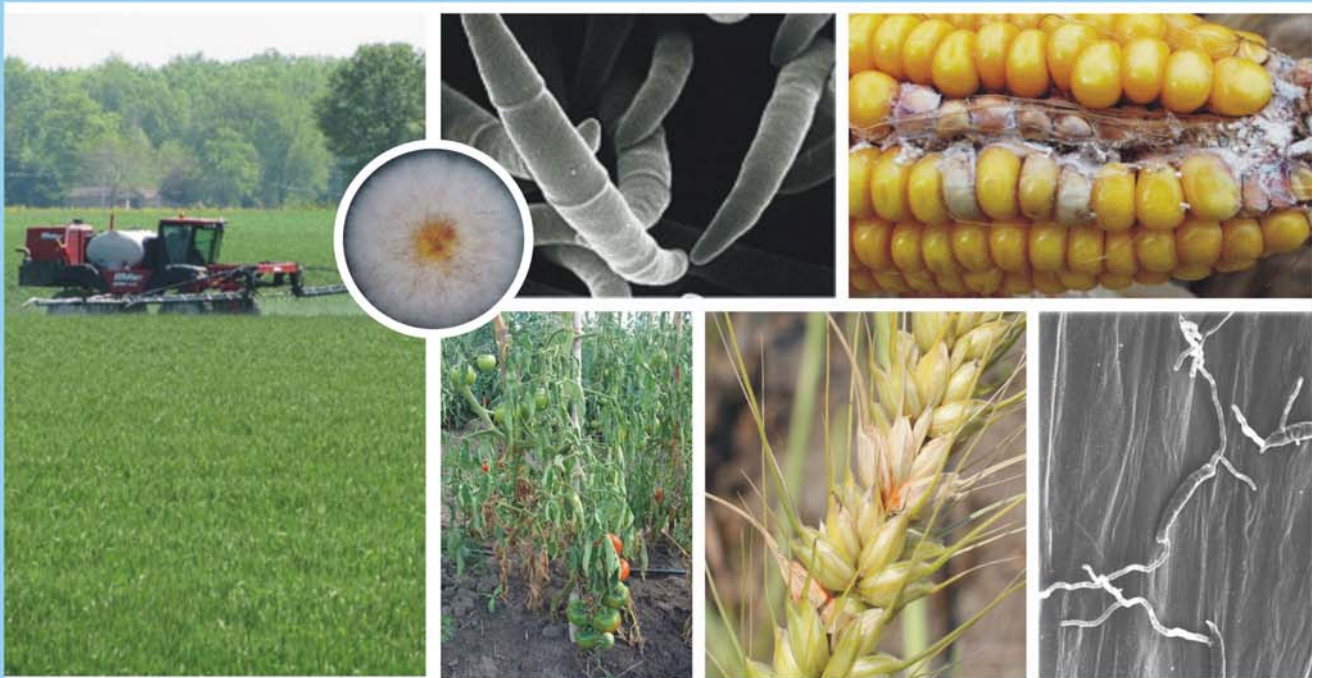


**A Workshop of International Congress of Plant Pathology, 2013**

# **11<sup>th</sup> International Fusarium Workshop**

## **Program Resource Book**



**August 20-24, 2013, Hangzhou, China**

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**The 11<sup>th</sup> International Fusarium Workshop, August 20-24,  
Hangzhou, China**

**Welcome!**

Dear Participants,

On the behalf of the entire organizing committee, I would like to welcome you to the 11<sup>th</sup> International Fusarium Workshop! The international Fusarium Workshops have provided a unique forum for Fusarium scientists to share experiences, exchange ideas and plan future collaborations during last decade.

The 11<sup>th</sup> International Fusarium Workshop continues to be a successful meeting due to the enthusiastic participation by scientists like you who consistently attend and present your research. In this workshop, the scientific program includes four sessions: Genetics and System Biology of Fusaria, Host Resistance and Fusarium-host Interactions, Population Biology, Epidemiology and Toxins, Future Challenges in Fusaria. More than eighty participants from 11 countries will attend this workshop. We hope that you enjoy the 11<sup>th</sup> International Fusarium Workshop and have a wonderful time in the beautiful city, Hangzhou.

Ulf Thrane



**Chair of International Society for Plant Pathology (ISPP) Fusarium Committee**

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## **International Fusarium Committee**

### **Ulf Thrane, Chair of ISPP Fusarium Committee**

Technical University of Denmark, Denmark

### **David Geiser, Vice-chair of ISPP Fusarium Committee**

University Park, Pennsylvania, USA

### **Jin-rong Xu, Vice-chair of ICPP Scientific Committee**

Purdue University, Indiana, USA

## **Local Organizers of 11<sup>th</sup> International Fusarium Workshop**

### **Zhonghua Ma, Chair**

Zhejiang University, China

### **Bin Li, Secretary**

Zhejiang University, China

### **Yanni Yin, Secretary**

Zhejiang University, China

### **Yun Chen, Secretary**

Zhejiang University, China

## **Acknowledgments**

The 11th International Fusarium Workshop Organizers would like to acknowledge the following for their financial contributions to this workshop:



国家自然科学基金委员会  
National Natural Science Foundation of China



syngenta

**National Natural Science Foundation of China**

**Novozymes A/S**

**Syngenta Crop Protection AG**

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## **General Information**

### **General Sessions**

All scientific sessions will be held in the **Huagang Salon** (Fourth floor) at the Hangzhou Huagang HNA Hotel. A name badge is required for entry into all sessions, meals and social events. The workshop staff will be available at reception or buffet place during the sessions.

### **Poster Sessions**

Posters boards are located at the back of Huagang Salon. The poster should be mounted before the poster session, 16:25-18:00, 21<sup>st</sup> AUG. No special order will be provide for your poster, you can use any available board in the room. During the poster session, the authors are to stand by the poster board to present the work.

### **Phones, Cameras and Recordings**

Please silence your cell phones during the meeting. Digital recorders, cameras (including camera phones) and video cameras are prohibited in the oral and poster sessions. Thanks for your collaboration.

### **Child Policy**

Children are not permitted in the session rooms. Please contact the hotel to arrange for babysitting services in your hotel room.

### **Guest Registration**

As noted in the program schedule, certain meals and social events are included in the registration fee for workshop participants. Registered participants may also purchase tickets for an accompanying guest (age 16 and older) to attend the welcome reception for an additional fee of 300 RMB and /or the buffet for a fee of 125 RMB. Guests are not permitted in the general sessions or poster sessions. Non-registered guests are not permitted to attend any part of the workshop or social events.

### **Contact information:**

If you have any question, please contact with  
Yun Chen, 18768403005;  
Yanni Yin, 15968864043.

## Scientific Program

Wednesday, August 21, 2013; Huagang Salon, Fourth floor

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8:30- 9:00	Welcome Remarks
9:00-11:40	<b>Keynote lectures</b> Session Chair: <i>Yucai Liao</i>
9:00-9:35	Fusarium pathogenomics: understanding fungal pathogenicity through genomics <b>Li-jun Ma</b> ; University of Massachusetts Amherst, MA, USA
9:35-10:10	Functional characterization of cell cycle related protein kinase genes in <i>Fusarium graminearum</i> <b>Jin-rong Xu</b> ; Purdue University, IN, USA
10:10-10:40	Coffee Break, Photograph
10:40-11:15	How do Fusarium pathogens overcome cereal defences? <b>Donald Gardiner</b> ; CSIRO, Australia
11:15-11:50	Management of Fusarium wheat head blight in China <b>Zhonghua Ma</b> ; Zhejiang University, China
11:50-13:30	<b>Lunch</b> (Caesars Palace, First floor, Buffet)
13:30-16:25	<b>Session I: Genetics and System Biology of Fusaria</b> Session Chair: <i>Lijun Ma, Emma Steenkamp</i>
13:30-13:50	Strawberry Fusarium wilt pathogen <i>Fusarium oxysporum</i> F. sp. <i>Fragariae</i> shows differential protein expression in isolates that differ in virulence <b>Xiangling Fang</b> ; University of Western Australia, Australia
13:50-14:10	Genus-specific CYP51C of <i>Fusarium graminearum</i> identified as a novel CYP51 rather than azole fungicide target sterol 14 $\alpha$ -demethylases <b>Jieru Fan</b> ; Chinese Academy of Agricultural Sciences
14:10-14:30	The gene FoOCH1 encoding a putative $\alpha$ -1, 6-mannosyltransferase in <i>Fusarium oxysporum</i> f. sp. <i>cubense</i> is required for virulence on banana plants <b>Minhui Li</b> ; South China Agricultural University, China
14:30-14:45	Coffee Break

- 14:45-15:05      Involvement of *FgERG4* in ergostrol biosynthesis, vegetative differentiation and virulence in *Fusarium graminearum*  
***Xin Liu***; Jiangsu Academy of Agricultural Sciences, China
- 15:05-15:25      Genetic analysis of TOR signaling pathway in *Fusarium graminearum*  
***Fangwei Yu***; Zhejiang University, China
- 15:40-16:05      Toxicology of the fungicide JS399-19 against *Fusarium asiaticum* and analysis of its resistance  
***Yipin Hou***; Nanjing Agricultural University, China
- 16:05-16:25      The MAPKK *FgMkk1* of *Fusarium graminearum* governs vegetative differentiation, multiple stress response, and virulence via the cell wall integrity and HOG signaling pathways  
***Yingzi Yun***; Zhejiang University, China
- 16:25-18:00      **Poster Presentation**
- 18:00-20:00      **Conference Dinner Reception** (GuiYu Salon, Third floor)

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**Thursday, August 22, 2013; Huagang Salon, Fourth floor**


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- 8:30-11:45      **Session II: Host Resistance and Fusarium-host Interactions**  
 Session Chair: *Donald Gardiner, Xiquan Gao*
- 8:30-8:50      A study in evolution of races in tomato wilt fungus  
*Takeshi Kashiwa*; Tokyo Univ. of Agriculture and Technology, Japan
- 8:50-9:10      Oxylipins-mediated signaling in plant-Fusarium interaction  
*Xiquan Gao*; Nanjing Agricultural University, China
- 9:10-9:30      Resistance to Fusarium head blight caused by Fusarium isolates with  
 three chemotypes in wheat varieties  
*Xu Zhang*; Jiangsu Academy of Agricultural Sciences, China
- 9:30-9:50      KIN1 regulates *Fusarium graminearum* morphology and  
 cytoskeleton differently between sexual and asexual life cycles  
*Yongping Luo*; Northwest A&F University, China
- 9:50-10:05      Coffee Break
- 10:05-10:25      Oat resistance to HT2 and T2 producing *Fusarium langsethiae*  
*Tijana Stancic*; Harper Adams University College, UK
- 10:25-10:45      Characterization of Fusarium head blight resistance in  
 a CIMMYT synthetic-derived bread wheat line  
*Zhanwang Zhu*; Hubei Academy of Agricultural Sciences, China
- 10:45-11:05      Distribution of the QTL associated with Fusarium head blight  
 resistance in breeding population of wheat and their genetic effects  
*Tao Li*; Yangzhou University, China
- 11:05-11:25      Nitrate nutrition enhanced the resistance of cucumber plants to  
 Fusarium wilt  
*Min Wang*; Nanjing Agricultural University, China
- 11:25-11:45      The scaffold protein FgSte50 regulates vegetative differentiation,  
 secondary metabolism and virulence via the Gpmk1 and HOG  
 MAPK pathways in *Fusarium graminearum*  
*Qin Gu*; Zhejiang University, China
- 11:45-13:30      **Lunch** (Caesars Palace, First floor, Buffet)
- 13:30-16:25      **Session III: Population Biology, Epidemiology and Toxins**  
 Session Chair: *Teis Søndergaard, Zhonghua Ma*



- 13:30-13:50 Population structures of *Fusarium* species isolated from wheat and maize in China  
**Yucai Liao**; Huazhong Agricultural University, China
- 13:50-14:10 Fusarilins  
**Teis Søndergaard**; Aalborg University, Denmark
- 14:10-14:30 Multi-component analyses and risk assessment of *Fusarium* mycotoxins in agricultural products  
**Aibo Wu**; Shanghai Academy of Agricultural Sciences, China
- 14:30-14:50 Study on the differentiation of physiological races, vegetative compatibility groups and infection process of *Fusarium oxysporum* f.sp. *niveum* in Jiangsu province  
**Haiyan Sun**; Jiangsu Academy of Agricultural Sciences, China
- 14:50-15:05 Coffee Break
- 15:05-15:25 Evidence for birth-and-death evolution and horizontal transfer of a mycotoxin biosynthetic gene cluster in *Fusarium*  
**François Van Hove**; Université catholique de Louvain, Belgium
- 15:25-15:45 Contamination of bananas with beauvericin and fusaric acid produced by *Fusarium oxysporum* f. sp. *cubense*  
**ChunYu Li**; Guangdong Academy of Agricultural Sciences, China
- 15:45-16:05 Population analysis of the *Fusarium graminearum* species complex from wheat in China  
**Hao Zhang**; Chinese Academy of Agricultural Sciences, China
- 16:05-16:25 Timing and efficacy of fungicides against *Fusarium* head blight in malting barley  
**Linda K Nielsen**; University of Nottingham, United Kingdom
- 16:25-18:00 **Session IV: Future Challenges in Fusaria**  
Session Chair: *Ulf Thrane*
- 16:25-17:00 Fusarium-Now, Future Challenges and Opportunities  
**Ulf Thrane**; Technical University of Denmark, Denmark
- 17:00-18:00 Discussion and Closing Remarks
- 18:00- Buffet dinner (Caesars Palace, First floor, Buffet)

## Speaker Abstracts

### Keynote lecture 1

#### FUSARIUM PATHOGENOMICS: UNDERSTANDING FUNGAL PATHOGENICITY THROUGH GENOMICS

Li-Jun Ma

Biochemistry and Molecular Biology, University of Massachusetts Amherst, MA

E-mail: [lijun@biochem.umass.edu](mailto:lijun@biochem.umass.edu)

Since the publication of the first fungal genome, *Saccharomyces cerevisiae*, in 1996, more than a thousand fungal genomes have been sequenced, which makes the kingdom of fungi the most sequenced eukaryotic taxon. Correlating genotypes based on sequence information to interesting phenotypes, such as pathogenesis, is one of many challenges associated with all genome projects. This presentation will use *Fusarium* comparative genomics as an example to study genetic mechanisms that contribute to pathogenicity among a group of important phytopathogens in *Fusarium oxysporum* species complex. Members of this species complex are responsible for destructive and intractable wilt diseases in over 100 diverse plant hosts, including the recent outbreak of Panama disease of banana that destroyed more than 70% crop in disease manifested areas. In contrast to the broad host range, a single pathogenic form within this species complex usually infects a single plant host, exhibiting strong host specificity. The *Fusarium* comparative genomic study demonstrated that horizontally acquired pathogenicity chromosomes convey host-specific pathogenicity. The genome of the pathogen can be divided into **core genomic regions**: responsible for essential biological processes, and **adaptive genomic regions**: contributing to pathogen virulence and host specialization. The application of the genome structure compartmentalization enabled the identification of candidate effectors among the newly sequenced genomes of pathogenic isolates. These candidate effectors hold the potential for monitoring the spread of a particular disease and for the development of management plans.

