

# INTERNATIONAL WORKSHOP

## Plant Health: Challenges and Solutions



**23-28**

**April, 2017**

Wind of Lara Hotel

**Antalya, TURKEY**



# ABSTRACT BOOK

# **International Workshop Plant Health: Challenges and Solutions**

**ISBN: 978-2-88945-218-7**

**DOI: 10.3389/978-2-88945-218-7**

April 23-28, 2017, Antalya, Turkey

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This workshop highlighted current developments in the field of plant protection against pests and pathogens. This included the current problems in the field, latest tools and developments in detection and diagnosis, smart biologics, emerging and re-emerging microbial pathogens and resistance breeding.

The workshop aimed to bring together leading researchers, young scientists and industrialists from the plant protection sectors to exchange knowledge and share best practice in integrated pest management towards food security.

Specifically, we aimed to: highlight the most recent pathogen detection and diagnostic tools; discuss the benefits of using smart biologics including beneficial microorganisms; discuss new technologies that could help to deliver faster pathogen and pest control strategies; consolidate existing and develop new connections between the plant protection research communities; enhance the application of molecular and immunochemical detection technologies within the plant health industry; explore different ways to translate laboratory-based research into application, especially in biological control and reduction of mycotoxins; increase the awareness of problems that the plant health industry faces; discuss the latest developments in the field and disseminate these cutting-edge scientific outcomes to a wider community; provide a platform for knowledge exchange between academics and industry; foster new research collaborations that are focused on delivering benefits for plant health improvement; and enable participants to develop links to assist their professional development.

Presentations from experts in the field provided delegates with an understanding of the latest problems in plant health control. This provided an opportunity for delegates to identify and exploit key technologies across the plant protection industry.

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**INTERNATIONAL WORKSHOP  
PLANT HEALTH: CHALLENGES AND SOLUTIONS**



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The first author/primary contact has responsibility for the content on behalf of co-authors.

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**INTERNATIONAL WORKSHOP**  
**PLANT HEALTH: CHALLENGES AND SOLUTIONS**

**WORKSHOP COORDINATORS**

Prof. Dr. Mahmut TÖR (United Kingdom)  
Assoc Prof. Dr. Kubilay Kurtuluş BAŞTAŞ (Turkey)

**WORKSHOP SCIENTIFIC COMMITTEE**

Prof. John MANSFIELD (Imperial College)  
Prof. Matthew DICKINSON (Nottingham University)  
Prof. Ömür BAYSAL (Mugla University)  
Prof. Yiguo HONG (University of Warwick)  
Prof. Carston SKJOTH (University of Worcester)  
Assoc. Prof. David STUDHOLME (University of Exeter)  
Dr Alison Woods-TÖR (University of Worcester)

**WORKSHOP MENTORS**

Prof. Dr. Mahmut TÖR (University of Worcester)  
Prof. John MANSFIELD (Imperial College)  
Prof. Matthew DICKINSON (Nottingham University)  
Prof. Ömür BAYSAL (Mugla University)  
Assoc Prof. Dr. Kubilay Kurtuluş BAŞTAŞ (Selcuk University)

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## **Acknowledgements**

**Plant Health: Challenges and Solutions workshop is funded by the British Council under the Newton Fund Researcher Links scheme.**

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## Welcome Letter From The Organisers

### Welcome to Antalya, Turkey 23-28 April 2017

On behalf of the Organising and Scientific Committee, we are pleased to welcome you to Antalya, Turkey and offer you the opportunity to present your current research work, participate in the scientific activities (oral and posters), and interact with each other during this workshop, "Plant Health: Challenges and Solutions".

We have put together an excellent programme, including keynote lectures on plant health challenges, diagnostics and impacts of diseases on plants, and a special section on bioinformatics, mycotoxins and agriculturally beneficial microorganisms. There will also be plenty of time for networking, information exchange during poster presentations and exploring collaborative research opportunities. The research funding session should give you an idea of different funding resources. Mentor-led discussions are designed to stimulate both awareness of current problems and new ideas towards future policy development.

We are very pleased to see that some M.Sc. and Ph.D. students have chosen to join us and participate with their great enthusiasm. Beyond doubt, face-to-face meetings with scientists in the field are more critical than ever and will help ensure that these young scientists' careers are on the right path.

In the programme, we have also included a one-day excursion, visiting some of the ancient historical sites and the old town of Antalya. In this way, we hope that participants can also gain some cultural and traditional experience of Turkey from this workshop.

We hope that you will enjoy both the scientific and social activities, and will have plenty of opportunities to catch up with old friends and colleagues as well as meet new ones.

With best regards,



**Mahmut TÖR**  
Organiser



**Kubilay Kurtuluş BAŞTAŞ**  
Organiser

## “PLANT HEALTH: CHALLENGES AND SOLUTIONS” WORKSHOP PROGRAMME

<b>SUNDAY, APRIL 23, 2017</b>	
16:00–17:30	REGISTRATION
20:30–22:00	WELCOME COCKTAIL
<b>MONDAY, APRIL 24, 2017</b>	
8:30–9:30	<b>Registration</b>
9:30–9:45	<b>Opening Talk</b> <b>Prof Dr. Mahmut TÖR</b> (University of Worcester)
9:45–10:30	<b>Key Note Speaker: Prof John Mansfield</b> (Imperial College): <b>Current global challenges for plant health.</b> <b>Session Chair: Prof Mahmut Tör</b>
10:30–11:00	Coffee break
11:00–13:00	<b>1<sup>st</sup> Session: Networking</b> Participants spend time in a group before moving to a new group. This will introduce them to each other and enable them to learn about each other’s work.
13:00–14:00	Lunch & Networking
14:00–14:40	<b>Key Note Speaker: Prof Salih Maden</b> (Ankara University) <b>Impact of diseases on agricultural crops in Turkey</b> <b>Session Chair: Assoc Prof Kubilay Kurtuluş Baştaş</b> (Selçuk University)
	<b>2<sup>nd</sup> Session: Bioinformatics for plant health</b> <b>Session Chair: Assoc Prof Kubilay Kurtuluş Baştaş</b> (Selçuk University)

14:40–15:00	Genomics and Bioinformatics for plant health <b>Dr Konrad Paszkiewicz</b> (University of Exeter)
15:00–15:20	Pycits: An easy to use metabarcoding “pipeline” to identify species based on Illumina sequence data and a user defined sequence database <b>Dr Peter Thorpe</b> (James Hutton Institute)
15:20–15:45	Use of Imaging technology for plant health problems <b>Dr Bo Li</b> (NIAB-EMR)
15:45–16:15	Coffee Break
16:15–18:00	<b>Poster Session</b>
19:00	Dinner
<b>TUESDAY, APRIL 25, 2017</b>	
09:15–10:00	<b>Key Note Speaker: Prof Matthew Dickinson</b> (University of Nottingham): <b>Diagnostics of Plant Pathogens</b> <b>Session Chair: Prof Dr. Mahmut TÖR</b> (University of Worcester)
10:00–10:30	Live Diagnostic demonstration using LAMP technology <b>Prof Matthew Dickinson</b> (University of Nottingham)
10:30–11:00	<b>Coffee break</b>
	<b>3<sup>rd</sup> Session: Mycotoxins and their detection</b> <b>Session Chair: Dr Yasemin Bektas</b>
11:00–11:30	Effects of environmental factors on mycotoxigenic fungi and mycotoxin production: Do we know enough? <b>Dr Angel Medina-Vaya</b> (Cranfield University)

11:30–12:00	Use of Immunodiagnostics for mycotoxin detection <b>Dr Özlem Ertekin</b> (TUBITAK MAM)
12:00–12:30	RNAi in Agriculture <b>Dr Johnathan Dalzell</b> (Queen's University Belfast)
12:30–14:00	Lunch and Networking
	<b>4<sup>th</sup> Session: Smart Biologics.</b> <b>Session Chair: Dr Seçkin Eroğlu</b> (Izmir Economy University)
14:00–14:30	Agriculturally Beneficiary Microorganisms <b>Prof Ömür Baysal</b> (Muğla University)
14:30–14:50	Use of Actinobacteria has a source of biocontrol on maize <b>Dr Carol Verheecke</b> (Cranfield University)
14:50–15:10	Capturing microbial co-symbiosis to sustain plant productivity <b>Dr. Fraz Hussain</b> (University of Warwick)
15:10–15:30	Application of biocontrol in horticultural crops <b>Dr Robert Saville</b> (NIAB-EMR)
15:30–16:00	Coffee Break
	<b>5<sup>th</sup> Session: Case studies:</b> <b>Session Chair: Dr Maryam Rafiqi</b> (Royal Botanic Gardens, Kew)
16:00–16:20	An interdisciplinary Approach to monitor the development of the ash dieback disease <b>Dr Thor-Bjorn Ottosen</b> (University of Worcester)
16:20–16:40	Biological clocks and disease control <b>Osman Telli</b> (University of Worcester)

16:40–17:00	Effector-triggered immunity in plants: ATR2/RPP2 gene pairs. <b>Dr DaeSung Kim</b> (The Sainsbury Laboratory)
17:00–17:20	Micronutrient deficiencies in plants <b>Dr Seçkin Eroğlu</b> (Izmir University of Economics)
17:00–18:30	<b>Poster Session</b>
19:00	Dinner
<b>WEDNESDAY, APRIL 26, 2017</b>	
	<b>6<sup>th</sup> Session: Research Funding</b>
09:30–10:30	Funding opportunities-TR-UK and others
10:30–11:00	Coffee break
11:00–13:00	<b>7<sup>th</sup> session: Mentor-led discussion</b> – Major challenges in diagnostics – <b>Profs Matt Dickinson</b> – Use of biological control agents - <b>Prof Ömür Baysal, Assoc. Prof. Kubilay Kurtuluş Baştaş</b> – Technology transfer from institute to farmers- <b>Profs John Mansfield &amp; Mahmut Tör</b>
13:00–14:00	Lunch & Networking
14:00–15:00	<b>8<sup>th</sup> Session: Networking possible areas of collaboration</b>
15:00–15:30	Information feedback from putative collaboration
15:30–16:00	Coffee break
16:00–17:30	<b>9<sup>th</sup> session. Future directions</b> <b>Prof Mahmut Tör (University of Worcester)</b>
19:00–20:00	Workshop Dinner & GALA NIGHT



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<b>THURSDAY, APRIL 27, 2017</b>	
09:00–17:30	Technical Trip and Excursion in Antalya
18:00–19:00	Closing Ceromony
19:00	Dinner
<b>FRIDAY, APRIL 28, 2017</b>	
	Checking out of Hotel

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## Speaker Abstracts

# Current Global Challenges for Plant Health – Emerging Threats to Crops and the Environment

### John Mansfield

Imperial College, London, United Kingdom  
jwm17@hotmail.com

The outbreak of economically and environmentally damaging disease is a recurring theme in plant pathology. International and national agencies provide valuable, updated details about potential threats, but why do epidemics occur and how should we prevent their occurrence? Several factors including pathogenic variability, reduced host diversity, trade in plants and, potentially, climate change, interact to create the conditions for damaging disease outbreaks. The impact of these factors will be discussed with reference to research on recent epidemics including ash dieback, kiwifruit canker, wheat rust UG99 and wheat blast. Genomics and metabolomics are being applied to analyse the sources of infections and accelerate the search for resistant genotypes. In order to avoid the breakdown of control, whether through plant resistance or chemical applications, the 3Ds approach is recommended using improvements to *Detection*, *Diversity* and *Durable Resistance*.



## Impact of Diseases on Agricultural Crops in Turkey

### Salih Maden

*Agricultural Faculty, Ankara University, Ankara, Turkey*  
*salihmaden@hotmail.com*

Impact of the diseases occurring on economically important crops of Turkey was briefly outlined in this presentation. Diseases of field crops reported on wheat, barley, maize, sunflower, cotton, sugar beet; of vegetables reported on tomatoes, cucumbers, melon, watermelon, and onions; of fruit crops reported on grapes, apples, olives, oranges, hazelnut, and green tea were reviewed. Important bacterial, fungal and viral diseases of the above mentioned crops were listed and their effects on crop productivity were discussed. The data relevant for the diseases were obtained by a literature survey and comments of the author on the control of the diseases was presented.



## Genomics for Plant Biology

### Konrad Paszkiewicz

Biosciences, University of Exeter, Exeter, United Kingdom  
k.h.paszkiwicz@exeter.ac.uk

Genomics is now a standard part of many projects in the field of plant health. Here we briefly review recent advances in DNA sequencing technology, especially with regard to long single molecule data and particular challenges associated with plant genome sequencing. In addition we examine case studies in the fungal rice pathogen *Magnaporthe oryzae* and the *Ensete ventricosum* and a bacterial pathogen *Xanthomonas campestris*.





## **Pycits or METAPY: An Easy to Use Metabarcoding “Pipeline” to Identify Species Based on Illumina Sequence Data and a User Defined Sequence Database**

**Peter Thorpe<sup>1</sup>, Leighton Pritchard<sup>1</sup>, Sarah Green<sup>2</sup> and David Cooke<sup>1</sup>**

<sup>1</sup>The James Hutton Institute, Invergowrie, Dundee, Scotland, United Kingdom

<sup>2</sup>Forest Research, Northern Research Station, Roslin, Scotland, United Kingdom

*peter.thorpe@hutton.ac.uk*

As global warming occurs, changes in the ecological niche may favour new invasive species. Such invasive species could pose huge risks to agriculture or the wider environment. For example, forests in the west of Scotland are being felled due to the invasion of *Phytophthora ramorum*. Therefore rapid, robust and fast identification methods are required to identify species which may be present. Such information can be used to make an informed decision/action regarding protection.

Here we present an updated sampling method to detect *Phytophthora* species and a bioinformatic analysis pipeline. The analysis pipeline is user friendly and unique as it performs analysis with multiple clustering tools and presents a comparison of these results to the user. We currently use the ITS1 region(s) to classify species, however the pipeline can take any custom database, thus being flexible to the user's requirements. Moreover, we have performed analysis on the variation and copy number of the ITS1 within *Phytophthora* genomes which may cast doubt on the suitability of this as a robust marker for metabarcoding.

The pipeline is engineered under Travis testing with Codecov and pep8 code quality tests to help ensure the software is of high standard: <https://github.com/widdowquinn/THAPBI-pycits>.



## Use of Imaging Technology for Plant Health Problems

### **Bo Li**

NIAB EMR, Kent, UK  
bo.li@emr.ac.uk

NIAB is an internationally recognised centre for crop research, and a wide range of advanced imaging techniques including 2D visible imaging, hyperspectral imaging and UAV imaging have been applied for the monitoring of plant health and imaging data were processed by using advanced statistical analysis and machine learning models for the plant phenotyping and pest control.

