SESSION 2:

PATHOGEN BIOLOGY AND GENETICS

Chairperson: David Schmale

GENETIC BASIS FOR THE 3-ADON AND 15-ADON TRICHOTHECENE CHEMOTYPES IN *FUSARIUM*

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ABSTRACT

Fungi in the *Fusarium graminearum* species complex (FGSC) and the related species *F. cerealis* (synonym *F. crookwellense*) and *F. culmorum* can cause *Fusarium* head blight (FHB) of wheat, barley, and other small cereal grain crops worldwide and contaminate grain with trichothecene mycotoxins. In general, *Fusarium* species that cause FHB exhibit three trichothecene production phenotypes (chemotypes): nivalenol (NIV) production, 3-acetyldeoxynivalenol (3-ADON) production, or 15-acetyldeoxynivalenol (15-ADON) production. The genetic basis for the NIV versus 3-ADON/15ADON chemotypes has been demonstrated previously. However, until now, the genetic basis for the 3-ADON and 15ADON chemotypes has not been identified. Two genes, *TRI3* and *TRI8*, have been proposed to affect 3-ADON and 15-ADON production based on functional analysis of the genes in 15-ADON strains of the FHB pathogen *F. graminearum sensu stricto* and in *F. sporotrichioides*, which produces another type of trichothecene, T-2 toxin. The analyses indicate that *TRI3* encodes an enzyme that catalyzes acetylation of trichothecenes at carbon atom 15 (C-15) and that *TRI8* encodes an enzyme that deacetylases trichothecenes at C-3. Here, we identified consistent DNA sequence differences in the coding region of the trichothecene biosynthetic gene *TRI8* in 3-ADON and 15-ADON strains. Functional analyses of the *TRI8* enzyme (Tri8), including gene disruption, cell-free feeding, yeast expression, and fungal transgenic expression, revealed that Tri8 from 3-ADON strains catalyzes deacetylation of the trichothecene biosynthetic intermediate 3,15-diacetyldeoxynivalenol at C-15 to yield 3-ADON, whereas Tri8 from 15-ADON strains catalyzes deacetylation of 3,15-diacetyldeoxynivalenol at carbon 3 to yield 15-ADON. In contrast, the function of *TRI3* was the same in NIV, 3-ADON and 15-ADON strains, and the function of *TRI8* was the same in NIV strains as it was in 15-ADON strains. Together, our data indicate that differential activity of Tri8 determines the 3-ADON and 15-ADON chemotypes in *Fusarium*.

A CROSS BETWEEN TWO GENETICALLY SIMILAR *FUSARIUM GRAMINEARUM* STRAINS PRODUCES STABLE TRANSGRESSIVE SEGREGANTS FOR FHB PATHOGENICITY RELATED TRAITS

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ABSTRACT

Epidemics of Fusarium Head Blight reduce the production and quality of wheat and small grains grown in North America and worldwide. *Fusarium graminearum (*teleomorph*: Gibberella zeae)* is the principal causal agent of FHB in the United States. Population studies have demonstrated that there are high levels of genetic diversity among North American *F. graminearum* field isolates. However, almost all isolates belong to a single genetic lineage known as lineage seven. *F. graminearum* is a homothallic ascomycete with the ability to outcross in culture and in nature. Sexual recombination is an important source of genetic diversity. The objective of this study was to study the genetic regulation of traits impacting fertility, pathogenicity, and aggressiveness among progeny generated by crossing two closely related lineage 7 *F. graminearum* strains. The *F. graminearum* strains chosen as parents, PH-1 (NRRL 31084) and Gz3639, have been used as models by different laboratories studying FHB, and both have available genome sequences. A GFP-tagged strain of Gz3639 was used to aid in screening for heterothallic perithecia. Segregation of SNPs and polymorphic repetitive DNA sequences was used to identify perithecia that resulted from heterothallic matings. Ninety-four progeny strains were isolated from four of these perithecia. The progeny isolates varied significantly in spore production and fertility, and in aggressiveness on the susceptible wheat variety Pioneer 2555. Transgressive segregants were observed for each phenotype. Four isolates with significantly higher, and four with significantly lower levels of aggressiveness, compared with the parental strains, were chosen for further analysis. Preliminary mycotoxin measurements demonstrated a correlation between DON production in planta and the severity of FHB symptoms. Fecundity, aggressiveness and DON production traits in the selected progeny strains were heritable among single-spored progeny of the transgressive segregant strains. These results demonstrate that crosses among phenotypically and genotypically similar strains have the potential to produce a highly diverse progeny population, including strains that are significantly more aggressive and toxigenic than either parent, although the significance of this in the field is unknown.

