

**SESSION 4:**

**PATHOGEN BIOLOGY**

**AND GENETICS**

Chairperson: Frances Trail



---

---

B-TRICHOHECENE GENOTYPES OF *FUSARIUM GRAMINEARUM*  
STRAINS FROM ACROSS BARLEY PRODUCTION REGIONS  
AND GROWING SEASONS IN SOUTHERN BRAZIL

P. Astolfi<sup>1</sup>, L. Schneider<sup>1</sup>, L. Simon<sup>1</sup>, T. Alves<sup>2</sup>,  
D.J. Tessmann<sup>2</sup> and E.M. Del Ponte<sup>1\*</sup>

---

<sup>1</sup>UFRGS, Faculdade de Agronomia, Porto Alegre, RS, Brazil; and

<sup>2</sup>UEM, Departamento de Agronomia, Maringá, PR, Brazil

\*Corresponding Author: PH: (55) 51-33086908; E-mail: emerson.delponte@ufrgs.br

---

**ABSTRACT**

Fusarium head blight (FHB) is a serious disease affecting barley in Brazil, especially for causing a significant impact to the malt industry due to mycotoxin contamination. Current information concerning the genetic diversity of Brazilian *F. graminearum* populations is limited to isolates obtained from wheat. Attempting to characterize the B-trichothecene profile of *F. graminearum* isolates from barley in Southern Brazil, fungal colonies were obtained from monitored commercial fields across 21 municipalities during the 2007 and 2008 growing seasons. Following fungal isolation from grains cultured on selective media, a total of 57 single-spore isolates were obtained. Pure fungal cultures were grown in complete liquid media for biomass production and further DNA extraction. PCR assays were conducted first using Fg16F/R primer pair target to *F. graminearum* complex, and, second, a multiplex reaction with portions of the *Tri3* and *Tri12* genes in which amplifications are predictive of Nivalenol (NIV) and Deoxynivalenol acetylates (3ADON and 15ADON). In both years, the 15ADON genotype was predominant (18/24 in 2007 and 17/33 in 2008). One 3ADON isolate was detected in each year. The NIV genotype was also detected in both years in relatively high proportions: 5/24 in 2007 and 15/33 in 2008 isolates. This was the first survey concerning trichothecene profile of regional *F. graminearum* barley populations in Brazil. The high number of NIV isolates, in a much greater proportion than those found in wheat based on our previous findings, suggest that monitoring nivalenol in barley grains may be needed due to its higher toxicological implications compared to deoxynivalenol.

STUDIES ON THE *FUSARIUM GRAMINEARUM* COMPLEX  
AFFECTING WHEAT IN SOUTHERN BRAZIL SUGGEST  
A PHYLOGENETIC SPECIES-SPECIFIC  
B-TRICHOHECENE PROFILE

P. Astolfi<sup>1</sup>, L. Schneider<sup>1</sup>, L.L. Simon<sup>1</sup>, E.M. Del Ponte<sup>1</sup>, T.C.A. Alves<sup>2</sup>,  
D.J. Tessmann<sup>2</sup>, M.M. Reynoso<sup>3</sup>, M.L. Ramirez<sup>3</sup>,  
A. Torres<sup>3</sup>, C. Farnochi<sup>3</sup> and S.N. Chulze<sup>3</sup>

---

<sup>1</sup>UFRGS, Faculdade de Agronomia, Porto Alegre, RS, Brazil; <sup>2</sup>UEM, Departamento de Agronomia, Maringá, PR, Brazil; and <sup>3</sup>UNRC, Departamento de Microbiología e Inmunología, Córdoba, Argentina