Cherry cankers cause girdling of branches and may result in dieback or eventual death of the tree when affecting the main trunk. Due to the variation in disease susceptibility, breeding approaches could be successful. Therefore, a rapid disease screening method would be highly beneficial in **Prunus** breeding programmes, to allow the identification of resistant genotypes. Automated imaging analysis software was developed for the quantification of canker and artificial neural network (ANN) was used to pixels correspondent to disease.

The UAV-based disease monitoring attracted many interests in recent years due to the advantage of less labour cost, high-throughput and objective assessment. Hyperspectral imaging sensor was fitted on drone and screen regularly over the wheat crop. Good correlation was found between the predicted disease level and manual assessment after two weeks and it was shown that UAV-based sensing was a promising technique for evaluating disease severity and susceptibility.

Verticillium wilt is one of the primary soil-borne pathogens of strawberry in the UK and across Western Europe. Below ground root traits screening is extremely challenging to understand the plant resistance. Lab-based hyperspectral imaging with image analysis was applied to discriminate the susceptible and resistant cultivars of strawberry, and with the statistical selection of key wavelengths, software was developed for the rapid quantitative assessment of root infection.



## Diagnostics of Plant Pathogens

### Matthew Dickinson

*University of Nottingham, School of Biosciences, Loughborough, United Kingdom  
matthew.dickinson@nottingham.ac.uk*

Nucleic acid-based techniques are being increasingly used to aid the diagnostics of plant pathogens, not only to identify pathogens to species level, but also to identify specific traits about the organisms involved. These techniques range from large-scale laboratory based methods to rapid point-of-care diagnostics. In this talk, I will discuss the advantages and disadvantages of PCR and Loop Mediated Amplification (LAMP) methods for plant pathogen diagnostics, including the advantages of real-time methods over conventional approaches, both in terms of speed and accuracy of diagnosis. A particular focus of the presentation will be on diagnostics of phytoplasma diseases, which have been a major focus of our work at the University of Nottingham, but the application of the techniques to detection and diagnosis of viruses and fungi will also be discussed. In addition, rapid methods of nucleic acid extraction from test samples will be discussed, and a real-time LAMP assay will be demonstrated during the course of the talk to indicate the power of this technique for rapid in-field diagnostics.



## Effects of Environmental Factors on Mycotoxigenic Fungi and Mycotoxin Production: Do We Know Enough?

Angel Medina<sup>1</sup>, Matthew K. Gilbert<sup>2</sup>, Brian M. Mack<sup>2</sup>,  
Maria Gutierrez-Pozo<sup>1</sup>, Greg Obrian<sup>3</sup>, Alicia Rodriguez<sup>4</sup>,  
Deepak Bhatnagar<sup>2</sup>, Gary Payne<sup>3</sup> and Naresh Magan<sup>1</sup>

<sup>1</sup>Applied Mycology Group, Cranfield University, Cranfield, United Kingdom

<sup>2</sup>USDA, Agricultural Research Service, New Orleans, LA, United States

<sup>3</sup>Department of Plant Pathology, North Carolina State University, Raleigh, NC, United States

<sup>4</sup>University of Extramadura, Badajoz, Spain

a.medinavaya@cranfield.ac.uk

There is a significant interest in the impact that climate change factors may have on mycotoxigenic fungi. We have, for the first time, examined on conducive media and maize grain the impact that three way interactions between water availability, temperature, and elevated CO<sub>2</sub> have on: (i) growth, (ii) the relative expression of all genes in the aflatoxin gene cluster using both RT-qPCR and RNAseq, and (iii) the phenotypic aflatoxin B<sub>1</sub> production by *Aspergillus flavus*. On conducive media, interactions between water stress (water activity, a<sub>w</sub>; 0.97, 0.95, and 0.92), temperature (34 and 37°C), and CO<sub>2</sub> exposure (350, 650, and 1000 ppm) were considered and the growth, AFB<sub>1</sub> production and expression of biosynthetic genes (aflD, aflR) studied. For maize grains, interactions between water stress (water activity, a<sub>w</sub>; 0.99 and 0.91), temperature (30 and 37°C), and CO<sub>2</sub> exposure (350, 650, and 1000 ppm) were included. Fungal growth, AFB<sub>1</sub> production and expression of the all genes in the aflatoxin gene cluster by RNAseq were studied. The results showed that for growth there was relatively little effect. In contrast, the three-way interacting conditions (elevated CO<sub>2</sub>, water, and temperature stress) had a profound effect on aflatoxin B<sub>1</sub> production both in media and maize grains. Under slightly elevated CO<sub>2</sub> conditions there was a stimulation of aflatoxin B<sub>1</sub> production.

With regard to gene expression in conducive media results show that at 37°C, there was a significant increase in expression of both aflD and aflR at 0.95 and 0.92 a<sub>w</sub> and 650 and 1000 ppm CO<sub>2</sub>. There was an associated increase in AFB<sub>1</sub> in these treatments. In contrast at 34°C, there were no significant differences for interacting treatments. In stored maize grain differential expression of several genes in the aflatoxin gene cluster were found in relation with these interacting factors. Aflatoxin B<sub>1</sub> production increased under elevated CO<sub>2</sub> conditions at both temperatures and a<sub>w</sub> tested. This is the first study to examine these three-way interacting climatic factors on growth and mycotoxin production in different species including *A. flavus*. This provides data, which are necessary to help predict the real impacts of climate change on mycotoxigenic fungi.



## Use of Immunodiagnosics for Mycotoxin Detection

**Özlem Ertekin<sup>1</sup>, Şeyda Şerife Pirinççi<sup>2</sup>, Esin Akcael<sup>1</sup> and Selma Öztürk<sup>1</sup>**

<sup>1</sup>TÜBİTAK, The Scientific and Technological Research Council of Turkey, Marmara Research Center, Genetic Engineering and Biotechnology Institute, Gebze, Kocaeli, Turkey

<sup>2</sup>Department of Medical Genetics and Molecular Biology, Kocaeli University, Umuttepe, Kocaeli, Turkey  
ozlem.ertekin@gmail.com

The word mycotoxin mostly refers to the toxic chemicals produced by fungi that colonize crops. Mycotoxins enter the food chain by animal feeding or direct consumption of the crops by human. They may cause severe health effects which may lead to death. Mycotoxin contamination may occur both pre- and post-harvest in the crops where pre-harvest contamination is not only a threat to human health, but also a factor reducing the productivity. Due to their toxic effects, mycotoxins are regulated in many countries and several methods are devised to fulfil the requirements of the regulations. Liquid chromatography based methods including high pressure liquid chromatography (HPLC) or liquid chromatography-tandem mass spectrometry (LC-MS/MS); and enzyme based immunological test methods including enzyme linked immunosorbent assay (ELISA) are most commonly used and internationally accepted methods for AF quantification. Instrumental AF analysis requires an extract cleanup step with immunoaffinity chromatography in order to concentrate and remove the AFs from complex extract matrix. Both immunoaffinity columns (IACs) and ELISA systems utilize the ability of anti-mycotoxin antibodies to specifically bind designated mycotoxins. In addition to the mentioned laboratory based standard methods, there is a requirement for on-site analysis of aflatoxins to provide rapid risk assessment. Biosensors and strip tests, which also rely on specific antibodies, are used to address this requirement. Hence, antibodies and antibody based assays are the core components of mycotoxin analysis. In the presented work, antibody development and development of IAC, ELISA and biosensors for aflatoxins, the most dangerous and common mycotoxin, will be discussed.



## Agriculturally Beneficiary Microorganisms: Smart Biologics

### Ömür Baysal

*Department of Molecular Biology and Genetics, Muğla Sıtkı Koçman, University Faculty of Science,  
Muğla, Turkey  
omurbaysal@mu.edu.tr*

Sustainable agricultural production has been affected by serious damage and losses caused by pests and pathogens. Breakdown of pathogen sensitivity to overused pesticides gives rise to occurrence of new issues related to environmental and health concerns. Biologics seem as alternative way, if we are able to utilize of their potential properties that, therefore; we can also attribute them as “smart biologics” in all types of agricultural systems. In recent studies showed that testing of biologic agents efficiency to target microorganism *in vitro* conditions is not sufficient, in assessment of a bio-control agent molecular studies and omics technologies (genomics, transcriptome, proteomics, and metagenomics) clearly reveal the nature of antibiotics, secreted enzymes and inhibitory compounds and other properties of microorganism related to population dynamism in microflora related to its suppression effect on target pathogen. We believe that genetic manipulations and gene-editing techniques will also provide unique opportunity to create multifunctional bio-agent individuals and to compile of all prominent characteristic properties on all in one in further studies.

**Keywords:** smart biologics, omics science, bio-control, gene manipulation



## Use of Actinobacteria as a Source of Biocontrol on Maize

**C. Verheecke<sup>1,2</sup>, P. Anson<sup>2</sup>, D. Caron<sup>2</sup>, T. Liboz<sup>2</sup> and F. Mathieu<sup>2</sup>**

<sup>1</sup>Applied Mycology Group, School of Water Energy and Environment, Cranfield University, Cranfield, Bedfordshire, United Kingdom

<sup>2</sup>Laboratoire de Génie Chimique, LGC, Université de Toulouse, UMR 5503 (CNRS/INPT/UPS), Toulouse, France

c.verheecke@cranfield.ac.uk

The main objective was to explore the concept of using Actinobacteria as a source of biocontrol to reduce aflatoxins content in maize crop. The experiments were conducted *in vitro* and in greenhouse.

The *in vitro* experiments focused on the reduction of aflatoxin B1 and B2 production in mutual antagonism on contact with *A. flavus*. Among the 37 Actinobacteria tested 11 strains showed less than 18% residual concentration of AFB1 and B2. Among those, four strains were able to reduce the concentration of pure-AFB1 by more than 60% (Verheecke et al., 2014). Further investigation revealed that prevention of aflatoxins production (RT-qPCR) was achieved by some strains (Verheecke et al., 2015a), that no strain was able to adsorb aflatoxin B1 (Verheecke et al., 2015b) and preliminary results are suggesting a potential enzymatic degradation in some cases (unpublished data).

Concerning the greenhouse experiments, the Actinobacteria strains from *in vitro* tests having a higher power to reduce fungal growth and aflatoxins content were chosen for further study. Three consecutive trials on maize plants were conducted. 17 days after flowering, we carried out artificial inoculations of Actinobacteria strains. *A. flavus* was inoculated with two modalities: jointly the same day or 7 days later. In these two modalities, one of our strains got a very significant aflatoxin B1 decrease of 50% on inoculated kernels (Caron et al., 2016).

### Acknowledgment

ANR Aflafree 2011-2015 N°11 003 01, Agrimip Sud Ouest Innovation, Naresh Magan.



## REFERENCES

- Verheecke, C., Liboz, T., Darriet, M., Sabaou, N., and Mathieu, F. (2014). In vitro interaction of actinomycetes isolates with *Aspergillus flavus*: impact on aflatoxins B1 and B2 production. *Lett. Appl. Microbiol.* 58(6), 597–603. doi:10.1111/lam.12233.
- Verheecke, C., Liboz, T., Anson, P., Zhu, Y., and Mathieu, F. (2015). *Streptomyces-Aspergillus Flavus* interactions: impact on aflatoxin B accumulation. *Food Addit. Contam. Part A Chem. Anal. Control Expo. Risk. Assess.* 32(4), 572–76. doi:10.1080/19440049.2014.1003336.
- Verheecke, C., Liboz, T., Anson, P., Diaz, R., and Mathieu, F. (2015). Reduction of aflatoxin production by *Aspergillus flavus* and *Aspergillus parasiticus* in interaction with *Streptomyces*. *Microbiol. Soc.* 161(5), 967–72. doi:10.1099/mic.0.000070.
- Caron, D., Anson, P., Jara, P., Verheecke, C., and Mathieu, F. (2016). Utilisation D'actinomycètes En Biocontrôle de Laflatoxine B1 Produite Par *Aspergillus flavus* Sur Épis de Mais En Serre.” in *Annales de La 11ème Conférence Sur Les Maladies Des Plantes 7, 8 et 9 Décembre 2015 À Tours, France*, AFPP, 197–206.





## Capturing Microbial Co-Symbiosis to Sustain Plant Productivity

**Rana Muhammad Fraz Hussain, Beatriz Lagunas, Chrysa Sergaki, Patrick Schäfer and Miriam Gifford**

*r.hussain.1@warwick.ac.uk*

Nitrogen and phosphorus are critical nutrients for plant health. Currently farmers spend millions of pounds to add these nutrients in the form of fertilizers. However, production of nitrogen (N) fertilizer is highly energy intensive, and phosphorus (P) is a non-renewable resource. Thus, there are very good economic and environmental incentives for reducing fertilizer use in agriculture. One strategy is to exploit the use of mutualistic microbes that can enable plants to obtain non-mineral or otherwise unavailable sources of N and P. Moreover, these mutualists protect plants against root stresses (e.g., diseases, drought, and salinity) that are devastating in crop production. In the rhizosphere, plants can influence the competition among soil microbes in any given soil type in order to establish a root-associated microbiota enriched in mutualists. Our work is analysing a co-symbiosis with the specialist N-fixing microbe *Sinorhizobium meliloti* and the P-supplying and disease resistance mediating fungus *Serendipita indica*. We found that this microbial combination not only increases P supply and disease resistance, but also enhances rhizobial nodulation in the legume *Medicago truncatula*. Thus, the *S. indica*-*S. meliloti* co-symbiosis represents a way to sustainably improve crop yield. The effect of these individual mutualists on plant performance was previously known, but the beneficial synergisms originating from this mutualistic co-symbiosis and their persistence in microbiota of different soil types was not known. This knowledge is critical, if we want to exploit mutualistic co-symbioses for agriculture. Moreover, these analyses might identify mechanisms that underlie the establishment of “mutualistic soils” that have beneficial activities in plant nutrition and stress resistance, in analogy to disease suppressive soils that provide stress protection for crops.



## Application of Biocontrol in Horticultural Crops

**Robert Saville, Gurkan Tut, Wei Feng<sup>†</sup>, Leone Olivieri and Xiangming Xu**

*NIAB EMR, New Road, East Malling, Kent, ME19 6BJ, United Kingdom*

*<sup>†</sup>Current address: CAAS, Zhongguancun South St., Haidian District, Beijing, China*

*robert.saville@emr.ac.uk*

NIAB EMR (formerly East Malling Research) have been developing and incorporating components of IPM for tree fruit diseases over our 104 year history. In this presentation, I will illustrate our current knowledge of the application of biocontrol in horticultural fruit crops and identify our research focus for the coming years.