INFECTION STRUCTURES AND MYCOTOXIN INDUCTION OF *FUSARIUM GRAMINEARUM* ON WHEAT FLORETS

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ABSTRACT

F. graminearum is one of the best investigated phytopathogens however detailed information about fungal development on host surfaces and the penetration strategy of the pathogen is limited. Therefore, a bioassay was established to investigate inoculated floret tissues of wheat. Detection of mycelium was facilitated by constitutive expression of the *dsRed* reporter gene. Bright field, fluorescence microscopy, and confocal laser microscopy was used to study fungal development and host symptoms during infection. Trichothecene production of the fungus was monitored by a *GFP* coupled *TRI5*-promoter. Combining bioimaging with scanning electron microscopy we identified different infection structures and subcuticular growth. In addition to short infection hyphae foot-like structures, lobate appressoria, and infection cushions were observed. Monitoring of *GFP* fluorescence visualized a specific induction of *TRI5* gene expression in infection structures. Interestingly, a *TRI5* deletion mutant exhibits the same infection strategy and efficacy. We conclude that trichothecene biosynthesis is specifically induced in infection structures, but is neither necessary for their development nor for disease symptoms of wheat florets.

A SUBSET OF THE NEWLY DISCOVERED NORTHLAND POPULATION OF *FUSARIUM GRAMINEARUM* FROM THE U.S. DOES NOT PRODUCE THE B-TYPE TRICHOTHECENES DON, 15ADON, 3ADON OR NIV

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ABSTRACT

Between 2003 and 2006, large-scale population surveys of *Fusarium graminearum* from North Dakota (ND), South Dakota (SD) and Minnesota (MN) were conducted to determine the spatial and temporal dynamics of the emergent population of *F. graminearum* that has been determined to be more toxigenic than the pre-existing and widespread Midwestern (MW) 15ADON population. To efficiently determine population membership, we developed three VNTR markers with alleles that are specific to the known populations. After genotyping and determination of trichothecene type of more than 6,000 isolates, and after identifying and excluding species other than *F. graminearum* (e.g. *F. culmorum*, *F. poae*) and clones from repeat isolations from the same wheat head, we identified roughly 400 *F. graminearum* isolates that did not display population specific VNTR patterns. Further genotyping these with PCR-RFLPs, together with members from known populations for comparison, and subsequent analysis with STRUCTURE, a Bayesian model-based clustering software that assigns multilocus genotypes probabilistically to *K* populations, revealed the presence of three populations, the MW15ADON population, the emergent population (including 3ADON and 15ADON types) and a newly identified population that we named the Northland population. We currently have identified 176 isolates in our collection that belong to this population, whereby the majority originated from MN (77%), followed by ND (19%), and SD and WI (2% each). Isolates were not only recovered from wheat, but also from grasses in non-agricultural regions such as the Arrowhead region of MN (extreme Northeast MN). About 2/3 of these isolates were typed as 15ADON and 1/3 as 3ADON. Twelve isolates of each trichothecene type were then examined for aggressiveness and mycotoxin potential on the susceptible wheat cultivar Norm in the greenhouse. While all spikelets inoculated with the 15ADON types of the Northland population contained as excepted DON>15ADON>3ADON, spikelets inoculated with eleven of the twelve 3ADON isolates of the Northland population did not contain detectable levels of any of the common trichothecenes. These 3ADON type isolates all had different PCR-RFLP genotypes, and were therefore not clonally related; they were also geographically widespread. These observations lead us to hypothesize that this phenotype is heritable and that there may not be a selective disadvantage against this phenotype. After point-inoculation into a central spikelet, these isolates also spread in the spike and symptoms caused by these isolates could visually not be distinguished from isolates that produce the common trichothecene toxins. Inoculations with only one of the 3ADON Northland isolates yielded plant material with the expected trichothecenes DON>3ADON>15ADON. We sequenced most of the TRI cluster (*TRI*8-*TRI*13) from one of the 3ADON Northland isolate that did not produce the common trichothecene toxins and compared that sequence to the previously published sequence (*TRI*3-*TRI*12) for NRRL 28336, a 3ADON isolate from Ohio. There were less than 70 SNPs over the 18.5kb of sequence and the very few indels were all in intergenic regions or introns. As expected, *TRI*7 was missing and *TRI*13 was a pseudogene. All other genes in the gene cluster had complete ORFs and appear to code for normal