\*Corresponding Author: PH: (55) 51-33086908; E-mail: emerson.delponte@ufrgs.br

---

**ABSTRACT**

Several members within the *Fusarium graminearum* species complex (*Fg complex*) have been reported in association with some host crops in Brazil. Our previous studies on a set of wheat isolates from regional populations have revealed the predominance of lineage 7 (*F. graminearum sensu stricto*) possessing a consistent 15ADON trichothecene genotype, whereas a small proportion of isolates belonged to lineage 2 (*F. meridionale*) and presented a nivalenol (NIV) genotype. We advanced our studies on three local populations of strains isolated from wheat, to test our hypothesis that multiple phylogenetic species are present and may have a species-specific B-trichothecene profile. A sample of 55 strains were obtained from symptomatic kernels collected at Cruz Alta (n=19) Ernestina (n=19) and Nonoai (n=17) municipalities during 2007 growing season. We used AFLP markers to determine the similarity among the isolates of Brazilian populations with members of the *Fg complex*. A total of 150 AFLP bands were identified in the 200–500 bp range when using three primer pair combinations (*EcoRI-AA/MseI-AT*, *EcoRI-CC/MseI-CG*, *EcoRI-TG/MseI-TT*). Representatives of members of *Fg complex* (lineages 1 to 9) were included in the study to compare with our isolates. A multiplex PCR was used to determine the trichothecene genotypes with sequence primers targeting portions of *Tri3* and *Tri12* genes that are predictive of 15ADON, 3ADON and NIV chemotypes. Our results confirms the predominance of the 15-ADON genotypes (48/55) all grouping with lineage 7. The NIV genotype was also found (06/55) and, in agreement with our previous findings, most belonged to lineage 2. However, one NIV isolated grouped with lineage 5 (*F. acaciae-mearnsii*). The only 3-ADON, detected in Ernestina population, grouped with lineage 8 (*F. cortaderiae*). In spite of the relative low number of strains analyzed this far, we confirm that FHB in Brazil is caused by multiple phylogenetic species and suggest a species-specific B-trichothecene profile, especially for the predominant species (*F. graminearum sensu stricto*).

---

---

WITHIN-FIELD PATTERNS OF B-TRICHOHECENE GENOTYPES  
IN THE *FUSARIUM GRAMINEARUM* COMPLEX  
AFFECTING WHEAT IN SOUTHERN BRAZIL

P. Astolfi<sup>1</sup>, L.L. Simon<sup>1</sup>, L. Schneider<sup>1</sup>, T.C.A. Alves<sup>2</sup>,  
D.J. Tessmann<sup>2</sup> and E.M. Del Ponte<sup>1\*</sup>

---

<sup>1</sup>UFRGS, Agronomia, Laboratório de Epidemiologia de Plantas, Porto Alegre,  
RS, Brazil; and <sup>2</sup>UEM, Departamento de Agronomia, Maringá, PR, Brazil

\*Corresponding Author: PH: (55) 51-33086908; E-mail: emerson.delponte@ufrgs.br

---

**ABSTRACT**

In our previous studies we have shown that Southern Brazilian pathogenic populations of *Fusarium graminearum* species complex obtained from *Fusarium*-damaged wheat kernels were consistently either DON/15-ADON (lineage 7) or Nivalenol (NIV) (lineage 2) regarding to the B-trichothecene genotype. The objective of this study was to assess whether the different lineages/trichothecene genotypes are present and how they are distributed in intensively sampled wheat fields. Two fields located at two production regions in Rio Grande do Sul State, Brazil, and distant 192 km apart from each other (field A = Vacaria; B = Sarandi), were assessed at early soft dough stage showing moderate FHB incidence levels. Each field was visually divided in four sections; five georeferenced sampling points were randomly defined within a section. At each sampling point, four adjacent symptomatic heads in a 0.2m x 0.2m area were collected. A total of 80 symptomatic heads were collected in each field. In the laboratory, heads were disinfested and sections of the head were plated on selective media for recovery of fungal isolates (one isolate per head). All isolates that showed typical *F. graminearum* characteristics were purified by a single spore technique and the resulting mycelium was further grown on liquid media for biomass production. Mycelium DNA was extracted and PCR assays were conducted using Fg16F/R primer to confirm isolate identity and sequence primers targeted at *Tri3* and *Tri12* genes predictive of NIV, 3ADON and 15ADON in a multiplex reaction. In field A, 75 isolates were obtained and 15ADON (72), 3ADON (2) and NIV (1) genotypes were detected. The 3ADON and NIV isolates were found at different field sections and sampling points. In field B, from a sample of 35 isolates obtained, 15ADON was the predominant type (33/35) and two NIV types were also detected. A polymorphism detected in Fg16 primer amplifications for all NIV types were indicative of lineage 2, in agreement with our previous findings. The identity of the 3ADON type is under investigation. Our advanced population studies suggest that *Fusarium* head blight of wheat in Brazil is caused by distinct members of the *F. graminearum* complex that show distinct trichothecene profiles and co-occur at the field scale, where the lineage 7/15ADON genotype is predominant at the spatial hierarchies studied.

PEPTIDE TECHNOLOGIES FOR MANAGEMENT  
OF FUSARIUM HEAD BLIGHT

James T. English<sup>1\*</sup>, Francis J. Schmidt<sup>2</sup>, Nathan Gross<sup>1</sup>,  
John Leslie<sup>3</sup>, Gary Yuen<sup>4</sup> and James E. Schoelz<sup>1</sup>

---

<sup>1</sup>Div. of Plant Sciences, and <sup>2</sup>Div. of Biochemistry, University of Missouri, Columbia, MO 65211;

<sup>3</sup>Dept. Plant Pathology, Kansas State University, Manhattan, KS 66506; and <sup>4</sup>Dept.

