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Abstracts of presentations at the 41st and 42nd Congresses of the Israeli Phytopathological Society

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Edited by Victor Gaba

Abstracts of presentations at the 41st Congress

Harnessing the genetic variation of an endophytic bacterium to control vector-borne bacterial plant pathogens

S. Sabach[1,2*], N. Mozes-Daube[3], E. Zchori-Fein[3] and O. Bahar[1]
[1]*Dept. of Plant Pathology and Weed Research, Agricultural Research Organization—Volcani Institute, Rishon LeZion, 7505101, Israel*
[2]*The Robert H. Smith Faculty of Agriculture, Food and Environment, The Hebrew University of Jerusalem, Rehovot, 7610001, Israel*
[*e-mail: shiramaryash@gmail.com]
[3]*Department of Entomology, Agricultural Research Organization, Newe-Ya'ar Research Center, Ramat-Yishai, 30095, Israel*

Vector-borne bacterial plant pathogens have attracted much attention in recent years following devastating disease outbreaks related to these pathogens and their spread to new niches. Among these diseases are *Candidatus* Liberibacter asiaticus, which causes the citrus greening disease, and the olive quick decline syndrome caused by *Xylella fastidiosa*. These pathogens are transmitted by the insect vector directly to the plant's vascular tissue, where they are protected from foliar applications of pesticides, making control difficult using conventional means. The bacterium *Frateuria defendens* (Frd), an endophyte of *Vitex agnus-castus*, was first isolated in 2011 from the planthopper *Hyalesthes obsoletus*, the vector of *Ca*. Phytoplasma Bois Noir disease of grapevines. Frd can be grown *in vitro*, applied to a variety of plants by foliar spray and was shown to access the vascular tissue of plants and to provide protection against phloem-limited disease agents. In the current study, we aimed to broaden our collection of Frd isolates to identify isolates with enhanced plant colonization and disease protective capabilities. Therefore, during 2019-2020 we collected insect and plant samples from different locations in Israel and obtained 16 new Frd isolates. The inhibition activity of the new isolates was examined in an *in vitro* model system and five isolates, including the original isolate, showed significant inhibition activity compared with the control. Further examination is needed to test the new isolates in host plants of the target pathogens.

Testing and improving the analytical sensitivity of the PCR detection method of the ergot disease agent *Claviceps africana* in sorghum seeds

A. Ruimy[1], M. Mirilashvili[2] and Y.M. Harel[1]
[1]*Laboratory of Plant Pathology*
[2]*Laboratory of Chemistry, Plant Protection and Inspection Services (PPIS), Ministry of Agriculture and Rural Development, Bet Dagan 50250, Israel*

In Israel, the ergot disease in *Sorghum bicolor* (L.) Moench caused by the fungus *Claviceps africana* is a quarantine disease. The disease develops when fungal windborne conidia germinate on the female flower. Hyphae penetrate and colonize the unfertilized ovaries, replacing the plant tissue with fungal structures (sphacelium and sclerotium), and asexual conidia are excreted in the honeydew.
Seeds of special varieties are imported from abroad to grow forage sorghum in Israel. Classical morphological examination of the seeds during routine purity tests allows the detection of the disease in cases where sclerotia are present. Following an ergot disease outbreak in Israel in 2015, a PCR test was developed, allowing identification of the fungal DNA on sorghum seeds. In the current work, the analytical sensitivity of the PCR test was measured and improved. Infected STT13 sorghum seeds imported from Texas in 2015 were used as the reference level of ergot infection. Concentration of DNA extracts by nitrogen evaporation allowed improvement of the test's sensitivity. In the improved test the pathogen could be detected at infection levels at least 64 times lower than the reference level.

The new Pepino mosaic virus virus in Israel in combination with Tomato brown rugose fruit virus leads to severe damage to tomato plants and fruit

C. Klap[1,2,*], N. Luria[1], E. Smith[1], L. Hadad[1,2], E. Bakelman[1], N. Sela[1], E. Belausov[3], O. Laskar[4], O. Lachman[1], D. Leibman[1], A. Gal-On[1] and A. Dombrovsky[1]
[1]*Dept. of Plant Pathology and Weed Research, Agricultural Research Organization—the Volcani Center, Rishon LeZion 7505101, Israel*
[*e-mail: chen.klap@mail.huji.ac.il]
[2]*The Robert H. Smith Faculty of Agriculture, Food and Environment, The Hebrew University of Jerusalem, Rehovot 761001, Israel*
[3]*Dept. of Ornamental Plants and Agricultural Biotechnology, Agricultural Research Organization, The Volcani Center, 68 HaMaccabim Road, P.O.B 15159 Rishon LeZion 7505101, Israel*
[4]*Israel Institute for Biological Research (IIBR), Ness-Ziona, Israel*

Tomato brown rugose fruit virus (ToBRFV) was first detected in 2014 in commercial tomato and was established in all tomato growing areas in greenhouses in Israel, even though the plants carry the *Tm-2*^{2} allele that confers resistance to viruses of the *Tobamovirus* genus (such as Tobacco mosaic virus and Tomato mosaic virus). As of November 2018, a new phenotype of ToBRFV disease, showing very strong and diverse symptoms in market produce and in greenhouse-tomato crops. Serological tests and transmission electron microscope scan of samples from symptomatic plants and fruits showed the presence of ToBRFV and a mild Pepino mosaic virus (PepMV) isolate. PepMV (*Potexvirus* genus) is a new virus in the Israel and, similarly to tobamoviruses) is transmitted effectively through mechanical infection. Serological and biological tests for the presence of ToBRFV and PepMV in the mesocarp, exocarp, juice and seeds of suspected symptomatic fruits disclosed the presence of both ToBRFV and PepMV in all tissues except the exocarp (the fruit skin), suggesting that ToBRFV and PepMV infected tomato fruits are an effective source of infection for tomato plants. These results have contributed to an understanding of the epidemiology of ToBRFV and PepMV and increased worldwide awareness of the necessity for hygienic conduct. Furthermore, combined inoculation of tomato plants with ToBRFV and PepMV causes a statistically significant increase in PepMV titer compared to PepMV-only treatment, with ToBRFV levels almost unchanged, which indicates a synergistic effect that exacerbates the intensity of disease symptoms and damage.

Bio-control of *Fusarium oxysporum f. sp. cubense (Foc)* by soil bacteria

D.-D. Cohen[1,2*] and D. Minz[1]
[1]*Institute for Soil, Water & Environmental Sciences, The Volcani Center, Rishon LeZion, Israel* [*e-mail: davidda.cohen@mail.huji.ac.il]
[2]*Robert H. Smith Faculty of Agriculture, Food and Environment, The Hebrew University of Jerusalem, Rehovot, Israel*

Bananas are large monocotyledonous herbs of the genus *Musa* from Asia. Panama disease, also known as *Fusarium wilt*, is a lethal infection that destroys banana plants, and has spread across Asia, Australia, Africa and the Middle East. The disease was first reported in Australia in 1876. The cause is a soil-borne fungus, *Fusarium oxysporum f. sp. cubense (FOC)*, that colonizes and obstructs the xylem of banana plants and causes wilting. Bananas of the Cavendish variety are clones, all of the same genetic makeup. Therefore, if one plant is infected, then all other plants in the area are potentially doomed. This means that it is easy for a Panama disease epidemic to spread in huge plantations of vulnerable host plants. Furthermore soil-borne pathogens are difficult to control, as they survive for long periods in soil, spread by wind, water, inoculated seeds and by soil clinging to farm equipment. Obviously, the simplest response is quarantine. Quarantine can slow down the progression of *FOC*, but in the long-term, it is necessary to find a way to fight this pathogen. Therefore, we developed a method to confront the disease, using soil-dwelling banana microbes for biological control. We isolated more than 400 rhizosphere bacterial strains from healthy banana trees. After testing these strains against the pathogen in the laboratory we discovered about 50 bacteria with effective inhibitory effects against *FOC in vitro.* Ten of these bacteria had a positive impact in the greenhouse on young banana plantlets in the presence of *FOC*, reducing disease severity between 15-35% and reducing the mortality of the treated banana plantlets compared to the positive control (infected banana plantlet without bacterial treatment).