proteins. Therefore, the cause of repression of common trichothecene production may reside outside of the trichothecene gene cluster. Current efforts focus on determining whether any other and potentially unknown toxins are produced by this group of isolates and what molecular mechanisms are responsible to generate this unusual phenotype.

DON BIOSYNTHESIS IN WHEAT

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ABSTRACT

Deoxynivalenol (DON) is a potent mycotoxin and a virulence factor and thus causes great concern in wheat and barley cultivation. Treatment of wheat with fungicides offers some relief from scab disease, although use of strobilurins has been reported to increase DON accumulation in wheat. The DON biosynthetic pathway is well characterized, with the core cluster gene, *Tri5,* catalyzing the formation of trichodiene, the first pathway-specific intermediate. Few studies have focused on the expression of *Tri5* during infection and colonization of wheat heads, despite its importance in DON accumulation. Using quantitative RT-PCR, we examined the expression of *Tri5* during wheat head infection and colonization of susceptible and resistant cultivars and susceptible cultivars treated with strobilurin fungicides. DON was then also quantified to correlate expression with toxin accumulation. The highest *Tri5* expression relative to housekeeping genes was observed in asymptomatic kernels at the infection front and immediately behind the infection front. As infection progressed, kernels closest to the inoculation point showed diminished *Tri5* expression relative to housekeeping gene expression, but *Tri5* expression never ceased during the 21 days observed. Relative *Tri5* expression in Quadris-treated Wheaten did not differ significantly from that in untreated Wheaten. In resistant cultivar Alsen, there was a reduced presence of the fungus and of *Tri5* expression. Interestingly, for the final day of the time-course, 21 dpi, no fungus was detected in Alsen. Importantly, *Tri5* continues to be transcribed even in fully senesced kernels 21 dpi. In addition, strobilurin treatment did not increase *tri5* expression or alter DON accumulation in treated plants in comparison to untreated plants.

TRACKING RELEASED CLONES OF *GIBBERELLA ZEAE* WITHIN WHEAT AND BARLEY FIELDS

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ABSTRACT

Previous research has made significant progress in defining the spatial dissemination of inoculum sources of *G. zeae* within agricultural fields, but it has been unable to unambiguously distinguish between within-field and background sources. We used a technique known as amplified fragment length polymorphisms (AFLPs) to track released clones of *G. zeae* within commercial wheat fields. This strategy allowed us to determine the contribution of released clones to FHB, compared to that of background inocula. Corn stalk pieces infested with clones of *G. zeae* were released in winter wheat fields in New York and Virginia in 2007 and 2008. Recovery of released clones decreased an average of 90% between 3 and 6 m from area sources of inoculum. In 2008-2010, varying amounts of corn stalk pieces (45, 200, or 400 g) infested with a single clone of *G. zeae* were released in winter wheat and barley fields in Virginia. The influence of the infested corn residues was dependent upon the environmental conditions within the growing season. Our work contributes to an increased understanding of the influence of overwintered corn residues to FHB and DON. Understanding the contribution of *Fusarium*-infested corn residues will enable future research to reduce the inoculum potential from within-field sources.