Plant Pathology, University of Nebraska, Lincoln, NE 68588, USA

\*Corresponding Author: PH: (573) 882-1472; E-mail: englishj@missouri.edu

---

**ABSTRACT**

We are developing two strategies for selection and deployment of peptides to protect wheat from infection by *Fusarium graminearum*. One strategy is based on the identification of small, combinatorially selected peptides that function as inhibitory ligand mimics for factors involved in pathogen growth and development. The three steps of this strategy include (1) selection of peptides with affinity for pathogen infectious structures, (2) assessment of impacts of affinity-selected peptides on pathogen development and, (3) delivery of bioactive peptides in susceptible tissues of host plants. We previously applied this strategy to successfully limit tomato root infection by the oomycetous pathogen, *Phytophthora capsici*, and to reduce the infection efficiency of spores produced by *Phakopsora pachyrhizi*, the fungus that causes Asian soybean rust. In our initial studies with *F. graminearum*, we have defined populations of peptides from two types of combinatorial libraries that bind to germinating macroconidia. Some of these peptides slow the rate of germ tube development. In addition to continuing assessments of peptides for inhibition, we are assessing germination of macroconidia and ascospores in relation to peptide concentration. A second strategy being pursued is the use of peptides that function as mating pheromones for *F. graminearum*. These peptides and their derivatives have been shown to inhibit germination of macroconidia. We are producing these peptides via yeast fermentation and by chemical synthesis. We are preparing to apply these peptides to plants to examine their potential for protection against infection by *F. graminearum* ascospores and macroconidia.

**ACKNOWLEDGEMENT AND DISCLAIMER**

This material is based upon work supported by the U.S. Department of Agriculture, under Agreement No. 59-0790-7-073. This is a cooperative project with the U.S. Wheat & Barley Scab Initiative. Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the authors and do not necessarily reflect the view of the U.S. Department of Agriculture.

---

---

AGGRESSIVENESS AND MYCOTOXIN POTENTIAL OF U.S.  
*FUSARIUM GRAMINEARUM* POPULATIONS IN  
FIELD-GROWN WHEAT AND BARLEY

Liane R. Gale<sup>1\*</sup>, Ruth Dill-Macky<sup>1</sup>, James A. Anderson<sup>2</sup>,  
Kevin P. Smith<sup>2</sup> and H. Corby Kistler<sup>1,3</sup>

---

<sup>1</sup>Dept. of Plant Pathology, University of Minnesota, St. Paul, MN; <sup>2</sup>Dept. of Agronomy and Plant Genetics, University of Minnesota, St. Paul, MN; and <sup>3</sup>USDA-ARS, Cereal Disease Laboratory, St. Paul, MN

\*Corresponding Author: PH: (612) 625-9266; E-mail: lianeg@umn.edu

---

## ABSTRACT

Previous greenhouse experiments have demonstrated increased toxigenic potential of certain populations of *Fusarium graminearum*. Inoculated and mist-irrigated field experiments were conducted in St. Paul, MN in 2008 & 2009 to test whether significant differences in aggressiveness and/or toxigenic potential exist between pathogen populations under field conditions and if so, whether aggressiveness and/or toxigenic potential on specific varieties or lines is correlated with specific pathogen populations. Twelve cultivars/lines of each wheat and barley were independently inoculated with three (2008) or four (2009) *F. graminearum* populations in addition to a water control. The fungal “populations” were generated by mixing conidia of 15-20 well-characterized strains that have been shown to belong to specific and genetically distinct populations (emergent (E)3ADON, E15ADON, midwestern (MW)15ADON and emergent (mix of E3ADON & E15ADON)). Experimental procedures were otherwise standard. FHB severity (SEV) in the field (proportion of infected spikelets), and visually scabby kernels (VSK; wheat only) were determined as measurements of aggressiveness. Toxigenic potential was determined by measuring mycotoxin concentrations (DON and derivatives) by GC/MS. Mycotoxin analysis was performed at the Mycotoxin Lab of the University of Minnesota (Dr. Yanhong Dong, director). Statistical analyses were performed using JMP software. For wheat, overall VSK and DON levels were much lower in 2009 than for 2008, although the same trends were observed in both years. No differences between populations were observed for VSK and SEV in wheat, and significantly higher levels of DON were again obtained for the emergent population. The added treatment in 2009 (E15ADON) also had significantly elevated levels of DON compared to MW15ADON and E3ADON. In contrast, for barley, the results from the two years were not consistent. While in 2008, there was no effect of population treatment on SEV and DON, significant population effects were evident in 2009. Inoculation with the E3ADON population resulted in significantly higher SEV than with the other three populations, while DON was significantly different between all four treatments (E3ADON > Emergent > E15ADON > MW15ADON). Overall, these latter results cannot be adequately interpreted without a third year of field experimentation. Nevertheless, we tentatively conclude at this time that the pathogen population may have an effect on FHB development and toxin accumulation in the field, which indicates that knowledge of the genetic composition of the inoculum in field trials is advisable.

