance of pumpkin rootstocks. Subsequently, tests were conducted to identify the presence of the virus in inoculated plants. Two potential resistant rootstocks were identified and their ability to protect the grafted plants will be tested by root inoculation. In addition, effective disinfectants for contaminated grafting knifes were determined. In conclusion, resistant rootstocks may protect grafted plants from contaminated soil infection. Moreover, using efficient disinfectants to decontaminate grafting knifes may help to reduce the primary infection sources and will minimize the virus spread in commercial farms.

STORAGE OF AGRICULTURAL PRODUCTS

Carbon regulation of environmental pH by secreted small molecules that modulate pathogenicity in phytopathogenic fungi

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Fruit pathogens can contribute to acidification or alkalization of the host environment. This capability has been used to divide fungal pathogens into acidifying and/or alkalizing classes. Here we show that diverse classes of fungal pathogens—*Colletotrichum gloeosporioides*, *Penicillium expansum*, *Aspergillus nidulans* and *Fusarium oxysporum*—secrete small pH-affecting molecules. These molecules modify the environmental pH that dictates acidic or alkaline colonizing strategies and induce the expression of PACC-dependent genes. We show that in many organisms, acidification is induced under carbon excess, i.e. 175 mM sucrose (the most abundant sugar in fruits). In contrast, alkalization occurs under conditions of carbon deprivation, i.e., less than 15 mM sucrose. The carbon source is metabolized by glucose oxidase (*gox2*) to gluconic acid, contributing to medium acidification, whereas catalyzed deamination of non-preferred carbon sources, such as the amino acid glutamate, by glutamate dehydrogenase 2 (*gdh2*) results in the secretion of ammonia. Functional analyses of Δ*gdh2* mutants showed reduced alkalization and pathogenicity during growth under carbon deprivation, but not in high-carbon media or on fruit rich in sugar, whereas analysis of Δ*gox2* mutants showed reduced acidification and pathogenicity under conditions of excess carbon. The present results indicate that differential pH modulation by fruit fungal pathogens is a host-dependent mechanism, affected by host sugar content, which modulates environmental pH to enhance fruit colonization.

Effect of polyamide/halloysite nanotubes and carvacrol packaging on decay development during fresh produce storage

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The annual global loss to decay of fresh produce is approximately 1.3 billion tons, totaling nearly US $35 billion. Antimicrobials incorporated into food packaging could mitigate decay causing agents. Halloysite Nano Tubes (HNTs) were used to encapsulate the highly volatile essential oil carvacrol, creating HNTs/carvacrol hybrids. These hybrids were melt-compounded with polyamide 6 (PA6) and stabilized carvacrol to extruding temperatures of 240-250 °C, producing PA6 film blends with 2 and 4% carvacrol, without losing antimicrobial potency. *In vitro*, carvacrol amended PA6 films effectively inhibited hyphal growth and sporulation of *Alternaria alternata, Botrytis cinerea, Penicillium digitatum, Penicillium expansum and Aspergillus niger* upon direct contact. Hyphal growth rates were reduced by 23-80% and 76-89% for 2 and 4% carvacrol amended PA6 films, respectively, in vapor phase bioassays. *In vivo*, 2% carvacrol in the PA6 film reduced decay by 17-50% on cherry tomato, lychee and grapes, following 51, 29 and 38 days of storage at 10, 4 and 1°C, respectively. Films with 4% carvacrol decreased decay by 15 and 27% on lychee and grapes, respectively; but not on tomato, where decay was increased 58-100% due to phytotoxicity. Examination of carvacrol residue by GC-MS after 10 days storage recorded concentrations of 2-18 ppm in fruit tissue indicating that the essential oil was absorbed into fruit tissue at organoleptic levels. Further customization of this novel and innovative technology is desirable to attain a commercially viable product with limited phytotoxicity or organoleptic effects.