**Keynote lecture 2****FUNCTIONAL CHARACTERIZATION OF CELL CYCLE RELATED  
PROTEIN KINASE GENES IN *FUSARIUM GRAMINEARUM*****Jin-Rong Xu**

Department of Botany and Plant Pathology, Purdue University, West Lafayette, IN

E-mail: [jinrong@purdue.edu](mailto:jinrong@purdue.edu)

Eukaryotic cell cycle consists of a defined set of events during which nuclear DNA is replicated and segregated into two daughter cells. Many protein kinases important for cell cycle are well conserved from yeast to humans. In model fungi *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, and *Aspergillus nidulans*, the key regulator of cell cycle is the single copy *CDC2* (*CDC28*) gene. However, unlike these model fungi, the filamentous ascomycete *Fusarium graminearum*, the causal agent of wheat and barley head blight disease, has two *CDC2* orthologs, *CDC2A* and *CDC2B*. Whereas the *cdc2B* deletion mutant had no or only minor defects, deletion of *CDC2A* resulted in significant reduction in virulence. Ascospore formation but not perithecial formation and ascus development was blocked in the *cdc2A* mutant, which was normal in conidium germination and vegetative growth. Deletion of both *CDC2A* and *CDC2B* appears to be lethal because we failed to identify *cdc2A cdc2B* double mutants after repeated attempts. Therefore, *CDC2A* and *CDC2B* must have independent and overlapping functions in *F. graminearum*. Mutants deleted of the *FgCAK1* CDK kinase gene had similar phenotypes with the *cdc2A* mutant. In yeast two-hybrid assays, self-interaction was detected for both Cdc2A and Cdc2B, which also interacted with each other and with *FgCAK1*. These results indicate that homodimers or heterodimers of Cdc2A and Cdc2B may be involved in cell cycle regulation in different growth and developmental stages. It is likely that infectious growth and ascospore formation may specifically require *CDC2A*, which is activated by *FgCAK1*.

*F. graminearum* also uniquely has two Aurora protein kinase genes that differ at the amino acid residue known to be related to different subcellular localizations and functions of Aurora A and Aurora B paralogs in humans, further indicating that yeast and *F. graminearum* differ in some key protein kinases involved in mitosis. The same results were observed in comparative and functional analyses of other protein kinase genes known to be related to cell cycle in model fungi.

**Keynote lecture 3****HOW DO FUSARIUM PATHOGENS OVERCOME CEREAL DEFENCES?**

**Donald Gardiner**<sup>1</sup>, Kemal Kazan<sup>1</sup>, Andrew Kettle<sup>1,2</sup>, Lorenzo Covarelli<sup>1,3</sup>, Amber Stephens<sup>1</sup>, Alan Munn<sup>4</sup>, Jacqueline Batley<sup>2</sup>, John Manners<sup>1</sup>

<sup>1</sup>CSIRO Plant Industry, Brisbane and Canberra, Australia. <sup>2</sup>School of Agriculture and Food Science, University of Queensland, Brisbane, Australia. <sup>3</sup>Department of Agricultural and Environmental Sciences, University of Perugia, Perugia, Italy. <sup>4</sup>School of Medical Science, Griffith University, Gold Coast, Australia

E-mail: [donald.gardiner@csiro.au](mailto:donald.gardiner@csiro.au)

*Fusarium pseudograminearum* is the principle cause of crown rot disease of wheat and barley in Australia and other parts of the world. *F. pseudograminearum* can also cause Fusarium Head Blight. Through comparative genomic and functional analyses of *F. pseudograminearum* and other cereal-infecting *Fusarium* spp., we have identified a number of genes that show phylogenetic distributions suggestive of acquisition via horizontal gene transfers within or across kingdoms. Some of these horizontally acquired genes encode enzymatic functions with predicted roles in degradation of xenobiotics. Knockout mutations in a subset of these genes resulted in fungal isolates with reduced virulence towards wheat (Gardiner et al., 2012 PLoS Pathog). These observations have led us to analyse the other genes with predicted products involved in xenobiotic detoxification in *F. pseudograminearum* and *F. graminearum*. These include a conserved ABC transporter and homologues of genes involved in the detoxification of the antifungal wheat metabolite benzoxazolinone (BOA) in other *Fusarium* spp. The importance of each of these genes for Fusarium to overcome wheat chemical defences will be discussed.

**Keynote lecture 4****MANAGEMENT OF FUSARIUM WHEAT HEAD BLIGHT IN CHINA****Zhonghua Ma**, Yanni Yin, Yun Chen

Institute of Biotechnology, Zhejiang University, 866 Yuhangtang Road, Hangzhou 310058, P. R. China

E-mail: [zhma@zju.edu.cn](mailto:zhma@zju.edu.cn)

Fusarium head blight (FHB) is a devastating disease of cereal crops which can cause huge losses in epidemic years. In addition to the yield loss caused by the disease, the contamination of infected grains with mycotoxins poses a serious threat to human and animal health. Although a number of *Fusarium* spp. can cause FHB, *Fusarium asiaticum* (Fa) and *F. graminearum* (Fg) are the primary causal agents of FHB in China. In this presentation, we will review the management strategies and outreach implementation against FHB in China. Strategies include development of resistance varieties, the use of fungicides, and cultural practices. The application of fungicides during wheat anthesis has become one of primary methods for management of FHB in China since 1980s. Because of the heavy application of benzimidazole fungicides, particularly carbendazim (MBC), resistance of *Fa* to MBC has been detected in many locations of China. Thus, we will also review recent advances in monitoring fungicide resistance, resistance mechanisms and management strategies for fungicide resistance in FHB in China.

## Session I-1

**STRAWBERRY FUSARIUM WILT PATHOGEN *FUSARIUM OXYSPORUM* F. SP. *FRAGARIAE* SHOWS DIFFERENTIAL PROTEIN EXPRESSION IN ISOLATES THAT DIFFER IN VIRULENCE****Xiangling Fang<sup>1</sup>, Patrick M. Finnegan<sup>1,2</sup>, Martin J. Barbetti<sup>1,2</sup>**

<sup>1</sup>School of Plant Biology, Faculty of Science, The University of Western Australia, 35 Stirling Highway, Crawley, WA 6009, Australia;

<sup>2</sup>The UWA Institute of Agriculture, Faculty of Science, The University of Western Australia, 35 Stirling Highway, Crawley, WA 6009, Australia

E-mail: [xiangling.fang@student.uwa.edu.au](mailto:xiangling.fang@student.uwa.edu.au)

*Fusarium oxysporum* f. sp. *fragariae* (Fof) is the causal agent of Fusarium wilt on strawberry, a serious threat to commercial strawberry production worldwide. However, it remains unknown about the factors and mechanisms contributing to the virulence of Fof to strawberry. To reveal this, comparative proteome analyses were conducted to determine the differences in mycelia proteomes of highly, moderately and weakly virulent isolates of Fof, in combination with the growth and sporulation difference of these isolates. The growth of the highly virulent isolate Fof51 was significantly faster than that of the weakly virulent isolate Fof35 and/or the moderately virulent isolate Fof109 in both solid and liquid cultures during the time course studied. Analysis of proteins separated by two-dimensional gel electrophoresis revealed 54 differential expressed proteins in abundance between isolates, with 47 proteins showing the highest abundance in the highly virulent isolate (greater than 2-fold difference than the weakly virulent isolate and/or the moderately virulent isolate and  $P < 0.05$ ) and seven proteins unique to the highly virulent isolate. The 54 proteins were identified through MALDI-TOF/TOF MS/MS analysis, and these proteins were mainly involved in primary and protein metabolism, antioxidation, electron transport, cell cycle and transcription. These proteins are potential factors contributing to the virulence of Fof to strawberry. In addition, protein modification may also have an important contribution. This study provides the first insights into the molecular base of Fof virulence, opening novel avenues to develop effective and sustainable strategies in managing Fusarium wilt on strawberry.

## Session I-2

**GENUS-SPECIFIC *CYP51C* OF *FUSARIUM GRAMINEARUM* IDENTIFIED AS  
A NOVEL *CYP51* RATHER THAN AZOLE FUNGICIDE TARGET STEROL  
14 $\alpha$ -DEMETHYLASES**

**Jieru Fan**<sup>1,2</sup>, Martin Urban<sup>3</sup>, Josie E. Parker<sup>4</sup>, Helen C. Brewer<sup>3</sup>, Steven L. Kelly<sup>4</sup>,  
Kim E. Hammond-Kosack<sup>3</sup>, Bart A. Fraaije<sup>2</sup>, Xili Liu<sup>1</sup>, Hans J. Cools<sup>2\*</sup>

<sup>1</sup>Department of Plant Pathology, China Agricultural University, Beijing, 100193, P.R.China; <sup>2</sup>Department of Biological Chemistry and Crop Protection, Rothamsted Research, Harpenden, AL5 2JQ, UK; <sup>3</sup>Department of Plant Biology and Crop Science, Rothamsted Research, Harpenden, AL5 2JQ, UK; <sup>4</sup>Institute of Life Science and College of Medicine, Swansea University, Swansea, Wales, SA2 8PP, UK.

\*Corresponding author: Hans J. Cools, E-mail: [hans.cools@rothamsted.ac.uk](mailto:hans.cools@rothamsted.ac.uk)

The recent expansion of publically available fungal genome sequence data has revealed many ascomycete fungi carry more than one copy of the cytochrome P450 sterol 14 $\alpha$ -demethylase gene (*CYP51*, syn. *ERG11*), encoding the target for azole fungicides. In several *Fusarium* species, including pathogens of both humans and plants, three paralogous *CYP51* genes (*FgCYP51A*, *FgCYP51B*, *FgCYP51C*) have been identified. *FgCYP51C* is unique to the genus *Fusarium*. Currently, the functions of these three genes and the rationale for their conservation within the *Fusaria*, is unknown. In this study, by expression in *Saccharomyces cerevisiae*, we demonstrate that both *FgCYP51A* and *FgCYP51B* can complement yeast *CYP51* function, whereas *FgCYP51C* cannot. By generating both single ( $\Delta FgCYP51A$ ,  $\Delta FgCYP51B$ , and  $\Delta FgCYP51C$ ) and double ( $\Delta FgCYP51AC$  and  $\Delta FgCYP51BC$ ) *CYP51* gene deletion mutants we demonstrate the intrinsically low sensitivity of *F. graminearum* to some azole fungicides is conferred by the *FgCYP51A* paralogue. We show ascospores formation is blocked in the  $\Delta FgCYP51B$  and  $\Delta FgCYP51BC$  mutants, even though superficially normal perithecia develop. Specific accumulation of eburicol, a *CYP51* substrate in filamentous fungi, and two 14-methylated sterols occurred in the  $\Delta FgCYP51B$  and  $\Delta FgCYP51BC$  mutants, which suggests *FgCYP51B* is the primary sterol 14 $\alpha$ -demethylase. Various bioassays revealed the  $\Delta FgCYP51C$  mutants, unlike  $\Delta FgCYP51AC$  and  $\Delta FgCYP51BC$  mutants, are phenotypically indistinguishable from wild-type *in vitro* and during *Arabidopsis* infection. However, on host wheat ears, *FgCYP51C* is required for full virulence and DON production. This is the first example of a fungal *CYP51* gene with a function supplementary to primary sterol biosynthesis.

## Session I-3

**THE GENE FOCH1 ENCODING A PUTATIVE  $\alpha$ -1,  
6-MANNOSYLTRANSFERASE IN *FUSARIUM OXYSPORUM* F. SP. *CUBENSE*  
IS REQUIRED FOR VIRULENCE ON BANANA PLANTS**

**M.-H. Li**<sup>1</sup>, X.-L. Xie<sup>1</sup>, X.-F. Lin<sup>1</sup>, J.-X. Shi<sup>1</sup>, Z.-J. Ding<sup>1</sup>, J.-F. Ling<sup>1,3</sup>, P.-G. Xi<sup>1</sup>, J.-N. Zhou<sup>1</sup>, S. Zhong<sup>2</sup>, Z.-D. Jiang<sup>1</sup>

<sup>1</sup>Department of Plant Pathology, South China Agricultural University, Guangzhou, 510642, P. R. China; <sup>2</sup>Department of Plant Pathology, North Dakota State University, Fargo, ND 58108, USA; <sup>3</sup>Institute of Plant Protection, Guangdong Academy of Agricultural Sciences, Guangzhou, 510640, P. R. China

E-mail: [liminhui@scau.edu.cn](mailto:liminhui@scau.edu.cn)

*Fusarium oxysporum* f. sp. *cubense*, the causal agent of banana *Fusarium* wilt, is one of the most destructive pathogens threatening the banana production. However, the molecular mechanisms underlying the virulence and pathogenicity of this fungal pathogen are still poorly understood. In this study, we identified and characterized the disrupted gene in a T-DNA insertional mutant L953 of FOC with significantly reduced virulence on banana plants. The gene disrupted by T-DNA insertion in L953 harbors an open reading frame encoding a protein with homology to  $\alpha$ -1, 6-mannosyltransferase (OCH1) in fungi. The deletion mutant ( $\Delta FoOCH1$ ) of the OCH1 orthologue (*FoOCH1*) and L953 were impaired in fungal growth, exhibited a higher sensitivity to the cell wall perturbing agents, Calcofluor White and Congo Red, produced less cell wall proteins and released more secreted proteins into liquid media than the wild type. Furthermore, the mutation or deletion of *FoOCH1* led to decline in virulence to the banana host. The mutant phenotypes were fully restored by complementation with the wild type *FoOCH1* gene. Our data provide a first evidence for the critical role of *FoOCH1* in maintenance of the cell wall integrity and the virulence of *F. oxysporum* f. sp. *cubense*.



## Session I-4

**INVOLVEMENT OF FGERG4 IN ERGOSTEROL BIOSYNTHESIS,  
VEGETATIVE DIFFERENTIATION AND VIRULRNCE IN *FUSARIUM  
GRAMINEARUM***

**Xin Liu**, Jinghua Jiang, Yanni Yin, Zhonghua Ma\*

Institute of Biotechnology, Zhejiang University, 866 Yuhangtang Road, Hangzhou 310058, China

\* Corresponding author: Zhonghua Ma, E-mail: [zhma@zju.edu.cn](mailto:zhma@zju.edu.cn)

The ergosterol biosynthesis pathway has been well understood in *Saccharomyces cerevisiae*, but currently little is known about the pathway in plant pathogenic fungi. In this study, we characterized *Fusarium graminearum* *FgERG4* gene encoding sterol C-24 reductase, which catalyzes for the conversion of ergosta-5, 7, 22, 24-tetraenol to ergosterol in the final step of ergosterol biosynthesis. The *FgERG4* deletion mutant  $\Delta$ FgErg4-2 failed to synthesize ergosterol. The mutant exhibited a significant decrease in mycelial growth and in conidiation, and produced abnormal conidia. In addition, the mutant showed increased sensitivity to metal cations and to various cell stresses. Surprisingly, mycelia of  $\Delta$ FgErg4-2 revealed increased resistance to cell wall degrading enzymes. Fungicide sensitivity tests revealed that the  $\Delta$ FgErg4-2 showed increased resistance to various sterol biosynthesis inhibitors (SBI), which is consistent with the over-expression of SBI target genes in the mutant. The  $\Delta$ FgErg4-2 was impaired dramatically in virulence although it was able to successfully colonize flowering wheat head and tomato, which is in agreement with the observation that the mutant produced a significantly low level of trichothecene mycotoxins than the wild type progenitor. All of these phenotypic defects of  $\Delta$ FgErg4-2 were complemented by reintroduction of a full-length *FgERG4* gene. Additionally, *FgERG4* partially rescued the defect of ergosterol biosynthesis defect in *Saccharomyces cerevisiae* *ERG4* deletion mutant. Taken together, the results of this study indicate that *FgERG4* has a crucial role in ergosterol biosynthesis, vegetative differentiation and virulence in the filamentous fungus *F. graminearum*.