New etiology markers for cucumber green mottle mosaic virus disease progression in cucumber plants exposed to fluctuating extreme temperatures

O. Molad[1,2], N. Luria[1], E. Smith[1], N. Sela[1], D. Leibman[1], O. Lachman[1], E. Bakelman[1] and A. Dombrovsky[1]
[1]*Dept. of Plant Pathology and Weed Research, Agricultural Research Organization—the Volcani Center, Rishon LeZion 7505101, Israel* [*e-mail: aviv@volcani.agri.gov.il]
[2]*Institute of Chemistry, The Hebrew University of Jerusalem, Edmond J. Safra Campus, Givat Ram, Jerusalem 91904, Israel*

Disease etiology studies of cucumber green mottle mosaic virus (CGMMV) have recently focused on plant molecular responses. However, extreme diurnal environmental temperatures, characteristic of global climate change, could affect plant susceptibility and disease phenotype progression. Our studies of CGMMV disease etiology, under simulated extreme temperature waves, have revealed two new disease initiation phenotypes that developed gradually, preceding severe symptom manifestations during post-recovery CGMMV systemic infection. "Early post-recovery stage" bright yellow islands (BYIs) with defined boundaries amid asymptomatic leaf tissues first emerged followed by "late post-recovery stage" BYIs with diffused boundaries. A CGMMV disease etiology model, postulating BYI symptom occurrence in the field, revealed BYIs following extreme temperature waves. Profiling ontology of cucumber differentially expressed genes in BYIs *vs* the corresponding dark green surrounding tissues revealed the activation of the jasmonic acid (Ja) pathway in "early post-recovery stage" BYIs. The activation of the Ja pathway was followed by signaling inactivation in "late post-recovery stage" BYIs, concomitant with increasing expression of Ja signaling inhibitors and downregulation of the Ja responsive phenylpropanoid pathway. Our results disclosed a new phenotypic description of CGMMV disease initiation, characteristic of cucumber plants grown under extreme environmental temperature fluctuations. The BYI phenotypes could define a time window for CGMMV disease management applications.

Characterization of pACM6, a plasmid that occurs in several strains of the cucurbit pathogenic bacterium *Acidovorax citrulli*

R. Yang[1,*], F. Pérez-Montaño[1, 2], D. Santos-Garcia[3] and S. Burdman[1]
[1]*Dept. of Plant Pathology and Microbiology, The Robert H. Smith Faculty of Agriculture, Food and Environment, The Hebrew University of Jerusalem, Rehovot 7610001, Israel* [*e-mail: rongzhi.yang@mail.huji.ac.il]
[2]*Dept. of Microbiology, University of Seville, Seville 41012, Spain*
[3]*Dept. of Entomology, The Robert H. Smith Faculty of Agriculture, Food and Environment, The Hebrew University of Jerusalem, Rehovot 7610001, Israel*

Acidovorax citrulli causes bacterial fruit blotch (BFB), a disease that threatens cucurbit production worldwide. Genome sequencing of the *A. citrulli* model strain M6 revealed a ~53 kb plasmid. This plasmid, named pACM6, occurs at relatively low copy numbers, and contains typical plasmid-borne genes involved in plasmid maintenance, replication and transfer. Plasmids highly similar to pACM6 occur in other *A. citrulli* strains. Elimination of pACM6 from strain M6 could not be detected at normal growth conditions, but occurred at low frequencies at relatively high temperatures (35 and 41°C). Analyses of pACM6-cured strains revealed that the plasmid does not seem to confer any advantage in terms of virulence in leaf inoculation and seed transmission assays of melon. On the other hand, cured strains were significantly less virulent than wild-type M6 in stem inoculation assays with melon seedlings. An operon encoding homologs of a Fic-VbhA toxin-antitoxin (TA) module was found in pACM6. Transcriptome data of *A. citrulli* M6 grown in a minimal medium that mimics to some extent the plant apoplast revealed that, under tested conditions, the TA genes were among the highest expressed genes in pACM6. Heterologous expression of these genes in *Escherichia coli* showed strong inhibition of cell growth when the toxin gene (*fic*) was expressed alone, whereas no effects on bacterial growth were observed between control bacteria and bacteria expressing the antitoxin gene (*vbhA*) alone or in combination with the *fic* gene. These results demonstrate that the pACM6 *fic-vbhA* operon encodes a *bona-fide* TA system.

Sterilization of cannabis inflorescences using nonchemical methods

S. Jerushalmi*, M. Maymon and S. Freeman
Dept. of Plant Pathology and Weed Research, Agricultural Research Organization—the Volcani Center, Rishon LeZion 7505101, Israel [*e-mail: shacharje1@gmail.com]

The use of medical cannabis (MC) has expanded over the last decade, as more therapeutic beneficial properties of MC are discovered, ranging from general analgesics to anti-inflammatory and anti-bacterial effects. Together with the intensified utilization of MC, concerns regarding the safety of usage in patients have arisen due to the strict regulation of pesticide application in MC cultivation. Three different sterilization methods of inflorescences were examined in this research: beta irradiation (e-beam), cold plasma and steam, to determine their efficacy in reduction of fungal colony forming units (CFUs) in vivo. Furthermore, the effect of steam treatment on the profiles of bioactive compounds in the inflorescence was examined. E-beam treatments at 10.26 KGy reduced CFU levels from 10^6 CFU/g inflorescence to below the detection threshold. A 10 min plasma treatment caused a maximal decrease of four orders of magnitude in CFU levels, while a 10 sec steam treatment at 62.5° C (the shortest treatment tested) reduced CFU levels from $10^{5.8}$ CFU/g inflorescence, to below detection threshold levels. Additionally, a 15 sec steam treatment at 65° C had a moderate effect on profiles of bioactive compounds, within the allowed 20% deviation in declared bioactive concentrations, permitted by the United States Pharmacopeia. All three methods showed potential for effective sterilization, although testing the effect on bioactive compounds after treatment with e-beam and cold plasma is still required.

Macro- and micro-nutrients suppress powdery mildew development in grapevines and alter berry metabolites

L. Gur[1,2,3,*], Y. Cohen[2], O. Frenkel[3], R. Schweitzer[4] and M. Reuveni[1]
[1]*Shamir Research Institute, University of Haifa, P.O. Box 97 Katzrin 1290000, Israel* [e-mail: liogur@gmail.com]
[2]*Faculty of Life Sciences, Bar-Ilan University, Ramat Gan 5290000, Israel*
[3]*Dept. of Plant Pathology and Weed Research, Agricultural Research Organization—the Volcani Center, Rishon LeZion 7505101, Israel*
[4]*Tel-Hai College, Upper Galilee 12210, Israel*

Powdery mildew caused by the fungus *Erysiphe necator* is a major disease of grapes worldwide. This fungus attacks foliage and berries and reduces yield and wine quality. Fungicides are the mainly used for combating the disease, but their reduced efficacy became alarming recently due to the appearance of resistant pathogen isolates. The global requisite to reduce pesticide deployment encouraged us to develop a new management strategy that we report here. Field experiments conducted during 2018-20 proved that foliar application of the potassium phosphate fertilizer Top-KP (1-50-33 NKP) reduced disease incidence in treated vines by 15-65% and disease severity by 75-90%, compared to untreated vines. Top-KP, when mixed with micro-nutrients i.e., boron plus zinc, or with TruPhos Platinum (containing N, P_2O_5, K_2O, Mg, Fe, Mn, Zn, Cu, B, Mo, Co) further reduced disease incidence by 30-90% and disease severity by 85-95%. Fertilizer efficacy was similar to that of synthetic fungicides. Tank-mixtures of fertilizers and the fungicide tebuconazole further improved efficacy in the vineyards. Fertilizer mixtures showed prophylactic and curative effects in artificially inoculated grape plants in the greenhouse. LC-MS/MS metabolite analysis showed significant increase in antifungal peptides and amino-acids in grapes sprayed with fertilizers or fungicide, compared to untreated grapes. Interestingly, the concentration of 22 metabolites was significantly higher in grapes treated with fertilizers compared to treatments with fungicides. We suggest to integrate macro- and micro-nutrients in spray programs against powdery mildew in vineyards. This will reduce fungicide deployment and consequently the buildup of fungicide resistance.