Microbiome and stem end rot in mango

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During storage, mango fruits develop stem end rots (SER) that reduce fruit quality and cause a significant loss of fresh produce. Pathogenic fungi that colonize the stem, without causing any visible symptoms, awaken during fruit ripening and cause SER. The major mango SER pathogenic fungi are *Alternaria*, *Lasiodiplodia, Phomopsis, Colletotrichum* and *Fusarium*. Confocal microscopy analysis revealed that those fungi colonize mainly the phloem of the fruit stem end. The stem ends are colonized by other microorganisms including fungi, yeast and bacteria, which do not cause any apparent symptoms and are considered as endophytes. Preliminary results suggest that different treatments (light exposure in orchard, hot water brushing, postharvest application of hormones and fungicides, harvest with or without stem end,

fungicide spraying during flowering) affect the composition of the microbial population and percentage of SER. For example, it appears that sunlight exposure in orchard and fruit storage in different temperatures changed the microbial population and reduced SER in 'Shelly' mango. To further study these results genomic DNA was extracted from stem ends. The ITS and the 16S sequences of fungi and bacteria (respectively) were PCR-amplified and deep sequenced using Illumina MiSeq. The bioinformatic analysis of the data show that microbial population changes occur during storage, at different storage temperatures, and due to sunlight exposure in the orchard. In summary, our results show that pre- and post-harvest treatments modify the microbial population in the stem end and could reduce SER after storage.

DIAGNOSIS OF GENETIC DIFFERENCES IN PATHOGEN POPULATIONS

Studying the occurrence of *Meloidogyne incognita* populations on pepper carrying resistance genes

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Root knot nematodes (*Meloidogyne* spp.) are major pathogens affecting pepper (*Capsicum* spp.). Owing to the drastic reduction in the use of most nematicides, breeding for root knot nematode resistance is a major challenge for pepper breeders. In pepper, resistance to this pathogen is dominant and controlled by several *Me*- and *N* genes, however repeated use of resistant rootstocks or cultivars can bring about the selection of virulent populations capable of overcoming resistance. In this work, we evaluated pepper host suitability and durability of these resistance genes, alone or pyramided in different genetic backgrounds against *M. incognita* populations. A total of 30 samples of *M. incognita* collected from pepper greenhouses infested by *Meloidogyne* were tested to identify nematodes races using the North Carolina differential host test. According to that test, while all *M. incognita* populations have shown to reproduce on pepper, tobacco, melon and tomato plants, some were shown to reproduce on cotton as well, illustrating high variation among the populations. The response of three pepper lines carrying the respective resistance genes PI1094 (*Me1*/+), PI1093 (*Me3*/+), Nirwin (*N* gene) to all 30 populations was studied. Intraspecific variability for *M. incognita* resistance was found. According to gall index and reproduction index, the pepper line PI1094 carrying the *Me1* successfully suppress all tested populations. Unlike *Me1*, plants carrying the *Me3* or the *N* gene allow reproduction of most tested populations. Further experiments, in which the *Me1* gene was introduced in different genetic background, indicated the efficiency of the *Me1* gene to control *M. incognita* populations. Our results suggest that *M. incognita* populations occurring in Israel fields appeared to be unable to overcome the *Me1* gene and thus might be integrated in control management undertaken to combat root knot nematode disease in pepper.

Occurrence of *Venturia inaequalis* resistance to QoI fungicides in Israel apple orchards

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Apple scab caused by *Venturia inaequalis* is one of the most important diseases of apple. The fungus attacks mostly Red Delicious and Pink Lady cultivars and causes leaf defoliation, fruit malformation and significant yield loss. Disease control is based on fungicide applications in relation to phenological stage and rain events. Even though, there are still severe outbreaks of the disease. Reports from various countries have shown resistance of *V. inaequalis* to the Strobilurin (QoI) fungicide group. Field trials during 2011-2013 in Israel showed a significant reduction in susceptibility of apple scab to Strobilurins and suggested the development of pesticide resistance. Twenty eight isolates were collected from infected leaves and fruit from the Upper Galilee and the Golan regions. The fungicide Strobi (Kresoxim-methyl) at a concentration up to 150 ppm did not inhibit mycelial growth of each of 27 isolates grown in Petri dishes, growth being similar to that of the untreated control treatment. Amplification of the *CYTB* gene with a pair of specific primers and separation of the PCR products on agarose gel and sequencing of the region of the G143A mutation (related to QoI resistance), confirmed that the above mentioned 27 isolates are resistant to Strobilurin and only one isolate from a neglected orchard is sensitive. This is the first report that exhibits *V. inaequalis* resistance to QoI fungicides in Israel with evidence from the orchard, *in vitro* and molecular-based studies.