## Session I-5

**GENETIC ANALYSIS OF TOR SIGNALING PATHWAY IN  
*FUSARIUM GRAMINEARUM*****Fangwei Yu and Zhonghua Ma\***

Institute of Biotechnology, Zhejiang University, 866 Yuhangtang Road, Hangzhou 310058, P. R. China

\* Corresponding author: Zhonghua Ma, E-mail: [zhma@zju.edu.cn](mailto:zhma@zju.edu.cn)

The target of rapamycin (TOR) pathway is conserved for controlling cell growth in many eukaryotes, especially in response to nutrient availability, however, little is known about the pathway in filamentous fungi. In this study, we found that rapamycin is effective against hyphal growth and asexual development of *Fusarium graminearum*. In addition, this compound also caused accumulation of lipid droplets in *F. graminearum* hyphae. Based on these observations, we identified eight genes encoding components of the TOR signal pathway in *F. graminearum*. Biological functions of these genes were analyzed by target gene deletion and complementation. Deletion of *FgFKBP12* and single point mutation in the Fkbp12-rapamycin binding domain of FgTor result in a high level of resistance to rapamycin. Disruption of *FgTAP42* is lethal and GFP-tagged FgTap42 is cytoplasm-localized. Three type 2A protein phosphatases (FgPp2A-1, -2 and -3) were found to interact with FgTap42. The FgPp2A-2 might be essential in *F. graminearum*. FgPp2A-1 and -3 play important roles in mycelial growth, virulence, sexual reproduction and cell wall integrity. These PP2As play different roles in regulation of conidiation, deoxynivalenol (DON) and lipid droplet biosyntheses. In addition, both FgTip41 and FgAreA also are involved in multiple biological functions, including mycelial growth, DON biosynthesis, and virulence. FgTip41 does not interact with FgTap42 but it interacts with FgPp2A-3, suggesting the existence of FgTap42:FgPp2A-3:FgTip41 heterotrimer in *F. graminearum*, which contrasts with the model proposed for the budding yeast counterparts. Collectively, results of this study indicate that the TOR pathway plays important roles in regulation of various cellular processes in the filamentous fungus *F. graminearum*.

## Session I-6

**TOXICOLOGY OF THE FUNGICIDE JS399-19 AGAINST *FUSARIUM ASIATICUM* AND ANALYSIS OF ITS RESISTANCE****Yiping Hou**, Zhitian Zheng, Tao Gao, Mingguo Zhou\*

College of Plant Protection, Nanjing Agricultural University, Nanjing, 210095, China

\* Corresponding author: Mingguo Zhou, E-mail: [mgzhou@njau.edu.cn](mailto:mgzhou@njau.edu.cn)

JS399-19 (development code no.), 2-cyano-3-amino-3-phenylacrylic acetate, is a novel cyanoacrylate fungicide introduced by Jiangsu Branch of National Pesticide Research & Department South Center. The fungicide exhibited specific activity against *Fusarium* spp, especially for *Fusarium asiaticum*. The study reviewed toxicology of the fungicide against *F. asiaticum*, including the impacts on mycelial growth, conidia germination, germ tube growth of the conidia, proteome expression and so on. In laboratory, resistant mutants were obtained by fungicide training methods. A major gene was demonstrated to confer resistance of *F. asiaticum* to JS399-19 and some resistance-related genes were reported.

## Session I-7

**THE MAPKK FGMKK1 OF *FUSARIUM GRAMINEARUM* GOVERNS VEGETATIVE DIFFERENTIATION, MULTIPLE STRESS RESPONSE, AND VIRULENCE VIA THE CELL WALL INTEGRITY AND HOG SIGNALING PATHWAYS**

**Yingzi Yun**, Zunyong Liu, Jingze Zhang, Yun Chen and Zhonghua Ma\*

Institute of Biotechnology, Zhejiang University, Hangzhou 310058, China

\* Corresponding author: Zhonghua Ma, E-mail: [zhma@zju.edu.cn](mailto:zhma@zju.edu.cn)

The cell wall integrity (CWI) signaling pathway has been well characterized in *Saccharomyces cerevisiae*, however, surprisingly little is known about the signaling components other than for Slt2 orthologs in filamentous fungi. In this study, we determined the function of a mitogen-activated protein (MAP) kinase kinase FgMkk1 in *Fusarium graminearum*, the causal agent of wheat head blight. The *FgMkk1* deletion mutant ( $\Delta$ FgMkk1) grew significantly slower than the wild-type progenitor on solid media. The FgMkk1 is involved in the regulation of pigmentation, conidiation and deoxynivalenol (DON) biosynthesis in *F. graminearum*. More importantly,  $\Delta$ FgMkk1 showed dramatically increased sensitivity to cell wall stress agents, including cell wall degrading enzymes, congo red, calcofluor white, and a biocontrol agent *Trichoderma atroviride*. In addition, the mutant also revealed increased sensitivity to osmotic and oxidative stresses, but exhibited decreased sensitivity to the dicarboximide fungicide iprodione and the phenylpyrrole fungicide fludioxonil. Pathogenicity tests showed that  $\Delta$ FgMkk1 was impaired in virulence on flowering wheat head. Western blot assays showed that FgMkk1 positively regulates phosphorylation of the MAK kinases Mgv1 (the ortholog of *S. cerevisiae* Slt2) and FgOs-2 (the ortholog of *S. cerevisiae* Hog1), which are key components of the CWI and high-osmolarity glycerol (HOG) signaling pathway, respectively. These results indicate that FgMkk1 is involved in both the CWI and HOG pathways in *F. graminearum*. Moreover, we observed the direct interaction between Mgv1 and a transcription factor FgRlm1 in the yeast two-hybrid assays. The *FgRlm1* deletion mutant also showed increased sensitivity to cell wall-damaging agents, and exhibited decreased virulence on flowering wheat head. Taken together, our data indicated that the MAPKK FgMkk1 is an upstream component of Mgv1 and FgOs-2, and governs vegetative differentiation, multiple stress response and virulence via the cell wall integrity and HOG signaling pathways. FgRlm1 may be a downstream component of Mgv1 in the CWI pathway in *F. graminearum*.

## Session II-1

**INSERTION OF A TRANSPOSON IN AN AVIRULENCE GENE *AVR1* AT  
DIFFERENT POSITIONS GENERATED HOMOGENEOUS RACE  
DIFFERENTIATION IN THE TOMATO WILT FUNGUS *FUSARIUM*  
*OXYSPORUM* F. SP. *LYCOPERICISI***

**T. Kashiwa**<sup>1,2,3</sup>, A. Sato<sup>2</sup>, K. Inami<sup>2,6</sup>, T. Suzuki<sup>4</sup>, Y. Morita<sup>5</sup>, M. Yamasaki<sup>5</sup>,  
T. Teraoka<sup>2</sup>, T. Arie<sup>2</sup>

<sup>1</sup>United Graduate School of Agricultural Science, Tokyo University of Agriculture and Technology, Fuchu, Tokyo, Japan; <sup>2</sup>Laboratory of Plant Pathology, Tokyo University of Agriculture and Technology, Fuchu, Tokyo, Japan; <sup>3</sup>Research Fellow of Japan Society for the Promotion of Science; <sup>4</sup>Chiba Prefectural Agriculture and Forestry Research Center, Chiba, Chiba, Japan; <sup>5</sup>Kochi Agricultural Research Center, Nangoku, Kochi, Japan; <sup>6</sup>Present address: NR R&D Department, Central Research, Bridgestone Corporation, Kodaira, Tokyo, Japan

E-mail: [tkashiwa712@gmail.com](mailto:tkashiwa712@gmail.com)

Race emergence in plant pathogenic fungi is caused by the mutation of avirulence genes. Takken (2010) reported that in the tomato wilt fungus *Fusarium oxysporum* f. sp. *lycopersici* (*Fol*), deletion of *AVR1*, an avirulence gene corresponding to the tomato resistance gene *I*, resulted in the emergence of race 2, and the following point mutation in *AVR2*, corresponding to *I2*, generated race 3. However, we found that an isolate of race 3, KoChi-1, possessed an *AVR1* truncated by a transposon *Hormin* and an *AVR2* carrying a point mutation. This proposed a new insight of generation of races in *Fol* (Inami 2012). Although no isolate of race 2 carrying the *AVR1* truncated by a *Hormin* had been reported, so far, here we report a new isolate of race 2, Chiba-6, that possesses the *AVR1* truncated by a *Hormin*. *Hormin*-insertion occurs at the different position of *AVR1* in Chiba-6 and KoChi-1, and both of the truncated *AVR1* code defective Avr1 proteins. The isolates Chiba-6 and KoChi-1 belong to different phylogenetic clades A1 and A2 (Kawabe 2005), respectively. These suggest that insertion of *Hormin* in *AVR1* in Chiba-6 and KoChi-1 occurred as distinctive events at different positions in the phylogeny, both of which generated the homogeneous race differentiation in *Fol* in different clades.

## Session II-2

**OXYLIPINS-MEDIATED SIGNALING IN PLANT-FUSARIUM  
INTERACTION****Xiquan Gao**<sup>1,2</sup> and Michael Kolomiets<sup>2</sup>

<sup>1</sup>State Key Laboratory of Crop Genetics & Germplasm Improvement, College of Agriculture, Nanjing Agricultural University, Nanjing, China, 210095; <sup>2</sup>Department of Plant Pathology & Microbiology, Texas A&M University, 2132 TAMU, College Station, TX, 77843;

E-mail: [xgao@njau.edu.cn](mailto:xgao@njau.edu.cn)

Both plant and fungal lipoxygenases (LOXs) catalyze the formation of a large family of oxygenated lipids, collectively called oxylipins. Previous data showed that both host and fungal oxylipins play essential role in determining the outcome of host-pathogen interactions, while recent evidence suggested the involvement of jasmonate-signaling pathways in plant resistance to *Fusarium* spp. Using a group of maize *Mutator*-insertional lipoxygenase mutants as the tool, we found that specific LOXs and their oxylipin products, including jasmonates, are required by *Fusarium verticillioides* for normal pathogenic development (e.g. tissue colonization, conidia and mycotoxin production). This enables us to propose that host-derived oxylipins may mimic or interfere with fungal oxylipins to regulate these processes in *Fusarium* spp.. Therefore, interaction between maize-*Fusarium* indicates the existence of complex oxylipin-mediated signaling between the host and the pathogen that governs the outcomes of their interactions.

## Session II-3

**RESISTANCE TO FUSARIUM HEAD BLIGHT CAUSED BY FUSARIUM ISOLATES WITH THREE CHEMOTYPES IN WHEAT VARIETIES**

**Xu Zhang**, Yong-jin Zhou, Dan Yang, Hong-xiang Ma

Provincial Key Laborator of Agrobiolgy, Jiangsu Academy of Agricultural Sciences, Nanjing 210014, China

E-mail: [xuzhang@jaas.ac.cn](mailto:xuzhang@jaas.ac.cn)

Wheat *Fusarium* head blight (FHB) is one of the worldwide destructive diseases of wheat in the warm, semi-humid and humid regions. Apart from direct yield losses, the most serious concern about FHB is the contamination of the crop with mycotoxins, which poses a health risk to human and livestock. Based on the profile of trichothecenes produced, *Fusarium* isolates can be grouped into one of three chemotypes, which is 3ADON, 15ADON, or NIV chemotype. Different chemotype populations make the incidence and epidemic of FHB more comprehensive. Breeding for improved FHB resistance has become an important breeding goal for numerous cereal breeders. It has been reported that Asian wheat varieties resistant to FHB, including Sumai3, Wangshuibai and Wuhan 1, posses QTLs not loly for FHB severity but also for DON content.

To investigate FHB resistance and mycotoxin accumulation in wheat grains infected by different chemotypes of *Fusarium* species, 8 wheat varieties selected from 400 ones were inoculated by 3ADON, 15ADON and NIV chemotype isolates in 2010 and 2011, respectively. Twenty isolates of each chemotype were randomly selected and spores were mixed together to make the final inoculum. The single-floret injection method was used to inoculate wheat spikes. The proportion of scabbed spikelet (PSS) was measured at 21 days after inoculation. Data indicated that 8 Chinese varieties had significant difference on FHB severity. Although 3ADON isolates were more aggressive than other two chemotype isolates, variety Sumai 3 still had the best FHB resistance to all three chemotype populations. New released variety Shenxuan 6 also had better FHB type II resistance than other six wheat varieties.

Content of DON, 3ADON, 15ADON and NIV of the matured grain harvested from inoculated spikes were measured by HPLC. The total amount of DON, 15ADON, and 3ADON varied ranging from no detection to 2.25 µg/g. Grouping of the isolates based on the chemotypes showed that the 3ADON population produced more DON and 3ADON than the 15ADON population. In grain harvested from spikes inoculated by NIV chemotype isolates, DON, 3ADON and 15ADON other than NIV were detected.

Sumai 3, the best FHB resistant source, had lower mycotoxin content indicating resistance to DON content. Ningmai 9, which was moderately resistant to FHB spread, didn't have any 15ADON or NIV accumulation in the grain infected by either chemotype population. On the contrast, in grain of Shexuan 6, which had better type II FHB resistance, the DON accumulation was highest among all 8 genotypes. The information obtained in this study suggested that different mechanisms may operate for resistance to FHB severity and DON accumulation. Wheat genotypes with type II FHB resistance may react differentially to different chemotypes in PSS and DON accumulation. This highlights the need and importance of using different sources of host resistance in combating *Fusarium* head blight.