The biological control of arthropod pests has been hugely successful in reducing the reliance on synthetic pesticides and the adoption of IPM in the fruit sector whilst biological control of disease has been under exploited. In a regulatory climate in which current actives are threatened and new actives restricted it is more important than ever to find alternatives to conventional plant protection products. The main barriers to exploiting biocontrol for diseases have been the inconsistency of control efficacy coupled with high cost compared to conventional treatments. Here I will discuss the work we are doing to optimise the use of biocontrol in the field by understanding climatic variables, dose, morphology, and ecology of the crop, pathogen, and antagonist. Examples will be drawn from the work across the pathology group at NIAB EMR.



## **An Interdisciplinary Approach to Monitor the Development of the Ash Dieback Disease**

**Thor-Bjørn Ottosen, Geoffrey Petch, Mary Hanson and Carsten Skjøth**

*Institute of Science and the Environment, University of Worcester, Worcester, United Kingdom  
t.ottosen@worc.ac.uk*

The plant disease Ash Dieback; a disease attacking ash trees, causing defoliation and dieback of branches, and subsequent death of the tree, has wiped out the ash trees in large parts of Europe. The disease is caused by a fungus which infects the ash leaves and survives on infected leaf litter on the forest floor, and spreads through the atmospheric dispersion of fungal spores. The disease arrived in the United Kingdom (UK) in 2012. Given the economic and social value of the UK ash trees, a large research effort has since then been going on to protect the UK ash trees. Given that, at present, there is no cure for the disease the management strategy has been to monitor the development and to try to prevent further spreading. The aim of the present ongoing research project is to develop risk maps, based on atmospheric models, for the future spread of the disease. This is accomplished through a number of steps: The spatial distribution of the ash trees is mapped based on high-resolution satellite images combined with forest statistics. The seasonal development (the phenology) of the fungus is studied through manual counts of the fruiting bodies in a specific woodland combined with measurements of environmental variables. Atmospheric measurements have been made and a method for detecting the fungal spores using qPCR is underway, with the aim to derive a diurnal emission profile. The presentation will give an overview of the progress of the various steps and the interconnection between them.



## Biological Clocks and Disease Control

**Osman Telli and Mahmut Tör**

*Institute of Science and the Environment, University of Worcester, Henwick Grove, Worcester,  
United Kingdom  
o.telli@worc.ac.uk*

Most organisms have an internal circadian clock that also called the circadian rhythm, which regulates and imparts a survival advantage by enabling an organism to anticipate daily environmental changes. The biological clocks have three basic properties; a period length of about 24 h, can be reset by environmental factors such as light and temperature, and has at least one internal autonomous circadian oscillator. The biological clock mechanisms are outwardly very similar in all species but the genes that make up the clock mechanisms can be quite different.

The biologic clock regulation system controls different biotic factors across the kingdom such as sleep times, flowering times, body temperature, hormonal secretion in organisms. Recently, a link between the plant immune system and the biological clock has been identified. However, the questions then arise as to whether (a) the biologic clock has any effect on pathogenicity, (b) the time of the day is important for disease control; and (c) we can use biologic clock for our advantage in the field.

We have been working on plant pathogen interactions using downy mildew disease. We have been trying to find answers for some of the questions raised above. Latest data will be presented.



## Effector-Triggered Immunity in Plants: ATR2/RPP2 Gene Pairs

**Dae Sung Kim<sup>1</sup>, Mahmut Tör<sup>2</sup>, Volkan Cevik<sup>3</sup> and  
Jonathan D. G. Jones<sup>1</sup>**

<sup>1</sup>The Sainsbury Laboratory, Norwich Research Park, Colney Lane, Norwich, United Kingdom

<sup>2</sup>Institute of Science and the Environment, University of Worcester, Henwick Grove, Worcester,  
United Kingdom

<sup>3</sup>Department of Biology and Biochemistry, University of Bath, Bath, United Kingdom  
daesung.kim@tsl.ac.uk

The interaction between *Hyaloperonospora arabidopsidis* (*Hpa*), a downy mildew pathogen, and the model plant *Arabidopsis* is a useful system to unveil the molecular mechanisms of plant immunity. *Arabidopsis* RPP2A and RPP2B recognize ATR2 from *Hpa* Cala2 isolate (Sinapidou et al., 2014). To reveal ATR2, *Hpa* Cala2 was crossed with *Hpa* isolate Noks1 which lacks ATR2. We found a few ATR2 candidates (A2Cs). A2C1<sup>Cala2</sup> and A2C2<sup>Cala2</sup> are not recognized by RPP2. We found a non-canonical RxLR effector candidate (A2C3<sup>Cala2</sup>) that carries a signal peptide, a dEER motif, and WY domain, which are typical features in oomycete effectors. In the Emoy2 and Noks1 alleles, there is a frame-shift that inactivates. From 12 different Cala2-Noks1 F2 segregants, all avirulent F2 are homo- or heterozygous for A2C3<sup>Cala2</sup> while all virulent F2 are homozygous A2C3<sup>Emoy2/Noks1</sup>. We determined A2C3 is recognized in Col-0 but not in CW84 in which RPP2 is absent using biolistic bombardment. Without cognate R genes, A2C3 can increase susceptibility in *Nicotiana benthamiana* against *Phytophthora infestans*. Adjacent to RPP2A (At1g19500) and RPP2B (At1g19510), there are two additional TIR-NB-LRR type genes (At1g19520 and At4g19530, hereafter RPP2C and RPP2D). They are similar gene pairs to RRS1 and RPS4 (Narusaka et al., 2009), including C-terminal extended post-LRR domain and a head-to-head structure in the genome. After inoculation with Cala2 on *rpp2c* and *rpp2d* mutants, hyphal growth could be observed by trypan blue staining, indicating they are also required for full ATR2-dependent immunity.

### REFERENCES

Sinapidou, E., Williams, K., Nott, L., Bahkt, S., Tör, M., Bittner-Eddy, P., and Beynon, J. (2004). Two TIR:NB:LRR genes are required to specify resistance to *Peronospora parasitica* isolate Cala2 in *Arabidopsis*. *Plant J.* 38, 898–909.

Narusaka, M., Shirasu, K., Noutoshi, Y., Kubo, Y., Shiraishi, T., Iwabuchi, M., and Narusaka, Y. (2009). RRS1 and RPS4 provide a dual Resistance-gene system against fungal and bacterial pathogens. *Plant J.* 60, 218–226. doi:10.1111/j.1365-3113X.2009.03949.x.



## Micronutrient Deficiencies in Plants

### Seçkin Eroğlu

Department of Genetics and Bioengineering, Izmir University of Economics, Izmir, Turkey  
erogluseckin@gmail.com

Micronutrient deficiencies in plants are often limiting factors for plant growth and, as human diet is largely dependent on plants, exacerbate human micronutrient deficiencies. Iron (Fe) deficiency is one of the most widespread plant micronutrient deficiencies in the world, especially seen on plants, which are grown in calcareous soils. In this talk, micronutrient deficiencies will be discussed, with a strong emphasis on Fe in molecular level.

Fe-deficient plants risk accumulating potentially toxic amounts of zinc ( $Zn^{2+}$ ), copper ( $Cu^{2+}$ ), cobalt ( $Co^{2+}$ ), nickel ( $Ni^{2+}$ ), and manganese ( $Mn^{2+}$ ). The detoxification of excess Zn, Ni, and Co in Fe-deficient plants is mediated by sequestration into vacuoles, mainly of root epidermal and cortical cells, by means of cation diffusion facilitator (CDF)- or iron-regulated gene (IREG)-type transporters. The compartmentalization of heavy metals into the vacuole of root cells also prevents their excessive translocation to the shoot. Among the metals overaccumulating in Fe-deficient plants, Mn has been known for a long time to exert a negative effect on Fe nutrition, and for field-grown plants, high Mn availability is one of the major factors that induce Fe deficiency chlorosis. Despite this fact, the molecular basis of the antagonistic Fe–Mn interaction and the mechanism by which Mn is detoxified in Fe-deficient plants have remained unclear. A protein recently identified from metal tolerance protein (MTP) family has been shown to be a critical determinant for *Arabidopsis thaliana* plants to sustain Fe homeostasis under low availability of Fe and provides valuable insights on interaction of Fe and Mn.



## Poster Abstracts

### Omics Sciences and Plant Pathology

#### Ömür Baysal<sup>1</sup> and Kubilay Kurtuluş Baştaş<sup>2</sup>

<sup>1</sup>Faculty of Science, Department of Molecular Biology and Genetics, Mugla Sıtkı Kocman University, Mugla, Turkey

<sup>2</sup>Agriculture Faculty, Department of Plant Protection, Selcuk University, Konya, Turkey  
omurbaysal@mu.edu.tr and kbastas@selcuk.edu.tr

Technological improvements provide the sequencing of environmental DNA and RNA that increases knowledge on whole information based on genomic and transcriptomic level. Metagenomic and transcriptomic analysis of pathogenic communities and the assembly of population genomes of living microorganisms (plant pathogens) from microflora DNA are knowledge stores. Although the value of “omics” approach is limited by technical and bioinformatic difficulties, these obstacles can be eliminated and “perfect” metagenomes and transcriptomes data can be well constructed. These conceptual challenges related to use of omics in plant pathology are also relevant to the application of omics to soil microflora. Plant disease and immunity is benefiting tremendously from omics database. Research programs using model pathogens *in vitro*, pathogen-crop interactions are considered to be a model, which reveal remained unclear points between host and invading pathogens. In this presentation, we discuss the contribution of omics technologies to these advances based on model host-pathogen systems to highlight advantage of novel crop protection concepts.

**Keywords:** omic technology, plant pathogens, host pathogen interaction



## The Effects of Climate Change on Plant Diseases: Conclusions and Research Gaps

**Kubilay Kurtuluş Baştaş**

*Faculty of Agriculture, Department of Plant Protection, Selcuk University, Konya, Turkey  
kbastas@selcuk.edu.tr*

Climate change in the 21<sup>st</sup> century is a global phenomenon and although it is a world-wide recognised issue its effects vary by geographic location making regional studies of potential impact of high importance. Climate change influences infection, reproduction, dispersal, survival between seasons and other critical stages in the life cycle of a plant pathogen in both biotrophs and necrotrophs. Other major impacts on diseases are through changes in host physiology, host-pathogen interactions and disease resistance. Further researches are needed in the following areas: (i) for sustainable food production, more sophisticated, “big picture,” modelling studies of each individual crop-pathogen-projected climate system, (ii) characterization of factors limiting the survival of pathogens (e.g., temperature, humidity, CO<sub>2</sub>, O<sub>3</sub> and radiation) by inoculated outdoor and controlled environment experiments, (iii) changes in host-pathogen interactions particularly for important crop species, (iv) determination of quarantine measures and forecasting models to control emerging pathogens and the geographical distribution and modification of diseases for understanding epidemiology and trends in epidemics, (v) determination of a new fungicide and bactericide application calendar and favour of nonchemical methods for plant disease control, (vi) changes on biological control and useful effects of naturally-occurring microbes on phyllosphere and rhizosphere pathogens, (vii) alterations in relative humidity, particularly plant-pathogenic bacteria within the critical leaf surface microclimate, (viii) alterations on pathogen-host relationships genetic resistance to diseases, and (ix) modifying of soil aspects for microbial activity under a changing climate, including soil nutrient availability, soil temperature and soil water content. The analysis of the potential impacts of climate change on plant diseases is essential for the adoption of adaptation measures, as well as for the development of resistant cultivars, new control methods or adapted techniques, in order to avoid more serious crop losses.

**Keywords:** climate change, plant pathogen, genetic, control





## **A Specific and Sensitive Method for Detection of *Erwinia amylovora*, Loop-Mediated Isothermal Amplification (LAMP)**

**Kubilay Kurtuluş Baştaş**

Faculty of Agriculture, Department of Plant Protection, Selcuk University, Konya, Turkey  
kbastas@selcuk.edu.tr

*Erwinia amylovora*, the causative agent of fire blight, is the most devastating bacterial disease of rosaceous plants, primarily apple and pear, but also other fruit trees and ornamentals of economic importance throughout the world. An important step in control of the disease depends on the fast and reliable detection of the pathogen. Current approaches for the identification of fire blight include morphological, biochemical, serological, and DNA-based methods. However, most of these methods are fairly time consuming, and none of these methods is quantitative. A new molecular technology, LAMP, has an equivalent or greater performance regarding sensitivity, specificity, speed from *E. amylovora* DNA or plant extract concentration and more simplicity than real-time PCR, immunoassays and plating, demonstrating its utility for routine testing. The LAMP assay enables the fast detection of down to approximately 20 CFU of pure *E. amylovora* or 100 fg genomic DNA per reaction (25 µl) within 45 min. Detection of *E. amylovora* using LAMP is carried out with primers complementary to *amsH* gene located on chromosomal DNA. Because LAMP assay is designed on a chromosomal target, it detects all known *E. amylovora* strains including those lacking the plasmid pEA29. Validation of the developed LAMP assay on naturally infected field samples shows good correlation to existing methods and thus applicability of LAMP technology for monitoring infections in orchards and nurseries. The method allows for a detailed description of fire blight epidemiology and pathogenesis as well as for an integration of results into improved predictive forecasting models for sustainable fire blight management.

**Keywords:** *Erwinia amylovora*, detection, control, LAMP



## Molecular Characterization of *Pseudomonas savastanoi* pv. *phaseolicola*

Badel Uysal<sup>1</sup> and Kubilay Kurtuluş Baştaş<sup>2</sup>

<sup>1</sup>Faculty of Agriculture, Department of Plant Protection, Iğdir University, Iğdir, Turkey

<sup>2</sup>Faculty of Agriculture, Department of Plant Protection, Selçuk University, Konya, Turkey  
kbastas@selcuk.edu.tr

The causative agent of halo blight disease, *P. s. pv. phaseolicola* (Psp), is possibly the most significant bacterial pathogen of bean in many countries. The control of halo blight is difficult, and the only practical methods for disease management rely on the use of pathogen-free seeds, appropriate culture practices and the use of resistant cultivars. Therefore, the timely detection and appropriate identification of the pathogen in seeds are essential for effective control of the disease. Nine Psp races are characterized based on a series of eight differential *Phaseolus* genotypes having different combinations of resistance (R) genes. Till now, the pathogen races 1, 2, 6, and 7 have a global distribution; races 3, 4, 5, and 8 are found predominantly in East and Central Africa and race 9 has been identified in East Africa and South America. Molecular typing methods including separation of bacterial components using chromatography, proteomic techniques such as MALDI-TOF, PCR based identification methods (IS-RFLP, macro restriction-PFGE, RAPD, BOX-PCR, ERIC-PCR, rep-PCR, AFLP, Real Time PCR, and BIO-PCR) are very useful to study intra-specific diversity, to identify sources of inoculum and to track the spread of the infections by Psp, although phenotypic methods are also used. In addition, by *in silico* analysis, genes encoding pathogenicity-related secondary metabolites of Psp is identified as being pathovar specific and targeted for developing a LAMP protocol with multiple primers selected from gene regions identified as discriminatory by comparative genomic analyses. The rapid detection and identification methods of Psp are useful to determine whether an agricultural product meets the phytosanitary standard for export or import.

