### Abstracts of presentations at the 40th Congress of the Israeli Phytopathological Society

February 11–12, 2019. Cohen Auditorium, Agriculture Research Organization – the Volcani Center, Bet Dagan, Israel.

Published online: 27 March 2019 © Springer Nature B.V. 2019

#### **Invited lectures**

#### Phytophthora infestans: the master invader

W.E. Fry

Plant Pathology and Plant-Microbe Biology, School for Integrative Plant Sciences, Cornell University, Ithaca NY, 14853, USA [e-mail: wefl@cornell.edu]

In the USA and in most locations worldwide, major epidemics (pandemics) of late blight caused by Phytophthora infestans have been caused by the introduction of novel genotypes. In the USA (and in most locations around the world), the population structure of P. infestans over time is characterized by a series of genotypes that come to dominance and then disappear from the scene. The exceptions to this situation occur in central Mexico where sexual reproduction has been common for as long as we know, and in parts of northwest Europe where sexual reproduction has been common since the 1990s - again the result of a migration event in the late 1970s. Different genotypes of P. infestans can have very different phenotypes. Genotypes can be assessed in hours, whereas it takes months to assess a phenotype. If there are only a few genotypes in a region, and if the phenotypes of these genotypes are known, then rapid knowledge of genotype can provide information useful for disease suppression.

### Spread of *Fusarium oxysporum* f. sp. *cubense* tropical race 4 causing wilt of Cavendish bananas in Israel

M. Maymon<sup>1</sup>, U. Shpatz<sup>1</sup>, N. Sela<sup>1</sup>, Y. Meller Harel<sup>2</sup>, E. Levy<sup>2</sup>, G. Elkind<sup>2</sup>, E. Teverovsky<sup>2</sup>, R. Gofman<sup>2</sup>, A. Haberman<sup>2</sup>, R. Zemorski<sup>2</sup>, N. Ezra<sup>2</sup>, Y. Levi<sup>3</sup>, G. Or<sup>3</sup>, N. Galpaz<sup>4</sup>, Y. Israeli<sup>5</sup> and S. Freeman<sup>1,\*</sup>

<sup>1</sup>Dept. of Plant Pathology and Weed Research, Agricultural Research Organization—the Volcani Center, Rishon LeZion 7505101, Israel [e-mail: freeman@volcani.agri.gov.il]; <sup>2</sup>Plant Protection and Inspection Services, Ministry of Agriculture and Rural Development, Bet Dagan 50250, Israel; <sup>3</sup>Extension Service, *Ministry of Agriculture and Rural Development, Bet Dagan* 50250, Israel; <sup>4</sup>Northern R & D, Kiryat Shmona 11016, Israel; <sup>5</sup>Jordan Valley Banana Exp. Stn., Zemach 15132, Israel.

Panama disease, caused by the soilborne pathogen Fusarium oxysporum f. sp. cubense (Foc), is one of the most destructive fungal pathogens of banana worldwide. Foc race 1 severely affected the cultivar 'Gros Michel' that was replaced by 1960 with resistant Cavendish sub-group cultivars. However, a new race of Foc, tropical race 4 (TR4), causing mortality of Cavendish banana cultivars was identified in the early 1990's in southeast Asia (Indonesia, Philippines, Malaysia), Taiwan and Australia, and has since spread to most banana growing countries in the region, including China. Recently, TR4 was discovered in Mozambique (Africa), Oman, Pakistan, India, Cambodia, Myanmar and the Middle East (Jordan and Lebanon). During the summer of 2016, typical TR4 symptoms of leaf-yellowing and wilting, accompanied by internal vascular discolorations of rhizomes and pseudostems were observed in mature 'Grand Naine' Cavendish cultivar plants, from Shfeya, Carmel coastal plain and Kibbutz Ein Gev, on the eastern shores of Lake Galilee. Symptomatic tissues were sampled, plated on PDAC medium and white-colored colonies with floccose mycelia developed consistently from affected tissues. Single conidium cultures isolated from these tissues resembled Fusarium oxysporum morphologically, while PCR analyses and sequencing confirmed their identity as FOC race TR4. TR4 representative isolates were tested for pathogenicity and 4 weeks post-inoculation, mortality of TR4 treated plants developed while control treatments remained healthy. TR4 identification from symptomatic plants was reconfirmed by PCR.

#### Epidemiology and disease dynamics

# Population structure of *Phytophthora infestans* in Israel in 2017 and 2018

Y. Cohen $^{1\ast},$  A.E. Rubin $^1,$  M. Galperin $^1,$  U. Zig $^2$  and D.E.L.  $Cooke^3$ 

<sup>1</sup>Faculty of Life Sciences, Bar Ilan University, Ramat Gan, Israel 5290002 [\*e-mail: yigal.cohen1@gmail.com]; <sup>2</sup>Maon Enterprises Ltd., MP Negev 85465, Israel;

<sup>3</sup>The James Hutton Institute, Dundee, Scotland, UK

Late blight caused by Phytophthora infestans is a major disease of potato crops in Israel. Disease outbreaks initiate in November and terminate in June. Here we report on the phenotypic and genotypic traits of P. infestans in Israeli during the seasons of 2017 and 2018. A total of 42 and 116 isolates were collected during 2017 and 2018, respectively, from late blight-infected potato crops in the Western Negev region of Israel. In addition, 84 isolates were collected from a single organic field at Nirim, 38 in March 2017 and 46 in April 2017. Sporangia were collected from samples and tested at Bar Ilan University for sensitivity to mefenoxam (MFX) and virulence factors. DNA was extracted from sporangia and analysed for SSR markers (genotyping) in The James Hutton Institute. In 2017, disease outbreaks occurred as late as March due to unfavourable weather conditions. Of the isolates collected during March-May 2017, 8, 17 and 3 isolates were sensitive (S), intermediate (I) and resistant (R) to MFX, respectively. Of these strains, 29 were genotyped as 23A1 and one was 13A2. The mean number of virulence factors was  $4.8 \pm 1.5$ . Results from the organic field showed that in March, 1, 24 and 5 isolates were S, I and R to MFX, respectively; 10 belonged to 23A1 and 14 to 13A2, and the mean number of virulence factors was  $5.8 \pm 2.0$ . In April, a strong change occurred in the population structure: 0, 4 and 38 isolates were S, I and R to MFX, respectively; 24 belonged to 23A1 and 7 to 13A2. The mean number of virulence factors was  $8.7 \pm 2.5$ . In 2018, 11, 70 and 21 isolates were S, I and R to MFX, respectively; 86 isolates belonged to 23A1, two isolates belonged to a new genotype 36A2 and one was a mix of both genotypes. Most isolates carried five virulence factors (1,3,4,7,9), but towards the end of season, some isolates carried 8-11 virulence factors. The results indicate that population structure of P. infestans in Israel may change rapidly, not only between seasons but also within the season.

### Spatial and temporal dynamics of Mal Secco disease spread in lemon orchards in Israel

M. Ben-Hamo, D. Ezra and L. Blank\*

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

Mal Secco is a severe disease of citrus in which the pathogenic fungus *Plenodomus tracheiphilus* (formerly *Phoma tracheiphila*) penetrates the host vascular system. The disease has great economic implications for the production of lemons, the most susceptible species, and on the export of other citrus. In this study, we characterized the spatial dynamics of the disease within seven lemon orchards located in central and southern Israel. A representative block of trees from each orchard was evaluated monthly for three consecutive years. In addition, we assessed annually disease severity in seventy-five orchards from three different geographical regions and tested for association between disease severity and measures of orchard management, environmental factors, cultural practices and cultivar. We assessed disease incidence and

characterized the spatial pattern of disease severity and evaluated the spatial and temporal dynamics of disease progress. We found different rates of disease spread in different orchards, which might be the result of differences in orchard management practices. We also found indication for the spread of the disease within rows of trees. Our results confirm that 'Interdonato' lemon (*Citrus limon* L. Burm.f.) is a less susceptible cultivar and suggest that the density of urban terrain surrounding each orchard is positively correlated with the disease severity. In contrast to our expectations, we found no correlation between the density of lemon orchards surrounding a focal orchard and the severity of the disease in it, which strengthened previous findings regarding the limited range of distribution of the disease.

# Biogeography and molecular species delimitation of *Pratylenchus capsici* n. sp., a new-old root-lesion nematode in Israel on pepper (*Capsicum annuum*)

Q. Xue<sup>1</sup>, A. Gamliel<sup>2</sup>, P. Bucki<sup>1</sup>, S. Duvrinin<sup>3</sup>, O. Abraham<sup>1,2,4</sup> and S. Braun<sup>1\*</sup>

<sup>1</sup>Dept. of Entomology, Nematology and Chemistry Units, Agricultural Research Organization—the Volcani Center [\*e-mail: sigalhor@volcani.agri.gov.il]; and <sup>2</sup>Laboratory for Pest Management Research, Agricultural Research Organization—the Volcani Center, Rishon LeZion 7505101, Israel; <sup>3</sup>Extension Service, Ministry of Agriculture and Rural Development, Bet Dagan 50250, Israel; <sup>4</sup>Dept. of Plant Pathology and Microbiology, The Robert H. Smith Faculty of Agriculture, Food and Environment, The Hebrew University of Jerusalem, Rehovot 76100, Israel

Root-lesion nematodes of the genus Pratylenchus parasitize the roots of various plants and can cause severe damage and yield loss. Here, we report on a new species, Pratylenchus capsici n. sp., from the Arava rift valley, Israel, which was characterized by integrative methods including detailed morphology, molecular phylogeny, population genetics and biogeography. We found this species widely spread along the Arava rift, causing significant infections in pepper (Capsicum annuum) roots and inhibition of plant growth. Both morphological and molecular species delimitation support the recovered species as a new species. We recovered a high cytochrome oxidase subunit I haplotype diversity: biogeographical analysis suggests that contemporary gene flow was prevented among different farms while population dispersal from weeds to pepper was found on a relatively small scale. Our results suggest that weeds are important for the dispersal of P. capsici n. sp., either as the original nematode source or at least to maintain the population between growing seasons.