Abstracts of presentations at the 42nd Congress

Invited lecture

Plant diseases and their management in agricultural crops during the period between the First Aliyah and Fifth Aliyah (1881-1939)

Y. Elad
Dept. of Plant Pathology and Weed Research, Agricultural Research Organization—the Volcani Center, Rishon LeZion 7505101, Israel [e-mail: elady@volcani.agri.gov.il]

Agriculture has existed in our region for thousands of years. From the period of the first "Aliyah" (a major wave of Jewish immigration to Ottoman Syria between 1881 and 1903), settlements were mostly based on agricultural production. Diversified agriculture took place, which expanded over the years and with the increase in settlement size. Agriculture included vegetable gardens, vineyards, orchards and field crops, alongside animal husbandry and feed production for the animals. Citrus production expanded in the twentieth century and was established in the Arab and Jewish sectors for export. Tobacco cropping existed during the whole period. Establishment of cash crops for farmers occurred regularly, but may have collapsed as happened in silk production. During the British Mandate (between the years 1920 and 1948) forests were planted as well. Plant pests were the most important limiting factor of agriculture from the sowing to crop harvest, storage and processing. The pests were familiar to those engaged in agriculture, but because of technical limitations, in-depth recognition was dictated by limitations of the existing research tools, such as light microscopy. Important means later developed with the development of the natural sciences, microbiology and phytopathology. Experts in the fields of plant protection included experienced farmers and agronomists who acquired their education abroad. In the 1920s, the Zionist Organization's Experimental Station, later the Volcani Institute, was run by scientists, and included Pathology and Entomology Departments, run by internationally renowned scientists (Dr. Israel Richard and Dr. Shimon P. Budenheimer, respectively). Already in the first year of its operation, the station conducted experiments on 85-100 ha throughout the country. They identified and published details of pests and control measures. The causal agents were fungi, bacteria, viruses, nematodes, snails, parasitic plants and arthropod pests. A thick book from the 'Moshva' Rishon Lezion' Library (Viala P, 1893, Les Maladies de la Vigne), contains detailed knowledge on powdery and downy mildews, black rot, anthracnose, root rot, gray mold and more. Control means varied and allowed partial disease suppression and included many cultural means: clean propagation material, removal of diseased leaves and plants, application of plant nutrients and dusting and spraying of sulfur, lime, gypsum, burgundy and burgundy broth, iron and copper sulfur, formaldehyde, formalin, potassium permanganate, soap, kerosene soap and plant extracts.

Charcoal rot (*Macrophomina phaseolina*): Adjusting control and germplasm selection methods for different crops

R. Cohen[1*], M. Elkabetz[1], H.S. Paris[2], A. Gur[2], N. Dai[3], O. Rabinovitz[4], S. Freeman[5]

[1]*Dept. of Plant Pathology and Weed Research, Cucurbits Section, Newe Ya'ar Research Center, Ramat Yishay, Israel* [*e-mail: ronico@volcani.agri.gov.il]

[2]*Dept. of Vegetable Sciences, Cucurbits Section, Newe Ya'ar Research Center, Ramat Yishay, Israel*

[3]*Dept. of Vegetable Sciences, The Volcani Center, Rishon LeZiyyon, Israel*

[4]*Northern Agriculture Research and Development, Migal Building, P. O. Box 831, Qiryat Shemona, Israel*

[5]*Dept. of Plant Pathology and Weed Research, Agricultural Research Organization—the Volcani Center, Rishon LeZion 7505101, Israel*

Macrophomina phaseolina is a soil-borne fungal pathogen infecting many important crop plants. The fungus, which can survive on crop debris for a long period of time, causes charcoal rot disease by secreting a diverse array of cell-wall degrading enzymes and toxins. *M. phaseolina* thrives during periods of high temperatures and arid conditions, as typically occur in Israel and other countries with a Mediterranean climate. Crop losses due to charcoal rot can be expected to increase and spread in a warming global climate. Management of this pathogen is challenging, requiring an array of approaches for the various hosts. Approaches that have had some success in Israel include grafting of melons and watermelons on resistant squash rootstocks and soil application of fungicide to reduce disease incidence in melons, fumigation and alterations in planting date and mulching of strawberries, and alteration in irrigation regime of cotton. Elsewhere, these approaches, as well as soil amendments and addition of organisms antagonistic to *M. phaseolina* have been successful in some situations. Management through host resistance would be the most sustainable approach, but requires identifying resistant germplasm for each crop and introgressing the resistance into leading cultivars. Resistance to charcoal rot is under complex genetic control in most crops, posing a great challenge for its introgression into elite germplasm. Moreover, fast, reliable methods of screening for resistance would have to be developed for each crop. The toothpick-inoculation method we use holds great promise for selecting resistant germplasm for melon and possibly for sesame, but other methodologies have to be devised for each crop.

Epidemiology and disease dynamics

The combined effect of biotic and abiotic factors on mortality of mature avocado trees

L. Blank*, D. Ezra, H. Krasnov, Y. Fooks, R. Shulhani and D. Shtienberg

Dept. of Plant Pathology and Weed Research, Agricultural Research Organization—the Volcani Center, Rishon LeZion 7505101, Israel [*e-mail: liorb@volcani.agri.gov.il]

The growth and development of mature, fruit bearing, avocado trees in the Menashe Heights region of Israel was retarded from the late-2000s. The trees decayed gradually, leaves dropped off and eventually the trees died. Examination of the rooting system of symptomatic trees revealed that the roots were severely impaired. Their biomass was markedly reduced compared to near-by asymptomatic trees. The objectives of the study were to examine the effects of excess water and soil borne fungi on the biomass of avocado root and document the within-orchard spatial distribution of symptomatic avocado trees. Roots of nursery trees planted in soil taken from within the rows of the trees in damaged orchards developed poorly, a phenomenon that did not occur when nursery trees were planted in soil taken outside rows of trees or in sterilized soil. In addition, roots of nursery trees irrigated in excess were damaged regardless of the source of the soil. We isolated a large variety of fungi from damaged tree roots; however, these fungi were comparable to fungi isolated from roots sampled from asymptomatic avocado trees taken from 20 avocado orchards across the country. These findings support the hypothesis that the roots were impaired by combination of a biotic (presumably pathogenic fungi) and abiotic factors (presumably excess water).

New insights on the genetic structure of grape powdery mildew populations in Israel

L. Gur[1,2,3,*], Y. Cohen[2], M. Reuveni[1], S. Ovadia[4] and O. Frenkel[3]
[1]*Shamir Research Institute, University of Haifa, P.O. Box 97 Katzrin 1290000, Israel* [e-mail: liogur@gmail.com]
[2]*Faculty of Life Sciences, Bar-Ilan University, Ramat Gan 5290000, Israel*
[3]*Dept. of Plant Pathology and Weed Research, Agricultural Research Organization—the Volcani Center, Rishon LeZion 7505101, Israel*
[4]*S.H.F Ltd, Karmey-Yosef, Israel*

Plant pathogens usually originate and diversify in geographical regions where hosts and pathogens co-evolve. *Erysiphe necator*, the causal agent of grape powdery mildew, is a destructive pathogen of grapevines worldwide. Although the Eastern USA is considered the center of origin and diversity of *E. necator*, previous reports of resistant native wild and domesticated Asian grapevines suggest Asia as another possible origin of the pathogen. By using multi-locus sequencing, microsatellites and a novel application of amplicon sequencing (AmpSeq), we show that the population of *E. necator* in Israel is composed of three genetic groups: Groups A and B that are common worldwide, and a new group IL, which is genetically differentiated from any known group in Europe and Eastern USA. Group IL showed distinguishing ecological characteristics: it was dominant on wild and traditional vines (95%), its abundance increased during the season, and it was more aggressive than isolates of Groups A and B on both wild and domesticated vines. The low genetic diversity within Group IL suggests that it invaded Israel from elsewhere. Therefore, we suggest that the Israeli *E. necator* population was founded by at least two invasions, of which one could be from a non-East USA source, possibly Asian origin.