Characterizing fusarium species that cause castor bean (*Ricinus communis*) wilt

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Castor bean (*Ricinus communis*) cultivation is increasing in the last few years, mostly as a monoculture. Castor bean wilt has become a major concern in many growing areas but the causal agent(s) is still poorly characterized. In most cases, wilt is related to *Fusarium oxysporum* f. sp. *ricini*. However, a wide range of symptoms raises the hypothesis that more than one pathogen is involved in the epidemic. A survey of infected wild and cultivated *R. communis* plants included two major symptom types. The most common symptom was characterized by young seedling wilt (at the stage of 1-4 leaves). Very young seedlings were able to recover while older seedlings (3-4 leaves) usually died. Reddish fungal colonies were isolated from the lower plant organs and were identified with molecular tools *as Fusarium brachygibossum,* providing the first evidence for its involvement in castor bean wilt.

F. brachygibossum isolates were pathogenic not only to castor bean, but also to members of the *Leguminosae* and the *Euphorbiaceae.* The pathogen was recovered from ~10% of the seeds from infected castor bean plants. The second group of symptoms included collapse and necrosis of older plants, in some cases pink conidia appeared on the herbaceous branches and black lesions were observed in roots and stems. Isolates from infected plant organs were molecularly characterized as *F. oxysporum* f. sp *ricinii*. Those isolates were only pathogenic to *R. communis*, survived in soils, but were hardly recovered from seeds (>1%). Differential responses were detected between three castor bean cultivars and the two pathogenic species.

THE USE OF CHEMICAL CONTROL

Farmore 200 – new technology in seed care to replace Thiram

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During seed storage there are many pathogens that could impact seed health and quality. For that reason it is a common practice to use controlled climate conditions and pesticides as seed care. During the past 40 years the main product in use has been Thiram. Lately there is a trend in EU countries to phase out Thiram. Syngenta developed a new unique seed care technology called "Farmore" that is a mixture of two to four active ingredients that will replace the use of Thiram as seed care. The production and the R & D units of "Zeraim Gedara" at Revadim are performing a series of experiments with the aim of registering "Farmore 200" (a mixture of two active ingredients—Mefenoxan and Fludioxonil). The experiments measure the efficacy and the phytotoxicity of this technology on six crops: tomato, watermelon, pepper, melon, cucumber and squash. In the process there is a comparison of the efficacy of "Farmore 200" to Thiram and to control (untreated seeds with no active ingredient). The efficacy tests were conducted in Petri dishes contaminated with two pathogens: *Rhizoctonia* and *Pythium.* The phytotoxicity tests were measured on a controlled temperature germination test and on nursery-grown plants. Preliminary results show that "Farmore 200" is effective in avoiding and exterminating both pathogens and in many cases outperforms the Thiram commercial treatment. In addition, no phytotoxicity was measured so far compared to control. In the next year we will expand efficacy tests to even more crops.

