

# **PATHOGEN GENETICS AND GENOMICS**

Chairperson: Frances Trail



FUNCTIONAL ANALYSIS OF POLYKETIDE SYNTHASE  
GENES IN *GIBBERELLA ZEAE*

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**ABSTRACT**

Polyketides are a complex class of secondary metabolites that include some of the most potent mycotoxins (i.e. aflatoxin, zearalenone, fusarin). In fungi, polyketides are synthesized by large, multi-domain enzymes called polyketide synthases. We have identified from the genomic sequence 15 polyketide synthase genes and functionally disrupted them. Five of these genes are responsible for producing the mycotoxins zearalenone, aurofusarin, fusarin C and the black perithecial pigment. We have shown that each of the 15 genes show relatively unique expression patterns during grain colonization, plant colonization, sexual development, and mycelial growth. None of these is essential to pathogenicity, however, our results indicate that they play important roles in the life cycle of this fungus.

DISPLACEMENT OF THE NATIVE POPULATION OF *FUSARIUM GRAMINEARUM* IN NORTH DAKOTA AND PARTS OF MINNESOTA BY A GENETICALLY DIVERGENT AND MORE TOXIGENIC POPULATION

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**ABSTRACT**

Population genetic analyses of *Fusarium graminearum* that examined hundreds of strains collected from 13 mainly Midwestern states from 1999-2002 revealed the presence of a genetically distinct population of *F. graminearum* that was restricted in distribution to North Dakota (ND) and Minnesota (MN) and that was characterized by a 3ADON chemotype. Systematic sampling in 2003 and 2004 of more than 7,000 wheat heads from 83 fields in 51 counties of MN, ND and South Dakota (SD) resulted in 4,957 strains. While previous surveys from these states recovered strains with a 3ADON chemotype only occasionally, these more recent and extensive surveys show that its frequency has dramatically increased over just a few years. In the 2003 collection 3ADON strains were at frequencies of 19% and 23% in ND and MN, respectively, while in 2004, 3ADON strain frequencies were again higher, with averages of ca. 35% for both ND and regions of MN north of the counties of Ottertail and Wilkin. A field in Pembina County, ND showed the highest frequency of 3ADON strains at 59.6%. While combined data from 2003 and 2004 indicate that the 3ADON type is now widespread and frequent in most ND and MN areas surveyed, the 3ADON type is less common in MN for regions south of the Fargo/Moorhead area. Among nine southern MN counties surveyed in 2004, the average frequency of 3ADON strains was only 1%, though the 3ADON type still could be detected in seven out of the nine counties. Also, the 3ADON chemotype has so far not been detected in SD. We also discovered that the emerging population actually consists of both 3ADON and 15ADON chemotype strains. By use of three unlinked VNTR markers we identified 15ADON strains that otherwise could not be distinguished from the 3ADON strains, indicating that they belong to the same population. When the data are combined, the emergent population was at a frequency of about 45% both in ND and MN (north of the counties Wilkin and Ottertail) in 2004, up from about 30% in 2003. Preliminary data indicate that the emergent population has a higher toxigenic potential compared to the native population that consists of 15ADON strains only. Compared to strains of the native population on the susceptible cultivar Norm, 3ADON and 15ADON strains of the emergent population produced on average 68% and 32% higher DON concentrations, respectively in inoculated spikelets. We are currently in the process of further determining the selective advantages of this emergent population in addition to identifying recombinants and determining the amount of recombination between the two populations. Identification and study of recombinants will not only be valuable for potentially mapping regions of the genome that contribute to the higher toxigenic potential, but also for determining whether the high toxigenic potential displayed by the emergent population will be reduced in the future by recombination with the native, less toxigenic population.