Black root rot in Avocado in Israel: from the nursery to the orchard

M. Zavitan[1], A. Paradise[1], A. Danino[1], Y. Tyutyunik-Shapir[1], A. Ruimy[2], T. Gershon[2], G. Elkind[2] and Y.M. Harel[2,*]
[1]*Division of Planting and Propagation Material*
[2]*Division of Disease Diagnostics* [*e-mail: yaelm@moag.gov.il]*, Plant Protection and Inspection Services, Ministry of Agriculture and Rural Development, Bet Dagan 50200, Israel*

Black root rot (BRR) of avocado (*Persea americana*) is caused by soil-borne fungal pathogens and recognized world wide as a severe disease occurring in both nurseries and young orchards. The disease symptoms include blackening rot and reduction of the root system, leaf and branch wilt, leading to potential tree decline and death. Different genera of fungi, depending on the geographical location, belonging to the *Nectriaceae* have been shown to be responsible for the disease. In Israel, BRR was reported for the first time in 1995, and in 2007 the identification of a fungal pathogen was published. New fungi involved have been lately identified by the Plant Protection and Inspection Services and today the list includes species of *Dactylonectria*, *Ilyonectria*, *Calonectria* and *Gliocladiopsis*. In February 2021, a survey of BRR agents was conducted in avocado nurseries. It confirmed that roots host agents of BRR independently of visual symptoms, and revealed the influence of growth conditions on the presence of these fungi in the plantlet roots. These results shed a new light on the etiology of BRR and suggest that the fungi involved in the disease switch from being endophytes to pathogens when the physiological balance of their host is not maintained.

Epidemiology of Stemphylium Leaf Blight on Garlic incited by *Stemphylium vesicarium*

N. Nitzan* and Y. Giladi
The Valley of Springs Research & Extension Center, M.P. Beit She'an Valley, 11710 [*e-mail: nitzan_nadav@yahoo.com]

In Israel, garlic is an important crop yielding approximately ten thousand tons annually. Stemphylium Leaf Blight (SLB), incited by the fungus *Stemphylium vesicarium* has become in recent years a leading foliar disease, reducing yield of green-marketed garlic. The disease is known to be active in the spring. Yet, observations suggest its presence during autumn as well. The study tested the hypothesis that SLB is active during fall and spring, and its increase in incidence and severity is associated with reduced sensitivity to fungicides. A commercial field at the Valley of Springs was monitored during 2021-22 growing season. Disease on-set was recorded in mid-December 2021, reaching 6.5% incidence. SLB was absent from early January to mid-February, but intensified from mid-February to end of March, reaching 63.4% incidence. Exploration of environmental conditions identified that SLB was active when the Humid Thermal Ratio ranged between 5 and 8 ($P<0.0001$). As well, a correlation (r=0.986; P<0.0001) was recorded between SLB and accumulated number of rainy days, but not for amount of precipitation. Eleven mono-conidial isolates from various locations in Israel were tested for sensitivity to fungicides. The commercial mixtures Primium, Duet, Ortiva Top, Priori Extra and Comodor, which contain azoxystrobin mixed with boscalid, difenoconazole, difenoconazole, cyproconazole and chlorothalonil, respectively, were examined at field application rates of 250 to 4000 ppm. Isolates' growth was reduced between 71.3 and 92.3%. Yet, single isolates had demonstrated growth reduction of less than 50%, suggesting reduced sensitivity. The data obtained supported the test hypothesis. Further research will validate the findings.

Sour rot in the vineyard is an indicator of Botrytis rot in grapes after storage

O. Lapidot[1,3], A. Owoyemi[1], B. Kochanek[1], T. Zahavi[4], U. Hochberg[2] and A. Lichter[1]
[1]*Dept. of Postharvest Science* [*e-mail: lapidotor@gmail.com]
[2]*Dept. of Environmental Physics and Irrigation, Agricultural Research Organization-The Volcani Center, Bet Dagan 50250, Israel*
[3]*Robert H. Smith Faculty of Agriculture, The Hebrew University of Jerusalem, Rehovot 7610001*
[4]*Extension Service, Ministry of Agriculture and Rural Development, Qiryat Shemona 10200, Israel*

At harvest, table grape clusters may seem intact but can suffer from unacceptable level of decay by *Botrytis cinerea* after storage. Sour rot is a disease complex that initiates from cracked or injured berries in which yeasts and acetic acid bacteria develop, and the disease is propagated by *Drosophila* flies. Initial results indicated a possible relation between the presence of microcracks and postharvest decay. Subsequent evaluation of the incidence of sour rot in nine *Vitis vinifera* cv. Scarlotta vineyards showed a range of 2 to 41% after veraison, and 27 to 83% at the day of harvest. Clusters free of sour rot were stored for 9 weeks and 3 days. Decay levels after storage ranged from 3 to 15% and the highest incidence of decay was encountered in grapes from vineyards with the highest level of sour rot at harvest. The correlation coefficient between the sour rot index and Botrytis decay after storage was 0.32 and 0.25 for the evaluations at veraison and harvest, respectively, with significant *P* values. These results suggests that sour rot in the vineyard can be an important indicator for development of decay during storage.

New insights on the genetic structure of grape powdery mildew populations in Israel

L. Gur[1,2,3,*], Y. Cohen[2], M. Reuveni[1], S. Ovadia[4] and O. Frenkel[3]
[1]*Shamir Research Institute, University of Haifa, P.O. Box 97 Katzrin 1290000, Israel* [e- mail: liogur@gmail.com]
[2]*Faculty of Life Sciences, Bar-Ilan University, Ramat Gan 5290000, Israel*
[3]*Dept. of Plant Pathology and Weed Research, Agricultural Research Organization—the Volcani Center, Rishon LeZion 7505101, Israel*
[4]*S.H.F Ltd, Karmey-Yosef, Israel*

Plant pathogens usually originate and diversify in geographical regions where hosts and pathogens co-evolve. *Erysiphe necator*, the causal agent of grape powdery mildew, is a destructive pathogen of grapevines worldwide. Although the Eastern USA is considered the center of origin and diversity of *E. necator*, previous reports of resistant native wild and domesticated Asian grapevines suggest Asia as another possible origin of the pathogen. By using multilocus sequencing, microsatellites and a novel application of amplicon sequencing (AmpSeq), we show that the population of *E. necator* in Israel is composed of three genetic groups: Groups A and B that are common worldwide, and a new group IL, which is genetically differentiated from any known group in Europe and Eastern USA. Group IL showed distinguishing ecological characteristics: it was dominant on wild and traditional vines (95%), its abundance increased during the season, and it was more aggressive than isolates of Groups A and B on both wild and domesticated vines. The low genetic diversity within Group IL suggests that it invaded Israel from elsewhere. Therefore, we suggest that the Israeli *E. necator* population was founded by at least two invasions, of which one could be from a non-East USA source, possibly Asian origin.

Coping with plant disease agents

Not entirely unlike fertilizers, *Pseudozyma aphidis* promotes plant growth

A. Fennec[*], G. Breuer, D.A. Srivastava, N. Rotem and M. Levy
Dept. of Plant Pathology and Microbiology, Robert H. Smith Faculty of Agriculture, Food and Environment, the Hebrew University of Jerusalem, Rehovot 76100, Israel (*e-mail: f3nn3c@yahoo.com)

Increasing global population demands higher agricultural yield while avoiding harmful products usage in agriculture. Utilizing beneficial microorganisms is an eco-friendly approach to reduce the use of harmful fertilizers, pesticides and plant growth enhancing products. *Pseudozyma aphidis* is a yeast-like dimorphic fungus related to the phytopathogenic fungus *Ustilago*. Unlike its relative, *P. aphidis* is not only non-pathogenic but is rather beneficial to the host plant. In our laboratory, we isolated a unique *P. aphidis* strain, L12, that has shown significant anti-fungal activity against *Botrytis* and powdery mildew. Tomato plants treated with *P. aphidis* L12 expressed enhanced plant growth and yield. Furthermore, we found that secreted compounds from *P. aphidis* have shown bioactive properties. We made *P. aphidis* extracts and purified them to examine their activity on plants with emphasis on growth promotion. Our results indicated that the plant response to the L12 treatment resembles dose dependent response similar to phytohormones. Plants treated with *P. aphidis* extracts had longer roots, higher germination rate and the auxin activity in treated plants was enhanced up to two weeks post treatment. Following our results, we characterized the pharmacological parameters and found that *P. aphidis* secretes active derivatives of auxins into the medium. The characterization of secreted fungal auxins and additional plant growth enhancing compounds will enrich our knowledge of plant beneficial microorganisms. Additionally, uncovering the mechanisms employed by fungi such as *P. aphidis* to enhance plant growth can assist in developing environmentally friendly products for agriculture and reduce the use of harmful ones.