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UNDERSTANDING THE MOLECULAR MECHANISMS OF *FUSARIUM OXYSPORUM*-MEDIATED DEGRADATION OF WHEAT STRAW

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ABSTRACT

Fusarium oxysporum is a facultative pathogen that degrades lignin and complex carbohydrates in plants and plant debris, thus facilitating its pathogenicity and persistence in the environment. We found that although two genetically distinct strains of this fungus were equally efficient in colonizing wheat straw (glucosamine content), they differed with respect to their ability to degrade this substrate. The ability to release phenolics during colonization was 2 times higher in strain 11C, as compared to strain 7E. The specific activity of the cellulases [endoglucanase (EC 3.2.1.4), exoglucanase (EC 3.2.1.91), β-glucosidase (EC 3.2.1.21)] secreted by strain 11C was significantly higher than those of 7E. However, there was no difference in the specific activity of hemicellulases [endoxylanase (EC 3.2.1.8) and β-xylosidase (EC 3.2.1.37)] secreted by the two strains. **Suppression subtractive hybridization (SSH**) analysis of gene expression during wheat straw colonization identified several genes up-regulated in strain 11C, as compared to 7E, including those involved in gene transposition, saccharification of cellulose and hemicellulose, cellular transport of sugars and pentose catabolism. These results give insights into how plant pathogens have evolved and can be exploited and manipulated for the purposes of lignocellulose degradation.

THE *HDF1* HISTONE DEACETYLASE GENE IS IMPORTANT FOR CONIDIATION, SEXUAL REPRODUCTION, AND PATHOGENESIS IN *FUSARIUM GRAMINEARUM*

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ABSTRACT

Head blight caused by *Fusarium graminearum* is an important disease of wheat and barley. Its genome contains chromosomal regions with higher genetic variation and enriched for genes expressed *in planta*, suggesting a role of chromatin modification in the regulation of infection-related genes. In a previous study, the *FTL1* gene was characterized as a novel virulence factor in the head blight fungus. *FTL1* is homologous to yeast *SIF2*, which is a component of the Set3 complex. Many members of the yeast Set3 complex, including Hos2 histone deacetylase (HDAC), are conserved in *F. graminearum*. In this study, we characterized the *HDF1* gene that is orthologous to *HOS2*. *HDF1* physically interacted with *FTL1* in yeast two-hybrid assays. Deletion of *HDF1* resulted in a significant reduction in virulence and DON production. The Δ*hdf1* mutant failed to spread from the inoculation site to other parts of wheat heads or corn stalks. It was defective in sexual reproduction and significantly reduced in conidiation. Expression of *HDF1* was highest in conidia in comparison with germlings and hyphae. Deletion of *HDF1* also resulted in a 60% reduction in HDAC activity. Microarray analysis revealed that 149 and 253 genes were down- and up-regulated, respectively, over 5-fold in the Δ*hdf1* mutant. Consistent with up-regulation of putative catalase and peroxidase genes, the Δ*hdf1* mutant was more tolerant to H_2O_2 than the wild type. Deletion of the other two class II HDAC genes had no obvious effect on vegetative growth and resulted in only a minor reduction in conidiation and virulence in the Δ*hdf2* mutant. Overall, our results indicate that *HDF1* is the major class II HDAC gene in *F. graminearum*. It may interact with *FTL1* and function as a component in a well conserved HDAC complex in the regulation of conidiation, DON production, and pathogenesis.

FUSARIUM TRI8 DETERMINES 3-ACETYLDEOXYNIVALENOL (3ADON) OR 15ADON PRODUCTION

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ABSTRACT

Trichothecene mycotoxins produced by *Fusarium* species can promote disease in small grain crops such as wheat and barley. Two main trichothecene production phenotypes (chemotypes) have been identified among strains of *Fusarium graminearum* and closely related species: strains produce either deoxynivalenol (DON) or nivalenol (NIV) trichothecenes. The DON phenotype can be further subdivided into the 3-acetyldeoxynivalenol (3ADON) chemotype and the 15-acetyldeoxynivalenol (15ADON) chemotype. However, grain infected by strains with either the 3ADON or 15ADON chemotype is typically contaminated with DON rather than the acetylated derivatives.