## ACKNOWLEDGEMENT AND DISCLAIMER

This material is based upon work supported by the U.S. Department of Agriculture, under Agreement No. 59-0790-7-074. This is a cooperative project with the U.S. Wheat & Barley Scab Initiative. Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the authors and do not necessarily reflect the view of the U.S. Department of Agriculture.

---

INOCULATION AND RECOVERY OF *FUSARIUM GRAMINEARUM*  
CHEMOTYPES FROM THE FHB NURSERY AT GLENLEA,  
MANITOBA IN 2008 AND 2009  
J. Gilbert\*, R.M Clear and D. Gaba

---

Cereal Research Centre, AAFC, 195 Dafoe Road, Winnipeg, MB, Canada, R3T 3E5. (R.M.C and D.G) Grain Research Laboratory, Canadian Grain Commission, 1404-303 Main St. Winnipeg, MB, Canada, R3C3G8

\*Corresponding Author: PH: (204) 983-0891; E-mail: jeannie.gilbert@agr.gc.ca

---

**ABSTRACT**

Fusarium head blight (FHB) is a serious threat to the Canadian grain industry. Most isolates of *Fusarium graminearum* Schwabe, the principal cause of FHB in North America, produce the mycotoxin deoxynivalenol (DON) and one of its acetylated derivatives, 3- or 15-ADON. In North America, a rapid shift from the 15-ADON to 3-ADON chemotype has been documented. While the 3-ADON isolates are not more aggressive than the 15-ADON isolates, they produce significantly more DON. The wheat FHB screening nursery at Glenlea, MB was inoculated with a macroconidial suspension of both chemotypes. In 2008, 2 strains producing 3-ADON and 2 producing 15-ADON were combined for increase in carboxymethyl cellulose (CMC). In 2009, the isolates were increased separately and combined in equal ratio just before inoculation. The objective of this study was to determine if isolates of 3- or 15-ADON were recovered in the same ratio as applied under each set of increase conditions. A set of 6 check cultivars/lines, planted throughout the nursery, was sampled after harvest in each year. For each check variety, 100 seeds were surface-sterilized and plated on potato dextrose agar. The first 40 isolates of *Fusarium graminearum* recovered per check were single-spored and analysed for chemotype by PCR. In 2008, the ratio of 3-ADON to 15-ADON isolates recovered from seed was on average 4:1, respectively, for all 6 checks. However, in 2009, when the isolates were applied in a 1:1 ratio of 3- and 15-ADON, respectively, the ratio of recovered isolates was 1:1. Although inoculation methods and spore concentration were unchanged between the 2 years, DON levels were much higher in 2009 (ranging from 11 ppm to 71 ppm) than in 2008 (ranging from 5 ppm to 25 ppm). This may be due to a cooler and wetter growing season in 2009 allowing for greater fungal growth and toxin production. The difference in growing conditions and chemotype frequency did not alter the relative DON rankings of the 6 lines used. Those rated as most tolerant to FHB had the lowest levels of DON, and those most susceptible had the highest DON levels in both years.



---

---

A COMPARISON OF THE AGGRESSIVENESS AND  
DEOXYNIVALENOL CONTENT OF CANADIAN 3-ACETYL AND  
15-ACETYLDEOXYNIVALENOL PRODUCERS OF *FUSARIUM*  
*GRAMINEARUM* IN FIELD-GROWN SPRING WHEAT

C. Knopf<sup>1</sup>, V. Gauthier<sup>2</sup>, L. Tamburic-Ilincic<sup>3</sup>, A. Brule-Babel<sup>2</sup>,  
W.G.D. Fernando<sup>2</sup>, R. Clear<sup>4</sup>, T. Ward<sup>5</sup> and T. Miedaner<sup>1\*</sup>

---

<sup>1</sup>State Plant Breeding Institute, Universitaet Hohenheim, Fruwirthstr. 21, 70599 Stuttgart, Germany;  
<sup>2</sup>Department of Plant Science, University of Manitoba, Winnipeg, MB, Canada, R3T 2N2; <sup>3</sup>Department  
of Plant Agriculture, University of Guelph, Ridgetown, ON, Canada, N0P 2C0; <sup>4</sup>Grain Research  
Laboratory, Canadian Grain Commission, 1404-303 Main Street, Winnipeg, MB, Canada, R3C 3G8;  
and <sup>5</sup>Bacterial Foodborne Pathogens and Mycology, USDA-ARS, Peoria, IL 61604, USA  
\*Corresponding Author: PH: 49-711-459-22690; E-mail: miedaner@uni-hohenheim.de