Endophytic *Penicillium* species secretes mycophenolic acid that inhibits the growth of phytopathogenic fungi

O. Liarzi[1,*], N. Azar[1], M. Zavitan[1], M. Samara[2], A. Nasser[2] and D. Ezra[1]
[1]*Dept. of Plant Pathology and Weed Research, Agricultural Research Organization—the Volcani Center,* [*e-mail: ornal@volcani.agri.gov.il
[2]*The Interinstitutional Analytical Instrumentation Unit, Agricultural Research Organization—the Volcani Center, Rishon LeZion 7505101, Israel, Israel*

The worldwide demand for reduced and restricted use of pesticides in agriculture due to serious environmental effects, health risks and the development of pathogen resistance, calls for the discovery of new bioactive compounds. In the

medical field, antibiotic-resistant microorganisms have become a major threat to humans, increasing mortality. Endophytes are endosymbiotic microorganisms that inhabit plant tissues without causing any visible damage to their host. Many endophytes secrete secondary metabolites with biological activity against a broad range of pathogens, making them potential candidates for novel drugs and alternative pesticides of natural origin. We isolated endophytes from wild plants in Israel, focusing on endophytes that secrete secondary metabolites with biological activity. We isolated 302 different endophytes from 30 different wild plants; 70 of them exhibited biological activity against phytopathogens. One biologically active fungal endophyte from the genus *Penicillium*, isolated from a squill (*Urginea maritima*) leaf, was further examined. A chloroform-based extract of its growth medium was similarly active against phytopathogens. High-performance liquid chromatography separation following by gas chromatography/mass spectrometry analysis revealed a single compound—mycophenolic acid—as the main contributor to the biological activity of the organic extract.

Will cannabinoids be the next antifungal agents against postharvest pathogenic fungi?

D. Duanis-Assaf[1,2*], M. Feldman[3], D. Maurer[1], O. Feygenberg[1], R. Mechoulam[4], D. Steinberg[3] and N. Alkan[1]
[1]*Dept. of Postharvest Science of Fresh Produce, Agricultural Research Organization, Volcani Center, Rishon LeZion 7505101, Israel* [*e-mail: danielle.assaf@mail.huji.ac.il]
[2]*Robert H. Smith Faculty of Agriculture, Food and Environment, The Hebrew University of Jerusalem, Rehovot, 76100, Israel*
[3]*Biofilm Research laboratory, The Hebrew University of Jerusalem, Jerusalem 9112002, Israel*
[4]*Medicinal Chemistry Laboratory, The Institute for Drug Research, School of Pharmacy, Faculty of Medicine, The Hebrew University of Jerusalem, Jerusalem 9112002, Israel*

Postharvest loss is estimated to be more than 40%. Pathogenic fungi cause a major part of this loss. The most effective treatment against postharvest diseases is fungicides. However, due to growing concerns of the harmful influences of fungicides, there is a need to develop new strategies to control postharvest pathogens. One of the major strategies to control postharvest decay is to identify natural compounds with antifungal properties. Phytocannabinoids are natural lipid-soluble molecules that were extracted from *Cannabis sativa*. Although the most familiar cannabinoid is the psychotropic Δ^9-tetrahydrocannabinol (THC), many cannabinoids are non-psychotropic, among them cannabidiol (CBD) and cannabigerol (CBG). The benefit of Phytocannabinoids on human health has been studied for many years; however, in recent years there are growing number of studies on their antibacterial effects. In the current study, we investigated the effect of CBD and CBG on postharvest fungal pathogens. We found that CBD and CBG inhibit the growth of *Botrytis cinerea, Penicillium expansum,* and *Colletotrichum gloeosporioides* in a dose-dependent manner. In a study of their mode-of-action, we found that both CBD and CBG reduced the chitin content in the *B. cinerea* cell wall, and changed the cell membrane fluidity and permeability. CBD treatment caused hyperpolarization to the mitochondria membrane, which resulted in increased fungal ROS levels. Thus, the tested cannabinoids were able to decrease gray-mold development on harvested grape. Overall, this study demonstrates the antifungal activity of CBD and CBG and their mode-of-action, and their potential to serve as antifungal agents against postharvest pathogenic fungi.

Development of metallic composites with entrapped antimicrobial compounds for management of bacterial plant diseases

A. Brill-Sepkuty[1,2,*], B. Menagen[3], E. Malach[1], D. Avnir[3], S. Burdman[2] and Z. Hayouka[1]
[1]*Institute of Biochemistry, Food Science and Nutrition, The Robert H. Smith Faculty of Agriculture, Food and Environment, The Hebrew University of Jerusalem, Rehovot 7610001, Israel* [*e-mail: aya.brill@mail.huji.ac.il]
[2]*Dept. of Plant Pathology and Microbiology, The Robert H. Smith Faculty of Agriculture, Food and Environment, The Hebrew University of Jerusalem, Rehovot 7610001, Israel*
[3]*Institute of Chemistry, The Hebrew University of Jerusalem, Jerusalem, 9190401, Israel*

Agricultural yields are limited by damage caused by pathogens. Plant-pathogenic bacteria are important causal agents of plant diseases with almost all major crops being severely affected by one or more important bacterial diseases. The chemical control options for bacterial plant diseases are limited, and the strategies for their management are often inadequate. To date, chemical control of bacterial plant diseases in agriculture predominantly relies on copper-based bactericides. These compounds, however, possess limited efficacy. Therefore, there is an urgent need to develop novel technologies to manage bacterial plant diseases and reduce food loss. Here we report a new type of composites generated by entrapment of small organic molecules with antimicrobial activity in a matrix of a reduced metal. In the present study the chosen metal was copper due to its importance for management of bacterial plant diseases. As the organic molecule we chose lauryl arginine ethyl ester (LAE). LAE possesses strong antibacterial activity and is used as a preservative in the food industry. The new composites were shown to possess high antibacterial activity against a variety of phytopathogenic bacterial species. Furthermore, LAE were effective in reducing the severity of *Acidovorax citrulli* (bacterial fruit blotch of cucurbits) on melon leaves. The composites also reduced disease severity on melon seedlings that emerged from *A. citrulli*-infected seeds, after treating seeds infected with the bacterium. Overall, our findings strengthen the potential of application of these metalic composites for management of bacterial plant diseases in agriculture.

Ulvan extract from the macroalgae *Ulva rigida* (Chlorophyta) induces table grape resistance to *Botrytis*

A. Shomron[1,2], D. Duanis-Assaf[2,3], O. Galsurker[2], A. Golberg[1]* and N. Alkan[2]*
[1]*Porter School of Environment and Earth Sciences, Faculty of Exact Sciences, Tel Aviv University, Tel Aviv, Israel*
[2]*Dept. of Postharvest Science of Fresh Produce, Agricultural Research Organization-Volcani Center, Rishon LeZion 7505101, Israel* [*email: noamal@agri.gov.il]
[3]*Robert H. Smith Faculty of Agriculture, Food and Environment, the Hebrew University of Jerusalem, Rehovot, 76100, Israel*

Wastage of fruit and vegetables reaches more than 40%, which translates to a major squandering of resources. Fungal pathogens are a central cause of postharvest fruit and vegetable loss. The use of synthetic fungicides for controlling decay is common. The World Health Organization recommends against the use of synthetic chemicals postharvest and warns against their harmful effects on public health and the environment. Seaweeds from the genus *Ulva* are fast-growing edible green macroalgae which can be found on the shore of every continent, and therefore present a resource that can be utilized on a global scale. In this study, we found that the application of ulvan, a crude aqueous extract from *Ulva rigida* (1,000 mg/L), indirectly elicited the table grape defense response and reduced the incidence and decay area of *Botrytis cinerea* by 43% and 41%, respectively. In addition, compared to the control group two days post-treatment, ulvan-extract elicited a variety of defense-related biomarkers such as a 43% increase in the activity of reactive oxygen species, 4-fold increase in the activity of catalase, 2-fold increase in the activity of superoxide dismutase and 1.4-fold increase in the activity of chitinase. No increase was observed in phenylalanine ammonia-lyase activity and the treatment did not affect fruit quality parameters such as sugar levels, acidity or firmness. These results illustrate the potential of *Ulva rigida* extract to naturally induce the plant defense response and to reduce postharvest decay.