DON and NIV are identical in structure except for the presence (NIV) and absence (DON) of a hydroxyl function at carbon atom 4 (C-4) of the trichothecene molecule. The basis for DON and NIV chemotypes resides in the trichothecene C-4 hydroxylase gene *TRI13*. In DON-producing strains of *F. graminearum*, *TRI13* is nonfunctional because of multiple insertions and deletions within its protein coding region. As a result DON-producing strains are unable to hydroxylate trichothecenes at C-4. In contrast, NIV-producing strains have a functional *TRI13* and, therefore, can hydroxylate trichothecenes at C-4. For greater efficiency in chemotype classification, differences in *TRI13*, as well as the C-4 acetyl transferase gene (TRI7), sequences have been used to develop PCR markers to predict DON and NIV chemotypes.

During the last several years, PCR markers for *TRI3* and *TRI12* have been used to predict 3ADON and 15ADON chemotypes in *Fusarium graminearum*. In order to determine the genetic basis for these chemotypes, we examined differences in the sequences and functions of *TRI3* and *TRI8*, two trichothecene biosynthetic genes that have been proposed to play a role in production of 3ADON versus 15ADON in *Fusarium*. *TRI3* was functional in both 3ADON and 15ADON strains and had the same function, namely trichothecene C-15 acetyltransferase, in strains with either chemotype. *TRI8* was also functional in strains with both chemotypes; however, its function differed in the two types of strains. In 15ADON-producing strains, the TRI8 enzyme is a trichothecene C-3 esterase; it catalyzes removal of an acetyl group from the C-3 position. In contrast, in 3ADON strains, the *TRI8* enzyme homolog is a C-15 esterase; it catalyzes removal of an acetyl group from the C-15 position. These results indicate that *TRI8*, but not *TRI3*, determines whether Fusarium produces 3ADON or 15ADON. Furthermore, expression studies with *TRI8* chimeras containing a portion of *TRI8* from a 3ADON strain and a portion from a 15ADON strain indicated that sequence differences in the middle of the coding region are responsible for determining the 3ADON versus 15ADON chemotype. These results should contribute to understanding the role of the 3ADON and 15ADON chemotypes in the ecology of *Fusarium* species that cause wheat head blight.

FUSARIUM SPP. ASSOCIATED WITH HEAD BLIGHT IN SOUTH AFRICAN WHEAT PRODUCTION AREAS

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ABSTRACT

Fusarium head blight (FHB) of wheat is a complex disease caused by a number of *Fusarium* species. Several of these *Fusarium* species produce mycotoxins, mainly trichothecenes, which are associated with human and animal mycotoxicoses. In South Africa, FHB is primarily associated with wheat planted under irrigation and in fields where wheat is rotated with crops like barley, maize, soybean and sunflower. The aim of this study was to determine the distribution of *Fusarium* spp. associated with FHB in all wheat production areas of South Africa during the 2008 and 2009 growing seasons. Wheat heads showing FHB symptoms were collected from four cultivars in fields under irrigation in the Northern Cape, KwaZulu-Natal (KZN), the Bushveld and the Free State, and under dry land conditions in the Western Cape. Twenty diseased heads from each cultivar were collected per location, and kernels from each head were plated on potato dextrose agar and selective *Fusarium* media. Single-spore isolates representing each *Fusarium* colony were identified using morphological and molecular techniques. *Fusarium graminearum* species complex was most commonly associated with FHB of wheat in South Africa, and was found in 75.6% of the kernels analysed in 2008, and in 86.2% in 2009. Other known *Fusarium* spp., such as *F. avenaceum*, *F. cerealis*, *F. culmorum*, *F. equiseti*, *F. lunulosporum*, *F. oxysporum*, *F. poae*, *F. pseudograminearum*, *F. sambucinum*, *F. scirpi*, *F. semitectum*, *F. solani* and *F. subglutinans*, were also isolated. At one location in the Free State, and also at the location in the Western Cape, the dominating species was *F. pseudograminearum*, occurring at higher frequencies than *F. graminearum* species complex. Chemotyping of the toxin-producing *Fusarium* isolates revealed that the 15-acetyl deoxynivalenol (15-ADON) chemotype was the most common in both years at most locations. However, in the Western Cape, and at one location in the Free State, the most common chemotype was 3-acetyl deoxynivalenol (3-ADON). The nivalenol (NIV) chemotype was most prevalent at one site in KZN (62.9%) in 2009. Information on the distribution of *Fusarium* species in South Africa, as well as the toxins they produce may be helpful for the future development of resistant wheat cultivars and sustainable disease management practices.