---

**ABSTRACT**

Twenty four isolates of *Fusarium graminearum* of Canadian origin half of which were 3-acetyldeoxynivalenol (3-ADON) and half 15-acetyldeoxynivalenol (15-ADON) producers were tested for their ability to cause Fusarium head blight (FHB), as measured by FHB index and production of deoxynivalenol (DON) in spring wheat. Objectives of this study were to determine (i) whether 3-ADON producers differ in aggressiveness and DON production from 15-ADON producers under field conditions and (ii) whether resistant host cultivars were stable in performance across isolates. Field tests of all isolates were conducted with three replications at each of two locations in Canada and Germany in 2008, with three host genotypes differing in FHB resistance level. Mean FHB indices and DON content were analysed. Mean FHB indices across locations ranged from 5.48 to 34.42%. The resistant host genotype showed resistance regardless of the isolate or location. The differences between mean FHB indices of 3-ADON and 15-ADON chemotypes were not significant. In contrast, DON production by the 3-ADON isolates was significantly ( $P < 0.05$ ) higher at three locations. Acetylated forms of DON accounted for only 2.5% (3-ADON) and 0.4% (15-ADON) of the total DON concentration across the two German locations. 3-ADON isolates may produce more DON depending on location than 15-ADON producers, but their mean aggressiveness is quite similar.

---

---

FUNCTIONAL CHARACTERIZATION OF HISTONE DEACETYLASE  
GENES IN *FUSARIUM GRAMINEARUM*

Yiming Li<sup>1,2</sup>, Chengfang Wang<sup>1</sup>, Wende Liu<sup>2</sup> and Jin-Rong Xu<sup>1,2\*</sup>

---

<sup>1</sup>Dept. of Plant Protection, Northwest Agricultural and Forestry University, Yangling, Shanxi, China;  
and <sup>2</sup>Dept. of Botany and Plant Pathology, Purdue University, West Lafayette, IN

\*Corresponding Author: PH: (765) 496-6918; E-mail: jinrong@purdue.edu

---

**ABSTRACT**

*Fusarium* head blight caused by *Fusarium graminearum* is an important disease of wheat and barley. In a previous study, the transducin-beta like gene *FTL1*, a component of a well-conserved histone deacetylase (HDAC) complex, was found to be essential for plant infection. The *F. graminearum* genome has one predicted class I HDAC gene that is homologous to yeast *RPD3*. It also contains three class II HDAC genes, FGSG\_01353, FGSG\_04324.3, FGSG\_05636.3, that are named *FgHOS2*, *FgHDA1*, and *FgHOS3*, respectively, in this study according to their homologs in *S. cerevisiae*. Mutants deleted for these four HDAC genes were generated with the split-marker approach. Infection assays with flowering wheat heads indicated that the *FgRPD3* and *FgHOS2* genes are important for plant infection. While the *fgrpd3* deletion mutant was severely reduced in vegetative growth, the *fghos2* mutant had relatively normal growth rate. The latter produced fewer conidia and shorter aerial hyphae. On mating plates, the *fghos2* mutant was sterile. Instead of forming protoperithecia or perithecia, the mutant produced abundant sporodochia with massive amount of macroconidia. In 16 h germlings, the mutant accumulated numerous lipid droplets. These data suggested that deletion of *FgHOS2* likely affected proper regulation of subsets of genes involved in sexual reproduction, conidiation, lipid metabolism, and plant infection. Microscopic examination of plant infection defects and expression profiles of the *fgrpd3* and *fghos2* are in the progress. Data on HDAC activity assays with these mutants and genes affected by deletion for *FgHOS2* also will be presented.

---

---

**TRI3, WHICH CONTROLS TRICHOHECENE C-15 ACETYLATION,  
IS FUNCTIONAL IN 3ADON CHEMOTYPE**

S.P. McCormick<sup>1\*</sup>, N.J. Alexander<sup>1</sup> and C. Waalwijk<sup>2</sup>

---

<sup>1</sup>USDA-ARS-NCAUR, Peoria IL 61604; and <sup>2</sup>Plant Research International, Wageningen, The Netherlands

\*Corresponding Author: PH: (309) 681-6381; E-mail: Susan.McCormick@ars.usda.gov

---

**ABSTRACT**

Three different trichothecene chemotypes have been identified in U.S. strains of *Fusarium graminearum*: 3-acetyldeoxynivalenol (3ADON), 15-acetyldeoxynivalenol (15ADON), and nivalenol (NIV), although grain is typically contaminated with deoxynivalenol (DON) or nivalenol rather than the acetylated derivatives.