FUSARIUM HEAD BLIGHT OF WHEAT IN LOUISIANA IS CAUSED  
LARGELY BY NIVALENOL PRODUCERS OF *FUSARIUM*  
*GRAMINEARUM* AND *FUSARIUM ASIATICUM*

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**ABSTRACT**

Annual collections of Fusarium head blight (FHB)-symptomatic wheat by the USDA-ARS Cereal Disease Laboratory (CDL) not only cover midwestern states with a history of FHB epidemics, but also southern states that are not generally considered epidemic regions. Since the year 2000, several hundred plant samples have been examined from Texas, Oklahoma, Alabama, Florida, Mississippi, and Louisiana. While *Fusarium* species other than *F. graminearum* are occasionally present in wheat seed, *F. graminearum* itself appears to be scarce in southern states. So far we have not encountered the species in samples from Oklahoma, Texas, and Florida, and our collections only hold one *F. graminearum* isolate each from Mississippi and Alabama. In contrast, genetically diverse populations of the *Fusarium graminearum* (Fg) species complex are present in Louisiana. Our collection of more than 150 strains from four parishes in Louisiana (from 2002, 2003, and 2005) consists predominantly of nivalenol (83%) and 3ADON (13%) chemotype strains; the 15ADON chemotype, which is otherwise predominant in the Midwest was only observed for seven strains. All strains also have been genotyped using five molecular markers based on variable number of tandem repeats (VNTRs) and species identification using the multilocus genotyping array has been performed for some VNTR genotypes (see T.J. Ward *et al.*, this Proceedings). Our preliminary analysis of the diversity in Louisiana indicates the following: the largest proportion of the population consists of *F. graminearum* isolates that may possibly belong to the same population as isolates commonly present in the Midwest, though nearly all strains of *F. graminearum* in Louisiana are of a nivalenol chemotype. A smaller proportion of isolates belong to a divergent lineage of *F. graminearum* that displays all three chemotypes. Nivalenol producers of *F. asiaticum* have also been identified that may represent about 25% of the population in Louisiana. The predominance of nivalenol producers from different Fg complex species in Louisiana strongly implies a selective advantage for this chemotype in this particular environment that will be addressed in future studies. Also, as nivalenol has a higher overall toxicity than deoxynivalenol, the risk of a potential spread of nivalenol producers to other U.S. wheat growing regions needs to be evaluated. While deoxynivalenol producers have been reported to have a selective advantage on wheat over nivalenol producers in Nepal this needs to be confirmed and evaluated taking into account the genetic background on which these chemotypes reside.

RELATIVE PATHOGENICITY OF 3-ADON AND 15-ADON ISOLATES  
OF *FUSARIUM GRAMINEARUM* FROM THE  
PRAIRIE PROVINCES OF CANADA

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**ABSTRACT**

Eighteen isolates of *Fusarium graminearum* Schwabe, 3 producing 15-ADON and 3 producing 3-ADON from each of the Canadian provinces of Manitoba, Saskatchewan and Alberta, were tested for relative pathogenicity and consistency of production of toxin, on two Canadian spring wheat cultivars, 'Roblin' (S) and '5602 HR' (MR). The experimental design was a 3-replicate randomized complete block. Each replicate consisted of a pot containing 2 or 3 plants of one cultivar, and were grown in a cooled greenhouse. At anthesis, 2 to 5 heads per pot were inoculated with one isolate and heads covered with glassine bags for 48 h to promote a favourable environment for disease development. Disease was scored at 14 d and 21 d after inoculation and recorded as percentage infected spikelets. These preliminary results showed no significant differences in pathogenicity among isolates from the three provinces and producing either 3-ADON or 15-ADON. Toxin analysis by GC/MS of seeds from the inoculated heads found higher levels of DON in the susceptible (45 ppm) vs resistant (14 ppm) wheat. All isolates formed their respective 3-ADON or 15-ADON analogs. The 3-ADON isolates formed, on average, 16.1 ppm DON on 5602HR and 56.9 ppm on Roblin, higher than the 15-ADON isolates which averaged 11.9 ppm and 33.6 ppm respectively.