## Session II-4

**KIN1 REGULATES *FUSARIUM GRAMINEARUM* MORPHOLOGY AND CYTOSKELETON DIFFERENTLY BETWEEN SEXUAL AND ASEQUAL LIFE CYCLES**

**Yongping Luo**<sup>1</sup>, Yimei Zhang<sup>1</sup>, Shulin Cao<sup>1</sup>, Chenfang Wang<sup>1</sup>, Jin-rong Xu<sup>1,2\*</sup>

<sup>1</sup> Purdue-NWAFU Joint Research Center and State Key Laboratory of Crop Stress Biology for Arid Areas, College of Plant Protection, Northwest A&F University, Yangling, Shanxi 712100, China; <sup>2</sup> Department of Botany and Plant Pathology, Purdue University, West Lafayette, Indiana 47907, United States of America;

\*Corresponding author: Jinrong Xu, E-mail: [jinrong@purdue.edu](mailto:jinrong@purdue.edu)

The KIN1/PAR-1/MARK protein family which is composed of eukaryotic serine/threonine protein kinases are conserved from yeasts to human, and involved in cell polarity, microtubule stability or cell cycle regulation. In the *Fusarium graminearum* *KIN1* knock-out mutant, ascospore release was blocked and ascospore germinated inside perithecia while the asci wall was dissolved much earlier or aborted. *FgKIN1* localized to the septal pore and like a spacer covered upon the septal pore. In the *Fgkin1* mutant conidia and vegetable hyphae, *F. graminearum*  $\beta$ -tubulin gene *FgTUB1* localized to and around the microtubule-organizing centers (MTOCs) area with a little spread into the nuclear which is near to the MTOCs. But in ascospores, the *TUB1* localized to microtubule cytoskeletons as that in the wide type PH-1. The *Fgkin1* mutant had fewer septa in conidia and delayed the germination of conidial middle compartments and hyphal branching. Similar to *KIN1* in *Schizosaccharomyces pombe*, the *Fgkin1* mutant has increased sensitivity to hyperosmotic stress, cell wall stress. Glycogen accumulation ability was apparently reduced in ungerminated conidia but increased in asci wall in the *Fgkin1* mutant. The *Fgkin1* mutant had about 50% reduction in virulence while assayed with flowering wheat heads, corn silks and corn stem. *FgKIN1* may promote cell energy metabolism and plasma membrane related cell wall remodel pathway in asexual cycle but inhibit in sexual cycle of *F. graminearum* via enhancing microtubule synthesis, organization, stability, transportation and material exchanging in the separation site between neighboring cells.

## Session II-5

**OAT RESISTANCE TO HT2 AND T2 PRODUCING FUSARIUM  
LANGSETHIAE****T. Stancic**<sup>1\*</sup>, A.A. Cowan<sup>2</sup>, S.G. Edwards<sup>1</sup>

<sup>1</sup>Harper Adams University College, Newport, Shropshire, TF10 8NB, UK; <sup>2</sup>IBERS, Aberystwyth University, Plas Gogerddan, Aberystwyth, SY23 3EB, UK,

E-mail: [tstancic@harper-adams.ac.uk](mailto:tstancic@harper-adams.ac.uk)

The European Commission is setting investigative limits for the combined concentration of *Fusarium* mycotoxins, HT2 and T2 (HT2+T2) in food and feed. In observational studies across the UK between 2002 and 2008 around 16% of oat samples collected at harvest exceeded the proposed investigative limit of 1000 µg/kg HT2+T2 for unprocessed oats intended for human consumption. From 2005 - 2011, oats from over 20 winter and more than 14 spring national Recommended List variety trials were analysed for the presence of HT2+T2 mycotoxins. All winter variety trials had higher levels of HT2+T2 compared to the spring variety trials. It is not clear whether the difference observed between winter and spring varieties is due to agronomic (ie drilling date) or genetic difference. To test the hypothesis that the difference observed were not due to agronomy, six spring and six winter varieties were drilled together in randomised block experiments at three sites in the UK in autumn 2011 and in spring 2012. Samples were collected before and after harvest and quantified for HT2 and T2. Preliminary results suggest that in field experiments where high levels of HT2+T2 were detected then some winter varieties had higher concentrations of HT2+T2 compared to other winter and spring varieties irrespective of if they were sown in the autumn or spring.

## Session II-6

**CHARACTERIZATION OF FUSARIUM HEAD BLIGHT RESISTANCE IN  
CIMMYT SYNTHETIC-DERIVED BREADWHEAT**

**Zhanwang Zhu**<sup>1,2</sup>, David Bonnett<sup>2\*</sup>, Marc Ellis<sup>2</sup>, Pawan Singh<sup>2</sup>, Nicolas Heslot<sup>3</sup>,  
Susanne Dreisigacker<sup>2</sup>, Chunbao Gao<sup>1</sup>

<sup>1</sup>Institute of Food Crops, Hubei Academy of Agricultural Sciences/Hubei Engineering and Technology Research Center of Wheat/Wheat Disease Biology Research Station for Central China, Wuhan 430064, China; <sup>2</sup>International Maize and Wheat Improvement Center (CIMMYT), Apdo. Postal 6-641, 06600, Mexico D.F., Mexico;

<sup>3</sup>Cornell University, Department of Plant Breeding and Genetics, 240 Emerson Hall, Ithaca, NY 14853, USA

\* Corresponding author: David Bonnett, E-mail: [d.bonnett@cgiar.org](mailto:d.bonnett@cgiar.org)

Fusarium head blight (FHB) is a devastating fungal disease of bread and durum wheat worldwide. It reduces wheat yield, lowers processing qualities, and makes grain not suitable for human or animal consumption due to mycotoxin contamination. Use of host resistance is an economically effective and environmentally friendly method to control FHB. The FHB resistant synthetic derivative ‘SYN1’ was developed from a cross between two resistance sources, Mayoor and the primary synthetic breadwheat Tksn1081/*Ae. tauschii* (222) that are likely to form an important component of resistance in many elite CIMMYT breadwheats. In order to map gene loci underlying FHB resistance of ‘SYN1’, a doubled haploid (DH) population produced from a cross between ‘SYN1’ and the susceptible CIMMYT-derived variety Ocoroni-86 was evaluated in artificially inoculated field nurseries in the 2010 and 2011 crop seasons at CIMMYT’s research station in El Batan, Mexico. Ten marked spikes from each plot were scored and subsequently FHB index calculated. Molecular data was obtained by genotyping by sequencing (GBS) and simple-sequence repeat (SSR) markers. Twenty eight linkage groups were constructed. These linkage groups were anchored by SSRs to most chromosomes, except 4D, 5D and 6D. Using inclusive composite interval mapping (ICIM), three genomic regions were found to have a significant effect on FHB index with phenotypic variation explained by *QFhs.cim\_1B* estimated at 4.7%, 25.0% by *QFhs.cim\_2D* and 4.2% by *QFhs.cim\_7A*. The QTL mapping results showed that the favorable alleles of *QFhs.cim\_1B*, *QFhs.cim\_2D* and *QFhs.cim\_7A* were derived from the synthetic-derived breadwheat ‘SYN1’. Genotypes of the parents of ‘SYN1’ indicated that the favourable alleles at these three QTLs were all inherited from Mayoor.

## Session II-7

**DISTRIBUTION OF THE QTL ASSOCIATED WITH FUSARIUM HEAD BLIGHT RESISTANCE IN BREEDING POPULATION OF WHEAT AND THEIR GENETIC EFFECTS**

**Tao Li**<sup>1\*</sup>, Xiali Zhou<sup>1†</sup>, Dadong Zhang<sup>2†</sup>, Guihua Bai<sup>2,3\*</sup>, Shiliang Gu<sup>1</sup>

<sup>1</sup>Jiangsu Provincial Key Laboratory of Crop Genetics and Physiology; Key Laboratory of Plant Functional Genomics of Ministry of Education, Yangzhou University, Yangzhou, 225009, China; <sup>2</sup>Department of Agronomy, Kansas State University, Manhattan, KS 66506, USA; <sup>3</sup>USDA-ARS Hard Winter Wheat Genetics Research Unit, Manhattan, KS 66506, USA

† These authors contributed to the equal work

\*Corresponding authors: Tao Li, [taoli@yzu.edu.cn](mailto:taoli@yzu.edu.cn);

Guihua Bai, [guihua.bai@ars.usda.gov](mailto:guihua.bai@ars.usda.gov)

Some of Chinese and Japan wheat varieties, particularly the landraces, might harbor unique resistance components. Investigation of these varieties may discover potential novel major QTL or favorable alleles for FHB resistance. In this study, 197 wheat varieties (or inbred lines) from different sources were genotyped using 370 genome-wide SSR and STS molecular markers and type II resistance to fusarium head blight (FHB) (the resistance to fungal spread within spike) were evaluated using single floret inoculation method both in the greenhouse for three seasons (or experiments) (E1, E2 and E3) and in the field for two seasons (E4 and E5). Marker-FHB resistance association was analyzed based on a mixed model via the TASSEL 2 pipeline. 18, 24, 20, 16 and 12 marker loci were identified to be significantly associated with FHB resistance in E1, E2, E3, E4 and E5, respectively, and these markers accounted for 0.7-4.7%, 0.2-3.9%, 0.4-7.3%, 1.1-11.8% and 2.1-9.9% of phenotypic variation, respectively. 12 markers were detected more than one experiment in the greenhouse whereas no markers were in common across the two experiments in the field. Xcfa2263, Xgdm138, Xumn10, Xgwm205, Xgwm261 and Xgdm35 were detected at least once in each environment (i.e. in the greenhouse and in the field). The allelic variation and the genetic effects of the key marker loci that were detected in two or more experiments were further untangled (two marker loci were presented here as examples only). Xgdm138 on 5DL had three statistically functional alleles, two were unfavorable and one favorable. The genetics effects of the favorable allele ranged from 0.32 to 0.34 across the experiments. Two Chinese varieties, Siyang117 and Huangcandou (landrace), six Japanese varieties and one American variety carry the favorable allele. Xgdm138 on 5DL might be a novel locus. Xbarc133 that is associated with Fhb1 locus had four statistically functional alleles. Three were unfavorable and

one favorable. Fifty-five varieties carry the favorable allele including: 19 Chinese commercial varieties, 11 Chinese landraces, 21 varieties from Japan, one from France, two varieties from South Korea and two from the USA. Cvs. Sumai3, Ning7840, Wanian2, Nyubai and Huangcandou carried the favorable allele, and the Fhb1 locus has been confirmed to be present in these varieties via multiple QTL mapping projects. Xbarc133 was a good but not a diagnostic marker for Fhb1 locus based on this study due to its false positive (cv. Chokwang) or false negative (cvs Wangshuibai and Huangfangzhu) presentation. Three markers including Xwsmv1.2, Xcfa2019 and Xgdm35 showed significant correlations to FHB resistance, the genetic effects, however, failed to be dissected probably due to the existence of multiple alleles but each allele was presented in few varieties (i.e. rare allele dilemma). The results may provide additional information to breeders and diversify the wheat FHB-resistance gene pool, thus are valuable for improvement of FHB resistance in wheat breeding program. Acknowledgement: We thank the financial support by the National Major Project of Breeding for New Transgenic Organisms (2012ZX08009003-004) and by NSFC (31171537).

## Session II-8

**NITRATE NUTRIENT ENHANCED THE RESISTANCE OF CUCUMBER PLANTS TO FUSARIUM WILT**

**Min Wang**, Ning Ling, Xiaokang Liu, Xian Dong, Qirong Shen, Shiwei Guo\*

Agricultural Ministry Key Lab of Plant Nutrition and Fertilization in Low-Middle Reaches of the Yangtze River; Jiangsu Key Lab and Engineering Center for Solid Organic Waste Utilization; Nanjing Agricultural University, 210095, China

\* Corresponding author: Shiwei Guo, E-mail: [sguo@njau.edu.cn](mailto:sguo@njau.edu.cn)

*Fusarium oxysporum* f. sp. *cucumerinum* (FOC) is the causal agent of cucumber (*Cucumis sativus* L.) Fusarium wilt, a soil-borne disease that causes severe losses in yield and quality. Mineral nutrition plays an important role in the management of a broad range of plant diseases. Nitrogen is one of the most important nutrients for plant growth and disease development. Not only the amount of nitrogen but also the form of nitrogen (ammonium or nitrate) available to plants and pathogens is critical in plant disease incidence. Nitrogen can affect the development of a disease by affecting plant physiological and biochemical processes or by affecting pathogens, or both of them. We investigate the effect of nitrogen forms on cucumber Fusarium wilt and to illustrate the possible mechanisms of cucumber resistance regulated by nitrogen forms. Compare to ammonium nutrition, nitrate nutrition significantly suppressed disease index of cucumber Fusarium wilt. Furthermore, the number of pathogens in nitrate grown plant was significantly lower than ammonium grown plant. To illustrate the resistance mechanism of nitrated grown plant, the component of root exudates from different treatments was identified. We found that higher concentration of citric acid, which was preferable to FOC spore germination, was accumulated in root exudates of ammonium grown plant resulting in significance increasing in the number of FOC in ammonium grown plant. In conclusion, nitrate nutrition enhanced the resistance of cucumber plants to Fusarium wilt. We should increase the nitrate fertilizer and decrease ammonium fertilizer input in cucumber seedlings to increase the resistance to Fusarium wilt.

## Session II-9

**THE SCAFFOLD PROTEIN FGSTE50 REGULATES VEGETATIVE  
DIFFERENTIATION, SECONDARY METABOLISM AND VIRULENCE VIA  
THE GPMK1 AND HOG MAPK PATHWAYS IN *FUSARIUM  
GRAMINEARUM***

**Qin Gu** and Zhonghua Ma\*

Key Laboratory of Molecular Biology of Crop Pathogens and Insects, Institute of Biotechnology, Zhejiang University, Hangzhou, 310058, China;

\* Corresponding author: Zhonghua Ma, E-mail: [zhma@zju.edu.cn](mailto:zhma@zju.edu.cn)

The mitogen-activated protein kinase (MAPK) signaling pathways commonly exist in all eukaryotes and play important roles in cell growth, differentiation and stress response. Although some of basic signaling components of the MAPK pathways have been characterized, little is known about the cross-talk among MAPK pathways in filamentous fungi. In this study, we characterized an adaptor protein FgSte50 of *Fusarium graminearum*, which is homologous to the Ste50 of *Saccharomyces cerevisiae*. The *FgSTE50* deletion mutant  $\Delta$ FgSte50 grew significantly slower than the wild-type progenitor on solid media. The mutant exhibited a significant decrease in conidiation. In addition,  $\Delta$ FgSte50 lost the ability to form perithecia *in vitro*. Pathogenicity tests showed that  $\Delta$ FgSte50 was non-pathogenic on flowering wheat heads, which may result from the multiple defects of the mutant in mycotoxin production, secretion of the extracellular lipase and formation of penetration structure. In yeast two-hybrid assays, FgSte50 interacts not only with FgSte11 and FgSte7, the key components of Gpmk1 MAPK pathway, but also with FgHog1 of the HOG pathway. Furthermore, FgSte50 positively regulates phosphorylation of FgGpmk1 and FgHog1 in *F. graminearum*. Similar to the *FgSTE50* deletion mutant, the *FgSTE11* deletion mutant reveals various defects in vegetative differentiation and plant infection. In addition, FgSte50 and FgHog1 share similar function in the regulation of DON biosynthesis. Our data indicate that FgSte50 plays important roles in multiple developmental processes related to vegetative differentiation, plant infection and secondary metabolism via the Gpmk1 and HOG MAPK pathways in *F. graminearum*.