In DON-producing strains of *F. graminearum*, two of the trichothecene cluster genes, *TRI7* and *TRI13*, are nonfunctional as a result of multiple insertions and deletions within their coding regions, whereas in NIV-producing strains, *TRI7* and *TRI13* are functional (Lee et al., 2002). These differences, combined with the finding that *TRI13* is responsible for trichothecene C-4 hydroxylation, identified the basis for NIV versus DON chemotypes in *F. graminearum*. Differences in *TRI7* and *TRI13* were used to develop PCR markers to predict DON and NIV chemotypes (Chandler et al., 2003).

PCR markers for *TRI3* and *TRI12* have been used to predict 3ADON and 15ADON chemotypes in *Fusarium graminearum* (Ward et al., 2008). In order to determine the genetic basis for these chemotypes, we looked at differences in the function of *TRI3* in 3ADON, 15ADON and NIV strains. *TRI3* controls the addition of an acetyl group at the C-15 of trichothecenes in *Fusarium sporotrichioides* (McCormick et al. 1996, Garvey et al. 2009). A group of sixty *Fusarium* strains were analyzed for production of trichothecenes in liquid culture and on rice to confirm the chemotype predicted with PCR markers for *TRI3* and *TRI12* (Ward et al., 2008). *TRI3* from representative strains of each chemotype were expressed in yeast and the transformants were fed possible Tri3 substrates (15-decalonecetrin, DON). Tri3 from all three chemotypes converted 15-decalonecetrin to calonecetrin indicating that Tri3 is functional, even in the 3ADON chemotype. DON was not a good substrate for Tri3 which supports the addition of the C-15 acetyl group earlier in the biosynthesis of 3ADON. Cell free extracts were also prepared from representative strains of each chemotype and fed 3,15-diADON. Cell-free extracts of 15-ADON and NIV strains converted 3,15-diADON to 15-ADON; cell-free extracts of 3ADON strains converted 3,15-diADON to 3ADON. The Tri8 esterase removes the acetyl group from the C-3 position in 15ADON strains (McCormick and Alexander, 2003). The results indicate that a C-15 esterase is required to produce 3ADON. Efforts to characterize this esterase are ongoing.

**REFERENCES**

Chandler, E.A., Simpson, D.R., Thomsett, M.A., Nicholson, P. (2003) Development of PCR assays to *Tri7* and *Tri13* trichothecene biosynthetic genes, and characterisation of chemotypes of *Fusarium graminearum*, *Fusarium culmorum* and *Fusarium cerealis*. *Physiol. Mol. Plant Pathol.* 62: 355–367.

Graeme S. Garvey, McCormick, S.P. Alexander, N.J. and Rayment, I. (2009) . Structural and functional characterization of TRI3 acetyltransferase from *Fusarium sporotrichioides*. *Protein Science* 18 (4): 747-761.

#### Session 4: Pathogen Biology & Genetics

---

Lee T, Han Y-K, Kim K-H, Yun S-H, Lee Y-W. (2002) *Tri13* and *Tri7* determine deoxynivalenol- and nivalenol-producing chemotypes of *Gibberella zeae*. Appl. Environ. Microbiol. 68: 2148–2154.

McCormick S.P, Hohn T.M., Desjardins, A.E. (1996) Isolation and characterization of *Tri3*, a gene encoding 15-O-acetyltransferase from *Fusarium sporotrichioides*. Appl. Environ. Microbiol. 62: 353–359.

McCormick, S.P. and Alexander, N.A. (2002) *Fusarium* TRI8 encodes a trichothecene C-3 esterase. Appl. Environ. Microbiol. 68: 2959–2964.

Ward T.J., Clear, R.M., Rooney, A.P., O'Donnell, K., Gaba, D., Patrick, S., Starkey, D.E., Gilbert, J., Geiser DM, Nowicki TW. (2008). An adaptive evolutionary shift in *Fusarium* head blight pathogen populations is driving the rapid spread of more Toxigenic *Fusarium graminearum* in North America. Fungal Genet. Biol. 45: 473–484.

---

---

MULTIPLEX QUANTITATIVE ANALYSIS FOR TRICHOHECENE  
GENES EXPRESSION OF *FUSARIUM GRAMINEARUM* IN  
DIFFERENT GENOTYPES OF WHEAT SPIKES

T. Miyazaki and T. Ban\*

---

Kihara Institute for Biological Research, Yokohama City University, Japan

\*Corresponding Author: PH: 81-45-820-2404; E-mail: tban@yokohama-cu.ac.jp

---

**ABSTRACT**

Wheat resistance level to mycotoxin contamination varies among genotypes, however it remains incompletely understood whether resistance to fungal invasion produces secondary effect or specific genes works on low level accumulation of the mycotoxins. We investigated trichothecene gene (*Tri* genes) expression of *F. graminearum* in the infested wheat genotypes, using newly developed multiplex quantitative PCR method.