Bacterial diseases of plants

Tomato brassinosteroid-signaling kinase 830 interacts with flagellin receptors and regulates stomatal immunity

G. Sobol[1,*], B.B. Majhi[1], N. Zhang[2], H.M. Roberts[2], G.B. Martin[2] and G. Sessa[1]
[1]*School of Plant Sciences and Food Security, Tel-Aviv University, 69978 Tel-Aviv, Israel.* [*e-mail: guysobol@mail.tau.ac.il]
[2]*Boyce Thompson Institute for Plant Research, Ithaca, NY 14853, U.S.A.*

Detection of bacterial flagellin by the tomato receptors Flagellin sensing2 (Fls2) and Fls3 triggers activation of defense responses collectively referred to as pattern-triggered immunity (PTI). Tomato signaling components acting in association or downstream of flagellin receptors are largely unknown. Here, we investigated the involvement of tomato brassinosteroid-signaling kinase 830 (BSK830) in PTI triggered by flagellin perception. BSK830 localized to the plasma membrane and interacted in yeast and *in planta* with flagellin receptors. Consistent with a role in Fls2 and Fls3 signaling, CRISPR/Cas9-generated tomato *BSK830* mutant lines were impaired in ROS accumulation induced by the flg22 and flgII-28 flagellin epitopes. In addition, in wild-type plants the flagellin deficient *Pseudomonas syringae* pv. *tomato* (*Pst*) strain Δ*fliC* developed larger populations than wild-type *Pst* bacteria possibly due to its lower immunogenicity, whereas in *BSK830* mutants *Pst* wild-type and Δ*fliC* bacteria displayed a similar growth. Remarkably, in contrast to wild-type plants, *BSK830* mutants failed to close stomata when infected with *Pst* bacteria suggesting a role for BSK830 in pre-invasion immunity. In agreement with this notion, *BSK830* mutants were more susceptible to *Pst* than wild-type plants when infected by dip inoculation, but not by vacuum-infiltration. Analysis and validation of transcriptome and proteome profiles of *BSK830* mutants during PTI revealed differential expression of genes/proteins related to jasmonic acid metabolism and signaling, ABA perception and signaling, and regulation of stomata aperture. These results demonstrate that tomato BSK830 participates in PTI triggered by flagellin perception and regulates stomatal aperture by modulating jasmonic acid and ABA metabolism and signaling.

Bacterial plant diseases: difficulties, dangers and opportunities

S. Burdman
Dept. of Plant Pathology and Microbiology, The Robert H. Smith Faculty of Agriculture, Food and Environment, The Hebrew University of Jerusalem, Rehovot 7610001, Israel [*e-mail: saul.burdman@mail.huji.ac.il]

Compared to medicine where bacteria are dominant disease causal agents, most plant diseases in agriculture are caused by fungi, oomycetes and viruses. However, bacterial diseases cause tremendous damage to agricultural yields in several regions of the world, and almost every crop is affected by at least one or few major bacterial disease. For examples of devastating plant bacterial diseases, one only has to ask a citrus grower in Florida, an olive grove owner in southern Italy, a watermelon grower in Georgia or a pear grower in the north of Israel. Most phytopathogenic bacteria are transmitted through seeds and other plant materials, and this enhances their spread to new regions and countries. In addition, the means of dealing with bacterial diseases are limited. Control of most bacterial diseases relies on copper-based bactericides, which possess limited effectiveness. These are protective compounds, meaning they can help reduce infection but are not a cure after the pathogen has penetrated into the plant. These compounds are also easily washed away from the plant surface following rain or irrigation from above, which are factors that increase spread and establishment of phytopathogenic bacteria. Consequently, high frequency of spraying is required, which increases production costs, environmental contamination and the risk of emergence of copper-resistant strains. Moreover, given the protective nature of these chemicals, they cannot be used at all to control bacteria that are transmitted directly into the plant organs by insects. In this talk I will give an overview of important bacterial diseases in the world, including some that occur in Israel as well as others that do not, and it would be good if such stay outside the country. I will also refer to additional approaches that can be exploited for management of bacterial plant diseases.

Endoplasmic reticulum (ER)-associated molecular responses underlying *Liberibacter solanacearum* interactions with the carrot psyllid

O. Jassar[1, 2,*] and M. Ghanim[1].
[1]*Dept. of Entomology, Agricultural Research Organization—the Volcani Center, Rishon LeZion 7505101, Israel* [*e-mail: olajassar@gmail.com]
[2]*Dept. of Agroecology and Plant Health, The Hebrew University of Jerusalem. Rehovot 7610001*

Carrot Yellows (CY) is a devastating disease which has been a major constraint to carrot cultivation in Israel and around the world, causing significant yield losses. *Candidatus* Liberibacter solancearum (CLso) is the causative agent of CY and it is transmitted by the carrot psyllid *Bactericera trigonica*. Management of diseases caused by Liberibacter species depends on chemical control for lowering the insect vector populations, leading to health, economic and environmental problems as a result of using these chemicals. Understanding the molecular mechanisms underlying the transmission of Liberibacter species by their psyllid vectors can pave the way for devising novel and ecologically friendly approaches for controlling the transmission. It has been shown that many pathogens can recruit their host endoplasmic reticulum (ER) for replication and thereby avoiding the host immune responses. Recently, CLso was associated with the ER in the midgut of its psyllid vector, and microscopic analysis has shown that the ER might be the site for CLso replication. Our project extended these studies and we show that genes associated with the ER-associated protein degradation (ERAD) machinery, which act as a quality-control mechanism for clearing unfolded/ misfolded proteins from the ER, are involved in CLso-ER interactions and CLso transmission. Targeting those genes may lead to developing new approaches for disease control.

New and old diseases and Plant resistance to disease and pathogenesis

Roles for cytokinin in host-pathogen interactions

R. Gupta, G. Anand, M. Leibman-Markus, D. Elkabetz and M. Bar*
Dept. of Plant Pathology and Weed Research, Agricultural Research Organization—the Volcani Center, Rishon LeZion 7505101, Israel [*e-mail: mayabar@volcani.agri.gov.il]

The plant hormone cytokinin (CK) is an important developmental regulator. CK is central in plant life from developmental processes, to growth and stress tolerance. CK is known to induce host plant resistance to several classes of phytopathogens. Recently, we uncovered a direct effect of CK on fungi. We found that CK directly inhibits the growth, development, and virulence of fungal phytopathogens. CK influences fungal development, by altering the cell cycle, the cytoskeleton and cellular trafficking. We also investigated the roles of CK in shaping the host plant microbiome. We examined the relationship between CK and the phyllosphere microbiome, finding that CK acts as a selective force in microbiome assembly, increasing richness, and promoting the presence of Firmicutes. CK-mediated immunity was found to partially depend on the microbial community, and bacilli isolated from CK-rich plant genotypes induced plant immunity and promoted disease resistance. Using a biomimetic system, we investigated the relationship between leaf microstructure, which is differentially patterned upon changes in CK content or signaling, and the growth of different phyllosphere microbes. Leaf structures derived from CK-rich plant genotypes support bacilli in the biomimetic system. Overall, our results indicate that host genotype and hormonal profiles can act as a strong selective force in microbiome assembly, underlying differential immunity profiles, and pathogen resistance as a result.

Isolation, identification and control of *Fusarium* spp., the causal agents of onion (*Allium cepa*) basal rot in Northeastern Israel

B. Kalman[1,2], D. Abraham[1], S. Graph[1], R. Perl-Treves[2], Y.M. Harel[3] and O. Degani[1,4,*]
[1]*Plant Sciences Dept., Migal–Galilee Research Institute, Tarshish 2, Kiryat Shmona 11016, Israel* [*e-mail: d-ofir@migal.org.il, ofird@telhai.ac.il]
[2]*The Mina and Everard Goodman Faculty of Life Sciences, Bar-Ilan University, Ramat Gan 52900, Israel*
[3]*Division for Plant Pests and Diseases, Plant Protection and Inspection Services, Ministry of Agriculture and Rural Development, Bet Dagan 50200, Israel*
[4]*Faculty of Sciences, Tel-Hai College, Upper Galilee, Tel-Hai 12210, Israel*

Over the past decade, reports have accumulated on the increasing incidence and spread of onion (*Allium cepa*) *Fusarium* basal rot disease in northern Israel. Rotting onion bulbs were sampled from fields in the Golan Heights during 2017 and 2018. Tissue samples were used to isolate and identify the infecting fungal species using colony and microscopic morphology, and DNA-based PCR amplification and sequencing using specific primers. Four *Fusarium* spp. isolates were identified: *F. proliferatum*, *F. oxysporum* f. sp. *cepae*, and two species less familiar as causative agents of this disease, *F. acutatum* and *F. anthophilium*. Phylogenetic analysis revealed that these species are subdivided into two populations: a group isolated near Meitsar from white (Riverside cv.) onion bulbs; and a group isolated near Eliad from red (565/505 cv.) bulbs. Pathogenicity tests conducted with seedlings and bulbs under moist conditions proved that all species could cause the disease symptoms, but with different degrees of virulence. Commercial chemical fungicides were evaluated against the basal rot disease pathogens in culture plates, a seed assay and potted 30-day-old sprouts. Prochloraz (0.3% *w/w* seed coating) reduced the harmful impact of *F. oxysporum* f. sp. *cepae*. on Noam (a red cv.) sprouts, but was not effective against this pathogen in cv. Riverside or against *F. acutatum*. Revealing the mycoflora of diseased onions in northern Israel constitutes the first step in developing a preventive program.