"Orvego"- a new Fungicide from BASF for the control of Late Blight and downy mildew

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The new fungicide Orvego developed by BASF contains two active ingredients with two different modes of action. The active ingredient Ametoctradin (commercial name Initium) was introduced in 2009 and is the only example of the triazolo-pyrimidylamine group (FRAC Code 45, Target Site C8). Ametoctradin inhibits the ubiquinone reductase enzyme in complex III in the fungal mitochondrial respiration process. Ametoctradin is rapidly adsorbed into the epicuticular wax layer of foliage, ensuring good rainfastness. The second active ingredient in Orvego is Dimethomorph in the cinnamic acid amides group which inhibits the cellulose synthase enzyme. Orvego has recently been registered in Israel for late blight (*Phytophthora infestans*) control in potatoes and tomatoes, downy mildew (*Plasmopara viticola*) control in grapes and downy mildew (*Peronospora destructor*) control in onions. The two active ingredients in suspension concentrate formulation prevent the development of fungal resistance and control the diseases at multiple sites of action and at different stages of the fungal life cycle. Extensive field trials in many crops, conducted during the last few years have demonstrated the efficacy of Orvego in downy mildew and late blight control.

ORONDIS™ Opti, a new anti-oomycete fungicide active against late blight and downy mildews

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Previously I reported on the strong efficacy of Oxathiapiprolin against downy mildew in cucurbits. I now report on the efficacy of ORONDIS™ Opti against late blight in tomato, downy mildew in cucurbits and downy mildew in basil. ORONDIS™ Opti is a new pre-packed fungicide mixture by Syngenta composed of 6 g oxathiapiprolin and 400 g chlorothalonil per 1 kg product. Growth chamber experiments showed that a foliar spray of 10 ppm (ai) ORONDIS™ Opti fully controlled late blight in potted tomato plants. Efficacy was similarly high regardless of whether the isolates of *Phytophthora infestans* used for inoculation were sensitive or resistant to mefenoxam, belonged to the A1 or the A2 mating type or carried simple or complex virulence factors. Activity of ORONDIS™ Opti against late blight in tomato persisted for ~10 days. Curative spray application at 1 or 2 days per inoculation with 100 ppm suppressed the appearance of the disease, but curative applications are not recommended by the manufacturer. Similar high efficacy was obtained with preventive or curative sprays applied to potted cucumber, melon, squash, butternut gourd or pumpkin plants inoculated with the downy mildew agent *Pseudoperonospora cubensis*, or potted basil plants inoculated with the downy mildew agent *Peronospora belbahrii*. Interestingly, isolates of *P. cubensis* resistant to mefenoxam, dimethomorph or azoxystrobin were all sensitive to ORONDIS™ Opti. Field experiments showed that 3 sprays of 0.05% ORONDIS™ Opti effectively controlled downy mildew in cucurbits and downy mildew in basil.

DEVELOPMENT OF BIOLOGICAL CONTROL AGENTS

Fungal endophyte diversity in orange (*Citrus sinensis*) trees irrigated with fresh water and treated sewage water

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The use of treated sewage water for agriculture is increasing. Trees watered for long periods with sewage water display leaf defoliation, decrease in fruit quantity and quality and death. Similar trees watered with fresh water do not express these symptoms. We examined the influence of water type on the diversity of endophytic fungi in trees. Endophytic fungi are known to be beneficial to the host tree, but under stress conditions some may become weak pathogens. Two orchards of 'Shamoti orange' on sour orange *(Citrus sinensis*) rootstock differing only in water quality were used. During four seasons of the year samples of leaves, branches and roots were collected from 3 trees in each orchard. The samples were used for fungi isolation and identification by phenotypic, microscopic and molecular (ITS) methods. Another molecular method based on Next Generation Sequencing ("deep sequencing") was used. This latter method differs from the former by its ability to compare the endophytic fungi communities independently of the ability to isolate them. Statistical analysis of the results from all methods demonstrated differences among endophytic fungi communities between plant tissues and particularly between the leaves and roots. Dissimilarity among samples from trees watered with fresh or sewage water was found mainly in the root tissue. In conclusion, our hypothesis was partially fulfilled, in that the most influential factor on endophytic fungal diversity in the trees examined was the tissue type, while water quality had a secondary influence with the greatest differences demonstrated in the roots.