GENES IMPORTANT IN ASCOSPORE DEVELOPMENT  
IN *GIBBERELLA ZEA*

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**ABSTRACT**

With the availability of the genome sequence and the Affymetrix Genome Chip for gene identification and gene expression, our ability to identify genes important to ascospore development and perithecium function has greatly increased. We have targeted several gene families using these methods and are now characterizing their role in inoculum development. Myosins are proteins found in a wide variety of organisms and are important in cellular transport and cellular movement. As asci must stretch at the appropriate time to discharge their contents, we hypothesize that they are important in ascus function. Fueled by ATP hydrolysis, myosins move along actin filaments and cause cell movement. In this study 3 myosin genes were deleted from the genome of *G. zea* and their phenotypic characterization will be presented. Several other groups of genes have been targeted for gene deletion and the results of these experiments will also be presented. Through this process, we have identified several genes whose products are vital to the development of asci and ascospores.

TRIACYLGLYCERIDE ACCUMULATION IN ANTICIPATION OF  
SEXUAL DEVELOPMENT IN *GIBBERELLA ZEA*

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**ABSTRACT**

*Gibberella zeae* accumulates fats in dikaryotic hyphae prior to host plant senescence and in anticipation of over-wintering and sexual reproduction. Using NMR and gas chromatography, we have analyzed the lipid profile from haploid mycelium to perithecium initials both in culture and *in planta*. Results show that the major sequestered lipid storage products are triacylglycerides. These lipid stores increase dramatically following perithecium induction in culture. In plants, they increase as the fungus approaches sexual development. The triacylglycerides consist mainly of C18 fatty acids with varying degrees of saturation. These results show that lipids play an integral role in survival and reproduction by this fungus. The ability of the fungus to colonize senescing vegetative tissue and accumulate fats has strong implications for the following seasons epidemic.

DELETION OF THE TRICHOHECENE GENE  
CLUSTER OF *FUSARIUM GRAMINEARUM*

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**ABSTRACT**

The trichothecene gene cluster (~ 27 kb) of *Fusarium graminearum* is comprised of genes involved in the synthesis of trichothecene mycotoxins. Strains of *F. graminearum* differ in the chemical profile of trichothecene derivatives produced. These different profiles, known as chemotypes, correspond to strains that produce predominantly 1) deoxynivalenol (DON) and 15-acetyl DON, 2) DON and 3-acetyl DON or 3) nivalenol. Our objective is to develop isogenic lines of *F. graminearum* that differ only in the toxin biosynthesis cluster. These lines may be used to ascertain whether chemotype is determined solely by genes within the cluster, and will be essential for understanding how trichothecenes influence fungal pathogenicity, selection and fitness. As the first step towards creating isogenic lines, the trichothecene gene cluster has been deleted from *F. graminearum* strain PH-1. Split-marker recombination was used for targeted deletion of the trichothecene gene cluster via homologous recombination, resulting in replacement of the cluster with a hygromycin resistance gene. Hygromycin resistant mutants were screened for deletion of the cluster using PCR for genes in the cluster as well as Southern hybridization using as a probe a BAC containing the trichothecene gene cluster.

STRATEGIES TO CONTROL SCAB USING RNAI TECHNOLOGY:  
PROGRESS AND OBSTACLES

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**ABSTRACT**

RNA interference (RNAi) technology takes advantage of a conserved eukaryotic mechanism that degrades mRNAs. A key component of this mechanism is the production of double stranded RNA (dsRNA) which can be digested into small interfering RNAs (siRNAs) 21-26 nt in length by a RNase III helicase-containing protein called Dicer. siRNAs enter into a Dicer containing complex known as RISC (RNA induced silencing complex) and anneal to same sequence mRNAs. These mRNAs are cleaved by the RISC complex leading to the degradation of mRNA. Here we demonstrate that trichothecene production can be down regulated by RNAi technology in the scab pathogen *Fusarium graminearum*. Current studies focus on (a) investigating the ability of fungi to uptake and amplify siRNAs in the cell through activity of RNA-dependent RNA polymerases and (b) creating transgenic siRNA producing wheat lines that will target *Fusarium* virulence genes.