We applied this method to analyze *Tri* genes expression dynamism at early infection stage to reveal effective wheat genotypes that suppress trichothecene biosynthesis. We analyzed the relative expression level of *Tri* genes (*Tri5*, *Tri6*, *Tri8*, *Tri10* and *Tri11*) per fungal cell using actin and  $\beta$ -tubulin as internal standards. It is a more cost-effective way to analyze *Fusarium*-wheat gene expression crosstalk than the microarray analyses and higher throughput than the real time PCR methods. FHB resistance cv. Sumai 3 and susceptible cv. Gamenya were infected 10<sup>6</sup> unit/ml spore of *F. graminearum* 132-9 (DON producer) injected to the first and second floret in central spikelet of the spike at flowering time. Infested spikes were maintained at 22°C and kept wet 48hrs for FHB initial penetration. Then, the relative humidity was kept around 60% and the three infested spikes were sampled together to extract total RNA at 5days after inoculation (DAI), 10DAI, 15DAI and 20DAI. The extracted total RNA was used for the multiplex quantitative PCR analysis with chimeric primers consisting of *Tri* gene specific sequences with a universal tail designed to amplify different size of each *Tri* gene. We quantified the expression and calculated average of the *Tri* genes expression level with triplication per one sample. Analyzed expression level of the *Tri* genes of *F. graminearum* in Sumai 3 at 5DAI was 73% higher than that at 10DAI, 15DAI and 20DAI, excepting constant level of *Tri 11*. On the other hand, no change of the *Tri* genes expression level was found in Gamenya at 5DAI was the same as 10DAI, and 54% higher than that at 15DAI, 20DAI. Comparison with the two varietal differences, *Tri* genes expression level in Sumai 3 at 5DAI was 49% higher than that Gamenya, despite that in Sumai 3 at 10DAI was lower than Gamenya. *Tri* genes expression level of *F. graminearum* varied in the infested wheat genotype, and its expression level decrease with time.

**ACKNOWLEDGEMENT**

This work was supported by a grant from the Ministry of Agriculture, Forestry and Fisheries of Japan (Genomics for Agricultural Innovation, TRC-1005).

THE NEWLY EMERGING 3ADON POPULATION OF *FUSARIUM GRAMINEARUM* IS MORE AGGRESSIVE AND PRODUCES A HIGHER LEVEL OF DON THAN THE PREVALENT 15ADON POPULATION IN NORTH DAKOTA

Krishna D. Puri and Shaobin Zhong\*

---

Department of Plant Pathology, North Dakota State University, Fargo, ND 58108-6050

\*Corresponding Author: PH: (701) 231-7427; E-mail: shaobin.zhong@ndsu.edu

---

**ABSTRACT**

Fusarium head blight (FHB) is primarily caused by *Fusarium graminearum* in North America. The fungal pathogen produces various types of trichothecenes, including deoxynivalenol (DON), 3-acetyl-Deoxynivalenol (3ADON), 15-acetyl-Deoxynivalenol (15ADON) and nivalenol (NIV). Based on the trichothecene profile, isolates of *F. graminearum* can be identified as one of the three chemotypes, i.e, 15ADON, 3ADON, and NIV. Population studies indicated that *F. graminearum* isolates with a 3ADON chemotype were rare in North America before 1998, but the frequency of 3ADON isolates has increased dramatically in Canada and the Upper Midwest of the United States in recent years. However, little information is available on the aggressiveness and DON production of the newly emerging 3ADON population in wheat genotypes with different sources of FHB resistance. In this study, we characterized *F. graminearum* isolates collected from 1980 to 2000 (old collection) and in 2008 (new collection) and found that the frequency of 3ADON isolates was very low (3%) in the old collection but it accounted for 44% in the new collection. Evaluation of fourteen 3ADON isolates and fourteen 15ADON isolates randomly selected from the collections by single-floret inoculation on three spring wheat genotypes (Grandin, Steele-ND and ND2710) showed that the 3ADON population caused a significantly higher level of disease severity and produced more DON accumulation than the 15ADON population on Grandin (susceptible to FHB) and ND2710 (with FHB resistance from Sumai 3). However, no significant differences in disease severity and DON production were observed between the two populations on Steele-ND (with moderate resistance from *Triticum dicoccoides*). The 3ADON isolates also exhibited a higher DON productivity in rice culture and produced more spores on agar media than the 15ADON isolates, suggesting a fitness advantage of the newly emerging 3ADON population over the prevalent 15ADON population. The information obtained could have a significant impact on FHB management and host resistance deployment.