## Session III-1

**POPULATION STRUCTURES OF FUSARIUM SPECIES ISOLATED FROM  
WHEAT AND MAIZE IN CHINA**

Jing-Bo Zhang<sup>1,2</sup>, He-Ping Li<sup>1,3</sup>, Jian-Hua Wang<sup>1,2</sup>, Wei Cheng<sup>1,2</sup>, Peng Yang<sup>1,2</sup>, Sheng  
Xue<sup>1,2</sup>, An-Dong Gong<sup>1,2</sup>, Xiu-Shi Song<sup>1,2</sup>, Dong-Yun Zuo<sup>1,3</sup>, Bo Qu<sup>1,2</sup>, **Yu-Cai**  
**Liao**<sup>1,2\*</sup>

<sup>1</sup>Molecular Biotechnology Laboratory of Triticeae Crops, Huazhong Agricultural University, Wuhan 430070, Hubei, China; <sup>2</sup>College of Plant Science and Technology, Huazhong Agricultural University, Wuhan 430070, Hubei, China; <sup>3</sup>College of Life Science and Technology, Huazhong Agricultural University, Wuhan 430070, Hubei, China

\*Corresponding author: Yu-Cai Liao, E-mail: [yucailiao@mail.hzau.edu.cn](mailto:yucailiao@mail.hzau.edu.cn)

*Fusarium* pathogens are responsible for devastating diseases such as *Fusarium* head blight (FHB) of wheat and *Gibberella* ear rot (GER) in maize worldwide. FHB epidemics in wheat occur frequently in the middle and lower regions of the Yangtze River, and in northeast areas covering more than ten provinces in China, whereas GER in maize is even more widely present in maize growing regions. Single spore isolates from FHB-related wheat spikes and GER-associated maize grains in all the regions throughout China were subjected to analysis of species identification, geographical distribution, mycotoxin chemotyping and pathogenicity. *F. graminearum* clade complex (Fg complex) is the main species causing FHB in wheat, in which two phylogenetic species, *F. asiaticum*, and *F. graminearum sensu stricto*, co-existed, with the former being the predominant one. Non-Fg complex *Fusarium* species such as *F. poae*, *F. proliferatum* and *F. tricinctum* were only minority. Genetic chemotyping together with chemical determination revealed that *F. asiaticum* produced deoxynivalenol (DON), 3-acetyldeoxynivalenol (3-ADON), 15-acetyldeoxynivalenol (15-ADON), and nivalenol (NIV) mycotoxins, with 3-ADON being the main chemotype. *F. graminearum* only contained DON and 15-ADON producers. A further shift towards 3-ADON producers in the field populations of *Fusarium* species from wheat took place during the last decade. No correlation was seen between phylogenetic species and pathogenicity. In GER in maize, Fg complex also contained the two species, *F. asiaticum*, and *F. graminearum*. However, a different proportion of these two species and a distinct chemotype pattern were observed in GER-associated Fg complex in maize. Moreover, non-Fg complex *Fusarium* species accounted for a larger proportion, including several new reported species. These results provide information for monitoring and controlling *Fusarium* pathogens and associated mycotoxins in agriculture and food/feed samples.



## Session III-2

## FUSARIELINS

**Teis Esben Sondergaard**, Aida Droce, Wagma Saei, Henriette Giese, Jens L. Sørensen

Department of Biotechnology, Chemistry and Environmental Engineering, Aalborg University, Sohngaardsholmsvej 49, DK-9000 Aalborg, Denmark.

E-mail: [tes@bio.aau.dk](mailto:tes@bio.aau.dk)

Fusarielins are produced by the fusarielin cluster harboring the polyketide synthase 9 (PKS9). By overexpressing the cluster specific transcription factor for PKS9 we successfully activated the cluster, which led to production of three novel fusarielins from *Fusarium graminearum* F, G and H. Fusarielin A-F have previously been identified in two unidentified *Fusarium* species and from a *F. tricinctum* strain, but the ability of other *Fusarium* species to produce fusarielins is unknown. To examine the distribution of fusarielin in the *Fusarium* genus we found that only *F. graminearum* and *F. tricinctum* were able to produce fusarielins. In order to identify the synthase pathway for fusarielins, we have generated knock out mutants of every gene in the cluster in *F. graminearum* and analyzed intermediated products by HPLC and NMR.

Fusarielins showed a weak toxic effect against bacteria and most human cell lines except human breast cancer cells. Rather, fusarielins proved able to stimulate MCF7 cells through a binding to the estrogen receptor. Because of this stimulation, fusarielins are categorized as mycoestrogens in spite of the fact that fusarielins have very little structural similarity to estrogen.

To examine possible implications of fusarielins for human health, we quantified fusarielins in six wheat spikes inoculated with *F. graminearum*. The predominant analogue, fusarielin H, was found in all six heavily infected spikes at levels ranging from 392 – 1865 µg/kg (mean: 989 µg/kg). This shows that fusarielins can be produced during infection, although only at relatively small levels.

## Session III-3

**MULTI-COMPONENT LC-MS/MS ANALYSIS FOR SIMULTANEOUS DETERMINATION OF FUSARIUM MYCOTOXINS IN AGRICULTURAL PRODUCTS**Zheng Han, Suquan Song, Jianhua Wang, **Aibo Wu**\*

Institute for Agri-Food Standards and Testing Technology, Laboratory of Quality and Safety Risk Assessment for Agro-Products (Shanghai), Ministry of Agriculture, Shanghai Academy of Agricultural Sciences, 1000 Jinqi Road, Shanghai 201403, PR China

\* Corresponding author: Aibo Wu, E-mail: wuaibo@saas.sh.cn

Fusarium mycotoxins, a series of secondary metabolites, are produced by various *Fusarium* species growing on the plant origin product either in the field or during storage. Hitherto, more than 200 Fusarium mycotoxins have been identified, which can be categorized into different groups on the basis of their different sources. For example, trichothecenes represent a group of secondary metabolites produced by toxigenic *Fusarium* species, especially *Fusarium graminearum*, *Fusarium sporotrichioides*, *Fusarium poae*, and *Fusarium equiseti*. Acute and chronic ingestion of trichothecenes exemplified as deoxynivalenol by humans and animals can elicit a variety of toxic effects, i.e., feed refusal, weight loss, vomiting necrosis of the myeloid tissue, and hemorrhage of visceral organs. Zearalenone and its derivatives are a group of phenolic compounds produced mainly by *Fusarium graminearum* and *Fusarium culmorum*, may damage the reproduction of mammals. A generic procedure, which involved accelerated solvent extraction and homemade cleanup cartridges, has been developed for the extraction and purification of more than 30 mycotoxins in various grain matrixes, i.e., wheat, maize, traditional Chinese medicines, and various vegetables for subsequent analysis by LC-MS/MS.

## Session III-4

**STUDY ON THE DIFFERENTIATION OF PHYSIOLOGICAL RACES,  
VEGETATIVE COMPATIBILITY GROUPS AND INFECTION PROCESS OF  
*FUSARIUM OXYSPORUM* F.SP. *NIVEUM* IN JIANGSU PROVINCE**

**Haiyan Sun**

Institute of Plant Protection, Jiangsu Academy of Agriculture Sciences, Nanjing,  
210014, P. R. China

E-mail: [haiyansun@jaas.ac.cn](mailto:haiyansun@jaas.ac.cn)

*Fusarium oxysporum* f. sp. *niveum* (E.F.Sm.) W.C. Snyder & H.N. Han., the casual agent of Fusarium wilt of watermelon, is widespread in watermelon growing regions of Jiangsu province in China. The promotion and use of resistant cultivars is the most economical and effective way to control the disease. Differences in virulence among isolates of *F. oxysporum* f.sp. *niveum* have long been recognized and isolates are subdivided into three races on the basis of virulence on watermelon cultivars that vary in their level of resistance. In order to investigate the differentiation of physiological races, vegetative compatibility groups of *F. oxysporum* f.sp. *niveum* in Jiangsu province in China, and also the relation between them, fifteen isolates of *F. oxysporum* f.sp. *niveum* were collected from different fields in Jiangsu province, physiological races of which were identified by inoculation on identified host (Sumi No1, Charleston Gray and Calhoun Gray) and vegetative compatibility of which were determined by the technology of nitrate reductase deficiency. Race 0, race 1 and race 2 were detected among 15 isolates, the frequencies of which were 6.7%, 73.3% and 20%, respectively. All isolates belong to one VCG, but each of them showed differences in the capacity to compatibility, which was not associated with Physiological races and locations. Isolates belonged to different races were vegetative compatible to each other. Confocal laser scanning microscopy (CLSM) images of watermelon roots infected with the highly virulent isolate GY16-GFP demonstrated that fungal penetration of watermelon roots occurs simultaneously at several random sites, but it occurs preferentially in the junctions between epidermal cells. And fungal hyphae were able to penetrate cell walls directly to grow inside and outside cells. *F. oxysporum* f.sp. *niveum* hyphae were observed primarily invading the xylem vessel, and than invading in epidermal and cortical rhizome cells.

## Session III-5

**EVIDENCE FOR BIRTH-AND-DEATH EVOLUTION AND HORIZONTAL TRANSFER OF A MYCOTOXIN BIOSYNTHETIC GENE CLUSTER IN FUSARIUM**

Robert H. Proctor<sup>1</sup>, **François Van Hove**<sup>2</sup>, Antonia Susca<sup>3</sup>, Gaetano Stea<sup>3</sup>,

Mark Busman<sup>1</sup>, Theo van der Lee<sup>4</sup>, Cees Waalwijk<sup>4</sup>, Todd J. Ward<sup>1</sup>, Antonio Moretti<sup>3</sup>

<sup>1</sup>United States Department of Agriculture, Agriculture Research Service, National Center for Agricultural Utilization Research, Peoria, Illinois, USA; <sup>2</sup>Université catholique de Louvain, Earth and Life Institute, Applied Microbiology, Mycology, Mycothèque de l'Université catholique de Louvain (BCCM<sup>TM</sup>/MUCL), Louvain-la-Neuve, Belgium; <sup>3</sup>National Research Council, Institute of Sciences of Food Production, Bari, Italy; <sup>4</sup>Plant Research International B.V., Wageningen, The Netherlands.

E-mail: [francois.vanhove@uclouvain.be](mailto:francois.vanhove@uclouvain.be)

Fumonisin are a family of carcinogenic secondary metabolites produced by members of the *Fusarium fujikuroi* species complex (FFSC) and rare strains of *F. oxysporum*. In *Fusarium*, fumonisin biosynthetic genes (*FUM*) are clustered, and the *FUM* cluster is uniform in gene organization. Here, sequence analyses indicated that the cluster exists in five different genomic contexts, defining five cluster types. In *FUM*-gene genealogies, evolutionary relationships between fusaria with different cluster types were largely incongruent with species relationships inferred from analyses of primary-metabolism (PM) genes, and *FUM* cluster types are not trans-specific. In addition, synonymous site divergence analyses indicated that three *FUM* cluster types predate diversification of FFSC. The data are not consistent with balancing selection or interspecific hybridization, but they are consistent with two competing hypotheses: multiple horizontal transfers of the cluster from unknown donors to FFSC recipients versus cluster duplication and loss (birth and death). Furthermore, low levels of *FUM*-gene divergence in *F. bulbicola*, an FFSC species, and *F. oxysporum* provide evidence for horizontal transfer of the cluster from the former, or a closely related species, to the latter. Thus, uniform gene organization within the *FUM* cluster belies a complex evolutionary history that has not always paralleled the evolution of *Fusarium*.

## Session III-6

**CONTAMINATION OF BANANAS WITH BEAUVERICIN AND FUSARIC ACID PRODUCED BY *FUSARIUM OXYSPORUM* F. SP. *CUBENSE***

**Chunyu Li**\*, Cunwu Zuo, Jing Yang, Ruibin Kuang, Yuerong Wei, Qiaosong Yang, ChunhuaHu, Ou Sheng, Guiming Deng, Siwen Liu, Ganjun Yi

Institution of Fruit Tree Research, Guangdong Academy of Agricultural Sciences, Guangzhou, 510640, Guangdong Province, China; Key Laboratory of South Subtropical Fruit Biology and Genetic Resource Utilization, Ministry of Agriculture, China

\* Corresponding author: Ganjun Yi, E-mail: [yiganjun@vip.163.com](mailto:yiganjun@vip.163.com)

Fusarium wilt, caused by the fungal pathogen *Fusarium oxysporum* f. sp. *cubense* (*Foc*), is one of the most destructive diseases of banana. Toxins produced by *Foc* have been proposed to play an important role during the pathogenic process. The objectives of this study were to investigate the contamination of banana with toxins produced by *Foc*, and to elucidate their role in pathogenesis. Twenty strains of *Foc* representing races 1 and 4 were isolated from diseased bananas in five Chinese provinces. Two toxins were consistently associated with *Foc*, fusaric acid (FA) and beauvericin (BEA). Cytotoxicity of the two toxins on banana protoplast was determined using the Alamar Blue assay. The virulence of 20 *Foc* strains was further tested by inoculating tissue culture banana plantlets, and the contents of toxins determined in banana roots, pseudostems and leaves. Virulence of *Foc* isolates correlated well with toxin deposition in the host plant. To determine the natural occurrence of the two toxins in banana plants with Fusarium wilt symptoms, samples were collected before harvest from the pseudostems, fruit and leaves from 10 Pisang Awak ‘Guangfen #1’ and 10 Cavendish ‘Brazilian’ plants. FA and BEA were detected in all the tissues, including the fruits. The current study provides the first investigation of toxins produced by *Foc* in banana. The toxins produced by *Foc*, and their levels of contamination of banana fruits, however, were too low to be of concern to human and animal health. Rather, these toxins appear to contribute to the pathogenicity of the fungus during infection of banana plants.

## Session III-7

**POPULATION ANALYSIS OF THE *FUSARIUM GRAMINEARUM*  
SPECIES COMPLEX FROM WHEAT IN CHINA****Zhang Hao**

State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agriculture Sciences, Beijing, China

E-mail: hzhang@ippcaas.cn

A large number of *Fusarium* isolates was collected from blighted wheat spikes originating from 175 sampling sites, covering 15 provinces in China. Species and tricothecene chemotype determination by multilocus genotyping (MLGT) indicated that *F. graminearum* s. str. with the 15-acetyl deoxynivalenol (15ADON) chemotype and *F. asiaticum* with either the nivalenol (NIV) or the 3-acetyl deoxynivalenol (3ADON) chemotype were the dominant causal agents. Bayesian model-based clustering with allele data obtained with 12 variable number of tandem repeats (VNTR) markers, detected three genetic clusters that also show distinct chemotypes. High levels of population genetic differentiation and low levels of effective number of migrants were observed between these three clusters. Additional genotypic analyses revealed that *F. graminearum* s. str. and *F. asiaticum* are sympatric. In addition, composition analysis of these clusters indicated a biased gene flow from 3ADON to NIV producers in *F. asiaticum*. In phenotypic analyses, *F. asiaticum* that produce 3ADON revealed significant advantages over *F. asiaticum* that produce NIV in pathogenicity, growth rate, fecundity, conidial length, tricothecene accumulation and resistance to benzimidazole. These results suggest that natural selection drives the spread of a more vigorous, more toxigenic pathogen population which also shows higher levels of fungicide resistance.