**Keywords:** bean, *P. s. pv. phaseolicola*, molecular detection, race, genom



## Identification of Drought Stress Related Genes in Bread Wheat (*Triticum aestivum* L.) Using Next Generation Sequencing Technology

Birsen Cevher-Keskin<sup>1</sup>, Yasemin Yildizhan<sup>1</sup>, Buğra Özer<sup>2</sup>,  
Uğur Sezerman<sup>3</sup>, Oktay Kulen<sup>1</sup>, Selma Onarici<sup>1</sup>, Bayram Yüksel<sup>1</sup>,  
A. Hediye Sekmen<sup>3</sup> and İsmail Türkan<sup>3</sup>

<sup>1</sup>TUBITAK, Marmara Research Center, Genetic Engineering and Biotechnology Institute, Gebze, Kocaeli, Turkey

<sup>2</sup>TUBITAK, National Research Institute of Electronics and Cryptology, Gebze, Kocaeli, Turkey

<sup>3</sup>Faculty of Medicine, Department of Biostatistics and Medical Informatics, Acibadem University, Istanbul, Turkey

<sup>4</sup>Faculty of Science, Department of Biology, Ege University, Bornova, İzmir, Turkey  
yasemin.yildizhan@tubitak.gov.tr

Bread wheat, is one of the main staple crops for many countries including Turkey. Yield losses could reach up to 80% in some years, especially in central Turkey where groundwater resources have been nearly depleted due to the excessive use for the irrigation, further exacerbating the problem. Hence, finding new approaches to improve wheat productivity under water-limited conditions is an urgent priority. Identification of novel stress-responsive genes and their role in drought response is an important research area for the improvement of the crops. Since the functions of some genes have not been completely identified yet, the knowledge of genes involved in drought response mechanism is still incomplete. Drought-stress related genes were identified for drought tolerant and non-tolerant *T. aestivum* cultivars after different drought stress treatments by Next Generation Sequencing technology. For RNAseq, fifty-bp Illumina paired-end reads from a transcriptome library was constructed from hexaploid wheat poly (A) RNA. *De novo* assembly for 311 Gb was applied to short-read transcriptome data. For comparative bioinformatics analysis, *de novo* assembly was used as a reference genome. Selected differentially expressed genes were confirmed by qRT-PCR. Root proteome profiling was performed by LC-MS/MS to get a lot of details of the molecular mechanisms of bread wheat in response to drought. The activities of antioxidant enzymes and peroxidation levels of lipids in cell membranes (TBARS content) of drought tolerant and non-tolerant cultivars were also investigated for different drought stress. All these analyses will allow us to get a better idea about the possible role of these genes in the drought-response mechanism.

### Acknowledgement

This work was supported by grand CRP/TUR09-03 from ICGEB to B. C-K.



## The Potential Using of Trans-Generational Resistance Against Common Bean Blight Caused by *Xanthomonas axonopodis* pv. *phaseoli*

**Ahmet Akköprü**

Agriculture Faculty, Plant Protection Department, Yuzuncu Yil University, Van, Turkey  
ahmetakkopru@yyu.edu.tr

The aim of this study was to investigate whether it is possible to control the common blight disease of bean caused by *Xanthomonas axonopodis* pv. *phaseoli* (*Xap*) by trans-generational resistance provided by chemical sitimulants; acibenzolar-S-methyl (ASM). In addition, the effects of the treatment on to disease progress, *Xap* population and plant growth were determined in climate chamber. Inoculation with ASM (50 ppm) was performed four times by spraying to parent plants at intervals of 3–5 days in climate chamber. Control plants (C) were treated with water. The seedlings were transplanted into field to obtain P1 and C1 seeds (offspring). *Xap* suspension was inoculated 3 days after application of ASM with 1/1, 1/4, and 1/8 doses to P1 and C1 seedlings. *Xap* population (with semi selective MXP medium) and disease severity (with 1–5 scale) were determined at specific intervals (3, 7, 14, and 21 days). Bean growth was evaluated by weighing fresh and dried roots and shoots 21 days with *Xap*. P1 seedlings significantly reduced disease severity up to at rates of 28% compared to C1 plants. A decrease of *Xap* population was observed in P1 plants until seventh day but it was not statistically significant. On the other hand, bean growth parameters of P1 plants significantly increased compared to C1 plants when both plant groups under the pathogen or ASM stress. This approach might have be a high potential to reduce using pesticides and improve the effectiveness of biological control.

**Keywords:** trans-generational resistance, epigenetic, *Xanthomonas axonopodis* pv. *phaseoli*, common bean blight



## Determination Aflatoxins and Cyclopiazonic Acid Production Ability of *Aspergillus flavus*

### Işılav Lavkor

Biological Control Research Institute, Adana, Turkey  
isilav.lavkor@tarim.gov.tr

Mycotoxins such as aflatoxins (AFs) and cyclopiazonic acid (CPA) are secondary metabolites produced by *Aspergillus flavus*. The aim of the study was to detect occurrence of AFs (type B and G) and cyclopiazonic acid (CPA) in *A. flavus* isolates from soil, air, and also from infected peanut plants from Adana in 2016. Totally 173 isolates were identified as *A. flavus*. AFs occurrence on *A. flavus* isolates were analyzed by immunoaffinity chromatography-reversed-phase high-performance liquid chromatography (IAC-HPLC) analysis and CPA occurrence on *A. flavus* isolates analyzed by thin layer chromatography (TLC). Only 121 (69.9%) of *A. flavus* isolates were found aflatoxigenic (B<sub>1</sub> or B<sub>2</sub> or B<sub>1</sub> and B<sub>2</sub>) also 29 (16.8%) of *A. flavus* isolates were producing CPA. It is concluded that, large number of isolates contaminated with AFs and the simultaneous detection of AFs and CPA. In addition, strategies should be developed to control production of these mycotoxins produced by *A. flavus*.

**Keywords:** *Aspergillus flavus*, aflatoxins, cyclopiazonic acid, soil, air, peanut

### **Acknowledgement**

This study was supported by TUBITAK (The Scientific and Technological Research Council of Turkey) within 115O007 project.



## **Yr15 Screening of Turkish Bread Wheat Varieties Using KASP Markers for Warrior, a New Race of Stripe Rust Pathogen (*Puccinia striiformis*) on Wheat in Turkey**

**Fatih Olmez<sup>1</sup>, Kadir Akan<sup>2</sup>, Fatmagül M. Vanlioglu<sup>1</sup>, Tugce Uslu<sup>1</sup> and Burcu G. Ergun<sup>1</sup>**

<sup>1</sup>Biotechnology Department, Field Crops Central Research Institute, Yenimahalle, Ankara, Turkey

<sup>2</sup>Plant Protection Department, University of Ahi Evran, Kirsehir, Turkey

olmez.fatih@tarim.gov.tr

Stripe Rust is one of the most devastating leaf diseases causing serious epidemics in some years and important yield losses on wheat throughout of Turkey. Although there are some effective chemicals against this pathogen, their usage is restricted by mostly economic reasons. The easiest way to struggle the pathogen epidemics is to use resistant wheat varieties. Resistant breeding is so problematic for this pathogen because new pathogenic variants of stripe rust may occur so fast in nature. Recently, newly emerged race of *P. striiformis* named Warrior have been determined in Europe and disseminated to some parts of Turkey. Yr15 dominant resistance gene of wheat can be used against not only Warrior but also lots of Stripe Rust races. Just after emergence of Warrior in Europe, some researchers developed a few SNP based molecular markers related to Yr15 to breed new varieties against new race of this pathogen. In this study to see if Yr15 is already exist in any registered Turkish bread varieties or not, we screened about 150 bread wheat varieties using KASP primers. Our results showed that there are no Turkish bread wheat varieties having Yr15 to be used for aims of Warrior resistance breeding and KASP primers can be successfully used to trace Yr15 gene throughout crossing populations in Turkey.



## Alien Invasive Forest Pests and Diseases in Turkey

**Funda Oskay<sup>1</sup> and Kahraman Ipekdal<sup>2</sup>**

<sup>1</sup>Faculty of Forestry, Department of Forest Entomology and Protection, Çankırı Karatekin University, Çankırı, Turkey

<sup>2</sup>Faculty of Science and Literature, Department of Biology, Ahi Evran University, Kırşehir, Turkey  
fundaoskay@karatekin.edu.tr

Alien invasive forest pests and pathogens are amongst the major threats to forest ecosystems. In Turkey, devastating impacts of alien invasive pests and pathogens have also been experienced almost simultaneously with Europe. Dutch elm disease and chestnut blight are notorious diseases caused by these pathogens which have induced immeasurable ecological and economic damage during the last 70–80 to Turkish forests. Beside, numerous pathogens and pests are still being introduced into urban areas and forest.

While *Cylindrocladium buxicola*, the causal agent of boxwood blight and *Ceratocystis platani*, the causal agent of canker stain are of examples for latest introduced and established pathogens, Chestnut gal wasp (*Dryocosmus kuriphilus*) and *Anoplophora chinensis* are of recently introduced forest pests. Strict attempts including removal of thousands of trees for eradication of the recent introductions, and quarantine as well as control measurements for preventing establishment or preventing their further spread are being applied for these pests and pathogens. On the other hand, oak and plane lace bug species (*Corythucha arcuata* and *C. ciliate*), Western conifer seed bug (*Leptoglossus occidentalis*) and box wood bugs (*Cydalima perspectalis*) represent the established and spreading invasive pests in the country.

In this study, invasive alien forest pest and pathogens especially those prompt enormous influence on urban areas and forest ecosystems were listed to briefly describe their means of introductions and establishment, and to give information on their impact and current situation.



## **Determination of Virulence Grades of *Fusarium* spp. Isolates Causes Wilt and Root Rot in Chickpea *In Vitro* Conditions**

**Gürkan Başbağcı<sup>1</sup> and F. Sara Dolar<sup>2</sup>**

<sup>1</sup>Transitional Zone Agricultural Research Institute, Eskişehir, Turkey

<sup>2</sup>Faculty of Agriculture, Department of Plant Protection, Ankara University, Dışkapı, Ankara, Turkey  
gurkanbasbagci07@hotmail.com

This study was carried out in order to determine the virulence grades of *Fusarium* spp. isolates causes root rot and wilt in chickpea in Eskişehir, Uşak, Kütahya, and Denizli provinces by pathogenicity tests. In this scope, first, survey studies were carried out during the 2015 chickpea production season. As a result of the surveys, a total of 892 plants showing disease symptoms were collected from 56 different fields, including 225 plants in 12 fields in Eskişehir, 243 plants in 16 fields in Uşak, 308 plants in 16 fields in Kütahya, and 116 plants in Denizli. As a result of isolation from these plants, a total of 714 *Fusarium* isolates were obtained, 178 of which were collected from Kütahya, 147 from Eskişehir, 167 from Denizli, and 222 isolates from Uşak provinces. In 2016, the isolates were tested for pathogenicity in Petri dishes *in vitro* conditions, then virulence grades were determined based on the percentage of disease severity. The results showed that 32 isolates had between 0 and 20, 113 isolates had between 20 and 40, 134 isolates had between 40 and 60, 271 isolates had between 60 and 80, and 164 isolates had between 80 and 100 percentage of disease severity. According to these values, high virulence isolate rate was determined as 60.9%.

**Keywords:** chickpea, *Fusarium*, root rot, wilt, virulence grade





## Antifungal Effects of Silicon Dioxide Nanoparticles (SiO<sub>2</sub>NPs) against Various Plant Pathogenic Fungi

Ilgin Akpınar<sup>1</sup>, Taner Şar<sup>1,2</sup> and Muammer Unal<sup>3</sup>

<sup>1</sup>Institute of Graduate Studies in Science and Engineering, Istanbul University, Istanbul, Turkey

<sup>2</sup>Department of Molecular Biology and Genetics, Gebze Technical University, Kocaeli, Turkey

<sup>3</sup>Science Faculty, Department of Botany, Istanbul University, Istanbul, Turkey  
akpinarilgin@gmail.com

This research is concerned with the fungicidal properties of nano-size silicon dioxide colloidal solution used as an agent for antifungal treatment of various tomato plant pathogens. Silicon (Si), which is the second most abundant element on the earth, is known to be absorbed into plants to increase disease and stress resistance. We used “amorphous, 26 wt%, 8–33 nm” silicon dioxide nanoparticles (SiO<sub>2</sub>NPs) at concentrations of 50, 100, 150, and 200 ppm. Two different plant pathogenic fungi were treated with these SiO<sub>2</sub>NPs on potato dextrose agar (PDA) plates. These pathogens are *Fusarium oxysporum* f. sp. *lycopersici* (FOL) and *Fusarium oxysporum* f. sp. *radicis lycopersici* (FORL). FOL and FORL, which are widely seen in tomato production, can be found in any soil type and live in different forms on organic materials. In our study, 6 isolates (AY5, H2, JR, SC, TR-1, and TA-4) from FORL and 4 isolates (EM2, FL, HT, and J2) from FOL were tested. We calculated fungal inhibition in order to evaluate the antifungal efficacy of silicon dioxide nanoparticles against pathogens. The results indicated that SiO<sub>2</sub>NPs possess antifungal properties against these plant pathogens at various levels. The highest inhibition rates were seen for SC and TA-4 from FORL isolates. This ratio is approximately 98%. Treatment with SiO<sub>2</sub>NPs resulted in maximum inhibition of FL and EM2 from FOL. Results also showed that the most significant inhibition of plant pathogenic fungi was observed on PDA and 400 ppm of SiO<sub>2</sub>NPs.