# The connection between environmental condition and the expression of charcoal rot (*Macrophomina phaseolina*) symptoms in cotton

#### R. Cohen\* and M. Elkabetz

Dept. of Plant Pathology and Weed Research, Agricultural Research Organization— Newe Ya'ar Research Center, Ramat Yishay 3657800, Israel [\*e-mail: ronico@volcani.agri.gov.il]

Macrophomina phaseolina is a fungus that attacks many summer crops, including cotton. The expression of disease symptoms can be affected by environmental factors such as soil type, previous crop, agro-technology, air and soil temperatures, and water economy. Previously it appeared that the extent of root colonization by the pathogen had only limited effect on disease expression. However, in field experiments conducted in the last two years, we noticed that effective inoculation accompanied by high levels of root colonization was easily achievable. Nevertheless, increased symptom development in the inoculated plants was not evident. Similar results were obtained following soil treatment with fungicides. The fungicides also reduced rot colonization after a single treatment applied as soil drench at sowing. Later in the growing season, when roots penetrated deep into the soil, the fungicide did not affect the whole root volume and the plant might exhibit disease symptoms. However, in some experiments azoxystrobin showed advantage over other fungicides tested; thus, the effect of fungicides as a control measure should be further investigated. It appears that the plants and the pathogen achieved a balance, and disease development occurred only after growing conditions changed, causing plant stress. Cotton growers reported higher disease incidence following leaf aphid attack. The irrigation economy had significant effect on disease incidence and severity. The timing of the first irrigation, dividing water volumes between the irrigations throughout the growing season and the position of the drip irrigation pipes in the field were critical to disease control and to the success of the crop.

#### New host range for the maize pathogen Harpophora maydis

O. Degani<sup>1,2,\*</sup>, S. Dor<sup>1,2</sup> and D. Regev<sup>1,2</sup>

<sup>1</sup>Migal–Galilee Research Institute, Tarshish 2, Kiryat Shmona 11,016, Israel [\*e-mail: d-ofir@bezeqint.net, ofird@telhai.ac.il]; <sup>2</sup>Tel-Hai College, Upper Galilee, Tel-Hai 12210, Israel

The late wilt disease causal agent, the fungus Harpophora maydis, is a vascular pathogen that causes severe damage to sensitive maize hybrids, at the ripeness stage, throughout Israel, Egypt, India, Spain and other countries. It can undergo pathogenic variations and survive as spores, sclerotia or mycelia on plant residues, in the soil, or inside seeds. Maize, Lupinus termis (lupine) and apparently cotton, are the only known hosts of H. maydis. Identification of other plant hosts that can assist in the survival of the pathogen is an important step in restricting disease outbreak and spread. Here, a field survey and growth chamber pathogenicity trial series accompanied by Real Time PCR tracking confirmed the presence of the fungus DNA inside the roots of cotton (cv. Pima) plants grown in infested soil. Moreover, we identified the presence of H. maydis DNA in the grass Setaria viridis (green foxtail) and watermelon (cv. Malali). Infected watermelon plants had delayed emergence and development, were shorter, and had reduced root and shoot biomass. H. maydis infection also damaged root biomass and phenological development of cotton plants but caused only mild symptoms in S. viridis. In liquid minimal medium, H. maydis growth was enhanced in the presence of maize or cotton root powder. While watermelon root powder had a minor influence on fungal dry weight, it caused a dramatic increase in pathogen laccase production. These findings are an important step towards uncovering the host range and endophytic behavior of *H. mavdis*, and encourage expanding this evaluation to other plant species.

Interception of *Ilyonectria liriodendri*, agent of black root disease, in raspberry from the United States

Y. Meller Harel<sup>1</sup>, G. Elkind<sup>1</sup>, E. Gomberg<sup>1</sup>, R. Gofman<sup>1</sup> and A. Shamai<sup>2</sup>

<sup>1</sup>Division for Plant Pests and Diseases [\*e-mail: yaelm@moag. Gov.il]; and <sup>2</sup>Division of Quarantine, Plant Protection and Inspection Services, Ministry of Agriculture and Rural Development, Bet Dagan 50250, Israel

*Cylindrocarpon spp* are soil fungi distributed worldwide which cause black root rot diseases in herbaceous plants and cankers in trees. The genus, defined in 1913, has undergone many taxonomic changes and has been enriched with new species in recent years. In a visual examination of raspberries varieties (*Rubus idaeus*) imported from the United States, conducted as part of the monitoring of imported material in post-entry quarantine conditions, signs of wilting and decline of individual plants were noticed. Following laboratory tests, an isolate from the root system of the suspected plants was identified by classical and molecular methods as *Ilyonectria liriodendri*. This fungus belongs to the *Cylindrocarpon* genus and causes grapevine black foot disease and was previously unknown in Israel. Pathogenicity of the fungus in raspberries was demonstrated for the first time by completing Koch's postulates and the plants were destroyed.

#### Mechanisms of plant-pathogen interactions

# The complex modes of action of the biocontrol agent *Pseudozyma aphidis*

C.E. Calderon, S. Alster, R. Haris, D. Vella-Corcia, A. Gafni, N. Rotem and M. Levy\*

Dept. of Plant Pathology and Microbiology, The Robert H. Smith Faculty of Agriculture, Food and Environment, The Hebrew University of Jerusalem, Rehovot 76100, Israel [E-mail: Maggie.Levy@mail.huji.ac.il]

Plant pathogens challenge our efforts to maximize crop production due to their ability to rapidly develop resistance to pesticides. Fungal biocontrol agents have become an important alternative to chemical fungicides as a result of environmental concerns regarding conventional pesticides. The complex modes of action of biocontrol agents reduces the likelihood that pathogens will develop resistance to them. We recently isolated a unique biologically active isolate of the epiphytic yeast-like fungus Pseudozyma aphidis. Application of P. aphidis spores to plants in the greenhouse significantly reduced different pathogen infections. P. aphidis was found to secrete extracellular metabolites that inhibit several plant pathogens. The secreted metabolites activate morphological alterations, reactive oxygen species accumulation and enhance programed cell death in Botrytis cinerea hyphae. We demonstrated that P. aphidis proliferates on infected leaves to which B. cinerea hyphae adhere and compete with B. cinerea for space and nutrients. During interactions with powdery mildew on cucumber plants, P. aphidis went through a morphological transition from yeast to hypha, to parasitize the pathogen by coiling around its hyphae. We also found that P. aphidis can activate an induced-resistance response in plants in a SA-, JA/ET- and NPR1independent manner. Moreover, *P. aphidis* activated microbeassociated molecular pattern-triggered immunity marker genes in *Arabidopsis* leaves, but suppressed the subsequent microbeassociated molecular pattern-triggered callose deposition. We demonstrate that *P. aphidis* uses complex modes of action to efficiently control plant pathogens.

#### Identification of new type III-secreted effectors of plant-pathogenic Acidovorax

I. Jiménez-Guerrero<sup>1</sup>, F. Pérez-Montaño<sup>1,2</sup> G. M. da Silva<sup>1</sup>, D. Shkedy<sup>3</sup>, N. Wagner<sup>3</sup>, T. Pupko<sup>3</sup>, G. Sessa<sup>4</sup> and S. Burdman<sup>1,\*</sup>

<sup>1</sup>Dept. of Plant Pathology and Microbiology, The Robert H. Smith Faculty of Agriculture, Food and Environment, The Hebrew University of Jerusalem, Rehovot 76100, Israel [\*e-mail: saul.burdman@mail.huji.ac.il]; <sup>2</sup>Dept. of Microbiology, Faculty of Biology, University of Sevilla, Sevilla, Spain; <sup>3</sup>Schools of Molecular Cell Biology and Biotechnology and <sup>4</sup>Plant Sciences and Food Security, George S. Wise Faculty of Life Sciences, Tel Aviv University, Tel Aviv, Israel

The Acidovorax genus includes several plant-pathogenic species. Among them, one of the most agricultural important species is Acidovorax citrulli, which causes bacterial fruit blotch of cucurbits. This disease is a serious concern for the cucurbit industry, mainly for watermelon and melon crops. In common with some other Gram-negative plant-pathogenic bacteria, A. citrulli pathogenicity relies on a functional type III secretion system. Through this secretion system, bacteria secrete tens of protein effectors into the host cell cytoplasm. Type III effectors (T3Es) collectively promote virulence through alteration of the host cell metabolism and/or suppression of defense responses. Plants evolved sets of proteins that are able to recognize the activity of some effectors, and elicit defense responses and resistance. Therefore, investigation of effectors has important implications for the development of crop varieties resistant to plant pathogens. In contrast to the accumulated knowledge about T3Es of several plant pathogenic bacteria like Pseudomonas syringae pathovars and Xanthomonas species, little is known about Acidovorax effectors. In recent years, several A. citrulli genomes have been sequenced. Based on similarity to known effectors from other bacteria, it is estimated that A. citrulli strains carry about 45 T3E genes. Our long-term goal is to understand the contribution of T3Es to A. citrulli virulence. We also hypothesize that plant-pathogenic Acidovorax species carry unique, as yet unidentified, T3E genes. Here we present a combination of transcriptomics and machine learning approaches to identify new T3E candidates of A. citrulli. We also show the adaptation of a translocation assay for validation of T3E candidates.

#### **Genomic Features of Bacterial Adaptation to Plants**

#### A. Levy

Dept. of Plant Pathology and Microbiology, The Robert H. Smith Faculty of Agriculture, Food and Environment, The Hebrew University of Jerusalem, POB 12, Rehovot 7610001, Israel [e-mail: alevy@mail.huji.ac.il] Plants intimately associate with diverse bacteria. Plantassociated bacteria have ostensibly evolved genes that enable them to adapt to plant environments. However, the identities of such genes are mostly unknown, and their functions are poorly characterized. We sequenced 484 genomes of bacterial isolates from roots of Brassicaceae, poplar and maize. We then compared 3837 bacterial genomes from different environments to identify thousands of plant-associated gene clusters. Genomes of plant-associated bacteria encode more carbohydrate metabolism functions and fewer mobile elements than do related non-plant-associated genomes. We experimentally validated a novel family of type VI secretion system effectors with potent antibacterial activity that allow Acidovorax citrulli to compete against plant-associated bacteria, commensal and pathogens. We also identified a novel bacterial secretion system that is likely directed against insects, enriched in the plant environment and horizontally transferred between plant-associated bacteria. Finally, we identified 64 plant-associated protein domains that potentially mimic plant domains; some are shared with plant-associated fungi and oomycetes. This work expands the genome-based understanding of plant-microbe interactions and provides potential leads for efficient and sustainable agriculture through microbiome engineering.