## Session III-8

**TIMING AND EFFICACY OF FUNGICIDES AGAINST FUSARIUM HEAD  
BLIGHT IN MALTING BARLEY****L. K. Nielsen**<sup>1</sup>, S. G. Edwards<sup>2</sup>, D. J. Cook<sup>1</sup>, R. V. Ray<sup>1</sup>

<sup>1</sup>University of Nottingham, School of Biosciences, Sutton Bonington Campus, Loughborough, Leicestershire, LE12 5RD, United Kingdom; <sup>2</sup>Harper Adams University, Newport, Shropshire, TF10 8NB, United Kingdom

E-mail: [linda.nielsen@nottingham.ac.uk](mailto:linda.nielsen@nottingham.ac.uk)

Fungicide efficacy and timing of application against Fusarium Head Blight (FHB) in UK malting barley have not been previously determined. The objectives of the present study were to evaluate a range of fungicide treatments and their timing of application against FHB in malting barley. Field experiments using the commercial cultivar Quench were conducted over two seasons (2011/12) at five different locations in England. Two locations (one misted and one non-misted) were artificially inoculated with a mixture of *F. poae*, *F. langsethiae* and *F. tricinctum*. Two more locations (one misted and one non-misted) were artificially inoculated with a mixture of *F. graminearum*, *F. culmorum*, *F. tricinctum*, *F. poae*, *F. langsethiae*, *F. avenaceum*, *M. majus* and *M. nivale*. One location relied on natural infection with *Fusarium* and *Microdochium* species. Nine fungicide combinations with four replicates were tested at each experimental location and year. The key treatments applied at GS 39/45 were a formulation of 263 g ha<sup>-1</sup> cyprodinil and 87 g ha<sup>-1</sup> isopyrazam or a formulation of 100 g ha<sup>-1</sup> fluoxastrobin and 100 g ha<sup>-1</sup> prothioconazole. At GS 59 the following fungicides were tested, 99 g ha<sup>-1</sup> prothioconazole, a formulation of 100 g ha<sup>-1</sup> fluoxastrobin and 100 g ha<sup>-1</sup> prothioconazole, or a mixture of 94 g ha<sup>-1</sup> isopyrazam and 99 g ha<sup>-1</sup> prothioconazole.

The incidence and severity of FHB and brown foot rot disease were assessed at GS 75. Fungicide application at GS 39/45 reduced brown foot rot severity by 17%. Reductions of more than 30% in FHB severity were achieved by all fungicide treatments applied at GS 59. The fungal biomass of *Fusarium* and *Microdochium* spp. were quantified in harvested grain and stems using species specific real-time PCR assays and will be used together with mycotoxin data and grain quality parameters to further evaluate the effects of chemical control of FHB.

**Session IV****FUSARIUM – NOW, FUTURE CHALLENGES AND OPPORTUNITIES****Ulf Thrane**

Fungal Physiology and Biotechnology, DTU Systems Biology, Technical University of Denmark, Kgs. Lyngby, Denmark

E-mail: [ut@bio.dtu.dk](mailto:ut@bio.dtu.dk)

The genus *Fusarium* is very important as a well-known plant pathogen, a mycotoxin producer, an opportunistic pathogen in animals and humans, a spoiler of food, feed as well as non-food products, but also as a production organism in biotech industries. This diversity is reflected by an overwhelming activity on a global scale resulting in an endless number of scientific publications, patents, technical reports and case stories in magazines. The palette of international activities is also visible at the many international and national *Fusarium* meetings that are held every year. In this last lecture at the 11<sup>th</sup> International *Fusarium* Workshop highlights of the presented papers will be summarized and put into a historical perspective to illustrate the recent movement and important achievement obtained within the *Fusarium* community.

The improvement of our body-of-knowledge of *Fusarium* seen in the light of the last decade's rapid development in biological sciences will be exemplified and discussed. The discussion will cover species concepts, ecological understanding, metabolism, and biotechnological exploitation. The discussion will also take a look into the future – what is coming up? What can we do with all the different kind of data that are stored? Will the technical development improve our knowledge on *Fusarium*? Can we eliminate the *Fusarium* problems? Will there be a super-biotech product from *Fusarium*? How will the World of *Fusarium* look like when we meet at the next International *Fusarium* Workshop?



## Poster Abstracts

### Poster-1

#### THE IMPORTANCE OF STYLAR CANAL ARCHITECTURE IN MEDIATING RESISTANCE TO FUSARIUM EAR ROT IN MAIZE

Vermeulen, M., Rose, L.J., Viljoen, A.

Department of Plant Pathology, Stellenbosch University, Private Bag X1, Matieland,  
7602, South Africa

E-mail: altus@sun.ac.za

*Fusarium verticillioides* is an important pathogen of maize that causes Fusarium ear rot (FER) and deposits toxic secondary metabolites, known as fumonisins, in maize grain. Infection by *F. verticillioides* in developing maize kernels has been shown to occur via the stylar canal, which is situated near the silk scar. The architecture of the stylar canal has been proposed as an important resistance barrier to *F. verticillioides* infection when closed, while facilitating infection when open. To determine its importance in providing structural resistance, the stylar canal of six resistant and three susceptible maize inbred lines were studied using scanning electron microscopy (SEM). Maize ovaries and kernels were harvested 1 to 2 weeks before pollination and 1 month after pollination respectively. The harvested samples were stored at -80°C, critical point dried, coated in gold and viewed using SEM. The ovaries of two resistant (CML 390 and US 2540) and one susceptible (I 137tnW) maize inbred lines exhibited closed stylar canals. The ovaries of the remaining resistant (CML 444, CML 182, VO 617Y-2, RO 549 W and R119W) and susceptible (RO 544 W and R 2565Y) maize inbred lines, however, had open stylar canals. The stylar canal of most of the maize kernels appeared to be covered by a raised ridge 1 month after pollination. A closed stylar canal architecture, therefore, could not be correlated with resistance to FER in all the maize inbred lines tested. While stylar canal architecture may provide a formidable barrier in mediating resistance to *F. verticillioides* infection, it cannot be considered the only mechanism of kernel resistance to the FER pathogen.

## Poster-2

**AN ANUCLEATE PRIMARY STERIGMATA PROTEIN FGAPSB INVOLVED  
IN REGULATING VEGETATIVE DIFFERENTIATION, ASEXUAL  
DEVELOPMENT, NUCLEAR MIGRATION AND VIRULENCE OF  
*FUSARIUM GRAMINEARUM***

**Zhitian Zheng**, Tao Gao, Yiping Hou, Jingxin Wang, Changjun Chen, Mingguo Zhou\*

College of Plant Protection, Nanjing Agricultural University, Nanjing 210095, China

\* Corresponding author: Mingguo Zhou, E-mail: [mgzhou@njau.edu.cn](mailto:mgzhou@njau.edu.cn)

An anucleate primary sterigmata protein ApsB has been shown to play critical roles in the migration and positioning of nuclei and in the development of conidiophores in *Aspergillus nidulans*. The functions of ApsB in *Fusarium graminearum*, a causal agent cause of Fusarium head blight in China, are largely unknown. In this study, we used the BLASTP program to identify FgApsB, an *F. graminearum* homolog of *A. nidulans* ApsB. The functions of FgApsB were evaluated by constructing a deletion mutant of FgApsB, designated  $\Delta$ FgApsB-28. Conidiation and mycelial growth rate are reduced in  $\Delta$ FgApsB-28. The hyphae of  $\Delta$ FgApsB-28 are thinner than those the wild type and have a reduced branching angle.  $\Delta$ FgApsB-28 exhibited reduced aerial hyphae formation but increased production of rubrofusarin. Whereas nuclei are evenly distributed in germ tubes and hyphae of the wild type, they are clustered and irregularly distributed in  $\Delta$ FgApsB-28. The mutant exhibited increased resistance to cell wall-damaging agents but reduced virulence on flowering wheat heads, which is consistent with its reduced production of the toxin deoxynivalenol. All of the defects in  $\Delta$ FgApsB-28 were restored by genetic complementation with the parental FgApsB gene. Taken together, the results indicate that FgApsB is important for vegetative differentiation, asexual development, nuclear migration, and virulence in *F. graminearum*.

## Poster-3

**COLONIZATION OF FUSARIUM WILT- RESISTANT AND SUSCEPTIBLE WATERMELON ROOTS BY GREEN FLUORESCENT PROTEIN-TAGGED ISOLATE OF *FUSARIUM OXYSPORUM* F. SP. *NIVEUM***

**Guiyun Lü**<sup>1</sup>, Shaogui Guo <sup>2</sup>, Haiying Zhang <sup>2</sup>, Lihua Geng<sup>2</sup> And Yong Xu<sup>2</sup>

<sup>1</sup>College of Horticulture, Agricultural University of Hebei, Baoding 071001, China;

<sup>2</sup>National Engineering Research Center for Vegetables, Beijing Academy of Agriculture and Forestry Sciences, 100097, China ;

Fusarium wilt of watermelon, caused by soilborne fungus *Fusarium oxysporum* f. sp. *niveum*(*Fusarium oxysporum* Schlechtend.:Fr. f. sp. *niveum* (E.F. Sm.) W.C. Snyder & H.H. Hans(FON), is one of the most severe vascular diseases worldwide. The disease is a yield-limiting factor in watermelon production and can affect all stages of plant growth. In this paper, we describe the *Agrobacterium tumefaciens*-mediated transformation of *F. oxysporum* f. sp. *niveum* race 1 with a plasmid-harboring *gfp* that results in stable expression of *gfp*, GFP is an excellent reporter in *Fusarium oxysporum* f. sp. *niveum*. Interactions between watermelon and a green fluorescent protein (GFP)-tagged isolate of FON1, were studied to determine differences in infection and colonization of watermelon roots in cultivars resistant and susceptible to Fusarium wilt. The roots of watermelon seedlings were inoculated with a conidial suspension of the GFP- tagged isolate and confocal laser scanning microscopy was used to visualize colonization, infection, and disease development. The initial infection stages were similar in both the resistant and susceptible cultivar but the resistant cultivar responded differentially after the pathogen had penetrated the root. The pathogen penetrated and colonized resistant watermelon roots but further fungal advance appeared to be halted and the fungus did not enter the taproot, suggesting that resistance is initiated post penetration. The initial infection zone for both the wilt-susceptible and – resistant watermelon roots appeared to be the epidermal cells of root hairs, which *F. o.* f. sp. *niveum* was able to penetrate directly after forming appressoria. Areas where secondary roots emerged and wounded root tissue were penetrated preferentially.

## Poster-4

**PHOSPHOLIPASE C4 IS INVOLVED IN SPORULATION AND PATHOGENICITY IN *FUSARIUM OXYSPORUM***Xiaojing Chu, Jiao Jiao, Hongbin Zhang, Ling Sun, **Yuancun Liang**\*

Department of Plant Pathology, Shandong Agricultural University, Taian 271018, China

\* Corresponding author: Yuancun Liang, E-mail: [liangyc@sdau.edu.cn](mailto:liangyc@sdau.edu.cn)

*Fusarium oxysporum* f. sp. *cucumerium* causes Fusarium wilt, an important disease of cucumber. Phospholipase C (PLC) enzymes hydrolyse the phosphodiester bond between the phosphate oxygen and the glycerol carbon of phospholipids, liberating a diacylglycerol molecule and head group-phosphate complex. Nine isoforms of *FoPLC* are identified in *F. oxysporum*, through a search of the *F. oxysporum* f. sp. *lycopersici* genome sequence database. Among of them, *FoPLC4* has the four basic domains. Sequence analysis of the *FoPLC4* gene indicates that its predicted protein contained 1092 aa. In order to investigate roles of *FoPLC4*, we constructed a deletion mutant  $\Delta FoPLC4$  based on the gene homologous combination theory and PEG-mediated gene transformation system. Although the wild-type and the mutant  $\Delta FoPLC4$  were similar in vegetative growth rate, the mutant  $\Delta FoPLC4$  exhibited sparse and fluffy aerial mycelium. The sporulation of  $\Delta FoPLC4$  reduced 82.2% compared with the wild-type.  $\Delta FoPLC4$  appeared to have distinct functions in the regulation of lytic enzymes and fusaric acid production. Furthermore, the  $\Delta FoPLC4$  strain displayed significantly reduced virulence on cucumber plants. Functional complementation of the  $\Delta FoPLC4$  restored the characterizations of the wild-type strain. These results suggest that *FoPLC4* gene regulates the sporulation, morphogenesis of conidia and pathogenicity.

## Poster-5

**TRANSCRIPTOME SEQUENCING ANALYSIS OF FUSARIUM  
WILT-RESISTANT AND SUSCEPTIBLE WATERMELONS**Ying-Hong Lin<sup>1</sup>, Tao-Ho Chang<sup>1</sup>, Kan-Shu Chen<sup>2</sup>, Jenn-Wen Huang<sup>1</sup>,**Pi-Fang Linda Chang**<sup>1,3\*</sup>

<sup>1</sup>Department of Plant Pathology, National Chung Hsing University, South District, Taichung City, Taiwan 40227, R.O.C.; <sup>2</sup>Fengshan Tropical Horticultural Experiment Branch, Agricultural Research Institute, Council of Agriculture, Fengshan District, Kaohsiung City, Taiwan 83052, R.O.C.; <sup>3</sup>Agricultural Biotechnology Center, National Chung Hsing University, South District, Taichung City, Taiwan 40227, R.O.C.

\*Corresponding author: [pfchang@nchu.edu.tw](mailto:pfchang@nchu.edu.tw)

*Fusarium oxysporum* f. sp. *niveum* (Fon), the Fusarium wilt pathogen of watermelon, is considered as one of the limiting factors for watermelon (*Citrullus lanatus*) production worldwide. Although watermelon is the third most important vegetable crop in the world, limited comparative transcriptome datasets between the Fusarium wilt-resistant and -susceptible watermelons are available for use in breeding programs for resistant cultivars. This study performed the whole transcriptome sequencing analysis of the resistant JSB and susceptible SB watermelons during interaction with Fon, including *de novo* assembling, functional annotation, differential gene expression, and Kyoto Encyclopedia of Gene and Genomes (KEGG) pathway analyses. Overall, this study provide the first available datasets of whole transcriptome sequencing of resistant and susceptible watermelons during interaction with Fon. The data presented here can facilitate future research towards understanding the resistance mechanisms of watermelon against Fon.

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**Poster-6****GLASS-BEADS CULTIVATION OF FUSARIUM**

Aida Droce, Jens Laurids Sørensen, Henriette Giese, **Teis Esben Sondergaard**

Department of Biotechnology, Chemistry and Environmental Engineering, Aalborg University, Sohngaardsholmsvej 49, 9000 Aalborg, Denmark.

E-mail: [tes@bio.aau.dk](mailto:tes@bio.aau.dk)

Easy laboratory cultivation methods that increase secondary metabolite production in filamentous fungi, yields high quality DNA and RNA and allow control of nutrient supply are needed. Liquid culture generally does not support secondary metabolite production and solid agar plates are laborious. Here we present a method for fungal cultivation on glass beads where secondary metabolite production resembles that of agar-cultivation and allows extraction of high quality RNA. The method allows complete control of nutrients which is important for the study of gene regulation in response to specific nutrient factors.