## Plant Growth Promoting Affect of Endophytic Bacteria

**Irmak Şah**

*Department of Molecular Biology and Genetics, Gebze Technical University, Kocaeli, Turkey  
isah@gtu.edu.tr*

Bacteria that promote plant growth use similar mechanisms. This bacteria include cyanobacteria and endophytic bacteria that can colonize in plant tissues, and bacteria that are able to make symbiotic relationship with plants. A microorganism is considered endophyte, if it is obtained by extraction from surface sterilized plant material or plant; the most basic feature that distinguishes endophytic organisms from pathogens is that they do not have any harmful effect on the host. Endophytes are microorganisms such as bacteria, actinomycetes, and fungi that maintain a symbiotic relationship in healthy plant tissues. While plants strictly limit the growth of endophytes, endophytes use many mechanisms that can be gradually adapted to their living conditions. They produce a large number of compounds that promote plant growth to maintain stable symbiosis and allow plants to better adapt to environmental conditions. Endophytic bacteria may promote plant growth directly or indirectly. Direct promotion is achieved by acquisition of essential nutrients to plant or regulation of hormone levels by the synthesis of auxin, cytokine, gibberellins, etc. Indirect promotion is by production of antagonistic compounds against bacterial, fungal pathogens, etc. This study describes how endophytic bacteria affect plant growth in a positive way; and gives information about how endophytic bacteria can be used in agriculture.

**Keywords:** endophytic bacteria, agriculture, plant growth promoting bacteria (PGPB)



## **Is the Non-Native Parasitoid, *Torymus sinensis*, Suitable as a Biological Control Agent of the Chestnut Gall Wasp, *Dryocosmus kuriphilus*, in the UK?**

**Matthew J. Everatt**

*Department for Environment Food & Rural Affairs, Fera Science Ltd (Fera), National Agri-Food Innovation Campus, Sand Hutton, York, United Kingdom  
Matthew.Everatt@defra.gsi.gov.uk*

The oriental chestnut gall wasp, *Dryocosmus kuriphilus*, is native to China and is a major pest of chestnut trees. In June 2015, it was discovered in the UK for the first time, in Farningham Wood, Kent, and in six trees in St Albans. The wasp has since been found in London and Surrey. Given its distribution, measures to eradicate the wasp are no longer being pursued and instead, long term management options are being considered. One of these options is the use of the non-native parasitoid wasp, *Torymus sinensis*, as a classical biological control agent.

While it has been successful in other countries, there are risks associated with releasing this non-native species into the UK, including its ability to parasitise species other than the chestnut gall wasp and its ability to hybridise with native wasps. I travelled to Italy, where the biological control agent is being used, as part of a Short Term Scientific Mission to gain a better understanding of the parasitoid and how effective and appropriate it might be for release into the UK. These findings, as well as further work to obtain a licence to release the parasitoid into the UK, are discussed.



## Evolution of Host Specificity and Virulence of *Pseudomonas syringae* on *Prunus*

Michelle T. Hulin, Andrew D. Armitage<sup>1</sup>, John W. Mansfield<sup>2</sup>,  
Robert W. Jackson<sup>3</sup> and Richard Harrison<sup>1</sup>

<sup>1</sup>NIAB EMR, East Malling, Kent, United Kingdom

<sup>2</sup>Department of Life Sciences, Imperial College London, South Kensington, London

<sup>3</sup>School of Biological Sciences, Whiteknights, University of Reading, Reading, Berkshire  
jwm17@hotmail.com

*Pseudomonas syringae* is a globally important plant pathogen, that includes pathovars that infect over 180 plant species. Individual pathovars are highly specialised and only infect one or a few hosts. *Pseudomonas syringae* uses a range of effector proteins to cause disease. However, plants have evolved to be able to detect these effectors and trigger immunity. Therefore, it is believed that a strain's repertoire of effectors dictates its host range and genetic alteration of these repertoires enables host range expansion. This topic was explored using comparative genomics of three divergent clades that have convergently evolved to cause bacterial canker on *Prunus* species such as cherry and plum. The clades include *P. syringae* pv. *morsprunorum* (*Psm*) (which is differentiated into two races based upon host response) and *P. syringae* pv. *syringae* (*Pss*). Three reference isolates of *Psm* R1, R2, and *Pss* were sequenced with PacBio and the genomes of a further fifteen isolates were sequenced using the Illumina MiSeq. Genomic analysis of the *Prunus* strains has revealed highly divergent effector and toxin repertoires within and between the different clades, indicating they may utilise distinct mechanisms to cause the same disease. A small number of conserved effectors, whose evolution is significantly associated with pathogenicity on *Prunus* were identified. Effectors that have been lost in these clades were expressed in pathogenic strains and found trigger immunity, leading to avirulence on cherry leaves. In conclusion, this work provides an insight into the convergent evolution of pathogenicity and mechanisms controlling the host specificity of bacteria.



## ***In vivo* Control Strategies of Walnut Blight**

**Mine Saraçođlu<sup>1</sup> and Hatice Özakta<sup>2</sup>**

<sup>1</sup>Republic of Turkey Ministry of Food, Agriculture and Livestock, Directorate of Plant Protection Center Institute, Ankara, Turkey

<sup>2</sup>Department of Plant Protection, Faculty of Agriculture, University of Ege, Bornova, Izmir, Turkey  
mine.erdal@tarim.gov.tr

Bacterial blight of walnut caused by *Xanthomonas arboricola* pv. *juglandis* (Xaj) is considered as economically important diseases in all walnut- producing fields, and the severity of outbreaks heavily depends on spring weather conditions. Turkey is one of the prominent country in terms of walnut production among the European Countries. Its production is mostly localized in Marmara and Aegean Regions in Turkey. Copper based compounds used to control Xaj for more than four decades have been resulted copper accumulation in soils, and caused adverse effects on the agricultural environment. The experiments have been conducted during 2007–2008 to control Xaj. Following *in vitro* tests, two main approaches have been examined *in vivo* tests: (i) efficacy of selected biological control agents (single and dual strain treatments) (ii) efficacy of innovative molecules (single or in combine with promising bacterial strains). Application of dual strain treatments of *P. fluorescens* strains WH-48/1a and WH-68 significantly reduced the incidence of bacterial blight of walnut compared with the single strain inoculant. *Pantoea vagans* strain C9-1, tested as reference biocontrol agent against Xaj, effectively inhibited the symptom development of bacterial blight of walnut on seedlings of three walnut cultivars. At the result of *in vivo* tests, Acibenzolar S-methyl (BION-SYGENTA), Prohexadione Ca (REGALIS-BASF) applications and their combined with bacterial strains successfully prevented the development of disease in the sense of integrated management of Xaj.



## Improvement of Phytoplasma Diagnostic Techniques and Use the Techniques at Evaluate Control Strategies

**Naofel Aljafer** and Matthew Dickinson

*School of Biosciences, University of Nottingham, Sutton Bonington, United Kingdom  
sbzna@nottingham.ac.uk*

In our project the aim is to improve the specificity and reliability of phytoplasma diagnostic techniques by using primers that detect specific genes in phytoplasma genomes, and designing new universal primers for conventional and real-time PCR. This has involved developing new assays for 16Sr groups II, III, V, VI, XI, and XII, to facilitate analysis of changes in levels of different phytoplasmas in mixed infections. In addition, this work will involve the evaluation of LAMP (loop mediated isothermal amplification) diagnostic assays for different phytoplasma groups (I, II, III, V, VI, X, and XII) and also validating a rapid DNA extraction method and whether this is effective for all plant species (i.e., Madagascan periwinkle versus Napier grass and other grasses).

The second main objective is to investigate the rate of evolution of phytoplasma genomes. For this, infected plants (of phytoplasma groups 16SrI, II, III, VI, and X) are grafted onto fresh plants at 3–4 month intervals throughout the project. Once the phytoplasma has re-established, DNA will be extracted and a range of genes including 16S rRNA, secA, tuf, and rp are amplified and sequenced. The aim is to determine whether there is any evidence of genome evolution over time.

Furthermore, I am planning to use micro-array experiment, using Affymetrix gene chip, to study the gene expression of phytoplasma disease versus healthy control rice plant by taking samples at different time intervals.



## Responses of Tomato Plants Carrying *Mi-1* Exposed to High Soil Temperatures for Varied Time Periods to *Meloidogyne incognita*

Tevfik Özalp and Zübeyir Devran

Department of Plant Protection, Faculty of Agriculture, University of Akdeniz, Antalya, Turkey  
zdevran@akdeniz.edu.tr

Responses to *M. incognita* of susceptible, heterozygote and homozygote resistant tomato varieties subjected to four different soil temperatures (25, 28, 30, and 32°C) for time periods (6, 12, 24, 48, 120, and 168 h) were separately investigated. First, plants were separately exposed to different soil temperatures for time periods mentioned above. Then, the plants were transferred to another growth chamber. When soil temperature was at 25°C, the plants were inoculated with *M. incognita*. Resistance of tomato varieties was not broken down in the tested all soil temperatures. Second, the plants were separately exposed to different soil temperatures mentioned above for time periods. When soil temperature reached separately at 25, 28, 30, and 32°C, plants were simultaneously inoculated with *M. incognita*. Inoculated plants were held in soil temperatures mentioned for time periods and then, transferred to another growth chamber at 25°C soil temperature. When the plants were held at 32°C soil temperature for 48 h, the number of juveniles in soil and gall, egg masses on root of them increased more than for 24 h. Pf/Pi ratio of heterozygote resistant plants subjected to at 32°C soil temperature for 48 h and more than 48 h was >1. However, Pf/Pi ratio of homozygote resistant plants subjected at 32°C soil temperature for 120 and 168 h was >1. This study showed that resistance was broken in plant held at 32°C soil temperature for 48 h and more. The findings could help efficiency use of tomatoes bearing *Mi-1* grown in fields which are at high soil temperatures ( $\geq 32^\circ\text{C}$ ).

**Keywords:** *Mi-1* gene, tomato, resistance, soil temperature, duration



## Genetic Diversity and Pathotype Differentiation of Pathogens *via* Soft Computing Model Approach

**Ragıp Soner Silme<sup>1</sup>, Ömür Baysal<sup>2</sup>, Hüseyin Gürüler<sup>3</sup> and Musa Peker<sup>3</sup>**

<sup>1</sup>Scientific Projects Department, Istanbul University, Istanbul, Turkey

<sup>2</sup>Department of Molecular Biology and Genetics, Faculty of Life Sciences, Mugla Sıtkı Kocman University, Mugla, Turkey

<sup>3</sup>Department of Information Systems Engineering, Faculty of Technology, Mugla Sıtkı Kocman University, Mugla, Turkey

rsoner@istanbul.edu.tr

Identifying and validating biomarkers' scores of polymorphic bands are important for studies related to the molecular diversity of pathogens. Although these validations provide more relevant results, the experiments are very complex and time-consuming. Besides rapid identification of plant pathogens causing disease, assessing genetic diversity and pathotype formation using automated soft computing methods are advantageous in terms of following genetic variation of pathogens on plants. This study presents a soft computing model for classifying plant pathogens and estimating pathotype differentiation with identification of fungicide resistance levels. In the present study, artificial neural network (ANN) as a soft computing method was applied to classify plant pathogen types and fungicide susceptibilities using the presence/absence of certain sequence markers as predictive features. A plant pathogen, causing downy mildew disease on cucurbits was considered as a model microorganism. Significant accuracy was achieved with PSO-based trained ANNs. Experiments to assess both resistance and pathotype were performed at three stages. First, with calculating of all raw data (of 800 samples) containing smeared and weak input or output values (which were detected by biomarkers during analysis) around 85% success rate was achieved. Secondly, input data containing SW were eliminated. At this stage, around 90% success rate was achieved. In the final step, both input and output data containing SW were eliminated, and 98% success rate was also obtained. This pioneer study for estimation of pathogen properties using molecular markers demonstrates that neural networks achieve good performance for the proposed application.





## **Epidemiology of Tomato Bacterial Wilt Disease (*Clavibacter michiganensis* subsp. *michiganensis*) in Tokat Province**

**Sabriye Belgüzar<sup>1</sup>, Yusuf Yanar<sup>1</sup> and Yeşim Aysan<sup>2</sup>**

<sup>1</sup>Faculty of Agriculture, Department of PlantProtection, Gaziosmanpaşa University, Tokat, Turkey

<sup>2</sup>Faculty of Agriculture, Department of PlantProtection, Çukurova University, Adana, Turkey  
sabriye.yazici@gop.edu.tr

This study was conducted to determine survival of *Clavibacter michiganensis* subsp. *michiganensis* in infested seed, soil, plant residues. Infested seeds rates were determined by plating seeds, which were infected with rifampicin-resistant *Cmm* isolate, on medium at monthly intervals. Also, seeds were planted and disease observations were made in plants to determine the ability of seed inocula to initiate disease. Infested soil was buried in test area within container and bacterial density was calculated in samples taken during summer and winter months. *Cmm* infected plant parts were buried in soil and bacterial density was calculated by sampling in monthly periods. Based on the results of the study, the survival rate of the pathogen in infected seed were decreased with increase in storage periods. The lowest survival rate of 17% was determined at the end of 370 day, but on the 400th day, *Cmm* was not recovered from infected seeds. It has been determined that pathogen could survive in infested soil for 15 and 30 days in summer and winter-periods, respectively. Also, *Cmm* could survive for 30 days in plant residues in the soil. As a result, it has been determined that *Cmm* could not survive in soil and plant residues in Tokat province and could not be transferred to next production season. However, it could be survive in infected seed sand has ability to initiate disease in seedlings. Therefore it is important to use disease free seed sand seedlings to reduce the initial inoculum source and the disease development under field conditions.

**Keywords:** epidemiology, *Clavibacter michiganensis* subsp. *michiganensis*, inoculum source, tomato bacterial wilt

## Reactions of Some Grape Varieties to Downy Mildew (*Plasmopara viticola* (Berk. et Curt.)) in Eğirdir Conditions

**Seçkin Gargin** and **Yusuf Öztürk**

General Directorate of Agricultural Research and Policies, Fruit Research Institute, Eğirdir, Isparta, Turkey  
secking32@hotmail.com

Downy mildew is the single most damaging disease of grapes due to the warm and wet climate during the vegetative growth of the vine. Downy mildew injures grapes by causing deformed shoots, tendrils and clusters. A major outbreak of this disease can cause severe losses in yield and quality. This study was conducted to determine tolerances of 10 different grape varieties tolerances to downy mildew (*Plasmopara Viticola*) in Eğirdir/Isparta/Turkey. It was done between 2010 and 2011 years at Eğirdir Fruit Research Institute vineyard area which was planted in 2005 year. Ten different local and popular grape varieties were evaluated for downy mildew (*Plasmopara Viticola*) in natural inoculation conditions. Local varieties Pembe Gemre, Siyah Gemre, Senirkent Dimridi, Burdur Dimridi, and popular varieties Trakya İlkeren, Sultani Çekirdeksiz, Alphonse Lavalee, Ata Sarısı, Italia, and Red Globe were evaluated. Counting was made in 12 grapevines with replicates. In each replicate 12 grapevine X 12 leaf samples were evaluated for disease index. 0–4 scale was used to determine “disease index”. “Index Formula” was used to evaluate disease index. All evaluations were done between 2010 and 2011 years. According to 2 years data were statistically analyzed. Red Globe, Burdur Dimridi, and Italia grape varieties were evaluated the most tolerant and Pembe Gemre, Siyah Gemre, and Trakya İlkeren grape varieties were evaluated the least tolerant varieties.