#### The role of *Penicillium expansum* carbon catabolite repressor CreA in regulation of fungal attack and host defense pathways in apples

J. Tannous<sup>1</sup>, D. Kumar<sup>2</sup>, N. Sela<sup>3</sup>, E. Sionov<sup>4</sup>, N.P. Keller<sup>1</sup> and D. Prusky<sup>2</sup>\*

<sup>1</sup>Dept. of Medical Microbiology and Immunology, University of Wisconsin – Madison; <sup>2</sup>Depts. of Postharvest Science of Fresh Produce [\*dovprusk@agri.gov.il]; <sup>3</sup>Plant Pathology and Weed Research; and <sup>4</sup>Food Storage, Agricultural Research Organization—the Volcani Center, Rishon LeZion 7505101, Israel

Penicillium expansum is a major concern during storage of apples, resulting in yield and quality losses because of the production of the mycotoxin patulin. Despite the characterization of the patulin biosynthetic gene cluster at both the molecular and chemical levels, the underlying regulation of patulin biosynthesis in P. expansum and the mechanisms of apple colonization remain largely obscure. Recent work has indicated that sucrose, a carbon catabolite repressive metabolite, is a critical factor in the regulation of patulin synthesis. In the present study, CreA, the global carbon catabolite regulator, was assessed for its involvement in fungal virulence and patulin synthesis both in vitro and in vivo. We showed that  $\Delta creA$  strains were nearly avirulent and did not produce patulin in apples. On the basis of RNA-sequencing (RNAseq) analysis and physiological experimentation, it was determined that these mutants were unable to successfully colonize apples for a multitude of potential mechanisms. These include, on the pathogen side, a decreased ability to produce proteolytic enzymes and to acidify the environment and impaired carbon/ nitrogen metabolism and, on the host side, an increase in the oxidative defense pathways. Our study defines CreA as a key factor for the development of strategies for virulence of this post-harvest pathogen.

#### Developmentally-regulated oscillations in the expression of UV repair genes in a soil-borne plant pathogen dictate repair efficiency

S. Milo Cochavi<sup>1,\*</sup>, S. Adar<sup>2</sup> and S. Covo<sup>1</sup>

<sup>1</sup>Dept. of Microbiology and Plant Pathology, The Robert H. Smith Faculty of Agriculture, Food and Environment, The Hebrew University of Jerusalem, Rehovot 7610001, Israel [\*e-mail: shira.milocochav@mail.huji.ac.il]; <sup>2</sup>Faculty of Medicine, The Hebrew University in Jerusalem, Jerusalem, Israel

Surprisingly, the soil-borne fungus Fusarium oxysporum sp. lycopersici is relatively UV-C resistant. We hypothesized that UV repair capacity is induced to meet irregular sun exposure. Unlike the SOS paradigm, our analysis revealed scarcely any increase and even a decrease in the expression of UV repair genes following UV-C irradiation. Strikingly, the major factor that controls the expression of UV repair genes is the developmental status of the fungus. Just after inoculation, expression of photolyase dramatically increases, while the expression of UV endonuclease decreases. During the switch from spores to germlings the trend is reversed. These changes are coupled to development as exposure to hydroxyurea disrupts the periodic expression. The contribution of photoreactivation to DNA repair capacity is much more significant at eight hours compared to 14 h post inoculation, in agreement with the expression pattern. The repair capacity is directly correlated to the vulnerability of the fungus to UV damage. We propose that the expression pattern reported here maximizes repair efficiency when the risk is high, while keeping the cost of gene expression as low as possible. We note that this solution to environmental UV could be found in other soil-borne microbes.

#### **Biological control**

### Biological control of plant pathogens: from soil, bacteria to bio-pesticide

E. Cytryn<sup>1</sup>\*, Y. Isack<sup>1</sup>, M. Moshe<sup>1,2,3</sup>, S. Croitoru<sup>1,2</sup>, D. Azoulay<sup>1,3,6</sup>, A. Tabib<sup>1,3,6</sup>, A. Gamliel<sup>4</sup>, M. Benichis<sup>4</sup>, R. Berger<sup>5</sup>, R. Afani<sup>5</sup>, S. Carmeli<sup>5</sup>, O. Frenkel<sup>3</sup> and D. Minz<sup>1</sup>

<sup>1</sup>Institute of Soil, Water and Environmental Sciences, Agricultural Research Organization—the Volcani Center; <sup>2</sup>The Faculty of Life Science, Bar Ilan University, Israel; <sup>3</sup>Dept. of Plant Pathology and Weed Research, Agricultural Research Organization—the Volcani Center; <sup>4</sup>Institute of Agricultural Engineering, Agricultural Research Organization—the Volcani Center, Rishon LeZion 7505101, Israel; <sup>5</sup>School of Chemistry, Tel Aviv University, Tel Aviv, Israel; <sup>6</sup>The Robert H. Smith Faculty of Agriculture, Food and Environment, The Hebrew University of Jerusalem, Rehovot 7610001, Israel

In recent years, there is increasing awareness regarding the potential detrimental environmental and public health effects of pesticides. Biological control applies microorganisms to inhibit plant pathogens by direct antagonism through antimicrobial activity, ecological exclusion or through stimulation of plant resistance. Although this approach is a safer and more environmentally sustainable method of plant protection, the efficacy of applying these microorganisms is often very low, and therefore, it currently does not provide a comprehensive alternative to chemical pesticides. Within the framework of this project, we are applying an interdisciplinary approach aimed at developing new and effective biocontrol agents that antagonize soil-borne fungal pathogens. The novelty of this project is that it integrates state-of-the-art chemical analyses with whole genome sequencing. Specifically, active metabolites from selected biocontrol agents that antagonize soilborne pathogens are isolated, purified and characterized. Concomitantly, the expression of secondary metabolite-encoding genes are monitored to determine optimal conditions for production of antagonistic metabolites by selected bacteria. Our efforts specifically focus on Rhizoctonia solani (Basidiomycetes), Pythium aphanidermatum (Oomycetes) and Fusarium oxysporum (Ascomycetes). To date, approximately 500 bacteria were isolated from various soil niches. We conducted in vitro assays against the three pathogens, and selected approximately 100 antagonistic bacteria for in planta tests. In tandem, we extracted, purified and chemically characterized metabolites from five bacterial isolates that showed especially high antagonistic activity towards the above phytopathogens. To date, this project has led to significant insight on the ecology and potential application of soilborne bacteria that can be applied as biological control agents.

# Using insect symbionts to control phloem-limited bacterial pathogens

#### D. Hamershlak<sup>1,2</sup>, O. Dror<sup>1</sup> and O. Bahar<sup>1,\*</sup>

<sup>1</sup>Dept. of Plant Pathology and Weed Research, Agricultural Research Organization—the Volcani Center, Rishon LeZion 7505101, Israel [\*e-mail: ofirb@agri.gov.il]; <sup>2</sup>Dept. of Plant Pathology and Microbiology, The Robert H. Smith Faculty of Agriculture, Food and Environment, the Hebrew University of Jerusalem, Rehovot 7610001, Israel

Phloem-restricted bacterial pathogens pose a major threat to many agricultural crops. These harmful bacteria are obligate plant pathogens, vectored by phloem-feeding insects. Application of chemical sprays to reduce vector populations have been largely inefficient at mitigating disease spread and reducing disease incidence. Additionally, the intracellular lifestyle of these pathogens provides enhanced protection from bactericide applications. The use of endophytes has therefore been suggested as a potential approach for disease control. We hypothesized that insect vectors of plant pathogens may harbor microbes that affect the disease agent. The current study focuses on a bacterium, Frateuria defendens, isolated from the planthopper Hyalesthes obsoletus, the insect vector of yellows disease of grapevines, caused by Candidatus phytoplasma. F. defendens was recently shown to significantly reduce grapevine yellows symptoms. Fluorescent labelling of F. defendens further revealed that it colonizes the plant's vascular tissues. The effectiveness of F. defendens to reduce carrot yellows symptoms, caused by the phloem-limited bacterium Ca. Liberibacter solanacearum, was also tested. F. defendens colonized carrot plants and systemically moved throughout the plant. In greenhouse and outdoor experiments, F. defendens application resulted in significant reduction in disease symptoms. However, when F. defendens was applied in a commercial carrot field the penetration rate was low and symptoms were not reduced. Taken together, our results demonstrate that *F. defendens* can serve as an effective biocontrol agent against phytoplasma and liberibacter. Nevertheless, additional work is needed to improve formulation and application practices to insure maximal effectiveness of *F. defendens* in field conditions.

#### Substance secreted by *Frateuria defendens* as a potential solution against yellows diseases

A. Naama<sup>1</sup>, S. Cohen<sup>1</sup>, V. Naor<sup>2</sup>, O. Dror<sup>3</sup>, O. Bahar<sup>3</sup>, E. Zchori-Fein<sup>4</sup> and L. Iasur-Kruh<sup>1,\*</sup>

<sup>1</sup>ORT Braude College of Engineering, Karmiel, Israel 2161002 [\*email: iasur@braude.ac.il]; <sup>2</sup>Shamir Research Institute, Katzrin 1290000, Israel; <sup>3</sup>Dept. of Plant Pathology and Weed Research, Agricultural Research Organization—the Volcani Center; Rishon LeZion 7505101, Israel; <sup>4</sup>Dept. of Entomology, Agricultural Research Organization—Newe Ya'ar Center; Ramat Yishay 3657800, Israel

Candidatus Liberibacter solanacearum (Lso) and Candidatus Phytoplasma, the causative agents of yellows diseases, inhabit the phloem of plants or the inner tissues of their insect vectors. These bacteria can cause substantial damage to several hundred plant species, including perennials like grapevines and citrus, and annual plants like periwinkle, sesame and carrots. In a previous study, we isolated Frateuria defendens, an insect vector endophyte that reduces the symptoms of grapevine yellows caused by phytoplasma. One of the modes of action suggested was that F. defendens secrets metabolites that act as antibiotics and inhibit the growth of phytoplasma and Lso in periwinkle and carrot, respectively. To test this possibility, the effect of F. defendens supernatant on periwinkle infected with phytoplasma was examined, and the substances secreted by the bacterium were identified by HPLC and GC-MS. Synthetic compounds, analogues to those identified by the biochemical analysis, were used to determine their influence on the titer of phytoplasma in periwinkle and Lso in carrot by qPCR. It was found that crude extracts of metabolites secreted by F. defendens reduced the symptoms of yellows diseases in periwinkle. Specific compounds, identified by the biochemical analysis, caused a significant reduction in the titer of phytoplasma in periwinkle and Lso in carrots in comparison to untreated infected plants. Further research is required to examine the potential of these compounds as a treatment against yellows disease, as well as other phloemrestricted phytopathogens.