**Poster-7****DIPEPTIDE TRANSPORTERS IN *FUSARIUM GRAMINEARUM***

Aida Drocea, Jens Laurids Sørensen, Henriette Giese, **Teis Esben Sondergaard**

Department of Biotechnology, Chemistry and Environmental Engineering, Aalborg University, Sohngaardsholmsvej 49, 9000 Aalborg, Denmark

E-mail: [tes@bio.aau.dk](mailto:tes@bio.aau.dk)

Di/tri peptide transporters 2 (PTR2) are reported to play essential roles in nutrient acquisition in plant pathogenic fungi by taking up di/tripeptides from the environment. Filamentous fungi have one to five PTR2s and their individual functions are unknown. Some PTR2s are active during the initial plant infection process, while others are constitutively expressed and involved in obtaining nitrogen from external sources. PTR2s may affect the global nitrogen regulation through alteration in the N status of the fungus and in this way influence the activation of secondary metabolite pathways, which could affect the toxin production. In this study, the role of four different PTR2s from *Fusarium graminearum* is evaluated through construction of knock out (KO) and double KO strains, yeast complementation and studies of plant pathogenesis.

## Poster-8

**THE INFLUENCE OF MAIZE KERNEL MATURATION ON FUMONISIN DEPOSITION BY *FUSARIUM VERTICILLIOIDES*****K. van Zyl**, D. Raubenheimer, L. J. Rose, A. Viljoen

Department of Plant Pathology, University of Stellenbosch, Stellenbosch, Western Cape, South Africa

E-mail: [karlienvz@sun.ac.za](mailto:karlienvz@sun.ac.za)

Fusarium ear rot of maize, caused by the fungal pathogen *Fusarium verticillioides*, affects maize producers and consumers worldwide. More importantly, *F. verticillioides* deposits a toxic secondary metabolite, known as fumonisin, in the maize kernels which causes serious illness in humans and animals. Factors such as pH, water availability and nutritional content in the infected kernel can serve as key components in the regulation of fumonisins. Understanding the roles of these micro-environmental factors in fumonisin deposition, individually or in combination, may assist researchers to develop novel mycotoxin management strategies. In this study, a susceptible maize variety was artificially inoculated with *F. verticillioides* and kernels collected at several time points ranging from before inoculation until biological maturity. The kernels were analyzed for pH, moisture content, sugar/starch ratio, and soluble nitrogen, and correlated with *F. verticillioides* infection (qPCR) and fumonisin production (LC-MS/MS). During kernel maturation the moisture content and the pH decreased, and soluble nitrogen content increased linearly. *F. verticillioides* biomass increased linearly at the early time points after which it remained constant. Fumonisin production increased during the dent stage with high levels of starch. Our findings suggest that the micro-environmental changes inside a maturing kernel may stimulate fumonisin deposition by *F. verticillioides*.



## Poster-9

**CHARACTERIZATION OF CARBENDAZIM SENSITIVITY AND TRICHOHECENE CHEMOTYPES OF *FUSARIUM GRAMINEARUM* IN JIANGSU PROVINCE OF CHINA**

**Leigang Zhang**, Xiaojing Jia, ChangJun Chen, MingGuo Zhou\*

Key Laboratory of Pesticides of Jiangsu Province, Nanjing Agricultural University, 210095, China;

\* Correspondence to: MingGuo Zhou, E-mail: [mgzhou@njau.edu.cn](mailto:mgzhou@njau.edu.cn)

Fusarium head blight (FHB), one of the most damaging plant diseases in Jiangsu province of China, is a leading cause of economic loss and toxin accumulation in wheat grain. Carbendazim (MBC) fungicide has been employed to control this disease since the 1970s and the resistance developed in the pathogen population. Our previous research revealed that mutation at the 167<sup>th</sup>, or 198<sup>th</sup> and or 200<sup>th</sup> codon of  $\beta_2$ -tubulin gene in *Fusarium graminearum* conferred moderate, high and moderate MBC-resistance, respectively. 7261 isolates collected in Jiangsu province between 2010 and 2012 were tested their sensitivity to MBC and 234 isolates (117 MBC-resistant and 117 MBC-sensitive isolates) were arbitrarily selected to determine trichothecene chemotypes. The relevance between trichothecene chemotype and MBC-sensitivity was found that (1) the MBC-sensitive populations occupied three chemotypes, including 76.9% for 3-AcDON, 7.7% for 15-AcDON and 15.4% for NIV; while the MBC-resistant populations consisted of only two chemotypes, 92.8% 3-AcDON and 7.2% NIV chemotype; (2) among the MBC-resistant populations, sum of 3-AcDON increased 197% in the population with mutation at the 167<sup>th</sup> codon, 65% for the mutation at the 198<sup>th</sup> codon and 28% for the mutation at the 200<sup>th</sup> codon when compared with the sum of 3-AcDON extracted from the same mycelial weigh of isolates selected from the MBC-sensitive population.

## Poster-10

**A POPULATION STUDY OF *FUSARIUM PSEUDOGRAMINEARUM*  
ASSOCIATED WITH FUSARIUM HEAD BLIGHT AND FUSARIUM CROWN  
ROT OF WHEAT IN SOUTH AFRICA**

**G.J. van Coller**<sup>1,2</sup>, A.E. van der Merwe<sup>3</sup>, J.A. Vervalle<sup>3</sup>, S.C. Lamprecht<sup>4</sup>, A. Viljoen<sup>2</sup>

<sup>1</sup> Directorate of Plant Science, Western Cape Department of Agriculture, Private Bag X1, Elsenburg 7607, South Africa; <sup>2</sup> University of Stellenbosch, Department of Plant Pathology, Private Bag X1, Matieland 7602, South Africa; <sup>3</sup> University of Stellenbosch, Department of Genetics, Private Bag X1, Matieland 7602, South Africa; <sup>4</sup> ARC-Plant Protection Research Institute, Private Bag X5017, Stellenbosch 7599, South Africa.

E-mail: gertvc@elsenburg.com

A recent comprehensive survey of *Fusarium* species associated with Fusarium head blight (FHB) and Fusarium crown rot (FCR) of wheat in South Africa revealed that *F. graminearum* and *F. pseudograminearum* are the main species associated with FHB and FCR, respectively. Surprisingly, *F. pseudograminearum* was the most common *Fusarium* species associated with FHB at one location in the Western Cape and one in the Free State Province. Since *F. pseudograminearum* is genetically a highly diverse organism, and because the climatic conditions required for FHB and FCR differs dramatically, the question arose whether the same *F. pseudograminearum* populations were responsible for both diseases. A simple sequence repeat analysis of *F. pseudograminearum* isolates associated with FHB and FCR from different locations in the Western Cape and Free State provinces were conducted, while the mating type of these isolates (*MAT-1* or *MAT-2*) were determined using PCR methodologies. Preliminary results indicate that certain haplotypes are associated with both diseases and at various locations. According to F-statistics and Factorial Correspondence Analysis, one sampling site in the Swartland production region of the Western Cape showed statistically significant differentiation from the rest. Mating type analysis indicated that both mating types occur at all sites, indicating that genetic recombination can occur when environmental conditions are favourable. These results highlight the importance of population studies when investigating the epidemiology of pathogens, and the use of good agricultural practices to limit their dissemination. Cross-pathogenicity studies will be performed to determine whether *F. pseudograminearum* isolates obtained from FCR can cause FHB and *vice versa*.

## Poster-11

**GENOME-WIDE MACROSYNTENY WITHIN THE *GIBBERELLA FUJIKUROI* SPECIES COMPLEX REVEALED BY AFLPS**

L. De Vos<sup>1</sup>, **E.T. Steenkamp**<sup>3</sup>, S.H. Martin<sup>2</sup>, Q.C. Santana<sup>1</sup>, G. Fourie<sup>3</sup>,  
N.A. van der Merwe<sup>1</sup>, M.J. Wingfield<sup>1</sup>, B.D. Wingfield<sup>1</sup>

<sup>1</sup>Department of Genetics, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa; <sup>2</sup>Department of Zoology, University of Cambridge, Cambridge, UK; <sup>3</sup>Department of Microbiology and Plant Pathology, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa.

E-mail: emma.steenkamp@fabi.up.ac.za

The *Gibberella fujikuroi* species complex includes many *Fusarium* species that are associated with significant losses in yield and quality of agricultural and forestry crops. Due to their economic importance, whole-genome sequence information is available for a number of species in the complex. However, no previous studies have explored the genomic commonalities and differences among these fungi. In this study, we utilized a previously completed genetic linkage map of an interspecific cross between *F. subglutinans* and *F. circinatum*, together with genome sequence data for *F. circinatum*, *F. fujikuroi* and *F. verticillioides*, to analyse their genomic synteny. Using *in silico*-generated AFLPs (Amplified Fragment Length Polymorphisms) from the *F. circinatum* genome, and BLAST results from pyrosequenced AFLP fragments, 933 homologous regions were aligned to *F. fujikuroi* and *F. verticillioides*. This also allowed us to assign the *F. subglutinans* and *F. circinatum* genetic linkage groups to their corresponding chromosomes in *F. verticillioides*, as well as to assign two previously unmapped supercontigs of *F. verticillioides* to chromosomes. We further found evidence of a translocation between the distal ends of chromosome 8 and 11, which apparently originated before the divergence of *F. circinatum* and *F. subglutinans*. Overall, a remarkable level of macrosynteny was observed among the three *Fusarium* genomes, which will undoubtedly aid in the genome assemblies of other *Fusarium* species. This study not only demonstrates how *in silico* AFLPs can aid whole-genome sequence assemblies, but it also highlights the benefits of using this tool to study genomic synteny and architecture.

## Poster-12

**REACTION OF WHEAT, BARLEY AND OAT TO FIELD INOCULATION  
WITH *FUSARIUM PSEUDOGRAMINEARUM*****S.C. Lamprecht**<sup>1</sup>, D. Lesch<sup>2</sup>, Y.T. Tewoldemedhin<sup>1</sup>, E. Retief<sup>1</sup> & G.J. Van Coller<sup>3</sup>

<sup>1</sup>Agricultural Research Council-Plant Protection Research Institute, Private Bag X5017, Stellenbosch 7599, South Africa; <sup>2</sup>Sensako, PO Box 133, Napier 7270; <sup>3</sup>Directorate of Plant Science, Western Cape Department of Agriculture, Private Bag X1, Elsenburg 7607, South Africa;

E-mail: [lamprechts@arc.agric.za](mailto:lamprechts@arc.agric.za)

Fusarium crown rot, caused by *Fusarium pseudograminearum*, is one of the most important soilborne diseases of small grain cereals in the Western Cape province of South Africa. Although crop rotation with non-host crops of *F. pseudograminearum* contributes significantly towards the management of this disease, an integrated management strategy, that includes the use of disease tolerant/resistant cultivars may be more effective in reducing losses caused by Fusarium crown rot. A study is currently being conducted to evaluate resistance/tolerance to *F. pseudograminearum* among cultivars of wheat, barley and oat at three experimental farms (Napier, Roodebloem and Tygerhoek) in the Western Cape province. Soil fumigation was also used as an experimental tool at the Roodebloem experimental farm to eliminate inoculum of the pathogen as well as inoculum of *Gaeumannomyces* spp., which can cause take-all on these crops. Fusarium crown rot and take-all were recorded at all localities, with the highest ratings (percentage infected tillers) for Fusarium crown rot on plants at the Napier, Roodebloem and Tygerhoek being 86.2%, 28.5% and 39.9%, respectively. Take-all was not recorded from plants collected from fumigated plots. There were no differences in disease severity among cultivars in non-inoculated plots at Roodebloem, but cultivars differed significantly after inoculation. Field inoculation with *F. pseudograminearum* often significantly increased the severity of Fusarium crown rot. Furthermore, inoculation also reduced yields, but these were often not significant. The relative response of cultivars in terms of disease susceptibility exhibited differential results across localities. These findings demonstrate the need for including field inoculation of the pathogen, soil fumigation as a positive control and challenges experienced in the conduct of field evaluations of small grain cereals for tolerance against Fusarium crown rot.

## Poster-13

**PHYLOGENETIC DIVERSITY OF FUSARIUM ISOLATES FROM WILD BANANA FRUITS IN CHINA - DISTRIBUTION OF THE FUMONISIN GENE CLUSTER AMONG IDENTIFIED SPECIES****François Van Hove**<sup>1\*</sup>, Sarah De Saeger<sup>2</sup>, Irene Lazzaro<sup>1</sup>, Yanxiang Qi<sup>3</sup>,Dabing Zhang<sup>4</sup>, Aibo Wu<sup>4</sup>, Françoise Munaut<sup>1</sup>

<sup>1</sup>Université catholique de Louvain, Earth and Life Institute, Applied Microbiology, Mycology, Mycothèque de l'Université catholique de Louvain (BCCM<sup>TM</sup>/MUCL), Louvain-la-Neuve, Belgium; <sup>2</sup>Laboratory of Food Analysis, Faculty of Pharmaceutical Sciences, Ghent University, Belgium; <sup>3</sup>Environment and Plant Protection Institute, Chinese Academy of Tropical Agricultural Sciences (CATAS), China; <sup>4</sup>College of Life Science and Biotechnology, Shanghai Jiao Tong University (SJTU), China.

E-mail: [francois.vanhove@uclouvain.be](mailto:francois.vanhove@uclouvain.be)

Banana fruit is one of the most important crops and is currently the second most important fruit produced all over the world. Several *Fusarium* species have been identified as important pre- and post-harvest pathogens of cultivated banana. However, there are no studies that have been conducted to identify *Fusarium* contaminants on wild banana fruits.

The first objective of this work was to study the phylogenetic and genetic diversity of *Fusarium* isolates from the *F. fujikuroi* species complex (FFSC) contaminating fruits of wild banana plants growing in two southern Chinese Provinces, Hainan and Yunnan. The second objective was to study the distribution and configuration of the fumonisin gene cluster (FGC) within these *Fusarium* species.

Phylogenetic results demonstrated important species diversity (8 distinct species) amongst *Fusarium* isolates contaminating fruits of wild banana. Very interestingly, two new phylogenetically distinct species, *Fusarium* sp. nov. A and B, were identified beside six other known *Fusarium* species. While the occurrence of *F. proliferatum* isolates is not really surprising because of its notorious large host specificity, the occurrence of other species is more intriguing as they are generally renowned as being more host specific, especially *F. mangiferae*.

The results also demonstrated the presence of seven *FUM* genes within all isolates of the first new phylogenetic species (*Fusarium* sp. nov. A), as well as within two *F. mangiferae* isolates, which are strong indications of the presence of an intact FGC. Very interestingly, only the last gene of the FGC, *FUM19*, was detected within the second new species (*Fusarium* sp. nov. B). This is most probably indicative of a truncated FGC and would be the first observation of another FGC excision event after the one described in *F. musae*.

## Poster-14

***FUSARIUM VERTICILLIOIDES*-MAIZE: A GENE EXPRESSION APPROACH TO STUDY THE FUNGUS-PLANTA INTERACTION****Lazzaro I.**<sup>1</sup>, Lanubile A.<sup>2</sup>, Marocco A.<sup>2</sup>, Battilani P.<sup>1</sup>

<sup>1</sup>Institute of Entomology and Plant Pathology; <sup>2</sup>Institute of Agronomy, Genetics and Field Crops, University Cattolica del Sacro Cuore, Via Emilia Parmense 84, 29122 Piacenza, Italy.