## EXPRESSION ANALYSIS OF DEFENSE-RELATED GENES IN WHEAT IN RESPONSE TO INFECTION BY *FUSARIUM GRAMINEARUM*

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### ABSTRACT

Fusarium head blight (FHB), caused by the fungus *Fusarium* species, is a worldwide disease of wheat (*Triticum aestivum* L.). The Chinese cultivar, Ning7840, is one of few wheat cultivars with resistance to FHB. GeneCalling, an open-architecture, mRNA-profiling technology, was used to identify differentially expressed genes induced or suppressed in spikes after fungal infection in FHB resistant cultivar Ning7840-*Fusarium graminearum* interaction. Over 150 individual cDNA fragments representing different transcripts expressed in wheat spikes were examined and sequenced, and putative functions assigned to some of the unigenes based on BLASTN and BLASTTX. Of the unigenes identified, 28 were assigned function in primary metabolism and photosynthesis, 7 were involved in defense response, 14 were in gene expression and regulation, 25 encoded proteins associated with plant cell wall degradation, 42 were without a known function with sequences in the database, and interestingly, 3 genes showed similarities to cloned disease resistance proteins. Of particular interest in this study were genes associated with resistance and defense genes to pathogen infection. Real-time quantitative reverse-transcriptase PCR indicated that, of 51 genes tested, 19 genes showed 2-fold or greater induction in the FHB resistant wheat lines KS24-2 containing FHB resistance derived from *Lophopyrum elongatum* and Ning7840, in contrast to susceptible wheat line Len, while another 32 genes were not significantly induced in either of the FHB resistant wheat lines compared with susceptible Len. Characterization of these genes, whose activity is correlated with FHB resistance and may be involved in wheat-fungal interactions, is ongoing in this study.

## FIELD POPULATIONS OF *GIBBERELLA ZEA*

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### ABSTRACT

*Gibberella zeae* is homothallic and the sexual stage occurs commonly where the fungus is found as part of wheat/maize cropping systems. In the northern United States the amount of genetic diversity is large, most individuals are unique (not clones) and linkage disequilibrium is detectable only at levels approaching the error rate associated with the statistical analysis. These patterns hold over wide distances, *i.e.* Virginia to Montana, and across years. In limited samples, isolates associated with maize fields are not significantly different from those associated with wheat fields. Outside the United States, patterns are different and sometimes more complex. Although isolates in the United States differ greatly, all of the isolates we analyzed can be associated with lineage 7 *sensu* O'Donnell *et al.* (2000). Near CIMMYT in Mexico, field isolates belong to lineage 3. In Uruguay and southern Brazil lineage 7 again dominates the field collections, but other lineages are present as are putative interlineage hybrids. In Australia and Viet Nam, the genetic boundaries for the lineages are not distinct with AFLP markers, but we have not analyzed diagnostic sequences to determine if interlineage hybrids are present. In Korea, the dominant lineage present depends on the host, with lineages 7 and 3 dominating in maize and lineage 6 dominating in rice/barley rotations. There is evidence for putative interlineage hybrids occurring in Korea as well. Thus, genetic variation in *G. zeae* occurs at all levels measured. The genetic isolation of the lineages remains an open question, but laboratory crosses and the existence of putative hybrids under field conditions suggest that these differences are not sufficient to stop significant gene flow between the lineages if more than one lineage is present at the same location.