#### Field application of the bacterium *Frateuria defendens* to reduce yellows disease symptoms in wine grapes

V.  $\mathsf{Naor}^{1,2,*},$  L. Iasur-Kruh², T. Zahavi³, Y. Kapulnik⁴ and E. Zchori-Fein⁴

<sup>1</sup>Golan Research Institute, POB 97, Katsrin 12900, Israel, [\*email: vered.spielmann@gmail.com]; <sup>2</sup>Ort Brude, Carmiel 2161002, Israel; <sup>3</sup>Extension Service, Ministry of Agriculture and Rural Development, Qiryat Shemona 10200, Israel; <sup>4</sup>Dept. of Entomology, Agricultural Research Organization—Newe Ya'ar Center, Ramat Yishay 3657800, Israel In Israel, yellows disease in grapevines is caused by Candidatus Phytoplasma of the stolbur-group resulting in a severe decrease in vine biomass and yield. It is transmitted by a polyphagous planthopper via feeding. Currently, there is no control measure against the disease. The bacterium Frateuria defendens (Fd), isolated from the insect vector, was successfully introduced into ex vitro plantlet and field grown vines resulting in a reduction of yellows disease symptoms under laboratory conditions. A field trial was conducted to examine the effect of Fd foliar spray to reduce yellows disease symptoms under field conditions. The experiment was carried on for two consecutive years in a Chardonnay plot in northern Israel, with ca. 35% initial rate of yellows disease infection. Vines were sprayed with 10<sup>8</sup> cfu/ml suspension at two week intervals from bud burst to leaf senescence, whereas control plots were not sprayed or water sprayed. Leaf samples were collected 7 days post each spray and the presence of Fd was confirmed by plating and by PCR analyses. Disease and yield parameters were evaluated at harvest. High penetration rates were observed during vigorous growth phases while no penetration was observed during leaf senescence. Fd spraying increased the spontaneous recovery by 60% but did not prevent new infections. In the second year, the infection rate in treated plots increased by 4.8% compared to 12% in the control. Spraying with Fd improved the yield of symptomatic vines by 18%-20% but did not affect yield quality. Ceasing spray of Fd four weeks prior to harvest avoided the presence of Fd in the must. In summary: spraying with Fd reduced the rate of disease distribution and improved the yield of symptomatic vines.

#### Development of a new biological control product based on soil borne bacterial consortia against plant parasitic nematodes

P. Bucki<sup>1</sup>, A. Gamliel<sup>2</sup>, D. Minz<sup>3</sup>, B.Chippandi<sup>4</sup>, G. Inbar<sup>1</sup> and S. Braun<sup>1,\*</sup>

<sup>1</sup>Dept. of Entomology, Nematology and Chemistry Units [\*e-mail: sigalhor@volcani.agri.gov.il]; <sup>2</sup>Laboratory for Pest Management Research, Institute of Agricultural Engineering; and <sup>3</sup>Dept. of Soil Chemistry, Plant Nutrition and Microbiology, Agricultural Research Organization—the Volcani Center, Rishon LeZion 7505101, Israel

Safety and environmental concerns related to the toxicity of nematocides along with a lack of natural resistance sources threaten many crops. This emphasizes the need to identify new alternatives to control devastating plant parasitic nematodes. We established a comprehensive screening system aimed to identify new bacterial agents possessing bionematicidial activity. Four screening methods were used to study bacteria bionematicidal activity against the Root Knot Nematode M. javanica: (i) in vitro activity in ELISA plates; (ii) in terra activity in small containers containing Hamra soil, nematodes and bacteria; (iii) on young tomato seedlings for short term experiments; and (iv) long term experiments with tomato plants in pots. So far, in vitro screening resulted in the selection of 40 potential bacteria possessing bionematicidal activity against second stage juveniles and eggs. In terra experiments indicate that, of the 40 studied bacteria, 13 have demonstrated bionematicidal activity against second stage juveniles within soil. Of these 13 potential bacteria, nine reduced gall appearance and egg production on tomato seedling roots. Seven bacterial combinations (consortia) were generated: CKD1, CKD2, CKD3, CKD4, CKP1, CKP2 and CKG and tested for their activity to reduce Root Knot Nematode disease occurrence on tomato plants in pot experiments. Of these consortia, CKD1, CKP1 and CKG consistently demonstrate activity in reducing galling and egg production. These potential consortia are being further developed to generate a formulation adjusted to field application.

#### Virology

#### The function of the ToBRFV movement protein in the breakdown of $Tm-2^2$ resistance in tomato

#### H. Hak and Z. Spiegelman\*

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

Viruses of the Tobamovirus genus, including Tobacco mosaic virus (TMV), can cause extensive damage to crops from the Solanaceae family. Resistance against tobamoviruses in tomato was developed over 50 years ago by the introgression of the  $Tm-2^2$ resistance gene from a related wild species. In recent years, a new  $Tm-2^2$  resistance-breaking tobamovirus emerged in Israel. This virus, Tomato brown rugose fruit virus (ToBRFV), severely limits Israeli tomato production. Successful viral infection depends on a viral-encoded movement protein, which enables the virus to move from cell to cell. To trigger resistance, the  $\text{Tm}-2^2$  protein binds to the movement protein. This interaction activates a signaling cascade that leads to the death of the infected cell in a process termed the hypersensitive response. Specific mutations in the movement protein can interfere with its recognition by Tm-2<sup>2</sup>, resulting in the breakdown of this resistance. In this study, we characterized the function of the ToBRFV movement protein in the breakdown of  $Tm-2^2$  resistance. Our results suggest that lack of movement protein recognition by  $\text{Tm}-2^2$  is the cause for resistance breakdown by ToBRFV.

# Virus-infected watermelon fruit are an important source of information on viruses transmitted by whiteflies

N. Luria<sup>1,\*</sup>, E. Smith<sup>1</sup>, O. Lachman<sup>1</sup>, N. Sela<sup>1</sup>, A. Koren<sup>2</sup> and A. Dombrovsky<sup>1</sup>

<sup>1</sup>Dept. of Plant Pathology and Weed Research, Agricultural Research Organization—the Volcani Center, Rishon LeZion 7505101, Israel [\*email: neta@volcani.agri.gov.il]; <sup>2</sup>Hishtil Nursery, Moshav Nahalim, Israel

Watermelon (*Citrullus lanatus Thunb.*) is one of the most important crops in Israeli agriculture. The crop is grown for most of the year in different regions in Israel in protected greenhouses or in the open field. Planting in the open field in the Dead Sea area is from the middle to the end of December, and harvest is in April and May. Generally, in this area in March increasing temperatures contribute to the proliferation and movement of vector insects to spread plant viruses. Different viruses infecting watermelon were previously known in Israel, e.g., Squash leaf curl virus, Watermelon chlorotic stunt virus and Squash vein yellowing virus, all of which cause a range of disease symptoms on watermelon fruit and leaves. In recent years, there has been an increase in symptomatic watermelon fruit being sent to market, and these fruits show more serious disease symptoms than in the past. Severe disease symptoms may be due to infection by multiple viruses. An advanced Next Generation Sequencing method was used to sequence the virus population (the virome) causing fruit symptoms in watermelon. For two growing seasons, watermelon fruit displaying serious disease symptoms were sampled from areas near the Dead Sea (Kalia and Mitzpe Shalem). Following the advanced sequencing of the samples from the above two sites, bioinformatic procedures were used to assemble full viral genomes from genome fragments of a number of viruses of different families causing the watermelon diseases. The watermelon virome in Israel comprised the following: the Criniviruses Cucurbit yellow stunting disorder virus and Cucurbit chlorotic yellows virus-the first identification of this virus in Israel; the Begomovirus Squash leaf curl virus; and the Ipomoviruses Cucumber vein yellowing virus and Squash vein yellowing virus.

#### **Pest Control Interface**

Biological and chemical applications during flowering in mango controlled *Botryosphaeria* penetration, which increase fruit count, yield and reduce postharvest fruit decay

O. Feygenberg, S. Diskin, D. Maurer and N. Alkan\*

Dept. of Postharvest Science of Fresh Produce, Agricultural Research Organization—the Volcani Center, Rishon LeZion 7505101, Israel [\*e-mail: noamal@volcani.agri.gov.il]

Pathogenic fungi such as *Botryosphaeria*, that endophytically colonize the mango tree stem without causing any visible symptoms, become active during fruit ripening or abiotic stress, and cause fruit stem-end rot and stem and inflorescence dieback. Preliminary results show that most of those pathogenic fungi penetrate during flowering. In this work, we show that biological treatments (Serenade [Bacillus subtilis]) or chemical treatments (Luna Tranquility [fluopyram and pyrimethanil]) or Switch [fludioxonil and cyprodinil]) during flowering significantly reduce the occurrence of pathogenic fungi within the microbial community of the fruit's stem-end. As mango orchards are commercially sprayed four times against powdery mildew during flowering, we combined two treatments against powdery mildew with two treatments against both powdery mildew and fruit stem-end rot-causing pathogens. This application of biological or chemical fungicides during flowering significantly reduced inflorescence/stem dieback and fruit drop, and significantly increased the number of fruit per tree, which led to significant increase in yield, up to 100% in heavily infected orchards. In addition, this application during flowering (March-April) affected postharvest fruit quality (August-September) by a significant reduction of incidence and severity of stem-end rots and even side decay after long storage. Thus, changing the anti-powdery mildew fungicide regime to control both Botryosphaeria and powdery mildew during mango orchard flowering, led to reduced inflorescence/stem dieback, reduced fruit drop and significant yield increase, while also reducing postharvest decay and fruit loss.