E-mail: [paola.battilani@unicatt.it](mailto:paola.battilani@unicatt.it)

*Fusarium verticillioides* is a filamentous fungus, worldwide pathogen of maize, on which it causes Fusarium ear rot and it is able to produce fumonisin. A study on the interaction between *F. verticillioides* and its main host-maize has been carried on, considering both *in vitro* and *in planta* perspectives. The former studied the effects of water activity ( $a_w$ ) and temperature (T) on fumonisin B (FB) production and expression of *FUM* genes (*FUM2-FUM3-FUM8-FUM13-FUM14-FUM21*) in *F. verticillioides* strains. The latter monitored which genes were differentially expressed in resistant and susceptible maize lines at several time points after inoculation by a fumonisin-producing strain of *F. verticillioides*.

The *in vitro* study showed that  $a_w$  had a significant influence on *FUM* gene expression rather than T, indicating that fungal secondary metabolism is more overturned by low  $a_w$  than by T decrease. Most of *FUM* genes were highly expressed at  $a_w=0.990$  compared to 0.955, and the same was observed for FB production. This common trend suggests that even if the expression of genes and the product of the metabolite are far events in terms of “biochemical times”, a close regulation of *FUM* gene expression and production of FB can subsist. At 21 days of incubation, *FUM14* and *FUM3* -regulating the production of FB<sub>1</sub> and FB<sub>2</sub> from FB<sub>3</sub> and FB<sub>4</sub>, respectively,- were maximally expressed. On the contrary *FUM21* -coding for a transcription factor for FB biosynthesis - was 10x less expressed.

The *in planta* study showed that in kernels at 48 h after inoculation (hai) about 800 genes were differentially regulated and nearly 10% assigned to the defence category. During the very early stages of incubation a small proportion of the host transcripts was induced and none of them was involved in defence processes. Early response genes encoded signalling or regulatory components. The highest number of differentially expressed genes was attained at 48 hai. The late response genes encoded effector proteins. When resistant and susceptible maize genotypes were compared, in the resistant line the expression of defence genes was detected before inoculation, while in the susceptible genotype they were induced only after pathogen inoculation. The identification of differentially expressed plant genes that interact with the fungus will produce useful tools to detect candidate genes, useful to select resistant maize genotypes by means of marker assisted selection.

## Poster-15

**CAN FUSARIUM SPECIES INFLUENCE THE COMPOSITION OF NEMATODE COMMUNITIES IN SUGARCANE ROOTS?****S.A. McFarlane**<sup>1</sup>, A. Viljoen<sup>3</sup>, and R.S. Rutherford<sup>1,2</sup>

<sup>1</sup>Crop Protection, South African Sugarcane Research Institute, Private Bag X02, Mount Edgecombe, 4300, KwaZulu-Natal, South Africa;<sup>2</sup>School of Life Sciences, College of Agriculture, Engineering and Science, University of KwaZulu-Natal, Pietermaritzburg, South Africa;<sup>3</sup>Department of Plant Pathology, Stellenbosch University, Private Bag X1, Matieland 7602, South Africa

E-mail: [sharon.mcfarlane@sugar.org.za](mailto:sharon.mcfarlane@sugar.org.za)

In South Africa, more than 90 species of nematodes feed on the roots of sugarcane. The most common damaging nematodes are *Pratylenchus zae*, *Meloidogyne javanica*, *Xiphinema elongatum* and *Paratrichodorus* species. While chemical control has been used effectively to reduce the impact of nematodes on production, novel management methods are being explored to facilitate sustainable integrated management of this deleterious pest. Plant roots emit volatiles that attract entomopathogenic nematodes (EPNs) either constitutively or in response to attack by insects. These chemical cues increase the likelihood of EPNs locating potential hosts but have also been shown to attract plant parasitic nematodes. A similar response has been demonstrated when plants are colonised by fungal pathogens, often in association with insects. In addition, certain bacteria and fungi, including *Fusarium* species, produce nematicidal toxins. A *Fusarium sacchari* isolate and a *F. pseudonygamai* isolate from sugarcane stalks that were found to influence the development and fecundity of the stalk borer *Eldana saccharina*, were tested for their effect on nematode communities in sugarcane roots. The *F. sacchari* isolate, which was antagonistic to *E. saccharina*, had little effect on nematode numbers *in vivo*. However, plants inoculated with the *F. pseudonygamai* isolate, which increased the rate of development and improved the fecundity of *E. saccharina* in earlier studies, supported significantly higher numbers of *Pratylenchus* and *Meloidogyne* than the uninoculated control in the early stages of plant growth. Volatiles such as 4-methyl phenol and p-benzoquinone, which have been associated with increased numbers of nematodes in other crops, were identified in sugarcane tissue inoculated with this fungus using GC-MS analysis. Norpinene, a monoterpeneoid closely related to  $\alpha$ -pinene, was also identified. Alpha-pinene has been shown to attract and improve the fecundity of nematodes and it is possible that norpinene would have a similar effect. The results indicate that sugarcane cultivars that are susceptible to *Fusarium* infection may in turn become more susceptible to plant parasitic nematodes when infected with the fungus. By improving resistance to *Fusarium* infection, damage by nematodes as well as *E. saccharina* may be reduced.

## 11<sup>th</sup> International Fusarium Workshop Attendees List

First name	Last name	Affiliation	Position	E-mail
Tania van	Antwerpen	South African Sugarcane Research Institute (SASRI)	Research Scientist	Tania.vanantwerpen@sugar.org.za
Erik De	Bruyne	Sesvanderhave N.V./S.A, Belgium	Research Scientist	erik.de-bruyne@sesvanderhave.com
Shulin	Cao	Northwest A&F University,China	Graduate Student	xyj100@yeah.net
Sha	Cao	Department of Plant Pathology, China Agricultural University	Graduate Student	sdbzcs@126.com
Pi-Fang Linda	Chang	National Chung Hsing University, Taiwan	Faculty	pfchang@nchu.edu.tw
Changjun	Chen	College of Plant Protection, Nanjing Agricultural University,China	Faculty	changjun-chen@njau.edu.cn
Yun	Chen	Institute of Biotechnology, Zhejiang University,China	Research Scientist	chenyun0927@zju.edu.cn
Xiang	Chen	Institute of Biotechnology, Zhejiang University,China	Graduate Student	chenxiangae86@sina.com
Jieru	Fan	Chinese Academy of Agricultural Sciences	Research Scientist	fanjieru1981@126.com
Xiangling	Fang	The University of Western Australia	Graduate Student	xiangling.fang@uwa.edu.a
Jie	Feng	Institute of Plant Protection, Chinese Academy of Agricultural Sciences,China	Faculty	Jfeng@ippcaas.cn
Xiquan	Gao	College of Agriculture, Nanjing Agricultural University, Nanjing, China	Faculty	xgao@njau.edu.cn
Qixun	Gao	Institute of Biotechnology, Zhejiang University,China	Graduate Student	gaoqixun@163.com
Donald	Gardiner	CSIRO, Australia	Research Scientist	donald.gardiner@csiro.au
PingPing	Gong	Fujian Agriculture and Forestry University, China	Graduate Student	649419474@qq.com
Qin	Gu	Institute of Biotechnology, Zhejiang University,China	Graduate Student	bbgugu.1988@163.com
Lihua	Guo	Institute of Plant Protection, Chinese Academy of Agricultural Sciences	Faculty	Guolihua1972@126.com
Yiping	Hou	College of Plant Protection, Nanjing Agricultural University,China	Research Scientist	houyiping@njau.edu.cn
Dongwei	Hu	Institute of Biotechnology, Zhejiang University,China	Faculty	hudw@zju.edu.cn
Weiqun	Hu	Zhejiang Institute of Chemical Industry	Faculty	huweiqun@soho.com
Qiwei	Huang	College of Resources and Environmental, Nanjing Agricultural University,China	Faculty	qw Huang@njau.edu.cn
Mengmeng	Huang	Institute of Biotechnology, Zhejiang University,China	Graduate Student	wza62085027@163.com
Jinhua	Jiang	Zhejiang Academy of Agricultural Sciences,China	Research Scientist	Jinhuaajh@sina.com
Zemin	Jiang	Institute of Biotechnology, Zhejiang University,China	Undergraduate Student	3110100438@zju.edu.cn
Takeshi	Kashiwa	Tokyo University of Agriculture and Technology,Japan	Graduate Student	tkashiwa712@gmail.com
Sandra	Lamprech	Agricultural Research Council Plant Protection Institute, South Africa	Research Scientist	lamprechts@arc.agric.za
Irene	Lazzaro	Université catholique de Louvain, Belgium	Post-Doc	irene.lazzaro@uclouvain.be
Andrew	Leadbeat	Syngenta Crop Protection AG, Switzerland	Research Scientist	andy.leadbeater@syngenta.com
Tao	Li	Yangzhou University,China	Faculty	taoli@yzu.edu.cn
Heping	Li	Huazhong Agricultural University,China	Faculty	hepingli@mail.hzau.edu.cn
ChunYu	Li	Institute of Fruit Science , Guangdong Academy of Agricultural Sciences,China	Faculty	Lichunyu881@163.com
Minhui	Li	Department of Plant Pathology, South China Agricultural University, China	Faculty	liminhui@scau.edu.cn
Bin	Li	Institute of Biotechnology, Zhejiang University,China	Faculty	libin0571@zju.edu.cn
Yucailiao	Liao	Huazhong Agricultural University,China	Faculty	yucailiao@mail.hzau.edu.cn
Ning	Ling	College of Resources and Environmental, Nanjing Agricultural University,China	Research Scientist	nling@njau.edu.cn
Xin	Liu	Jiangsu Academy of Agricultural Sciences,China	Research Scientist	moxiasang@126.com
Ye	Liu	Institute of Biotechnology, Zhejiang University,China	Graduate Student	11216077@zju.edu.cn
Zunyong	Liu	Institute of Biotechnology, Zhejiang University,China	Graduate Student	ponderliu@163.com
Na	Liu	Institute of Biotechnology, Zhejiang University,China	Graduate Student	liunalz@163.com
Yongping	Luo	Northwest A&F University,China	Graduate Student	Lyping1234@aliyun.com



WORKSHOP ATTENDEES LIST

Li-jun	Ma	University of Massachusetts Amherst, MA, USA	Faculty	lijun@biochem.umass.edu
Zhonghua	Ma	Institute of Biotechnology, Zhejiang University,China	Faculty	zhma@zju.edu.cn
Sharon	McFarlane	SA Sugarcane Research Institute, South Africa	Graduate Student	sharon.mcfarlane@sugar.org.za
Linda K	Nielsen	University of Nottingham, United Kingdom	Faculty	linda.nielsen@nottingham.ac.uk
Zhongju	Qi	Nunhems Beijing Seeds Co., LTD	Research Scientist	zhongju.qi@bayer.com
Biao	Shen	College of Resources and Environmental, Nanjing Agricultural University,China	Faculty	shenbiao@njau.edu.cn
Mohamed	Sobhy Hamada	Faculty of Agriculture, Mansoura University,Egypt	Faculty	m_sobhy@mans.edu.eg
Teis Esben	Søndergaard	Department of Biotechnology,, Aalborg University, Denmark.	Faculty	tes@bio.aau.dk
Rong	Song	Syngenta Crop Protection,Shanghai,China	Research Scientist	Rong.Song@syngenta.com
Tijana	Stancic	Harper Adams University College, UK	Graduate Student	tstancic@harper-adams.ac.uk
Emma	Steenkamp	University of Pretoria, South Africa	Faculty	emma.steenkamp@up.ac.za
Haiyan	Sun	Jiangsu Academy of Agricultural Sciences,China	Research Scientist	yumucun@126.com
Ulf	Thrane	Technical University of Denmark, Denmark	Faculty	ut@bio.dtu.dk
Gert	Van Coller	Stellenbosch University, South Africa	Research Scientist	gertvc@elsenburg.com
François	Van Hove	Université catholique de Louvain, Belgium	Faculty	francois.vanhove@uclouvain.be
Karlién	Van Zyl	University of Stellenbosch,South Africa	Graduate Student	karlienvz@sun.ac.za
Meagen	Vermeulen	University of Stellenbosch,South Africa	Graduate Student	meaganv@sun.ac.za
Altus	Viljoen	Stellenbosch University, South Africa	Faculty	altus@sun.ac.za
Shimei	Wang	College of Resources and Environmental, Nanjing Agricultural University,China	Faculty	smwang@njau.edu.cn
Zhuo	Wang	Chinese Academy of Tropical Agricultural Sciences ,China	Research Scientist	wangzhuocd@126.com
Min	Wang	College of Resources and Environmental, Nanjing Agricultural University,China	Graduate Student	2010203034@njau.edu.cn
Jianhua	Wang	Shanghai Academy of Agricultural Sciences, China	Faculty	jianhuawang163@163.com
Jianqiang	Wang	Fujian Agriculture and Forestry University, China	Graduate Student	wjq9042@163.com
Xuehong	Wu	Department of Plant Pathology, China Agricultural University	Faculty	wuxuehong@cau.edu.cn
Aibo	Wu	Shanghai Academy of Agricultural Sciences, China	Faculty	wuaibo@saas.sh.cn
Jin-rong	Xu	Purdue University, Indiana, USA	Faculty	jinrong@purdue.edu
Lijun	Yang	Hubei Academy of Agricultural Sciences,China	Faculty	yanglijun1993@163.com
Yanni	Yin	Institute of Biotechnology, Zhejiang University,China	Faculty	ynyin@zju.edu.cn
Dafang	Yin	Institute of Biotechnology, Zhejiang University,China	Graduate Student	21216096@zju.edu.cn
Fangwei	Yu	Institute of Biotechnology, Zhejiang University,China	Graduate Student	yfwhope@126.com
Yingzi	Yun	Institute of Biotechnology, Zhejiang University,China	Graduate Student	11016068@zju.edu.cn
Suwen	Zhan	Syngenta Crop Protection,Shanghai,China	Research Scientist	Evan.Zhan@syngenta.com
Nan	Zhang	College of Resources and Environmental, Nanjing Agricultural University,China	Research Scientist	nanzhang@njau.edu.cn
Hao	Zhang	Institute of Plant Protection, Chinese Academy of Agricultural Sciences,China	Research Scientist	hzhang@ippcaas.cn
Shijie	Zhang	Northwest A&F University,China	Research Scientist	zsjw4501@163.com
Yimei	Zhang	Northwest A&F University,China	Graduate Student	xinongyimei@163.com
Leigang	Zhang	College of Plant Protection, Nanjing Agricultural University,China	Graduate Student	leigang.zh@163.com
Xu	Zhang	Jiangsu Academy of Agricultural Sciences,China	Faculty	xuzhang@jaas.ac.cn
Chengqi	Zhang	Institute of Biotechnology, Zhejiang University,China	Graduate Student	11216078@zju.edu.cn
Xiaoping	Zhang	Institute of Biotechnology, Zhejiang University,China	Graduate Student	tao910629@sina.com
Zhihui	Zhao	Dalian Nationalities University, China	Research Scientist	zzh@dlnu.edu.cn
Zhanwang	Zhu	Hubei Academy of Agricultural Sciences,China	Research Scientist	zhuzhanwang@163.com