## DEVELOPMENT OF A NOVEL BIOASSAY SYSTEM FOR FHB MOLECULAR INTERACTIONS

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### ABSTRACT

*Fusarium* head blight (FHB) of wheat and barley, caused by *Fusarium graminearum*, is a destructive disease that occurs in warm and humid regions. FHB causes serious yield and quality losses, but of greater concern is the contamination of cereal food and feed with mycotoxins. Further investigations of the molecular interactions between the plant and pathogen are necessary to more effectively combat this disease. We developed a novel bioassay system using primary leaves to evaluate *Fusarium graminearum* pathogenicity and gene expression *in planta*. When a drop of the conidial suspension was placed on a wound (approximately 1 mm in diameter) of a primary leaf, the pathogen succeeded in infection and produced an oval lesion. The diameter of oval lesions produced on resistant cultivars (according to field data) was significantly less than the diameter of lesions produced on susceptible cultivars. When wounds were treated with purified toxin (DON) alone, water-soaked symptoms were observed. Lesion size was remarkably increased when wounds were treated with a mixture of toxin and conidial suspension. These results indicate that DON plays an important role in lesion development by the fungus. DON & NIV mycotoxins have been shown to be virulence factors in FHB and some toxin synthesis genes have already been cloned. However, their role in host-pathogen interactions is not clearly understood. Therefore, fungal gene expressions were investigated using total RNA prepared from infection sites produced by conidial inoculation of wounds on primary leaves. Transcript accumulation of two constitutive genes (Actin and  $\beta$ -tubulin) and two trichothecene genes (*Tri4* and *Tri5*) were detectable by RT-PCR no later than 24h after inoculation. Notably, Actin and  $\beta$ -tubulin gene expressions were observed even at 3h after inoculation. This suggests that the new bioassay system, using conidia, mycotoxin, or conidia plus mycotoxin, is useful for analyzing early mycotoxin regulation by *Fusarium* at the molecular level.

GENE EXPRESSION ANALYSIS OF CONIDIUM MATURATION  
AND GERMINATION ON *FUSARIUM GRAMINEARUM*  
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**ABSTRACT**

To understand the infection cycle of the head blight pathogen *F. graminearum*, gene expression profiles were monitored in newly formed conidia, conidia that had been desiccated for 10 days and germinating conidia using the 18K feature *F. graminearum* Affymetrix GeneChip. A total of 6,384 positive signals were detected in newly formed spores with detection p value <0.001. Enhanced expression of many genes involved in transcription or transcriptional regulation and metabolism such as glycolysis, the glyoxylate cycle, and  $\alpha$ -oxidation imply that newly formed conidia are not dormant cells but rather are metabolically active. Surprisingly, a total of 2,916 positive signals were detected even in mature conidia. Among 543 genes that were up-regulated more than 2-fold upon spore maturation were many genes involved in autophagy, proteolysis, protein secretion, and cell wall synthesis. After suspending conidia in liquid complete medium for 2h, a total of 5,587 signals were detected (p value <0.001) and 2,593 signals were up-regulated more than 2-fold in these swollen spores. Genes involved in transcription, RNA splicing, protein synthesis, and amino acid and nucleotide metabolism were highly induced during the initiation of spore germination. Up-regulation of proteasome components and secretory proteins was observed as germlings established polarized growth after 8h of incubation. Many stage-specific genes and events during spore maturation and germination were identified and will be discussed.