**Keywords:** Downy mildew, grape, variety, viticulture

## **Molecular Variability of CI Protein of *Zucchini yellow Mosaic virus* Isolates Affecting Cucurbits in Turkey**

**S. Topkaya<sup>1</sup>, F. Ertunç<sup>2</sup> and C. Desbiez<sup>3</sup>**

<sup>1</sup>Faculty of Agriculture, Department of Plant Protection, Gaziosmanpasa University, Tokat, Turkey

<sup>2</sup>Faculty of Agriculture, Department of Plant Protection, Ankara University, Ankara, Turkey

<sup>3</sup>INRA, UR407 Pathologie Végétale, Domaine Saint Maurice, Montfavet, France  
serife.topkaya@gop.edu.tr

*Zucchini yellow mosaic virus* (ZYMV) is economically the most important virus disease in cucurbit growing area in Turkey. This virus is a member of the genus Potyvirus of the family Potyviridae. ZYMV genome consists of a single polyprotein precursor that is subsequently processed into about ten proteins (P1 protein (P1), helper component protein (HC-Pro), P3, cylindrical inclusion (CI), NIa, NIb, and coat protein CP) by three virally encoded proteases. Encoded proteins have different functions in expression. CI protein is involved in RNA helicase activity, ATPase activity and cell to cell movement in potyviridae. In this study, the cylindrical inclusion body (CI) protein genes of ZYMV isolates provided from different cucurbit growing areas around Turkey were sequenced and compared with other sequences from GenBank. The CI protein of Turkish ZYMV was 888 nucleotide long and encoded 296 amino acids (aa). Phylogenetic analysis of nucleotide sequences of CI region revealed that majority of isolates belong to a major molecular subgroup (A1), the most common in Europe and in the world, and three isolates related to molecular group (A5). According to coat protein nucleotide analysis, however, these three isolates grouped with molecular subgroup A4, the emerging group recently in Europe. According to aa analysis of CI ZYMV nucleotide binding motif (NTBM) and RNA helicase activity region (five motifs) were conserved among the isolates.

**Keywords:** *Zucchini yellow mosaic virus*, cylindrical inclusion protein, potyviridae, Turkey



## New Generation Sequencing Technologies for Diagnosis of Viruses and Viroids in Grapevine

Serkan Önder<sup>1</sup>, Antonio Olmos<sup>2</sup>, Z. Çiğdem Ulubaş Serçe<sup>3</sup> and Neslihan Öztürk Gökçe<sup>4</sup>

<sup>1</sup>Manisa Viticulture Research Institute, Republic of Turkey Ministry of Food, Agriculture and Livestock, Yunusemre, Manisa, Turkey

<sup>2</sup>Instituto Valenciano de Investigaciones Agrarias, Moncada, Valencia, Spain

<sup>3</sup>Department of Plant Production and Technologies, Ayhan Şahenk Faculty of Agricultural Sciences and Technologies, Ömer Halisdemir University, Niğde, Turkey

<sup>4</sup>Department of Agricultural Genetic Engineering, Ayhan Şahenk Faculty of Agricultural Sciences and Technologies, Ömer Halisdemir University, Niğde, Turkey

onderserkan@gmail.com

In grapevine, 65 viruses and 5 viroids have been recorded until now which represents the highest number of viral agents ever found in a single crop. Consequently, the reliable and specific detection of all the viruses and viroids present in a sample are a major issue that can be difficult to solve by available serological and molecular methods. The new next generation sequencing (NGS) technologies has drastically changed the diagnostic field allowing the simultaneous sequencing of millions of nucleic acids present in a sample, including those derived from viral agents. In addition, NGS has importantly increased the knowledge of grapevine virome facilitating the discovery of new viruses. A grapevine sample exhibiting severe vein banding and mosaic symptoms was selected for NGS analysis. Total RNA was extracted and rRNA depletion was performed by treatment of Ribo-Zero rRNA Plant Removal Kit. NEBNext<sup>®</sup> Ultra<sup>™</sup> RNA Library Prep Kit was used for library preparation. Deep sequencing was performed using Illumina Hiseq2000 RNAseq technology with 2 × 150 read length and 40 million depths for each reads. Deep sequencing analysis yielded around 111 M sequences in total. Bioinformatic analysis was performed using Geneious R10 and CLC Genomic Workbench v.10 softwares. Following *de novo* assembling, contigs were analysed by blast against NCBI using blastn, to detect known viruses. Detected viruses were further studied by two different approaches (i) mapping all reads against reference sequences without host genome subtraction and (ii) mapping as previously but with host genome subtraction. *Grapevine Pinot gris virus* (GPGV), *Grapevine deformation virus* (GDefV), and *Grapevine yellow speckle viroid-1* (GYSVd-1) were detected with similar coverage in both cases. However, *Grapevine syrah virus-1* (GSYV-1), *Grapevine fanleaf virus* (GFLV), *Grapevine Roditis leaf discoloration-associated virus* (GRLDaV) and *Hop stunt viroid* (HSVd) had higher coverage ratios when host genome was present.



*Grapevine rupestris stem pitting associated virus* (GRSPaV) had higher coverage ratio without host genome subtraction. Although the presence of all these viruses and viroids should be confirmed by PCR-based methods and Sanger sequencing, these results show that the subtraction of host genome has to be carefully evaluated in order to optimize a reliable detection of viruses and viroids.

### **Acknowledgement**

This study supported by the project TUBITAK-115O014 and COST-DIVAS of COST FA-1407 Action.



## Weed Control with Total Herbicides in Conventional Crop Production System

**Ahmet Tansel Serim, Ünal Asav and Ercan Koca**

*Plant Protection Central Research Institute, Ankara, Turkey  
a\_serim@hotmail.com*

Controlling annual weeds, which compete with sunflower using selective herbicides, is possible in conventional crop production system (CCPS), but this opportunity is unlikely to be effective to control perennial weeds because of limited efficacy of these herbicides in many times. The troublesome weeds such as Bermuda grass, Canada thistle, field bindweed, and Russian knapweed cannot be controlled by selective herbicides in CCPS. Glufosinate and glyphosate, on the other hand, are effective herbicides approved for use by glyphosate-resistant or glufosinate-resistant crop system to control annual and perennial weeds; however, they can cause severe injury on crops if they are applied using traditional field sprayer in CCPS. Banded herbicide application (BHA) technique give a new opportunity to control these weed species using total herbicides in CCPS. The main objective of this study was to control inter-row weeds with glyphosate and glufosinate and intra-rows weeds with aclonifen in sunflower fields. Recommended rates of herbicides (glyphosate at 1.44 and 2.88 kg a.i. ha<sup>-1</sup>, glufosinate at 0.6 and 1.5 kg a.i. ha<sup>-1</sup>, and aclonifen at 0.75 kg a.i. ha<sup>-1</sup>) were used. Field studies were performed at two locations, Tokat and (Gölbaşı) Ankara, Turkey in 2016. Efficacy of herbicides on weed suppression and response of sunflower to them were determined 30 days after treatment and at harvest, respectively. All inter-rows weeds were effectively controlled by glyphosate and glufosinate at both rates while aclonifen solely controlled wild mustard, lamb's quarters and purple dead-nettle. Using BHA not only can help to control perennial weeds in CCPS but also give a new opportunity to control herbicide resistant weed species.

**Keywords:** banded herbicide application, glyphosate, glufosinate, sunflower



## Phytotoxic Effects of Foramsulfuron on Photosynthetic Pigments and Anthocyanin Contents in *Zea mays* L.

**Tuğçe Erürker and Gül Cevahir Öz**

*Institute of Graduate Studies in Science and Engineering, Istanbul University, Istanbul, Turkey*  
*tugce.erurker@gmail.com*

The purpose of this study is; the herbicide foramsulfuron, which is used extensively in the economically valuable maize plant, can exert its effect outside the target organism. Foramsulfuron, an acetolactate synthase (ALS) inhibitor, is a herbicide in the sulphonylurea family. Urea and triazine, which are found in the main structure of the sulphonylurea family, inhibit the primary effect of amino acid synthesis. Secondary effect is to stop photosynthesis, respiration, and protein synthesis. Foramsulfuron herbicide applications; (330  $\mu\text{M}$ ) for recommended dose, twice the recommended dose (660  $\mu\text{M}$ ), three times the recommended dose (990  $\mu\text{M}$ ), and four times the recommended dose (1320  $\mu\text{M}$ ). It was applied after germination of *Zea mays* L. plant. Chlorophyll a, chlorophyll b, carotenoid, and contents of total anthocyanin (on the 5<sup>th</sup>, 10<sup>th</sup>, and 15<sup>th</sup> days of foramsulfuron application) were investigated in corn plants after germination. Chlorophyll a and chlorophyll b levels were higher in the 5<sup>th</sup> day and 15<sup>th</sup> day of foramsulfuron application than in the control groups. However, on the 10<sup>th</sup> day, chlorophyll a increased, while chlorophyll b decreased. On the other hand, carotenoid amounts decreased remarkably in the same groups. The variation in the content of anthocyanins on different days are parallel to the increase in the amount of concentration. The highest amount of anthocyanin was determined at the recommended dose (330  $\mu\text{M}$ ) and on 15<sup>th</sup> day analysis.



## Synthetic Elicitor DPMP Increases Innate Plant Resistance to Pathogens

**Yasemin Bektas<sup>1,2</sup>, Melinda Rodriguez-Salus<sup>1</sup>, Mercedes Schroeder<sup>1,3</sup>, Adilene Gomez<sup>1</sup>, Isgoushi Kaloshian<sup>4</sup> and Thomas Eulgem<sup>1</sup>**

<sup>1</sup>Center for Plant Cell Biology, Department of Botany and Plant Sciences, University of California, Riverside, CA, United States

<sup>2</sup>ChemGen Integrative Graduate Student Education and Research Traineeship Program, Center for Plant Cell Biology, Department of Botany and Plant Sciences, University of California, Riverside, CA, United States

<sup>3</sup>Department of Molecular Biology and Genetics, Faculty of Science and Arts, Gaziosmanpaşa University, Tokat, Turkey

<sup>4</sup>Center for Plant Cell Biology, Department of Nematology, University of California, Riverside, CA, United States

yas.aktas@gmail.com

Plants serve as a source of nutrients for a wide variety of heterotrophic microorganisms that can cause diseases in their hosts. To defend themselves against invading pathogens plants utilize a complex regulatory network that coordinates extensive transcriptional and metabolic reprogramming. Although many of the key players of this immunity-associated network are known, the details of its topology and dynamics are still poorly understood. As an alternative to forward and reverse genetic studies, chemical genetics-related approaches based on bioactive small molecules have gained substantial popularity in the analysis of biological pathways and networks. Initiating a chemical genomics-related study on the plant immune system, we identified by high throughput screening 114 synthetic elicitors. Synthetic elicitors are small drug-like molecules that induce plant defense responses, but are distinct from known natural elicitors of plant immunity. They can protect plants from diseases by activating host immune responses and can serve as tools for the dissection of the plant immune system as well as leads for the development of environmentally-safe pesticide alternatives. Here, we report on the characterization of one of these synthetic elicitor identified by our previous high throughput screen, 2,4-dichloro-6-((E)-[(3-methoxyphenyl)imino]methyl)phenol (DPMP). DPMP strongly triggers disease resistance of Arabidopsis against bacterial and oomycete pathogens and has a unique mode of action. mRNA-seq analysis reveal transcriptional profiles triggered by DPMP to resemble typical defense-related responses.





## Development of Primary Genomic Resources for Securing Sustainable Hazelnut Production in Turkey

Andrew J. Helmstetter<sup>1</sup>, Nihal O. Erol<sup>2</sup>, Stuart J. Lucas<sup>2</sup> and Richard J. A. Buggs<sup>1,3</sup>

<sup>1</sup>Jodrell Laboratory, Royal Botanic Gardens, Kew, Richmond, Surrey, United Kingdom

<sup>2</sup>Sabancı University Nanotechnology Research and Application Center (SUNUM), Sabancı University, Orhanlı, Tuzla, Istanbul, Turkey

<sup>3</sup>School of Biological and Chemical Sciences, Queen Mary University of London, London, United Kingdom  
a.helmstetter@kew.org

The European hazelnut (*Corylus avellana*) is Turkey's most valuable agricultural export. 70–80% of the world's hazelnut market is produced in Turkey and a significant proportion of the rural Black Sea population rely on hazelnut for their primary income. Despite this, little work has been done to understand genomic variation within hazel. This project will generate genomic resources that will act as an initial step towards securing sustainable hazelnut production in Turkey. By using high-throughput, reduced-representation sequencing, we aim to lay the foundations for improving resistance to drought, frosts and an emerging disease in this relatively understudied yet economically important species. We aim to assess the genetic diversity within and among over 100 hazelnut cultivars and wild populations, and to determine whether any diversity uncovered is linked to cold/drought tolerance or resistance to an emerging powdery mildew threat. This disease, caused by a fungus of the genus *Erysiphe* (Section "Microsphaera"), is considered by Turkish producers to be the most significant immediate threat to hazelnut production today. This work will provide a platform for more targeted genomics research in the future that can enhance the speed at which trees can be bred, through selection of beneficial gene combinations in seedlings.