The effect of Beet Curly Top Virus (BCTV) on *Cannabis sativa*

Y. Giladi, L. Hadad, N. Luria, O. Lachman, W. Cranshaw and A. Dombrovsky*
Dept. of Plant Pathology and Weed Research, Agricultural Research Organization—the Volcani Center, Rishon LeZion 7505101, Israel [*email: aviv@volcani.agri.gov.il]

An Israeli-American research team has recently published that *Beet Curly Top Virus* (BCTV) infected *Cannabis sativa* plants. Infected plants exhibit stunted growth, inhibition of chlorophyll production and reduced number and size of flowers. Leaves of infected plants are pale green, narrow and curl sideways. The first publication of the destructive effects of BCTV occurred at the beginning of the 20th century, following devastating damage caused to sugar beet crops that led to abandonment of thousand acres of fields in California, Utah and Colorado, resulting in great income loss. It is considered to be one of the worst agricultural diseases in USA history. The viral variant that infects *Cannabis sativa* was identified by the Dumbrovsky Volcani Center laboratory, following isolation of RNA from infected leaves, followed by RNA sequencing by the Technion Genome Center, Israel. Analysis using the Virus Detect software revealed 31 contigs which shared 96.5 identity with the BCTV genome sequence. Validation of the presence of BCTV was obtained by antibody analysis performed at the Plant Pathology laboratory, Colorado University. The virus is transmitted by the beet leafhopper vector *Circulifer* (=*Neoaliturus*) *tenellus*. Research conducted by A. Gera, confirmed that this leafhopper vector exists in Israel. However, the viral disease was not detected in *Cannabis sativa* grown in Israel. We describe the discovery of this infectious virus, how to identify disease symptoms, describe the viral vector, and discuss ways to avoid the spread of this disease in Israel.

Efficacy of fire blight management in pome fruit in northern Israel: copper agents and their effect on yield parameters

M. Dafny-Yelin[1*]; J. Clara Moy[1,2]; O. Mairesse[1,2]; M. Silberstein[1]; G. Sapir[3]
[1]*Northern Agriculture Research & Development, Migal—Galilee Technology Center, P.O.B. 831, Kiryat Shemona 11016 Israel* [*email: merydy@gmail.com; merydy@migal.org.il]
[2]*Tel-Hai Academic College, Upper Galilee, Israel, 12208 Kiryat Shemona, Israel*
[3]*Edete precision technologies for Agriculture*

Erwinia amylovora causes fire blight, a bacterial disease of pome fruits. For 15 years, fire blight management in Israel was based solely on the antibiotic oxolinic acid but, in response to decreased disease-control efficacy, copper agents were added in 2014. However, concern was raised as to whether repeated applications of copper might have impaired pollen germination and thereby diminished yields. In Israel, apple cultivars, except for the early-blooming cultivar 'Cripps pink' (trademark Pink Lady), appeared to be markedly less sensitive to the infection than pear cultivars ('Spadona' and 'Costia'). The aims of the present study were to monitor the effects of treatments with copper-based agents during blossoming in reducing fire blight damage, while recording their effects on pollen-tube germination and fruit-yield parameters. Copper tribasic sulfate and copper hydroxide reduced disease levels by more than 70% compared with uninfected controls. Pollen-tube germination rates in both apples and pears were significantly reduced after two successive daily applications of copper tribasic sulfate, but usually they recovered to control levels after introduction of a single-day interval. Four to seven seasonal applications of copper agents on apple and pear blossoms did not significantly reduce fruit weight and size, seed number, seed-set or fruit-set. In summary, copper application against fire blight in pears and apples during their blooming in Israel is both effective and harmless to yields.

Biological soil crust microalgae as a novel source for the development of a biocontrol agent

D. Eckstein, N. Margolis and H. Raanan*
Dept. of Plant Pathology and Weed Research, Gilat Research Center, Agricultural Research Organization, Mobile Post Negev 2, 8531100, Israel [*e-mail: hagair@volcani.agri.gov.il]

Soil-borne pathogens cause severe diseases in many crops worldwide, which result in significant economic losses. In the absence of a suitable host, these pathogens produce resting structures that are tolerant to environmental stresses and pesticides. Furthermore, pesticides are toxic, and thus, their accumulation in plants and soil is a serious threat to humans and the environment. Unsurprisingly, extensive research has focused on developing sustainable solutions such as biocontrol agents. Nevertheless, the complex interaction of these agents with their biotic and abiotic environment challenges the development of novel effective agents, a critical but unmet need. Biological soil crusts (BSCs) are the natural cover of many arid and semi-arid lands, and their destruction is considered an important promoter of desertification. The microorganisms inhabiting BSCs developed unique mechanisms to survive the harsh environmental conditions of desiccation, heat and radiation. These microorganisms are unexplored gene pools. The green algae *Chlorella ohadii* isolated from desert BSCs shows a remarkable ability to withstand harsh environmental conditions. Surprisingly, it is also one of the fastest-growing phototrophs known. Preliminary results show that co-culturing of *C. ohadii* significantly inhibits the growth as well as the production and viability of resting structures of various soil-borne pathogenic fungi, including *Verticillium dahliae* and *Rhizoctonia solani*. The combination of (i) fast-growth, (ii) resilience and (iii) the observed antifungal activity make *C. ohadii* a promising candidate for the development of a biocontrol agent against soil-borne pathogenic fungi, specifically for arid and semi-arid regions and in the face of climate change.

The role of Target of Rapamycin (TOR) in tomato defense responses and disease resistance

I. Marash[1,2,*] M. Leibman-markus[1], R. Gupta[1] and M. Bar[1]
[1]*Dept. of Plant Pathology and Weed Research, Agricultural Research Organization—the Volcani Center, Rishon LeZion 7505101, Israel* [*e-mail: iftahmarash@mail.tau.ac.il]
[2]*School of Plant Science and Food Security, Tel-Aviv University, Tel-Aviv, 69978, Israel*

To determine the best time for growth and development all organisms need to sense and process information about the availability of nutrients, energy status and environmental cues. The conserved Target of Rapamycin (TOR) protein kinase has a central role in sensing and perceiving nutritional information. To maintain cellular homeostasis TOR connects environmental information about nutrient availability to developmental and metabolic processes. Under favorable energy conditions, TOR is activated and promotes anabolic processes such as cell division, while suppressing catabolic processes. Conversely, catabolic processes are promoted when nutrients are limited or environmental stresses are present, and TOR is inactivated. Given the central role of the TOR in regulating metabolism, several studies have examined whether TOR is wired to plant defense: the mechanisms by which TOR influences plant defense are unclear. Here, we addressed this question by testing the effect of inhibiting TOR on immunity and pathogen resistance in tomato. The hormonal defense pathways influenced by TOR were examined. We show that tomato defense responses and disease resistance to several pathogens increase upon TOR inhibition, and that TOR-inhibition-mediated resistance likely requires a functional salicylic acid pathway, but not a jasmonic acid pathway. Our results support the notion that TOR is a master regulator of the development-defense switch in plants.