A MULTILOCUS GENOTYPING ASSAY FOR IDENTIFICATION  
OF FUSARIUM HEAD BLIGHT SPECIES AND  
TRICHOHECENE TOXIN CHEMOTYPES

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**ABSTRACT**

Fusarium head blight (FHB) places a serious constraint on the production of wheat and barley worldwide. In addition to yield reductions, infested grains may be contaminated with trichothecene mycotoxins that pose a serious threat to human and animal health. Recent evolutionary analyses have revealed unexpected diversity among FHB pathogens. Fourteen different species within the B-trichothecene lineage (B-clade) of *Fusarium*, including nine within the *Fusarium graminearum* species complex (*Fg* complex), have been described. In addition, B-trichothecene toxin chemotype polymorphism (NIV, 3ADON, and 15ADON) was found to be transspecific and maintained by balancing selection. Using a unique multilocus DNA sequence database (13.6 kb of DNA sequence from 47 strains) we have developed a high-throughput single tube assay for the simultaneous identification of all described B-trichothecene species and chemotypes in order to improve pathogen surveillance and facilitate a greater understanding of the ecology and epidemiology of FHB pathogens. The multilocus genotyping (MLGT) array, consisting of 37 probes targeting single nucleotide differences unique to individual species or chemotypes, was validated using a panel of 218 isolates with known chemotype and species identity. Over 99.6% of the genotypes produced with the MLGT array matched expectations, and due to probe redundancy, chemotype and species identity was correctly determined for all 218 isolates. Use of this assay in our ongoing molecular surveillance has identified unexpected FHB species and chemotype diversity in North America (see related posters by Ward *et al.* and Gale *et al.*).

## FHB SPECIES AND TRICHOHECENE TOXIN DIVERSITY IN NORTH AMERICA

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### ABSTRACT

Our previous phylogenetic and molecular evolutionary analyses demonstrated that the primary etiological agent of FHB, the morphospecies *F. graminearum*, actually comprises at least nine distinct species (*Fg* species complex). In addition, we have demonstrated that the three trichothecene toxin chemotypes (NIV, 3ADON and 15ADON) segregating within these species have been maintained by balancing selection, indicating that these differences are adaptive. Until recently, it appeared that *F. graminearum* (*sensu stricto*) isolates with a 15ADON chemotype were the only significant cause of FHB in North America. However, molecular surveillance with a multilocus genotyping assay for FHB species and chemotype determination has revealed an East-West chemotype cline in Canada and evidence that *F. graminearum* isolates with a 3ADON chemotype may be displacing those with a 15ADON chemotype. In addition, 3ADON isolates have been found to produce significantly ( $P < 0.001$ ) higher levels of trichothecene than those with a 15ADON chemotype. Significant 3ADON populations have also been detected in the Northern Plains (Gale *et al.* poster) and within the Northeast U.S. We have also identified a novel member (*Fusarium gerlachii*) of the *Fg* species complex from the Northern Plains of the U.S. Isolates of this previously unrecognized species produce nivalenol (NIV chemotype), which has higher vertebrate toxicity than 15ADON. In addition, NIV-producing *F. asiaticum* as well as 3ADON and NIV-producing *F. graminearum* have been identified in the Southern U.S. (see Gale *et al.* poster for additional details), and a highly divergent evolutionary lineage of *F. graminearum* from the U.S. Gulf Coast has been found to segregate for all three B-trichothecene chemotypes. Taken together, these results indicate significant changes in B-FHB pathogen composition are taking place in North America, which could have significant implications for food safety, disease control efforts and regulatory policy regarding trichothecene-contaminated grain.

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## FUNCTIONAL GENOMIC STUDIES OF PATHOGENICITY IN *FUSARIUM GRAMINEARUM*

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### ASBTRACT

Head blight or scab caused by *Fusarium graminearum* is a disease of wheat and barley that occurs worldwide and that has great impact on U.S. agriculture and society. Infested cereals are often contaminated with trichothecene and estrogenic mycotoxins. To better understand fungal pathogenesis and development in this important pathogen, we have generated over 30,000 ESTs from three cDNA libraries and a draft sequence of the *F. graminearum* genome. A whole-genome *Fusarium* Affymetrix GeneChip (representing ~14000 putative genes) has been developed. Hybridizations with the *Fusarium* GeneChip were used to identify genes differentially expressed in cultures grown under different nutritional conditions or developmental stages. Transcript profiles of several mutants defective in plant infection have been generated and compared with that of the wild-type strain. Microarray analysis also was used to identify fungal genes differentially expressed during plant infection. In addition, a collection of over 10,000 random insertional mutants have been generated and screened for mutants defective in conidiation and pathogenicity. We also have constructed mutants deleted for over 50 candidate genes and thereby have identified novel virulence factors in *F. graminearum*. Details of the microarray data and phenotypes of selected mutants will be presented.