### Fungicide resistance in *Erysiphe necator* the causal agent of grape powdery mildew

L. Gur<sup>1,2,4,\*</sup>, M. Reuveni<sup>1</sup>, Y. Cohen<sup>2</sup>, S. Ovadia<sup>3</sup> and O. Frenkel<sup>4</sup>

<sup>1</sup>Shamir Research Institute, University of Haifa, P.O. Box 97 Katzrin 1290000, Israel [\*e-mail: liogur@gmail.com]; <sup>2</sup>Faculty of Life Sciences, Bar-Ilan University, Ramat Gan 5290000, Israel; <sup>3</sup>Carmel Winery, Israel; <sup>4</sup>Dept. of Plant Pathology and Weed Research, Agricultural Research Organization—the Volcani Center, Rishon LeZion 7505101, Israel

Powdery mildew caused by Erysiphe necator is one of the most destructive diseases of grapes in Israel and worldwide. The fungus attacks the green parts of the vine and causes great damage to yield and wine quality. Since 2004, low efficacy in disease control was observed after foliar applications of demethylation inhibitors (DMI). Since 2007, low efficacy of strobilurin (QoI) fungicides and indications of reduced efficacy of the azanapthalene (AZN) fungicide quinoxyfen was observed. Resistance of E. nector to DMI, QoI and AZN fungicides is well known worldwide, but was never determined in Israel in the laboratory or genetically. Field experiments during 2017-18 seasons were conducted in the Judaean-foothills, Galilee and Golan regions. Disease incidence and severity were evaluated, and reduced efficacy of some fungicides was observed. Resistance to DMI, QoI and AZN fungicides by the newly collected isolates of E. necator was documented on leaf discs. LD50 rates of resistant isolates were 100-1000, 2-8 and > 100 µg/mL, respectively, compared to 0.5-0.9, 0.04-0.4 and 0.002-0.09 µg/mL, respectively, in non-sprayed wild type isolates. Resistant isolates possessed genetic mutations for DMI and QoI resistance, with no apparent cost of fitness to QoI resistance. The results of this study demonstrated that reduced efficacy in disease control in Israel vineyards in recent years is related to fungicide resistance. This work will be used to develop a protocol for an early and rapid phenotypic and genetic profiling of isolates, and application of an effective control management while reducing fungicide resistance risk.

#### Foliage sprays of calcium during cultivation to control postharvest gray mold in bell pepper

C.  $Ziv^{1,*}$ , E. Fallik<sup>1</sup>, S. Alkalai-Tuvia<sup>1</sup>, D. Chalupowicz<sup>1</sup>, F. Yudelevich<sup>1</sup>, T. Alon<sup>2</sup>, D. Silverman<sup>2</sup>, S. Omar<sup>2</sup>, Z. Gilad<sup>3</sup> and Z. Kleinman<sup>3</sup>

<sup>1</sup>Dept. of Postharvest Science of Fresh Produce, Agricultural Research Organization—the Volcani Center, Rishon LeZion 7505101, Israel [\*e-mail: carmit.ziv@agri.gov.il]; <sup>2</sup>Extension Service, Ministry of Agriculture and Rural Development, Bet Dagan 50,250, Israel; <sup>3</sup>Jordan Valley R&D 91906, Israel

Gray mold disease caused by the phytopathogenic fungus *Botrytis* cinerea is a major source of postharvest loss of bell pepper (*Capsicum annuum*) during long-term cold storage. While infection of fruit with fungal spores occurs in the field, the disease symptoms

may develop only after harvest. The application of fungicides to control the disease is not adequate due to limited efficiency and in accordance with public demand to reduce the use of pesticides. Previous work indicated that application of calcium by fertigation can improve plant resistance to foliar pathogens, which may also be beneficial to the fruit. However, calcium translocation from the leaves to the fruit is limited. To increase fruit calcium levels, we examined the application of calcium by foliage sprays during bell pepper cultivation. Several commercial calcium formulations were examined, and compared with common fungicide treatments. Specifically, fruit calcium level at harvest, postharvest susceptibility of the fruit to Botrytis and fruit quality after cold storage were tested. Foliage sprays of CaO chelated with Diethylenetriaminepentaacetic acid (DTPA) was found to significantly increase calcium levels in the pulp, increase fruit firmness after storage and reduce postharvest gray mold rot of the fruit. Although the DTPA-Calcium complex was found to inhibit B. cinerea hyphal growth in vitro, the observed effect on the fruit cannot be attributed to direct inhibition, since the calcium level of the fruit peel was not altered. The results obtained may pave the way for the development of an environmental-friendly, fungicide-free control of gray mold disease during postharvest storage of peppers.

# Significance of molecule structure in controlling *Sclerotium* rolfsii

#### O. Liarzi<sup>1,\*</sup>, A. Ewenson<sup>2</sup> and D. Ezra<sup>1</sup>

<sup>1</sup>Dept. of Plant Pathology and Weed Research, Agricultural Research Organization—the Volcani Center, Rishon LeZion 7505101, Israel [\*e-mail: ornal@volcani.agri.gov.il]; <sup>2</sup>Luxembourg Industries Ltd., 27 Hamered St., P.O Box 13, Tel Aviv 6100001, Israel

The endophytic fungus Daldinia cf. concentrica emits biologically active volatile organic compounds. One of the compounds, trans-2-octenal, presents antifungal activity against various phytopathogenic fungi. This molecule is a linear unsaturated aldehyde, with eight carbons, and a double bond located at the alpha position of the carbonyl group. To examine the significance of the molecule structure (the length of the carbon chain and the presence of the double bond), we compared the effect of molecules with similar structures on the growth and viability of the fungus Sclerotium rolfsii (also called Athelia rolfsii). Comparing the activity of alkene aldehydes with 5 to 10 units in their carbon chain, revealed that the optimal length of the carbon chain, which facilitates full growth inhibition and fungal death at the lowest concentration, was eight carbons. Comparing the activity of aldehydes, with the same length of their carbon chain but without the double bond, revealed that the presence of the double bond was essential for the antifungal activity of the compound. These results suggest that there is a structure-function relationship in the antifungal activity of the molecule. A possible model is that a hypothesized fungal receptor binds eight carbons alkene aldehyde with the highest affinity. This binding may activate a signal transduction pathway, which results in inhibition of fungal growth and fungal death. Further studies to find the hypothetical fungal receptor will enable the development of additional approaches for the control of pathogenic fungi.

#### Plant resistance

## Generating pathogen resistant plants by exploiting immunity priming mechanisms

O. Gershony<sup>1</sup>, M. Leibman-Markus<sup>1,2</sup>, L. Pizarro<sup>1,2</sup>, R. Gupta<sup>1</sup>, D. Rav-David<sup>1</sup>, G. Lebedev<sup>3</sup>, M. Ghanim<sup>3</sup>, Y. Elad<sup>1</sup>, A. Avni<sup>2</sup> and M. Bar<sup>1,\*</sup>

<sup>1</sup>Dept. of Plant Pathology and Weed Research, Agricultural Research Organization—the Volcani Center [\*e-mail: mayabar@volcani.agri.gov.il]; <sup>2</sup>School of Plant Sciences and Food Security, Tel-Aviv University, Ramat Aviv 6997801, Israel; <sup>3</sup>Dept. of Entomology and the Nematology and Chemistry units, Agricultural Research Organization—the Volcani Center, Rishon LeZion 7505101, Israel

Fungal pathogens are the cause of devastating diseases and significant tomato crop losses worldwide, due to their extremely wide host range and lack of sufficiently effective fungicidal strategies. While it has been known for some time that resistance traits exist in wild tomato populations, transferal of this resistance to cultivated tomato has thus far not been successful. A well-known concept in the world of plant immunity is that of induced systemic resistance following pathogen exposure. Interestingly, some biocontrol microorganisms are known to operate by priming induced systemic resistance in the plant, subsequently allowing for increased resistance upon pathogen encounter. We propose that tapping into plant immune priming to generate constitutively immunoactivated plants can result in increased pathogen resistance, though it may have an agricultural cost. Here we present two examples of constitutively activating plant immunity using genome editing, one by increasing the inherent response to a biocontrol microorganism and the other by constitutively activating a plant immunity signaling hub, both resulting in broad spectrum resistance to plant pathogens at minimal agricultural cost.

### Functional validation of disease resistance genes in melon by CRISPR-CAS9 mutagenesis

S. Nizan<sup>1\*</sup>, A. Bar-Ziv<sup>1</sup>, T. Dahan<sup>2</sup> and R. Perl-Treves<sup>1</sup>

<sup>1</sup>The Mina and Everard Goodman Faculty of Life Sciences, Bar Ilan University, Ramat Gan 5290002, Israel [\*e-mail: rafi.perl@gmail.com]; <sup>2</sup>Dept. of Plant and Environmental Sciences, Weizmann Institute of Science, Rehovot 7610001, Israel

Our laboratory has identified a few melon disease resistance genes by positional cloning. Among these, *Fom-1* and *Prv* reside in a head-to head orientation in a single locus, and control resistance, respectively to, *Fusarium* races 0 and 2, and *Papaya ring spot virus*. The two genes encode TIR-nucleotide binding-leucine rich repeat (NBL) proteins; such paired R genes have been shown recently to form functional units in which the two proteins physically interact. To confirm their function we used CRISP-Cas9 mutagenesis. We employed the Golden Braid cloning system to clone, for each target, two guide-RNAs, the Cas-9 nuclease, and the NptII marker on a single binary plasmid. Transgenic melons from the appropriate resistant genotypes were regenerated with a high frequency of bi-allelic mutations in the target gene. We observed deletions of the region between the two targets, and even beyond that area. To our best knowledge, this is a first report of CRISPR mutants in melons. Plants were fertile, and their progeny are now being tested for breaking the resistance phenotype, and also for a possible functional interaction between the two paired *R*genes in the *Prv-Fom-1* locus.