Posters

Pathogenic variations and geographic distribution in Israel of *Magnaporthiopsis maydis*, the causal agent of late wilt of maize

G. Shofman[1,2] and O. Degani[1,2,*]
[1]*Plant Sciences Dept., Migal–Galilee Research Institute, Tarshish 2, Kiryat Shmona 11016, Israel* [*e-mail: d-ofir@migal.org.il, ofird@telhai.ac.il]
[2]*Faculty of Sciences, Tel-Hai College, Upper Galilee, Tel-Hai 12210, Israel*

The late wilt disease of maize causes significant damage to crops in Israel and other countries, and crop losses for food and feed may reach 100%. The disease is caused by the soil- and seed-borne fungus *Magnaporthiopsis maydis* and is characterized by rapid wilting of the plants near tasseling. The pathogen's soil durability makes it hard to control. Therefore, resistant maize cultivars are the preferred method to restrain the disease. The Spanish and Egyptian pathogen populations have varying degrees of aggressiveness, and virulent strains can overcome host resistance. In addition, competitive and antagonistic relations between pathogen strains can decrease disease severity and the pathogen infection of host plants. In recent years, 18 *M. maydis* strains were isolated from infected maize fields in Israel. The current study characterized the virulence difference among those fungal isolates – their effect on seed germination, plant development and disease symptom severity. The Israeli isolates display a diverse degree of aggressiveness that may be linked to their geographic distribution. The most virulent strains harmed plants and ears' development and caused severe wilting and death. In contrast, plants inoculated with the less virulent strains exhibited only mild dehydration symptoms, and crop yield was similar to that of the non-inoculated control. Future research will examine the effect of the most aggressive fungal strains on resistant maize cultivars and the effect of intra-species relationship on disease expression. The knowledge acquired in this study will further increase our understanding of the pathogen and our ability to control it.

Canon® controls late blight in processing tomato at the Valley of Springs

N. Nitzan*, Y. Giladi, S. Lahiani and M. Zoabi
The Valley of Springs Research & Extension Center; M.P. Beit She'an Valley, 11710 [*email: nitzan_nadav@yahoo.com]

Late blight, caused by *Phytophthora infestans*, may lead to losses of half a million US dollars for both the conventional and organic processing tomato farmers at the Valley of Springs. Canon® (a.i. K_2HPO_3) is a Plant Host Defense Inducer recognized for its ability to promote systemic acquired resistance in treated plants. As new and eco-friendly fungicides are sought, the present study tested the hypothesis that Canon® can control late blight in processing tomato under the local environmental conditions. Two field trials executed at Eden farm identified that Canon® controlled late blight in a similar manner ($P>0.05$) to the commercial benchmark fungicides when applied to the foliage preventively at 10,000, 7,500 and 5,000 mL/Ha every 10 days. Leaves of field-treated and -untreated plants removed 3 days-post-application (dpa) and inoculated with a local Mefenoxam-resistant isolate supported this outcome, signifying a residual inhibition efficacy of up to 9 dpa at 10,000 and 7,500, but not at 5,000 mL/Ha. The dynamics of late blight was evaluated in the laboratory by stem-inoculating tomatoes foliar-sprayed at a rate equivalent to 10,000 mL/Ha, 3 and 10 dpa. The outcome suggested that Canon® may limit infection occurrence 1 to 3 dpa, and reduce disease rate significantly ($P<0.05$) compared to the untreated control, up to 22 dpa. Hence, the study supported the hypothesis that Canon®, applied preventively, can manage late blight on processing tomato under the Valley of Springs conditions. Further study will evaluate Canon®'s efficacy at broader application intervals.

A platform for developing new molecules to inhibit infection mechanism and virulence of pathogenic bacteria

O. Galsurker[1*], M. Pun[1,2], N. Khazanov[3], H. Senderowitz[3] and I. Yedidia[1]
[1]*The Institute of Plant Sciences, Volcani Center-Agricultural Research Organization), Bet Dagan 50250, Israel* [*email: ortalg@volcani.agri.gov.il]
[2]*The Robert H. Smith Faculty of Agriculture, Food and Environment, The Hebrew University of Jerusalem, Rehovot 76100, Israel*
[3]*Dept. of Chemistry, Bar-Ilan University, Ramat Gan 52900, Israel*

Lack of approaches to control bacteria in agricultural crops, and the rise of bacterial antibiotic resistance, led to a search for alternative ideas for the control of bacteria. A new approach to reduce pathogenic bacteria is based on identifying unique targets in bacteria, to prevent infection. The quorum sensing (QS) system, which enables chemical communication between bacteria and activation of virulence, is a potential target. To inhibit QS activity, homology models of QS proteins were constructed based on their similarity to known proteins with a solved crystallographic structure, using a computational approach. These structures were used to detect unique molecules with high binding potential to the proteins' active sites. We focused on *Pectobacterium* (soft rot bacteria in potatoes and ornamental plants) and on the inhibition of its QS proteins to reduce infection. We developed a virtual screening system that detected new molecules that may specifically decrease the bacterial virulence activation in plants. Five target proteins were defined, and underwent molecular modeling, from which two pharmacophores were constructed. Broad screening of three databases was conducted to detect molecules with the potential to inhibit bacterial target proteins. Forty six million molecules were scanned for primary binding to target proteins. Among them, 1074 molecules were selected and underwent docking simulations on a target protein. Potential molecules were tested on *Pectobacterium*: three molecules inhibited the QS mechanism. The system has proven that computational screening can detect new molecules with high activity potential to inhibit QS. Further activity characterization for the molecules that exhibited high activity is required, and testing of additional molecules should continue.

High-density pear orchard shows reduced tree sensitivity to fire blight damage due to decreased tree vigor

M.D. Yelin[1*], J.C. Moy[1] R.A. Stern[1,2], I. Doron[1] and M. Silberstein[1]
[1]*Northern Agriculture Research & Development, MIGAL–Galilee Research Institute, P.O.B. 831, Kiryat Shmona 11016, Israel*
[2]*Dept. of Biotechnology, Faculty of Life Sciences, Tel-Hai College, Upper Galilee 12210, Israel* [*email: merydy@gmail.com]

Fire blight, caused by *Erwinia amylovora*, is a severe disease of pear. Highly vigorous trees are more sensitive to *E. amylovora* damage after summer pruning. Under the same growing conditions, trees grown in high-density orchards have lower vigor than those in low-density orchards. High-density orchards have fewer required inputs, such as pruning and tying, and increased yield and orchard profitability per dunam. In this study, we assessed tree damage due to fire blight in high-density pear orchards vs. the common Israeli low-density orchards. Pear trees of the fire blight-sensitive cultivar Spadona were planted at high density using the spindle system (250 trees/dunam), or at low density using the palmette system (100 trees/dunam). Four years after planting, both designs were similarly infected with fire blight (11–50 infected blossoms per tree), but 1 year post infection, trees in the high-density orchard had fewer infections in the main limbs and trunk bases compared to the low-density orchard. Three years post infection, no trees had died in the high-density orchard, whereas in the low-density orchard, 10% of the trees were wilted. Results indicate that for fire blight-susceptible pear cultivars, a high-density planting system associated with reduced tree vigor presents a decreased risk of fire blight damage.

Controlling sweet basil downy mildew with plant host defense inducers

N. Nitzan; Valley of Springs Research & Extension Center, Israel [email: nitzan_nadav@yahoo.com]

Sweet basil downy mildew (SBDM), caused by *Peronospora belbahrii*, is the most destructive disease of sweet basil (*Ocimum basilicum*) in Israel. Fungicide control is limited due to increased insensitivity, and pre-harvest interval limitations and reduced maximum residue limits. The commercial product Cordon-super (Fosfital; www.artal.net) and the experimental formulation AMF (Luxembourg Ind. Ltd., Israel) are phosphite-based Plant Host Defense Inducers (PHDIs) fortified with microelements. Growth chamber trials tested their efficacy to control SBDM protectively, curatively or in combinations by foliar application. Extended incubation post-inoculation and fogging events were used to promote infection, disease development and sporulation. In the growth chamber, both PHDIs reduced ($P<0.0001$) disease when applied protectively 3- and 7-days pre-inoculation, but demonstrated inconsistent efficacy as curatives. Nonetheless, disease control was most prominent ($P=0.009$) when the PHDIs were applied 1 h pre-inoculation and 7 days later. In the fall, under field conditions, both PHDIs reduced disease ($P=0.0084$) by nearly 96% in relation to the non-treated control, and similarly ($P>0.05$) to the commercial benchmarks. The study provided insight to the potentiality PHDIs to manage SBDM singly, or in combination with benchmark fungicides.