**ACKNOWLEDGEMENTS**

We thank Dr. Robert W. Stack (retired) for providing the *Fusarium graminearum* isolates collected before 2000 and Dr. Kelly Benson for analysis of the mycotoxins. We acknowledge Joe Mulins, Jana Hansen, Shaukat Ali and Yueqiang Leng for assistance in the greenhouse inoculation.

---

---

LINKING FIELD AND ATMOSPHERIC POPULATIONS  
OF TOXIGENIC FUSARIA  
David G. Schmale III

---

Department of Plant Pathology, Physiology, and Weed Science, Virginia Polytechnic  
Institute and State University, Blacksburg, VA 24061-0390  
Corresponding Author: PH: (540) 231-6943; E-mail: dschmale@vt.edu

---

**ABSTRACT**

*Fusarium* is arguably one of the most important fungal genera on the planet earth. Some fusaria are plant pathogens, others saprophytes, and still others producers of dangerous secondary metabolites. Many fusaria use the atmosphere to travel from one habitat to another. Their atmospheric transport is poorly understood, yet necessary to understand their ecological roles in agricultural ecosystems and evaluate risks posed by invasive fusaria in these habitats. We collected hundreds of fusaria with autonomous (self-controlling) unmanned aerial vehicles (UAVs) tens to hundreds of meters above of the surface of the earth at Virginia Tech's Kentland Farm. Partial translation elongation factor (TEF) DNA sequences were generated from a series of single-spored isolates, and BLAST queries were performed against a curated *Fusarium* TEF database and GenBank. At least 12 different species of *Fusarium* were present in samples collected from 75 different autonomous UAV flights. Most of the flight populations contained more than one *Fusarium* species, suggesting that these fungi are traveling together through the atmosphere as part of discrete assemblages. Strains of *Fusarium graminearum* collected with UAVs 40 to 300 meters above the ground during fall, winter, spring, and summer months were able to cause Fusarium head blight on a susceptible cultivar of spring wheat and produce a variety of trichothecene mycotoxins. A new framework for understanding punctuated changes in the population structure of atmospheric fusaria is being developed and tested at both local (individual farm) and regional (eastern U.S.) scales. This work aims to transform our knowledge of the atmospheric transport of microorganisms and develop new paradigms that link field and atmospheric populations of toxigenic fusaria.

**ACKNOWLEDGEMENT**

This material is based in part upon work supported by the National Science Foundation under award number 0919088.

AGGRESSIVENESS OF 15-ACETYL-DEOXYNIVALENOL AND  
NIVALENOL *FUSARIUM GRAMINEARUM* TRICHOTHECENE  
GENOTYPES TOWARDS WHEAT VARIETIES  
P. Spolti, L. Simon, J. Santos and E.M. Del Ponte\*

---

UFRGS, Faculdade de Agronomia, Porto Alegre, RS, Brazil

\*Corresponding Author: PH: (55) 51-33086908; E-mail: emerson.delponte@ufrgs.br

---

**ABSTRACT**

Fusarium head blight (FHB) of wheat in Brazil is caused mainly by *Fusarium graminearum* species complex members that possess a deoxynivalenol (DON) or nivalenol (NIV) B-trichothecene genotype. Our recent research showed that one acetylated form of DON (15ADON) seems to predominate over NIV across several wheat production regions. In this study we tested whether 15ADON strains have fitness advantages over NIV strains when inoculated onto two wheat varieties (Guamirim – moderate resistant and BRS194 - susceptible) of known reaction to FHB in the field. Two separate greenhouse experiments were conducted for each variety using four strains of distinct trichothecene genotype and isolated from wheat or barley (15ADON-wheat, 15ADON-barley, NIV-wheat and NIV-barley). Two inoculation methods were used for assessing 1) infection rate in excised head tissues (lemma and paella) following different incubation times and 2) rate of disease spread in the heads (mid-point inoculation). A significant difference for the infection and colonization rates among isolates was observed only for Guamirim while the disease developed similarly in BRS194, regardless of the strain type. Both rates were higher for 15ADON strains compared to NIV strains in Guamirim; head severity at 15 days after mid-point inoculation averaged 45% and 10% for 15ADON and NIV, respectively. Infection frequency on spikelet tissues was 50% and 5% for NIV-wheat and NIV-barley, respectively. Our results may help to explain the predominance of 15ADON over NIV genotypes in the field and suggest that they may play a differential role in pathogenesis depending on the resistance level of the wheat variety, which deserves further investigation.