## Determination of Mixed *Fusarium* spp. Infections Causing Dryings in Pepper Growing Areas in the GAP Region of Turkey

Aysin Bilgili<sup>1</sup>, Hakan Fidan<sup>2</sup>, Seral Yücel<sup>3</sup> and M. Ertugrul Güldür<sup>4</sup>

<sup>1</sup>Department of Plant Health, GAP Agricultural Research Institute, Sanliurfa, Turkey

<sup>2</sup>Faculty of Agriculture, Department of Plant Protection, Akdeniz University, Antalya, Turkey

<sup>3</sup>Department of Plant Pathology, Biological Control Research Institute, Adana, Turkey

<sup>4</sup>Faculty of Agriculture, Department of Plant Protection, Harran University, Sanliurfa, Turkey  
ahb4463@hotmail.com, aysin.bilgili@tarim.gov.tr

Pepper (*Capsicum annuum* L.) is a significant cultural crop grown in Turkey and especially in GAP region. Around 2457,822 tonnes is produced yearly in 815,632 da areas in Turkey. 121,368 da and 267,868 tonnes of this production is obtained from GAP region. Pepper production is economically a significant potential for the region. The aim of this study was to determine the root soil borne fungi diseases that cause commonly dryings in pepper grown areas. These disease factors cause reductions in quality and yield of pepper. With the purpose of identifying these disease agents in the region a survey study was performed in 2013 and 2014 years in a total of nine cities; Sanliurfa, Adiyaman, Mardin, Kilis, Gaziantep, Diyarbakır, Batman, Sırnak, and Siirt. The survey was performed in around 10% of the GAP region with a total pepper production. Isolation, pathogenicity and diagnosis studies of 104 and 193 pepper fields having disease symptoms were performed, respectively, in 2013 and 2014 years. As a result of survey studies morphological diagnoses of *Fusarium* species were made and *Fusarium solani*, *F. oxysporum*, and *F. oxysporum* f.sp. *vasinfectum* were commonly detected in the region. Molecular and scene analyses of some of these isolates whose morphological diagnoses were performed were made. ITS-Fuf-r, ITS-Fs5f-r and ITS-Fu2f-r, Clox 1-2, and Fov1egf-r primers were used, respectively, for *Fusarium* spp., *F. solani*, *F. oxysporum*, *F. oxysporum* f. sp. *vasinfectum*. The protocol of Thermo Scientific GeneJET Plant Genomic DNA Purification Mini Kit in Plant Genomic DNA purification was used. In the course of molecular studies, 2 samples from 2013 and 14 samples from 2014 were detected as “Mixed *Fusarium* spp. infections” showing positive response reactions performed with sample primers.

**Keywords:** pepper, *Fusarium* spp., mixed, GAP region, Turkey

### Acknowledgements

This study has been financially supported by TAGEM (General Directorate of Agricultural Researchers and Politics) and HUBAK (Harran University Scientific Researcher Commission) as a part of the Ph.D study.



## **COST Action FA1407: Empowering NGS Technologies for the Study and Diagnostic of Plant Viruses**

**Çiğdem Ulubaş Serçe, Serkan Önder, Antonio Olmos, Neil Boonham, Carmen Büttner, Thierry Candresse, Rosario Felix, Isabel Font, Miroslav Glasa, Risto Jalkanen, Petr Kominek, Margit Laimer, Tadeusz Malinowski, Varvara Maliogka, Angelanotio Minafra, Nelia Ortega Parra, Annalisa Poliverari, Maja Ravnikar, Dana Safarova, Rene Vandervlugt, Christina Varveri, Johanna Witzell, Ioan Zagrai, Thierry Wetzl, Massart Sebastien<sup>†</sup>**

*Laboratory of Phytopathology, University of Liege, Gembloux Agro-BioTech, Gembloux, Belgium  
sebastien.massart@ulg.ac.be*

<sup>†</sup>*The affiliations and addresses of the contributors of this communication are listed in the COST website at the following address: [http://www.cost.eu/COST\\_Actions/fa/Actions/FA1407?management](http://www.cost.eu/COST_Actions/fa/Actions/FA1407?management)*

Plant viral diseases are currently a major economic problem in agriculture throughout the world. The objective of the COST Action is to coordinate and raise the European capacity to apply Next Generation Sequencing (NGS) technologies for the study and diagnosis of viral diseases in vegetatively propagated plants, seeds and seedlings. NGS enables rapid and reliable holistic virus identification (indexing), which is needed for the development of innovative, knowledge-based solutions for plant production. By bringing together a multidisciplinary and multi-actor consortium, the action will ensure cost-effective research and build up a strong Pan-European knowledge-based network for better control of established, emerging and exotic viral plant diseases. The action will deliver new scientific knowledge about viral plant diseases that are currently poorly understood, and contribute to the development of more effective surveillance of stock material health and to the improvement of quarantine procedures. The Action thus implements the European strategy of integrated pest management and protection against harmful plant pathogens and contributes to the securement of food production. The impact of DIVAS will target three levels: 1. Improving the technological level: networking and developing a collaboration platform to produce validated protocols for specific NGS applications in diagnostics. 2. Streamlining the basic research: Associating on-going research projects to the Action. 3. Bringing technical and social innovations: Improving plant virus control at European borders and within European territories through scientific and evidence-based recommendations for a safer plant trade inside and outside Europe to Policy Makers, National and European Plant Protection Organization and diagnostic laboratories.



## Several Aspects of Harpin Signaling and Its Role in Plants

**Özlem Yildiranca<sup>1</sup> and Salih Canavar<sup>2</sup>**

<sup>1</sup>Plant Breeding and Genetic Department, Nazilli Cotton Research Institute, Aydin, Turkey

<sup>2</sup>ProGen Seed, Aydin, Turkey

yildiranca@gmail.com

Plant immunization is the activating natural defense system in plant induced by biotic or abiotic factors. Plants are treated with activating agents to stimulate plant defense responses that form physical or chemical barriers that are used from plant to ward off diseases. Inducers are used to give the signals to promulgates the enhanced defensive capacity throughout the plant defence genes ultimately resulting into induced the systemic resistance. Plant's natural defense mechanism, known as the systemic acquired resistance (SAR) pathway is a mechanism of induced defense that confers long-lasting protection against a broad spectrum of microorganisms. The initial component of the natural defense mechanism of higher plants, the hypersensitive response (HR), is associated with plant defense against many bacteria, fungi, viruses and nematodes. The HR is characterized by the rapid, localized death of tissues affected by a pathogen. Understanding of the biochemical pathway leading to this resistance could enable the development of plant protection compounds that act by stimulating the plant's inherent disease resistance mechanisms. Harpin proteins are elicitors produced by several gram-negative plant pathogenic bacteria, triggering multiple beneficial responses in plants, such as induction of defense response against diverse pathogens and insects, growth promotion, and drought tolerance. Some of the biotic or abiotic determinants induce systemic resistance in plants through Harpin protein dependent SAR pathway. These protein regulate the function of many structure including stress responsive structures, resulted the phenotypic response of stress tolerance. This review article describes several aspects of harpin signaling and its role in plants.

**Keywords:** natural defense system, systemic acquired resistance (SAR), hypersensitive response (HR), harpin, elicitors



## Inhibitory Effect of Antifungal Activity of Titanium Dioxide (TiO<sub>2</sub>) Nanoparticles on Some Pathogenic *Fusarium* Isolates

Taner Şar<sup>1,2</sup>, Iğın Akpınar<sup>2</sup> and Muammer Ünal<sup>3</sup>

<sup>1</sup>Department of Molecular Biology and Genetics, Gebze Technical University, Kocaeli, Turkey

<sup>2</sup>Institute of Graduate Studies in Science and Engineering, Istanbul University, Istanbul, Turkey

<sup>3</sup>Science Faculty, Department of Botany, Istanbul University, Istanbul, Turkey

st.taner@gmail.com

Emerging infectious diseases and the increase in incidence of pesticide resistance among plant pathogenic fungus have made the search for new antifungals inevitable. In the current situation, one of the most promising and novel therapeutic agents are the nanoparticles. Because of this reasons, this study examined the potential antifungal activity of titanium dioxide nanoparticles on *Fusarium* isolates, which can cause disease in tomato plants. Titanium dioxide (TiO<sub>2</sub>) nanoparticles are manufactured worldwide in large quantities for use in a wide range of applications including pesticide, pigment and cosmetic manufacturing. Recently, several studies have suggested that TiO<sub>2</sub> application suppresses bacterial and fungal pathogens of culture plants. We used “anatase, 16 wt%, 3–12 nm” titanium dioxide nanoparticles (TiO<sub>2</sub>NPs) at concentrations of 50, 100, 150, and 200 ppm. In our study, FORL (*Fusarium oxysporum f. sp. radialis lycopersici*) isolates and FOL (*Fusarium oxysporum f. sp. lycopersici*) isolates isolated from Turkey were used. Six isolates (AY5, H2, JR, SC, TR-1, and TA-4) from FORL and 4 isolates (EM2, FL, HT, and J2) from FOL were tested for the evaluation of antifungal activity of different concentration of titanium dioxide nanoparticles. The inhibitory effects of TiO<sub>2</sub> were determined as ranged from 43 to 93% for FORL isolates, and ranged from 59 to 97% for FOL isolates. Thus, it was determined that titanium dioxide showed antifungal activity against *Fusarium* isolates. According to the results obtained, titanium dioxide nanoparticles (TiO<sub>2</sub>) has been determined to be an important protective effect against fungal contaminants and could be used effectively in agricultural activities.

**Keywords:** antifungal activity, *Fusarium oxysporum*, titanium dioxide nanoparticles



## A Preliminary Study of *Pyrenophora teres* Mating Type Distribution in Turkey

**Arzu Çelik Oğuz<sup>1</sup>, Fatih Ölmez<sup>2</sup> and Aziz Karakaya<sup>1</sup>**

<sup>1</sup>Faculty of Agriculture, Department of Plant Protection, Ankara University, Dışkapı, Ankara, Turkey

<sup>2</sup>Central Research Institute for Field Crops, Yenimahalle, Ankara, Turkey

*Pyrenophora teres* f. *maculata* (*Ptm*) and *Pyrenophora teres* f. *teres* (*Ptt*) cause spot form and net form of net blotch diseases of barley, respectively. Although both forms of *Pyrenophora teres* are morphologically similar, their symptoms and genetic background are different from each other. In this study, 50 single spore isolates, obtained from different regions of Turkey, were evaluated for their mating type prevalence. Fungal isolates of both forms were differentiated using form-specific PCR primers and symptoms on the Susceptible cultivar Bülbül 89. For mating type determination studies, duplex PCR was performed using MAT-specific single nucleotide polymorphism primers. Fourteen and 16 of 30 *Ptm* isolates were found as MAT1-1 and MAT1-2 types, respectively and 15 and 5 of 20 *Ptt* isolates were found as MAT1-1 and MAT1-2 types, respectively. These results show the possibility of sexual reproduction in Turkey among the *Pyrenophora teres* isolates. As a result of new sexual combinations more virulent pathotypes may occur. This is the first study related to mating types of *Pyrenophora teres* isolates in Turkey.



## Random Mutagenesis Screen Shows That *Phytophthora capsici* CRN83\_152 Mediated Cell Death Is Not Required for Its Virulence Function(s)

Tiago M. M. Amaro<sup>1,2</sup>, Gaëtan J. A. Thilliez<sup>1,2,3</sup>, Rory A. Mcleod<sup>1,2</sup> and Edgar Huitema<sup>1,2</sup>

<sup>1</sup>Division of Plant Science, School of Life Sciences, University of Dundee at the James Hutton Institute (JHI), Invergowrie, Dundee, United Kingdom

<sup>2</sup>Dundee Effector Consortium, JHI, Invergowrie, Dundee, United Kingdom

<sup>3</sup>Cell and Molecular Sciences, JHI, Invergowrie, Dundee, United Kingdom

tiago.amaro@hutton.ac.uk

With the increasing availability of plant pathogen genomes, secreted proteins that aid infection (effectors) have emerged as key factors that help govern plant-microbe interactions. The conserved CRN (CRinkling and Necrosis) effector family was first described in oomycetes by their capacity to induce host cell death. Despite recent advances towards elucidation of CRN virulence functions, the relevance of CRN induced cell death remains unclear. *In planta* over-expression of PcCRN83\_152, a CRN effector from *P. capsici*, causes host cell death and boosts *P. capsici* virulence. We used these features to ask whether PcCRN83\_152 induced cell death is linked to its virulence function. By randomly mutating this effector, we generated PcCRN83\_152 variants with no cell death phenotypes (NCD), which were subsequently tested for activity towards enhanced virulence. We show that a subset of PcCRN83\_152 NCD variants retain their ability to boost *P. capsici* virulence. Moreover, NCD variants were shown to have a suppressive effect on PcCRN83\_152 mediated cell death. Our work shows that PcCRN83\_152 induced cell death and virulence function can be separated. This work in turn, will provide a framework for studies aimed at unveiling the virulence functions of cell death inducing CRN effectors.



## Morphological Characteristics of Black *Aspergillus* spp. Collected from Manisa Province and Their Sensitivity Against Some Fungicides

Berrin Alaca<sup>1</sup> and Pervin Kinay teksür<sup>2</sup>

<sup>1</sup>Çanakkale Directorate of Provincial Food Agriculture and Livestock, Çanakkale, Turkey

<sup>2</sup>Faculty of Agriculture, Department of Plant Protection, Ege University, Bornova, İzmir, Turkey

The major problem on raisins is not only residue but also ochratoxin A produced by black *Aspergillus* spp. Twenty *Aspergillus* spp. isolates were obtained from Sultani Seedless vineyards in Manisa province. The isolation of black *Aspergillus* spp. were obtained from different stages and plant parts such as buds, flowers, bunches and berries starting from pre-flowering period until the ripening period of the fruit periodically in the vineyards. The identification of 20 isolates of *Aspergillus* spp. was carried out by light microscopy in terms of their morphological characteristics. When the conidiophore structure, conidiomorph size, shape, roughness of the conidiomatic surface were examined, it was identified that 17 of isolates were *Aspergillus niger*, 1 was *A. carbonarius*, 2 isolate were *A. japonicus*. In the study on the sensitivity of isolates were tested against 8 doses of 17 different fungicides. The lowest ED<sub>50</sub> levels was obtained for prochloraz (0.01–0.08 ppm) followed by fludioxonil (0.01–0.09 ppm). Both fungicides are modern fungicides and sterol biosynthesis inhibitors. The isolate no. 14 was identified as the most sensitive isolate with the lowest dose of 0.01 ppm for the prochloraz, fludioxonil, and tebuconazole fungicides. When isolates are examined separately, they can be designated as isolate no. 1 resistant to almost to all tested fungicides. Trifloxystrobin, azoxystrobin and kresoxim methyl, thiophanate methyl had mean of 30 ppm ED<sub>50</sub> values for almost all isolates. From the classical fungicides, captan, mancozeb and metiram, ED<sub>50</sub> values were ranged from 1.78 to 5.27 ppm and the efficacy was lower compared to modern fungicides.