SYSTEMATIC CHARACTERIZATION OF THE KINOME OF THE WHEAT SCAB FUNGUS *FUSARIUM GRAMINEARUM*

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ABSTRACT

Wheat scab caused by *Fusarium graminearum* is one of the most important diseases of wheat. Beside yield losses, infested wheat kernels are often contaminated with mycotoxins. Like in many other eukaryotes, protein kinases play major regulatory roles in filamentous fungi. In *F. graminearum*, there are 126 predicted protein kinases that belong to different protein kinase groups and families. Like other fungi, *F. graminearum* lacks tyrosine kinase and kinases belonging to the **RGC and TKL group**. To determine their functions, we have undertaken a systematic approach to generate gene replacement mutants. To date, we have isolated mutants for over 100 kinase genes. For six of them, their orthologs are essential genes in the budding yeast. Sixteen of these predicted protein kinase genes appeared to be essential in *F. graminearum*. All the mutants have been assayed for their defects in wheat head infection, DON production, conidiogenesis, sexual reproduction, responses to various stresses, conidium germination, and hyphal growth. Twenty one of them were significantly reduced in virulence or non-pathogenic. Four of them did not produce DON in colonized wheat kernels. One of them produced predominantly single-celled conidia in CMC cultures. We also identified several mutants that germinated faster than the wild type or were more resistant to hyperosmotic or other stresses. Further characterization of these mutants is in progress. A database has been developed to include gene annotation and all the phenotypes of the *F. graminearum* kinase genes. In addition, the interaction among these protein kinases and their association with other *F. graminearum* proteins were predicted based on their yeast orthologs using the interlog approach. For a few predicted pathways or networks that are important for plant infection, affinity purification and co-immunoprecipitation assays will be used to determine their interactions *in vivo*. Overall, this is the first systematic characterization of protein kinase genes in filamentous fungi. We have identified protein kinase genes that regulate conidiogenesis, conidium germination, responses to hyperosmotic and ROS stresses, pathogenesis, sexual reproduction, hyphal growth, and mycotoxins production.

PREINOCULATION OF WHEAT HEADS WITH A NONTOXIGENIC *FUSARIUM* ISOLATE INHIBITS DEOXYNIVALENOL PRODUCTION BY A TOXIGENIC PATHOGEN

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OBJECTIVE

To evaluate the potential of using a naturally-occurring, nontoxigenic isolates of *Fusarium graminearuum* to reduce Fusarium head blight and deoxynivalenol accumulation.

INTRODUCTION

Despite the effectiveness of new scab resistant wheat cultivars and new fungicides in reducing the severity of Fusarium head blight (FHB) and reducing deoxynivalenol (DON) levels in grain, economic loss from DON accumulation can still occur under disease favorable conditions. Biocontrol agents are being investigated as a strategy to augment host resistance and fungicides, but they have not been consistently effective in the field. This may be related to the agents being very dissimilar ecologically to the pathogen *Fusarium graminearum* (*Fg*). A solution to finding more effective biological control agents against FHB may be to use agents that share the same physical niches and environment conditions as *Fg*. Many examples can be found in the biological control literature of related incompatible pathogens (i.e. pathogens of another host) or hypovirulent pathogen isolates being used to control a virulent pathogen (Cook and Baker, 1983). This strategy is being applied commercially in the management of aflatoxin in peanut and corn using aflatoxin-nonproducing isolates of *Aspergillus flavus* (EPA, 2010). Disease control using hypovirulent pathogen isolates is attributed to niche competition and induction of host resistance (Whipps, 2001). Infection by *Fg* activates plant resistance responses (Pritsch et al., 2000). Therefore, the ideal biological agent for FHB control and DON reduction would be a strain of *Fg* that is non-toxigenic (Tox-) and hypovirulent.