The use of the endophytic fungus *Daldinia concentrica* and its volatiles against nematodes

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Endophytic fungi are microorganisms that spend most of their life cycle inside plant tissues without causing any visible damage to the host plants. Some endophytic fungi are capable of emitting volatile organic compounds (VOCs), which may be biologically active and beneficial to their hosts. In a previous study we showed that the VOCs emitted by the fungus *Daldinia concentrica* isolated from an olive tree (*Olea europaea*) are active against various phytopathogenic fungi, as well as against the root knot nematode *Meloidogyne javanica*. Furthermore, a synthetic mixture, composed of four VOCs emitted by the fungus, showed even stronger nematicidal activity. Here we demonstrate a significant reduction in hatching ability of *M. javanica* by the synthetic mixture. Moreover, application of the synthetic mixture to inoculated soil prior to planting susceptible tomato plants resulted in significantly lower galling index and smaller number of eggs per gram soil with no effect on root weight. To understand further the mechanism of action of the VOCs against nematodes, we examined their effect on the model nematode *Caenorhabditis elegans*. Preliminary experiments show that similarly to the root-knot nematode, both the fungus and the volatiles possess a significant effect on the viability of *C. elegans* larvae. Future study will aim at elucidating the mechanism of these compounds to control nematodes by examining the response of mutant lines of *C. elegans* defective in neuronal pathways to the different VOCs. These results will be compared to the mechanism of action of compounds such as organophosphate and carbamate, which are currently in use.

Field trial to examine the use of endosymbiont/endophytic bacteria to reduce yellows disease symptoms in wine grapes

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In Israel, yellows disease in grapevines caused by the obligatory parasitic bacterium *Candidatus phytoplasma* results in heavy yield loss. It is transmitted to plants by *Hyalesthes obsoletus* (HB) where the bacterium resides only in phloem cells. Currently, there is no means to control the disease. An isolated bacterium from HB was characterized as a secondary endosymbiont and identified as a member of *Xanthomonadacea*. Assuming it was acquired by the vector from its host plants we concluded it can be reintroduced to grapevines. Following trials that indicated inhibition activity in a model system and a reduction of symptoms in diseased plantlets, introduction methods were studied and a field experiment was conducted to study the effect of spraying the isolate on reduction of yellows symptoms. A chardonnay plot of 468 vines in the Golan height with 35% infection was divided into untreated and treated plots which were sprayed once every two weeks in the growing season. The presence of the bacterium was examined 7 and 14 d from spraying by PCR analysis and isolation of bacteria from leaf samples. In sprayed vines, yield was increased by 12%, symptom severity was reduced by 25% and rate of symptom remission increased by ~20% compared to control. Sugar level was higher by 0.5 brix but no pH difference was detected. This is a novel trial where an endosymbiont/endophytic bacterium is used as a bio-control agent against yellows disease in grapevines. Currently, questions concerning improving the application and the effect of the bacterium need further investigation.

POSTERS

Scanning diversity of *Botrytis cinerea* isolates

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Botrytis cinerea (teleomorph: *Botryotinia fuckeliana*) is a broad host-range necrotroph, inflicting an extensive economic burden worldwide. A dominant factor hindering its control is a remarkable genetic variability resulting from mating capability and other sources of variation (e.g., transposons, variable chromosome number and vegetative compatibility), enabling broad adaptation capability. To characterize the variability of *B. cinerea*, we tested 30 isolates from different locations and crops in Israel. Isolates were characterized by traits associated with growth parameters and pathogenicity on tomato (i.e., growth rate on PDA, rate of development of necrotic lesions on leaves and stems, and rate of conidia germination on leaves). Genetic variability was further analyzed using polymorphic microsatellite markers. The variable in the collection isolates may: i) facilitate breeding programs developing sustainable resistance in crops, based on a "strain-mixture" that will represent diverse pathogenicity mechanisms; ii) serve as a base for future research of the underline genetic mechanisms based on differential virulent-phenotypes.