#### Immunity to PepMV in transgenic tomato

#### D. Leibman<sup>1,\*</sup>, D. Wolf<sup>2</sup>, M. Shtarkman<sup>1</sup> and A. Gal-On<sup>1</sup>

<sup>1</sup>Depts. of Plant Pathology and Weed Research and <sup>2</sup>Vegetable Research, Agricultural Research Organization—the Volcani Center, Rishon LeZion 7505101, Israel [\*e-mail: diana@agri.gov.il]

Pepino mosaic virus (PepMV) was detected for the first in Peru in 1974, and has since caused heavy yield losses of tomato crops in Europe and the US. The main damage is the uneven ripening of tomato fruit, which affects fruit quality. In the last year, the virus was observed in greenhouses in the Lachish region in Israel, with great homology to an Italian strain. There are no stable genetic resistance sources against this virus, so we have adopted a strategy of transgenic host plant resistance, as successfully performed in different crops against various viruses. Transgenic resistance against viruses is based in most cases on Post Transcriptional Gene Silencing, an endogenous mechanism that can destroy a virus genome. The present work was based on the use of this mechanism to develop resistance against PepMV in tomato. Tomato plants were transformed with a hairpin-silencing construct which expressed transgene-derived double-strand-RNA (tr-dsRNA). The construct contains three consecutive partial fragments of replicase genes from three different PepMV strains (Italian, Spanish and American) to provide broad virus resistance. The selected transgenic lines showed a high accumulation of tr-siRNA of 21-24 bases in length. The T2 transgenic lines showed complete immunity to mechanical inoculation of PepMV isolated in Israel.

# A new resistance gene *Pb2* against basil downy mildew *Peronospora belbahrii*

Y. Ben-Naim, O. Liberanz and Y. Cohen\*

Faculty of Life Sciences, Bar Ilan University, Ramat Gan 5290002, Israel [\*e-mail: yigal.cohen1@gmail.com]

Sweet basil (*Ocimum basilicum*) is susceptible to downy mildew caused by the oomycete foliar pathogen *Peronospora belbahrii*. After more than a decade only few tolerant commercial cultivars were developed. The first commercial resistant cultivar 'Prospera' carries the dominant *Pb1* resistance gene. This gene was derived from the wild basil *Ocimum americanum* var. *americanum* PI 500945. In the present study, the accession Op (*Ocimum americanum* var. *pilosum*) was compared with PI 500945 for resistance against a wide range of *P. belbahrii* isolates. It was found that Op and PI 500945 differ in their response to some isolates. An embryo-rescued population of 131 BCs1 plants derived from the interspecies cross between the resistant Op and the universally susceptible Sweet basil segregated 73:58 (1:1) resistant:susceptible, indicating that resistance is controlled by a

single, incompletely dominant gene. Knowing that resistance of Op and PI 500945 (*Pb1*) is isolate-dependent, an allelic test was performed to determine the allele relationships between these two accessions. We used *P. belbahrii* isolate M that overcome *Pb1*, and the avirulent isolate Knafo3 to inoculate both Op and PI 500945. All F1 plants derived from the cross between line BCs3F3(56) (derived from Op) and the line BCs6F3(12–4-6) (derived from PI 500945) showed overdominance complementation of resistance to both isolates. F2 plants segregated to inoculation by Knafo3 at a ratio 12: 3: 1 i.e., complete resistance, incomplete resistance and susceptible, respectively, but segregated to M isolate inoculation at a ratio 9: 3: 4. The results confirm that accession Op carries a new resistance gene (called *Pb2*) against downy mildew, which is not allelic with *Pb1*.

#### Development of resistance to *Tomato brown rugose fruit virus* in *Solanum lycopersicum*

M. Kravchik\*, D. Leibman, R. Kumari, Y. Shnaider and A. Gal-On

Dept. of Plant Pathology and Weed Research, Agricultural Research Organization—the Volcani Center, Rishon LeZion 7505101, Israel [\*e-mail: michael.kravchik@mail.huji.ac.il]

Due to the obligatory parasitic nature of viruses, their multiplication relies on interactions between viral and host susceptibility factors. Plant susceptibility genes facilitate infection and support virus replication. Tomato brown rugose fruit virus (TBRFV) encodes two replication proteins required for its replication, which interact with Solanum lycopersicum (tomato) susceptibility proteins SITOM1 and SITOM3. SITOM1 and SITOM3 form replication complexes together in association with intracellular membranes. We successfully disrupted the interaction between TBRFV replication and SITOM1 and SITOM3 proteins through targeted gene knockout by the CRISPR/Cas9 system. SITOM1 and SITOM3 mutant plant lines infected with TBRFV showed lower TBRFV accumulation 13 days post inoculation than in wild type (wt) plants by several orders of magnitude. Later, TBRFV accumulation remained significantly higher in wt plants compared to mutant plants. This shows that mutants were resistant, though time course analysis suggests higher resistance at early infection stages. As early as 21 days post inoculation the infected transgenic plants were more vigorous, stayed mostly symptomless and with no effect on total fruit weight and number in comparison to infected wt plants. When the mutant plants were infected with Tomato mosaic virus (ToMV), accumulation of ToMV was similar to wt plants. This suggests the involvement of different host factors during TBRFV and ToMV infections, despite the fact that the two viruses belong to the genus Tobamovirus. In other experiments, an additional host factor SITHH (SITOM1 homolog) was edited out to improve tomato resistance against TBRFV.

# The involvement of *Fusarium* species in late collapse of tomato crops

D. Rockenstin<sup>1,2,\*</sup>, O. Frenkel<sup>1</sup>, A. Dombrovsky<sup>1</sup>, J. Katan<sup>3</sup>, Y. Rekah<sup>3</sup>, S. Pivonia<sup>4</sup>, O. Guy<sup>4</sup>, Y. Key<sup>4</sup>, Y. Messika<sup>5</sup>, A. Koren<sup>6</sup>, S. Gunz<sup>7</sup>, N. Mor<sup>7</sup>, Y. Meller Harel<sup>8</sup>, O. Lachman<sup>1</sup> and L. Abraham<sup>7</sup>

<sup>1</sup>Dept. of Plant Pathology and Weed Research, Agricultural Research Organization—the Volcani Center, Rishon LeZion 7505101, Israel [\*e-mail: daniellerock26@gmail.com]; <sup>2</sup>Bar-Ilan University, Ramat-Gan 5290002, Israel; <sup>3</sup>The Hebrew University of Jerusalem, Faculty of Agriculture, Food and Environment, POB 12 Rehovot 7610001, Israel; <sup>4</sup>Ramat Negev Research and Development, Desert Agriculture Economic Development Co. Ramat HaNegev Lld., D.N Halutza 85515, Israel; <sup>5</sup>Shorashim Nurseries, Ein Habsor, Israel; <sup>6</sup>Hishtil Nurseries, Nehalim, Israel; <sup>7</sup>Extension Service, Ministry of Agriculture and Rural Development, Bet Dagan 50250, Israel; <sup>8</sup>Plant Protection and Inspection Services, Ministry of Agriculture and Rural Development, Bet Dagan 50250, Israel

The greenhouse tomato in southern Israel is a significant and profitable crop. In the last 3 years, an extensive late collapse phenomenon has become a major problem in this area. In most cases, this collapse is accompanied by reddish conidia on the collar region of the stem, which leads to the hypothesis that Fusarium solani might be involved. Since the phenomenon has expanded simultaneously with the distribution of the new Tomato brown rugose fruit virus throughout the country, and because F. solani is known in the literature mostly as a minor pathogen, it is suggested that the symptoms may be intensified due to the combination of the two pathogens. The objectives of the study were: (i) identifying the causal agents of the disease and developing a method for conducting inoculation tests; (ii) etiological study of the disease, focusing on the pathogen F. solani; and (iii) development of initial treatment methods to help reduce the damage caused by the disease. To assess which pathogens are involved, a survey in infected greenhouses in the various Negev regions was conducted. In Ramat HaNegev, the vast majority of samples were infected mostly with F. solani, whereas in the Besor region the phenomenon was caused by a wide range of pathogens. The F. solani EF1 a gene of the collected samples was sequenced, and clustered into a phylogenetic tree. This tree includes two main groups: one containing all the Ramat HaNegev and other isolates, while the other group includes samples from the western Negev. A method of infection was developed for F. solani, which simulates the disease development in the field.

#### Macrophomina phaseolina: Determining the genetic diversity in Israel and screening for resistance in strawberry (Fragaria×ananassa)

B. Pickel<sup>1, \*</sup>, N. Dai<sup>2</sup>, M. Maymon<sup>1</sup>, M. Elazar<sup>1</sup>, Z. Tanami<sup>2</sup>, O. Frenkel<sup>1</sup>, M. Abo Toamy<sup>3</sup>, N. Mor<sup>3</sup>, A. Sharon<sup>4</sup> and S. Freeman<sup>1</sup>

<sup>1</sup>Depts. of Plant Pathology and Weed Research [\*e-mail: barpickel@gmail.com] and <sup>2</sup>Vegetable and Field Crops, Agricultural Research Organization—the Volcani Center, Rishon LeZion 7505101; <sup>3</sup>Extension Service, Ministry of Agriculture and Rural Development, Bet Dagan 50250, Israel; <sup>4</sup>Dept. of Molecular Biology and Ecology of Plants, Tel Aviv University, Tel Aviv 6997801, Israel

*Macrophomina phaseolina* is a typical soil-borne pathogen causing crown and root rot, often referred to as charcoal rot, affecting more than 500 plant species including strawberry. The fungus has a wide geographical distribution worldwide, abundant especially

in tropical and subtropical regions. As an anamorphic fungus, M. phaseolina creates microsclerotia that serve as resting structures allowing the fungus to survive in the soil for extended periods. The disease is favored when plants are subjected to water stress and high temperature. In parallel to the methyl bromide phase-out, M. phaseolina has become the most significant soilborne pathogen of strawberry in Israel. To study the genetic diversity of the fungus, arbitrarily-primed PCR and SSR genotyping was performed on 198 isolates from strawberry and melon plants. Based on the high diversity found according to these methods, host specificity and pathogenicity tests were conducted with seven genetically diverse isolates, four from strawberry and three from melon. It was found that strawberry plants were significantly susceptible to isolates from strawberry compared to those from melon; in contrast, melon plants did not display specificity among the different pathogen isolates. Furthermore, isolates from strawberry showed differential pathogenicity towards strawberry. Screening for strawberry germplasm resistant to M. phaseolina has commenced, based on the assumption that resistant plant material has been detected in other botanical species and a high variance was found among 34 commercial cultivars.