A naturally-occurring, presumably Tox- isolate of *Fg* (WG-9) was isolated from a wild grass from a remote non-agricultural area in northern Minnesota by L. R. Gale and associates. Although DNA isolated from WG-9 amplified well for ten highly polymorphic PCR-RFLP primer pairs developed specifically for *F. graminearum sensu stricto* (Gale et al., 2010), the resulting genotype was not typical of any described U.S. population of *Fg*. Additional isolates that group with WG-9 have since been detected and found to represent a new population of *F. graminearum*, the Northland population (see abstract in this volume by Gale et al. "A Subset of the Newly Discovered Northland Population of *Fusarium graminearum* from the U.S. Does Not Produce the B-Type Trichothecenes DON, 15ADON, 3ADON or NIV"). In greenhouse experiments, WG-9 did not produce any detectable amounts of DON or other derivatives (3ADON, 15ADON, NIV) in inoculated spikelets. Furthermore spread of WG-9 on inoculated wheat heads from point inoculations at individual florets ranged from low to moderate compared to the standard virulent isolate PH-1. In this study, WG-9 was used to test the concept that preapplication of a Tox- hypovirulent strain to wheat heads can inhibit floret infection by a toxigenic (Tox+) virulent pathogen resulting in reduced DON accumulation in the grain.

MATERIALS AND METHODS

Two sets of experiments were conducted, the first involving preinoculation of Tox- isolate WG-9 onto the flowering heads followed by a challenge inocula-

tion with Tox+ isolate PH-1 one day later. Inoculations were made by spraying conidial suspensions containing 10^4 spores/ml. Water was applied as the preinoculation and challenge inoculation controls. In the second set of experiments, the Tox- and Tox+ isolates were co-inoculated by injecting 10 µl volumes of spore suspension into the center florets in each wheat head. The treatments tested included equal volume mixtures of Tox- and Tox+ spore suspensions (10^4 spores/ml), Tox- spore suspension diluted with water, Tox+ spore suspension diluted with water, and a water only control. Both sets of experiments were conducted on susceptible hard red spring wheat cultivars Wheaton and Bobwhite grown in 20 cm pots (6 plants per pot) in a greenhouse, with 6 replicate pots per treatment. Inoculated plants were kept in a humid growth chamber for 2 days and then moved to a greenhouse for symptom development. After 1 week, inoculated heads were rated for numbers of spikelets exhibiting scab lesions. Seed was harvested at maturity for determination of Fusarium diseased kernels (FDK). Diseased and asymptomatic seed were assayed separately for DON concentration by the University of Minnesota Diagnostic Lab. All data was analyzed with SAS ProcMixed, with the LSD test used to separate means. In addition, the proportion of kernels infected by WG-9, PH-1, or both isolates were determined in the first set of experiments. Infected seeds were cultured separately on half-strength PDA in 24-well plates for 2 days, and then the seed and mycelium extracted for DNA. The extracts were used as templates in multiplex PCR amplification using 3ADON and 15ADON chemotype-specific primers based on *TRI3* and *TRI12* gene sequences (Starkey et al. 2007). WG-9 and PH-1 were distinguished on the basis of amplicons produced in the *TRI3* and *TRI12* multiplexes, with WG-9 producing 243 and 410 bp amplicons and PH-1 yielding 610 and 670 bp amplicons, respectively (Figure 1).

RESULTS AND DISCUSSION

We found that sequential spray inoculation of wheat heads with Tox- isolate WG-9 followed by Tox+ isolate PH-1 or point inoculation with the two isolates together significantly reduced DON concentrations in the mature seed compared to inoculation with the Tox+ isolate alone (Tables 1 and 2). The reductions in DON concentrations were measured in asymptomatic and scabby wheat kernels. In the first set of experiments, preinoculation with WG-9 prior to challenge inoculation with PH-1 suppressed kernel infection by PH-1, as revealed by PCR analysis of fungi in individual seeds. Among seeds from heads inoculated with both isolates, more than of 50% were infected with WG-9 while only 5% were infected with PH-1 (Table1). Similar proportions of WG-9 and PH-1 infections were found in the WG-9/PH-1 treatment in other experiments in which nearly 100% of seeds from heads inoculated with one isolate alone were infected by the respective isolate (data not shown). However, measurements of scab severity and proportions of FDK in experiment set 1 revealed that preinoculation with WG-9 prior to inoculation with PH-1 was ineffective in suppressing total symptom development compared to inoculation with PH-1 or WG-9 alone (Table 1). It appeared that, although WG-9 is assumed to be Tox-, it is virulent in respects to infection of individual florets, supporting previous research on other DON nonproducing *Fg* (Bai et al., 2002). In contrast, the ability of WG-9 to spread through wheat heads from point inoculation was significantly reduced compared to PH-1 (Table 2), which is consistent with DON production being important in the spread of *Fg* through the rachis. But point inoculation with the two isolates together had no effect on subsequent symptom severity compared to inoculation with PH-1 alone. We speculate that DON produced by PH-1 in association with WG-9 might have allowed both fungi to spread through the rachis, but eventually, fewer florets were infected by PH-1 via the rachis. This would be consistent with lower DON concentrations being detected in the seed following coinoculation.