Occurrence of the A2 mating type of *Pseudoperonospora cubensis* in India

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Downy mildew is widespread in India, attacking various *Cucurbitaceae* species. Oospores of *P. cubensis* were found in infected leaves under field conditions in India, but their formation under laboratory conditions was not reported. The A2 mating type of *P. cubensis* was first discovered in Israel in 2010 and later found in China, U.S.A., Vietnam, Russia, Ukraine, France and Germany. The A1 mating type was associated with *Cucumis* species, whereas the A2 was associated with *Cucurbita* species. In a survey conducted in Bangalore, Karnataka, India (~800 km^2) in fall 2014, twenty one isolates of *P. cubensis* were collected from cucumber, ridge gourd, pumpkin, ash gourd, bitter gourd, spot gourd and snake gourd. The isolates were tested for mating type by co-inoculation with sporangia of either A1 or A2 reference isolates on detached melon leaves. All isolates were found to belong to the A2 mating type except one, from cucumber, that belonged to the A1 mating type. A2 mating type produced abundant oospores (~40 μm diameter) in melon leaves. This is the first report of the occurrence of the A2 mating type in India. It might occur that the recent resurgence of *P. cubensis* in Israel, the U.S.A. and Europe resulted from the migration of A2 from India by seeds or by man. In Israel, cucumber could become infected with A2 when infected *Cucurbita* plants were grown in its close vicinity. A1 and A2 isolates could be distinguished molecularly by using SSR markers.

Characterization of alternative hosts of *Spongospora subterranea*, the causal agent of powdery scab on potato

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Powdery scab caused by *Spongospora subterranea* f. sp. *subterranea* (Sss), is an important disease of potato reducing tuber quality and marketability. Symptoms include raised pustules on the tuber surface containing a powdery mass of thick-walled spore-balls (resting spores), which are highly resistant to environmental stress and remain dormant in the soil for long periods. In severe infections tubers may be malformed with ugly secondary growth. In addition, galls developed on roots and stolons may lead to yield reduction. The pathogen is both seed- and soilborne. Cool soil temperatures and wet conditions induce disease development. The host range of Sss has a great importance from the epidemiological aspect. Alternative hosts infected by Sss may either allow multiplication and survival of the pathogen over time, or serving as trapping plants which prevent the completion of the pathogen's life cycle, thus reducing soil inoculum levels. The objectives of the current study are to identify and characterize potential alternative hosts of Sss in Israel. Weeds and plants of rotational crops are being sampled in commercial farms with naturally infested soil and Sss presence is being detected by root staining followed by microscopic observations and by Real-Time PCR analysis.

Elucidating the mechanism by which plant derived small molecules affect virulence determinants of the genus *Pectobacterium*

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Pectobacterium (formerly *Erwinia*) causes soft rot on fruits, ornamentals and vegetables produced worldwide. Being a major threat for potato and ornamental plant industries, efforts were dedicated to control the disease in both field and storage with a limited degree of success. We focused on the potential of plant-derived molecules (mainly phenolics), to control the

disease. The antimicrobial potential of different phenolics groups was tested on *Pectobacterium* in search for a possible mode of action. Interestingly, biofilm formation and synthesis of exoenzymes were significantly impaired at compound concentrations that did not affect bacterial cell growth. These observations suggested a mechanism which specifically interferes with bacterial virulence. Since the major virulence determinants (biofilm and exoenzymes) in *Pectobacterium* are controlled by quorum-sensing (QS), we focused on the effect of specific molecules on the QS system in pectobacteria. The study revealed an inhibiting effect of the tested compounds on the expression level of central QS system and QS controlled genes, using qRT-PCR. Two reporter strains (CV026 and pSB401) were used to reveal a prominent reduction in the level of QS signal molecule N-acyl-homoserine lactone accumulation. Infection capability was strongly impaired by the compounds on potato, cabbage and calla lily. These were almost completely recovered by application of exogenous N-acyl-homoserine lactone. To support a potential interaction of plant phenolics with QS targets, Drug Discovery tools from SCHRODINGER® were used to reconstruct a computational model of the QS central proteins ExpI/ExpR and predict the potential of the compounds to bind to the active sites of these targets in *Pectobacterium*.