#### POSTERS

### Aerial dissemination of *Lasiodiplodia theobromae* and *L. pseudotheobromae* pycnidiospores

#### R. Shulhani and D. Shtienberg\*

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

Since 2013 branch dieback in mature trees and mortality of young plants were observed throughout Israel in avocado plantations. Two fungi belonging to the family Botryosphaeriaceae, Lasiodiplodia theobromae (LT) and L. pseudotheobromae (LPT), were routinely isolated from symptomatic tissues. The fungi were identified according to their morphology and by molecular markers. To verify the involvement of LT and LPT in the development of the symptoms, Koch postulate tests were successfully completed. LT and LPT infect many fruit trees including avocado, causing pre- and post-harvest symptoms. The teleomorph stages of LT and LPT were not yet found in Israel and it was believed that these pathogens spread by pycnidiospores, splashed short distances by raindrops during rain events. This is the common dissemination mechanism of fungi of the Botryosphaeriaceae and Dioporthaceae classes, such as Septortia, Ascochyta, Diplodia and others. Observations made in commercial avocado plantations raised the hypothesis that LT and LPT pycnidiospores disperse aerially to long distances, even on dry, rainless days. The formation, dissemination and dispersal of LT pycnidiospores were examined in a series of controlled environment experiments. Artificially inoculated avocado stem-segments (10 cm long) bearing pycnidia were placed in high relative humidity conditions. Chirri containing abundant numbers of pycnidiospores was secreted and when dried-out, the pycnidiospores remained attached in long threads and easily disseminated aerially. Effects of relative humidity (level and duration) and temperature on the formation, and the effect of wind speed on the dispersal of the pycnidiospores were quantified in a wind tunnel. In addition, the diurnal dispersal of LT and LPT pycnidiospores in a commercial avocado plantation was monitored using Burkard spore traps. The results confirmed the hypothesis that LT and LPT pycnidiospores disperse aerially on dry, rainless days. This finding has significant consequences in understanding the pathogen's epidemiology and for the development of means for its suppression.

### Interactions between *Harpophora maydis* and *Macrophomina phaseolina*, the causes of wilt diseases, in cotton and maize

S. Dor<sup>1,2,\*</sup>, Y. Kanter<sup>2</sup>, D. Regev<sup>1,2</sup>, R. Cohen<sup>3</sup>, O. Rabinovitz<sup>1</sup> and O. Degani<sup>1,2</sup>

<sup>1</sup>Migal–Galilee Research Institute, Kiryat Shmona 11016, Israel [\*email: dorshlomit@gmail.com]; <sup>2</sup>Tel-Hai College, Upper Galilee, Tel-Hai 12210, Israel; <sup>3</sup>Dept. of Plant Pathology, Agricultural Research Organization—Newe Ya'ar, Ramat Yishai 30095, Israel

Soil diseases may be caused by a pathogen complex. The soilborne fungus Harpophora maydis severely affects sensitive maize hybrids by causing rapid wilting of plants at the mature stage. Similarly, the soil fungus, Macrophomina phaseolina, develops hyphae, conidia and conidiospores inside the vascular system in a variety of host plants, which leads to plant dehydration. The presence of both pathogens together in diseased cotton plants in Yavne in 2017 suggested possible interactions between these pathogens. These relationships were tested in a series of experiments accompanied by Real Time PCR tracking in maize and cotton. The molecular analysis revealed that, compared to inoculation with each of the pathogens alone, infection by both pathogens in 40-day-old sprouts caused higher amounts of H. maydis DNA in maize and increased amounts of M. phaseolina DNA in cotton. Similar results were obtained in a full season experiment in maize, but an opposite tendency resulted in cotton-a reduction in the amount of M. phaseolina DNA in the presence of H. maydis. The double infection caused an increase in the growth parameters (plant height, root and shoot weight) in maize and cotton, but the same levels of dehydration in maize plants were accompanied by a decrease in yield production (cob weight). In cotton, the double infection did not affect yield (seed quantity). These findings suggest that both pathogens are involved in wilt diseases in maize and cotton, and they encourage expanding the study to additional hosts and examining the involvement of other pathogens, such as Fusarium verticillioides.

# Apple powdery mildew in Israel: sources of inoculum and susceptible phenological stages

A. Halperin<sup>1,2</sup>\*, M. Reuveni<sup>1</sup>, L. Gur<sup>1</sup>, A. Farber<sup>3</sup> and Y. Cohen<sup>2</sup>

<sup>1</sup>Shamir Research Institute, University of Haifa, P.O.B 97 Katzrin 1290000, Israel [\*e-mail: halperinadi@gmail.com]; <sup>2</sup>The Mina & Everard Goodman Faculty of Life Sciences, Bar-Ilan University, Ramat Gan 5290000, Israel; <sup>3</sup>Kiryat Shmona, Israel

Powdery mildew, caused by the fungus *Podosphaera leucotricha*, is a major disease of apple in Israel and worldwide. It attacks young shoots, leaves, blossoms and fruits and reduces yield. Control of the disease in Israel is poor, in spite of the numerous

sprays applied during the season. The objective of this study was to develop an effective control management strategy against the disease. For this purpose, we searched for (i) the primary sources of inoculum; (ii) the susceptible phenologic stages of the tree; and (iii) the appropriate timing of spray applications. Two main sources of asexual inoculum were found in the orchards: flagshoots and suckers. Winter pruning of infected twigs in the orchard reduced the number of flag-shoots developed by 65%, and disease severity by 50%, compared with non-pruned trees. Disease incidence in field and greenhouse-grown trees with suckers was 81% and 70% higher, respectively, compared to trees without suckers. No chesmothecia (sexual fruit bodies) of the fungus were found in Galilee and Golan orchards. Eight percent of infected leaves were counted in trees in which spraying started at either the green tip or at 50% bloom or petal fall as against 91.5% in untreated trees. A significantly lower disease incidence, but not disease severity, was observed in trees sprayed at 7 day intervals as compared to trees sprayed at 10 day intervals. These results and further studies may serve for developing an effective disease management strategy against apple powdery mildew.

#### Chemical control of potato late blight incited by mefenoxamresistant isolates of *Phytophthora infestans*

Y. Cohen\*, A. E. Rubin and M. Galperin

Faculty of Life Sciences, Bar Ilan University [\*e-mail: yigal.cohen1@gmail.com]

Potato late blight is one of the most destructive diseases of potato. It attacks leaves, stems and tubers, and causes losses in yield quantity and quality. In Israel, most isolates of the pathogen are resistant to mefenoxam (MFX) and therefore its use for disease control is ineffective. In the present study, we compared the efficacy of 15 fungicide(s) in controlling late blight in potato cv. Nicola. One month-old plants were sprayed manually once with each of 15 products at 0.01, 0.02 and 0.1% ai. The plants were inoculated the following night with a mixed sporangial suspension of two MFX-resistant isolates of *P. infestans*. Symptoms of late bight appeared in control untreated plots at 6 dpi (days post inoculation). Disease progress curves and AUDPC (area under disease progress curve) are shown. The results show that Oxathiapiprolin and its mixtures were the most effective products in suppressing the disease.

#### Endophytes from wild plants of Israel, as source for new active natural metabolites for agricultural and medicinal use against pathogens and pests

N. Azar\*, D. Ezra, O. Liarzi and Y. Elad

Dept. of Plant Pathology and Weed Research, Agricultural Research Organization—the Volcani Center, Rishon LeZion 7505101, Israel [\*e-mail: neriazar14@gmail.com]

Endophytes are endosymbionts that live within a plant for at least part of its life cycle without causing apparent disease. Some endophytes secrete secondary metabolites that have biological activities against other microorganisms. These substances may serve as new metabolites against fungi, bacteria, insects, etc. This study focuses on isolating endophytes from Israeli wild plants, with an emphasis on endophytes that secrete biologically active secondary metabolites. The goal is to find new natural materials that will serve as a basis for the development of innovative applied technologies in both agriculture and medicine. Over the past year, we isolated 298 different endophytes (156 fungi and 142 bacteria) from 27 different wild plants. Isolation was performed from all parts of the plant-roots, stem/branches, leaves, flowers, fruits and seeds. Seventy-two endophytes exhibited biological activity against different fungi and bacteria. Biological activity is defined as inhibition of growth of fungi/bacteria compared to controls (the absence of the endophyte or the secreted substances). Ten of the active endophytes were selected for chloroform-based extraction of their growth medium. The extracts were tested for biological activity. The active substances secreted by the endophytes are being isolated and identified by methods of analytical chemistry (HPLC, LC/MS, GC/MS). In addition, active endophytes will be identified using molecular methods.

### Entomopathogenic fungi – broad range biopesticide and its potential in pest management in Israel

D. Ment<sup>1,\*</sup>, E. Yossef<sup>1</sup>, A. Protasov<sup>1</sup>, Z. Mendel<sup>1</sup>, L. Mondaca<sup>2</sup>, S. Ben Yehuda<sup>3</sup>, C. Sofer-Arad<sup>4</sup>, R. Sharon<sup>4</sup>, A. Boimel<sup>5</sup>, E. Levin<sup>5</sup>, A. Alouch<sup>6</sup>, G. Yaacobi<sup>6</sup>, H. Kokiçi<sup>7</sup> and E. de-Lillo<sup>7</sup>

<sup>1</sup>Dept. of Entomology and the Nematology and Chemistry Units, Agricultural Research Organization—the Volcani Center, Rishon LeZion 7505101, Israel [\*e-mail: danam@volcani.agri.gov.il]; <sup>2</sup>Sapir Academic College, Israel;, <sup>3</sup>Extension Service, Israel; <sup>4</sup>Northern Agriculture R&D, Israel; <sup>5</sup>Moshavot Hashomron, Israel; <sup>6</sup>BioBee Sde Eliyahu, Israel; <sup>7</sup>University of Bari Aldo Moro, Italy

Entomopathogenic fungi (EPF), mainly from the genus Metarhizium and Beauveria, are the active ingredient of microbial biopesticides against arthropods. These EPF are ubiquitous in soil, within plants and are safe for vertebrates. The host range of EPF is typically wide and about 200 different susceptible hosts are documented worldwide. EPF exhibit a high diversity of specific characteristics such as degree of virulence and tolerance to high temperatures. Wise application of EPF-based commercial products may reduce, or substitute for, the use of synthetic chemical pesticides. EPF commercial products are mainly manufactured and sold as emulsions and wettable powders. These formulations are not optimally protective against abiotic stresses which characterize various habitats in Israel, such as high temperatures and high UV irradiation. Therefore, research on the application and efficacy of EPF commercial products in Israel should be studied along with research for novel formulation to enable use in the local habitats. We examined EPF commercial products and laboratory formulations for the management of four species of insect pests, all of which lack effective and significant biological control means, and thus management practices are based on synthetic pesticides. The field trials were: (i) Banana aphid in banana plantations; (ii) Pineapple mealybug in a commercial pineapple net house, and in laboratory situations mimicking field conditions; (iii) Capnodis larvae in stone-fruit rootstocks; and (iv) False codling moth eggs on citrus fruits. Each of the case studies requires a different concept for application. We will present the potential of EPF products for pest management in Israel and discuss the challenges and the research required for expanding and increasing the application of EPF in Israel.