**Keywords:** black *Aspergillus* spp., morphology, fungicide sensitivity, ED<sub>50</sub> values





## Walnut Blight: Control Strategies

**Mine Saraçođlu<sup>1</sup> and Hatice Özakta<sup>2</sup>**

<sup>1</sup>Republic of Turkey Ministry of Food, Agriculture and Livestock, Directorate of Plant Protection Center Institute, Ankara, Turkey

<sup>2</sup>Department of Plant protection, Faculty of Agriculture, University of Ege, Bornova, Izmir, Turkey  
mine.erdal@tarim.gov.tr

Bacterial blight of walnut caused by *Xanthomonas arboricola* pv. *juglandis* (Xaj) is considered as economically important diseases in all walnut orchards, and the severity of outbreaks heavily depends on spring weather conditions. Turkey is a major producer of walnuts and copper based compounds have been used to control Xaj for more than four decades which has resulted in copper accumulation in soils, and has caused adverse effects on the agricultural environment. Experiments were conducted during 2007–2008 to control Xaj. Following *in vitro* tests, two main approaches were examined *in vivo*: (i) efficacy of selected biological control agents (single and dual strain treatments) and (ii) efficacy of innovative molecules (single or in combine with promising bacterial strains). Application of dual strain treatments of *P. fluorescens* strains WH-48/1a and WH-68 significantly reduced the incidence of bacterial blight of walnut compared with the single strain inoculant. *Pantoea vagans* strain C9-1, tested as reference biocontrol agent against Xaj, effectively inhibited the symptom development of bacterial blight of walnut on seedlings of three walnut cultivars. Also *in vivo* tests showed Acibenzolar S- methyl (BION-SYGENTA), Prohexadione Ca (REGALIS-BASF) applications and their combined with bacterial strains successfully prevented the development of disease, so are useful for integrated management of Xaj.

## Net Blotch Resistant *Hordeum Spontaneum* Genotypes Identified

**Arzu Çelik Oğuz<sup>1</sup>, Aziz Karakaya<sup>1</sup>, Rukiye Murat Duran<sup>2</sup> and Kürşat Özbek<sup>2</sup>**

<sup>1</sup>Faculty of Agriculture, Plant Protection Department, Ankara University, Disıkapı, Ankara, Turkey

<sup>2</sup>Central Research Institute for Field Crops, Yenimahalle, Ankara, Turkey

acelik@agri.ankara.edu.tr

Wild barley (*Hordeum spontaneum*) is a progenitor of cultivated barley and naturally grows in Turkey. *Hordeum spontaneum* genotypes possess superior characteristics for biotic and abiotic stress tolerance factors. In this study, three virulent *Pyrenophora teres* f. *maculata* and 3 virulent *P. teres* f. *teres* isolates were tested under greenhouse conditions in order to find net blotch resistant *H. spontaneum* genotypes. A total of 104 *H. spontaneum* genotypes were used. Twenty-six *H. spontaneum* genotypes which corresponded to 25% of the genotypes (genotypes numbered 8, 13, 14, 16, 22, 24, 27, 31, 37, 44, 47, 54, 58, 62, 65, 66, 69, 74, 78, 81, 89, 94, 99, 102, 104, and 107) exhibited reactions classified the resistant group to 3 virulent *P. teres* f. *maculata* isolates. Eight *H. spontaneum* genotypes which corresponded to 7.6% of the genotypes (genotypes numbered 24, 27, 29, 33, 44, 54, 89 and 94) exhibited reactions classified the resistant group to 3 virulent *P. teres* f. *teres* isolates. Six *H. spontaneum* genotypes which corresponded to 5.7% of the genotypes (genotypes numbered 24, 27, 44, 54, 89, and 94) exhibited reactions in the resistant group to both 6 virulent *P. teres* f. *teres* and *P. teres* f. *maculata* isolates. In addition, a considerable number of genotypes exhibited resistant group reactions to one or two isolates of both forms of the pathogen. These genotypes could be used for developing net blotch resistant barley cultivars.

## Postharvest Decays on Pomegranate Fruit (*Punica granatum* L. var Hicaz) in Cold Storage Conditions

Pervin Kinay Teksür<sup>1</sup>, Fatih Şen<sup>2</sup> and Figen Yıldız<sup>1</sup>

<sup>1</sup>Faculty of Agriculture, Department of Plant Protection, Ege University, Bornova, İzmir, Turkey

<sup>2</sup>Faculty of Agriculture, Department of Horticulturae, Ege University, Bornova, İzmir, Turkey  
pervin.kinay@ege.edu.tr

As a result of this, the planting, growing and storing of pomegranate rate also has been increased. There are lots of fungal pathogens of pomegranate fruit that infected from orchard and caused important losses on storage period. The purpose of this study is to determine the postharvest diseases on pomegranate fruits stored in cold storage condition. In addition, it was studied to determine the relationships between the postharvest treatments and diseases. In this context, totally 33 cold storage facilities in Antalya, Manisa (Alaşehir), and İzmir were visited and samples were taken from cold storage rooms. The information sheet filled for each cold storage room. Fruit showing symptoms of the disease were brought to the laboratory as an example and isolations were made from them. The average incidence rate of the disease was ranged from 30 to 50% in cold storage rooms. As a result of counting from 33 cold storage rooms, it was found that *Botrytis cinerea* was the most dominant pathogen with average proportion of 40% diseases incidence. *Coniella granati* by 15%, *Penicillium* species by 12%, *Alternaria alternata* 12%, *Aspergillus niger* by 8%, Pathogenicity tests of these diseases agents were done. *B.cinerea* and *A. alternata* were found two major pathogens causing the most important postharvest losses due to infection in the orchards. In addition, *C. granati* becomes important at the end of storage period.

**Keywords:** cold storage, *Punica granatum*, *Botrytis cinerea*, *Alternaria* spp.

## Cadmium Treatment and Photosystem II Responses in Tropical Invasive Species

**Freddy Can Igiebor<sup>1</sup>, Gülriz Bayçu kahyaoğlu<sup>1</sup>, Michail Moustakas<sup>1</sup> and Utku Gültopu<sup>2</sup>**

<sup>1</sup>Faculty of Science, Department of Biology, Division of Botany, Istanbul University, Suleymaniye, Istanbul, Turkey

<sup>2</sup>Faculty of Engineering, Department of Computer Science, Koc University, Sariyer, Istanbul, Turkey  
freddy.igiebor@hotmail.com

*Ailanthus altissima* is a noxious weed and an invader in many countries. It tolerates broad amplitude of climatic conditions. Cadmium (Cd) is an effective inhibitor of plant metabolism. In this study, the relation between heavy metal accumulation rate and photosynthetic quantum yield of non-regulated energy dissipation (Y(NO)) were examined to understand the invasive plant health. 15-day-old plants grown in perlites transferred to the hydroponic culture for 11 day, then different concentrations of Cd (5, 10, 20, 40, and 80  $\mu\text{M}$ ) were applied to plants during 7, 15, and 30-day period. The amount of Cd in each plant group was measured by ICP-OES and compared with the efficiency of photosynthesis (measured by IMAGING-PAM) which is related to plant health.

*For 7-Day Plants:* The maximum Cd accumulation in leaf (1391 mg/kg) and the highest Y(NO) (0.671) has been observed in the group which has been applied 80  $\mu\text{M}$  Cd.

*For 15-Day Plants:* The maximum Cd accumulating leaf with 1143 mg/kg is in the group which has been applied 40  $\mu\text{M}$  Cd and the leaf with the highest Y(NO) (0.456) is in the group which has been applied 20  $\mu\text{M}$  Cd.

*For 30-Day Plants:* The maximum Cd accumulating leaf with 1851 mg/kg is the group which has been applied 40  $\mu\text{M}$  Cd and with the highest Y(NO) (0.419) is the control group which has not been applied Cd.

The obtained findings are expected to contribute to understand role of the quantum yield of nonregulated energy dissipation in abiotic stress.

**Keywords:** *Ailanthus altissima*, invasive species, photosynthesis response, heavy metal

## Effects of Some Antagonists as Seed Treatments on Biological Control of Watermelon Fruit Blotch

**Sumer Horuz<sup>1</sup> and Yesim Aysan<sup>2</sup>**

<sup>1</sup>Faculty of Agriculture, Plant Protection Department, Erciyes University, Kayseri, Turkey

<sup>2</sup>Faculty of Agriculture, Plant Protection Department, Cukurova University, Adana, Turkey  
sumer\_536@yahoo.com

Watermelon fruit blotch agent, a seed borne bacterium, *Acidovorax citrulli* is one of the destructive pathogen of watermelon growing areas in the world including Turkey. Cotyledon symptoms are water-soaking, brown, sunken, necrotic spots or large necrotic lesions. Control strategies of the disease should focus on using disease-free seeds. In this study, 322 candidate antagonists were isolated from healthy watermelon leaves, blossoms or soils. Of those, 14 antagonists were selected from the highest inhibition zone growth *in vitro* tests. Antagonists were tested for their ability to suppress the pathogen on seeds. Pathogen and antagonists treated seeds were sown in plastic containers, kept in 30°C and 85% relative humidity. A week after germinations, infected cotyledons were determined with 0–7 scale. 100 seeds were used for per treatment. In the experiment, antagonists reduced the pathogen incidence from 6 to 94% and disease severity was ranged from 10 to 93%. Seven antagonists (Antg-12, Antg-57, Antg-79, Antg-147, Antg-197, Antg-198, and Antg-273) were reduced the disease incidence and disease severity over 85 and 88%, respectively. This study demonstrated that antagonists are able to reduce disease occurrence and serves as our first attempts on biological control of watermelon fruit blotch for further studies.

**Keywords:** *Acidovorax citrulli*, biocontrol, cucurbit, management



## Genetic Differentiation of *Pseudomonas syringae* pv. *syringae* Isolates from Citrus and Stone Fruits in the Eastern Mediterranean Region, Turkey

Raziye Cetinkaya Yildiz<sup>1</sup>, Ali Karatas<sup>1</sup>, Sumer Horuz<sup>2</sup> and Yesim Aysan<sup>3</sup>

<sup>1</sup>Biological Control Research Institute, Adana, Turkey

<sup>2</sup>University of Erciyes, Kayseri, Turkey

<sup>3</sup>University of Cukurova, Adana, Turkey

raziye.yildiz@tarim.gov.tr

Citrus blast disease, caused by *Pseudomonas syringae* pv. *syringae* occasionally rise severe damages both on citrus and stone fruit trees in the Eastern Mediterranean region of Turkey. The spread of the bacterium in/around the orchards in Cukurova region increases due to closely planted citrus and stone fruit trees. In this study, 45 putative bacterial strains isolated among 2014–2015 from citrus and stone fruit trees. Immature raw peach and lemon fruits were used for the pathogenicity tests. Isolates were identified as *Pseudomonas syringae* pv. *syringae* according to pathogenicity, classical and molecular identification tests. Genetic relationship among isolates was determined using BOX, ERIC and REP-PCR tests. All isolates consisted of bands of different sizes in BOX-PCR from 0.3 to 3.0 kb, 0.15 to 3.0 kb in ERIC-PCR and 0.2 to 3.0 kb in REP-PCR. According to the constructed dendrogram, it was determined that four different inoculum sources are present in Cukurova region. The difference among those sources were 2.65%. This result showed that four different inoculum sources exist in Cukurova region.. Both isolates from citrus and stone fruit orchards were involved in the same groups. This finding indicated that, there is no specific relation between pathogen and host. Also isolates obtained from both two years were involved in same groups and determined that there is no specialization among pathogen and years. Citrus and stone fruit orchards were planted in Adana, Mersin and Hatay provinces side-by-side or nested. This situation leads to spread of the pathogen from one orchard to another easily. Due to pathogen spread via cultural practices and winds and lack of host specialization of the disease increases the risk of infection of other cultivated plants.

**Keywords:** blast disease, molecular characterization

### Acknowledgements

This study was supported with the project number 113O454 by TUBITAK.



## **Predictive Modelling as a Tool for Performance Objectives (PO) Achievement, Performance Criteria (PC) and Process/Product Criteria (PcC/PdC) Calculation for the Mycotoxin Hazard**

**Esther Garcia-Cela, Angel Medina and Naresh Magan**

*Applied Mycology Group, Cranfield University, Cranfield, United Kingdom  
m.e.garcia-cela@cranfield.ac.uk*

The ideal goal for controlling mycotoxins is to eliminate them from the food chain; however, on a practical level this is not possible and specific level for certain contaminants are set in the Legislation. Codex considered the food safety objective (FSO) is the maximum frequency and/or concentration of the hazard in a food at the time of consumption, but only apply this concept for microbial hazard.

The aim of this study was to apply the emerging risk management metric for food safety. We used the example of the fate of deoxynivalenol (DON) during the processing of wheat grains to flour. Therefore, only those lots of raw materials in which the initial level of contamination can be reduced to safe levels through processing would be accepted. The starting point was the maximum levels in Europe for DON (Commission, 2006). Having these values in mind, the process steps were individually considered and Performance Criteria (PCs) determined when required. According to the initial (1750 µg/kg) and final values (750 µg/kg) proposed by European legal limits, processing (either selection or selection plus milling) is expected to decrease in 57% the initial DON concentration in the raw wheat. According to these PCs, possible PcC and PdC were calculated, using previously published results. Hope et al. (2005), established 0.90  $a_w$  as a limit to *Fusarium culmorum* growth and DON production, therefore zero increments of this toxin could be expected under Good Storage Practices. On the other hand, 48, 15, and 35% of DON reduction were achieved in sorting and cleaning, debraning and milling step respectively (Cheli et al., 2010). Taking into account this percentage of reduction the FSO value for flour could be guaranty. Therefore, the food industry is responsible for setting up food safety management systems that deliver foodstuffs in compliance to the FSO.

The present study demonstrates the usefulness of predictive modelling as a management and prevention tool for mycotoxin hazards. The need for: (i) development of predictive models for mycotoxigenic fungi at the boundary storage conditions for growth and (ii) kinetics of mycotoxins reduction in food substrates during processing, have been demonstrated.



## REFERENCES

Cheli, F., Campagnoli, A., Ventura, V., Brera, C., Berdini, C., Palmaccio, E., and Dell'Orto, V. (2010). Effects of industrial processing on the distributions of deoxynivalenol, cadmium and lead in durum wheat milling fractions. *LWT Food Sci. Technol.* , 43(7), 1050–1057. doi.org/10.1016/j.lwt.2010.01.024.

Codex Alimentarius Commission. (2007). *Principles and Guidelines for the Conduct of Microbiological Risk Assessment (MRM), CAC/GL 63-2007*. doi.org/10.1016/S0015-6264(66)80695-8.

Hope, R., Aldred, D., and Magan, N. (2005). Comparison of environmental profiles for growth and deoxynivalenol production by *Fusarium culmorum* and *F. graminearum* on wheat grain. *Lett. Appl. Microbiol.* , 40(4), 295–300. doi.org/10.1111/j.1472-765X.2005.01674.x

Commission, E. (2006). Commission Regulation (EC) No. 1881/2006 of 19 December 2006, Setting maximum levels for certain contaminants in foodstuffs. *Off. J. Eur. Union*, 364, 5–24. Available at: <http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32006R1881&from=EN>







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