### The biocontrol potential of the bacterial predators *Bdellovibrio* and like organisms on potato bacterial soft rot disease

#### D. Youdkes\*, Y. Helman, S. Burdman and E. Jurkevitch

Dept. of Plant Pathology and Microbiology, the Robert H. Smith Faculty of Agriculture, Food and Environment, the Hebrew University of Jerusalem, Rehovot 7610001, Israel [\*email: daniel.youdkes@mail.huji.ac.il]

Pectobacterium spp. and Dickeya spp., are genera of gram-negative pectinolytic bacteria, and are the main agents for bacterial black leg and soft rot disease. Bacterial soft rot affects a wide range of fruits, vegetables, and ornamentals in a variety of climates, though most notably in potato-the fifth most economically important crop worldwide. A potential remedy to combat Pectobacterium spp. and Dickeya spp. may be found in the use of the bacterial predators Bdellovibrio and like organisms (BALOs). In this study we examined the *in situ* effectiveness of using these bacterial predators to reduce soft rot caused by Pectobacterium carotovorum subsp. brasilense on potato slices. We found that the BALO strains tested in situ were very effective at reducing disease in a concentrationwise manner, and the application of BALOs before infection with P. carotovorum subsp. brasilense performed significantly better than applying BALOs after, and, in the majority (82%) of cases, entirely suppressed disease development. In cases where disease was present, this was reduced by 95%.

#### The tomato NRC4a NB-LRR receptor activates plant immunity, generating broad spectrum pathogen resistance

M. Leibman-Markus<sup>1,\*</sup>, L. Pizarro<sup>1,2</sup>, O. Gershony<sup>1</sup>, R. Gupta<sup>1</sup>, I. Marash<sup>1</sup>, G. Lebedev<sup>3</sup>, M. Ghanim<sup>3</sup>, A. Avni<sup>2</sup> and M. Bar<sup>1</sup>

<sup>1</sup>Dept. of Plant Pathology and Weed Research, Agricultural Research Organization—the Volcani Center [\*e-mail: meiravleibman@gmail.com]; <sup>2</sup>School of Plant Sciences and Food Security, Tel-Aviv University, Ramat Aviv, 6997801, Israel; <sup>3</sup>Dept. of Entomology and the Nematology and Chemistry units, Agricultural Research Organization—Volcani Center, Bet Dagan 7505101, Israel

Pathogens are the cause of devastating diseases and significant tomato crop losses worldwide. While it has been known for some time that resistance traits exist in wild tomato populations, transfer of this resistance to cultivated tomato has thus far not been successful. A well-known concept in the world of plant immunity is that of induced systemic resistance following pathogen exposure. Previously we identified SINRC4a as a hub for immunity signaling through various pattern recognition receptors (PRRs). Coimmunoprecipitation demonstrates that the tomato gene SINRC4a is able to associate with different PRRs. Physiological assays with specific elicitors revealed that SINRC4a alters PRR-mediated responses. SINRC4a overexpression enhances defense responses while silencing SINRC4 reduces plant immunity. We found that tomato plants possessing elevated NRC4a expression to be resistant to a variety of pathogens, demonstrating that constitutively activating a plant immunity signaling hub results in broad spec-

### Barley Net-Blotch: screening for resistance in *Hordeum* vulgare germplasm and genetic mapping of resistance alleles

trum resistance to plant pathogens at minimal agricultural cost.

M. Ronen<sup>1,\*</sup>, A. Harel<sup>1</sup>, H. Sela<sup>2</sup>, E. Fridman<sup>1</sup> A. Moreau<sup>1</sup> and R. Ben-David<sup>1</sup>

<sup>1</sup>Dept. of Vegetable and Field Crops, Agricultural Research Organization—The Volcani Center, Rishon LeZion 7505101, Israel [\*e-mail: moshe744@gmail.com]; <sup>2</sup>Institute for Cereal Crop Improvement, Tel Aviv University, Tel Aviv 6997801, Israel

Barely (Hordeum vulgare L.) is the 4th most important cereal crop with worldwide distribution, yielding 140 million tonnes annually. Net Blotch (NB) is a major disease of barley caused by the necrotrophic fungus Pyrenophora teres f. sp. teres propagated mainly on leaf tissue and severely effecting yield. The wild progenitor of barley (H. vulgare ssp. spontaneum) prevails in natural habitats of Israel, and plays a key part in NB epidemiology at the center of origin of this crop. Here we report on: (i) field characterization of NB resistance in a cultivated barley collection over two consecutive seasons; and (ii) genetic mapping of NB quantitative resistant loci (QRLs) using three segregating families from the barley HEB-NAM population. The HEB-NAM families were characterized in the field as both seedlings and adult plants. We identified high variability in tolerance to the disease among cultivated barley varieties, a few of which demonstrated high resistance suitable for future breeding. QRL tassel-analysis of the reaction in the adult plant stage in the field (reaction type; and average ordinate) revealed a total of three significant QRLs with a LOD score range of 4.3–6.3 (located on chromosomes 4H, 5H and 6H). These QRLs contained at least one novel loci that was not previously reported as associated with NB resistance. In two identified QRLs, resistance was derived from the wild progenitor (previously reported as sensitive compared to cultivated barley).

# Modulation of pathogen triggered immunity by endomembrane trafficking: the tomato RLP LeEIX2 as a case study

L. Pizarro $^{1,2,\ast},$  M. Leibman-Markus $^2,$  S. Schuster $^1,$  M. Bar $^2$  and A. Avni $^1$ 

<sup>1</sup>School of Plant Sciences and Food Security, Tel-Aviv University, Ramat Aviv, 6997801, Israel; <sup>2</sup>Dept. of Plant Pathology and Weed Research, Agricultural Research Organization—the Volcani Center, Rishon LeZion 7505101, Israel [\*email: lorepizaro@gmail.com]

Plants encounter numerous potential pathogens and are able to respond through a set of defense mechanisms based upon the ability of every cell to initiate immune responses. Initiation of such defense responses relies on recognition of pathogen associated molecular patterns (PAMPs) by pattern recognition receptors (PRRs) at the plasma membrane. PRRs such as receptor-like kinases (RLK) and receptor-like proteins (RLP) interact with PAMPs, triggering a signal cascade and activating immune responses against pathogen

propagation, known as pattern triggered immunity (PTI). The tomato RLP LeEIX2 recognizes and binds to the endoxylanase EIX from Trichoderma spp. After EIX recognition, LeEIX2 triggers immune responses characterized by an oxidative burst, ethylene production and hypersensitive response. Endomembrane trafficking has an important role in PTI, ensuring PRR plasma membrane steady state localization and their internalization after PAMP recognition. Through co-immunoprecitation and split-ubiquitin Y2H, we identified a dynamin-related protein and a prenylated acceptor of Rab 1, called hereinafter SIDRP2A and SIPRA1A, as involved in the regulation of PTI mediated by LeEIX2 in tomato. Overexpression of SIDRP2A induces LeEIX2 endosomal residence and enhances EIX-mediated immune responses, indicating the importance of LeEIX2 internalization for the elicitation of EIX response, as we have previously shown. On the other hand, we show that SIPRA1A over-expression strongly induces LeEIX2 degradation in a specific and vacuolar dependent process, reducing the EIX response. Thus, we underscore the relevance of deciphering PRR trafficking-regulation to better understand plant immunity.

# Random peptide mixtures reduce symptoms of plant diseases caused by *Xanthomonas* with no toxic effect on honeybee survival

S. Topman<sup>1,2,\*</sup>, H. Tamir<sup>2</sup>, D. Tamir-Ariel<sup>1</sup>, S. Shafir<sup>3</sup>, Z. Hayouka<sup>2</sup> and S. Burdman<sup>1</sup>

<sup>1</sup>Dept. of Plant Pathology and Microbiology [\*e-mail: shiri.topman@mail.huji.ac.il]; <sup>2</sup>Inst. of Biochemistry, Food Science and Nutrition; and <sup>3</sup>Dept. of Entomology, Faculty of Agriculture, Food and Environment, The Hebrew University of Jerusalem, Rehovot Campus, 7610001, Israel Plant pathogenic bacteria are among the most important causal agents of plant diseases with almost all major crops being severely affected by one or more important bacterial diseases. The chemical control of bacterial plant diseases in the field vastly relies on copper-based bactericides, yet with limited efficacy. Moreover, the occurrence of copper-resistant strains has been reported. Antimicrobial peptides have been studied as potential crop protection agents. In the present study, we explored the potential of two random peptide mixture (RPM) models as novel crop protection agents. These unique peptide mixtures consist of random combination of L-phenylalanine and L/D-lysine (FK and FdK, respectively) along a 20-mer peptides chain length. Both RPMs displayed powerful bacteriostatic and bactericidal activities towards strains of several plant pathogenic bacterial genera including Xanthomonas, Clavibacter and Pseudomonas. In planta studies in the greenhouse revealed that RPMs significantly reduced disease severity of tomato and kohlrabi plants infected with Xanthomonas perforans and Xanthomonas campestris pv. campestris, respectively. Moreover, RPM effects on reduction of disease severity were similar to those exerted by the commercial, copper-based bactericide Kocide 2000, which was applied at a 12-fold concentration of the active compound relative to the RPM treatments. Importantly, the two tested RPM compounds had no toxic effect on survival of bees and Caco-2 mammalian cells. The present study demonstrates the potential of these innovative RPMs to serve as crop protection agents against crop diseases caused by Xanthomonas species and other phytopathogenic bacteria.