The results from this study support the hypothesis that a Tox- isolate of *Fg* could be used as a biological control agent to compete with or exclude Tox+ pathogens strains and, thus, reduce DON levels in the harvested grain. The high disease severity and expected yield loss caused by WG-9 alone is an obvious drawback to using WG-9 as a biocontrol agent. But we surmise this problem could possibly

be overcome by using Tox- strains of *Fg* with lower virulence than WG-9, by applying Tox- isolates at lower spore concentrations or at different time intervals relative to flowering, or by integrating applications of Tox- strains with scab resistant cultivars. Field experimentation also is essential to confirm the benefits of pretreatment with Tox- strains. Other alternatives might be identified when the mechanisms by which WG-9 and other Tox- isolates exert their suppressive effects become known.

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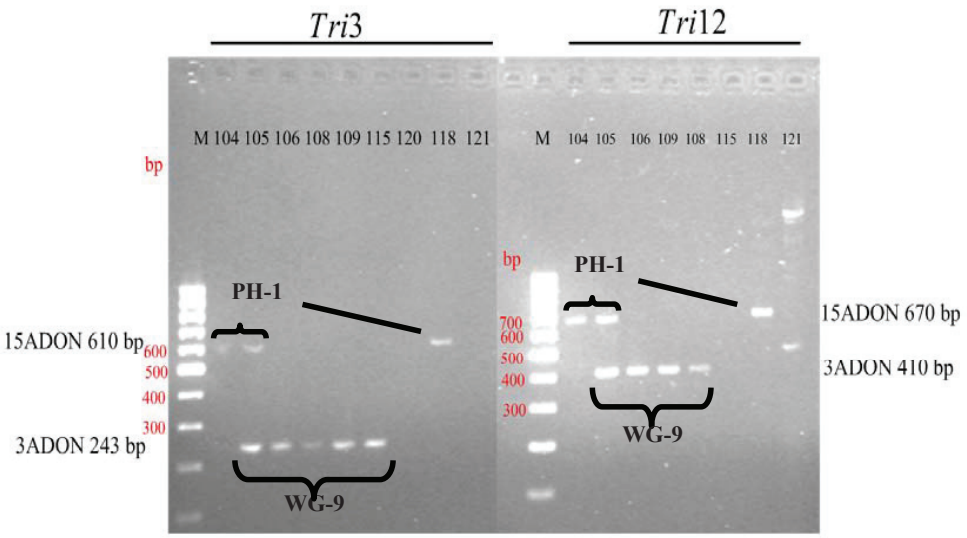

Figure 1. Representative electrophoresis results from *TRI3* (left) and *TRI12* (right) multiplex PCR of DNA extracted from plated seed and mycelia. *Fg* PH-1 (TOX+) and *Fg* WG-9 (TOX-) are indicated by amplicons of different size in each multiplex.

Table 1. Effects of preinoculation of wheat heads with Tox- WG-9 and challenge inoculation with Tox+ PH-1 on scab severity and DON.

Cultivar	Pretreatment/ challenge	% infected spikelets 1 week after challenge	Proportion of seed with: WG-9	PH-1	% FDK	DON in diseased kernels (ppm)	DON in asymptomatic kernels (ppm)
'Bobwhite'	WG-9/PH-1	70.7 A[†]	12/19	1/19	79.3 AB	24.9 B	0.8 B
	Water/PH-1	34.4 B	-*	-	65.6 B	176.0 A	3.1 A
	WG-9/water	78.0 A	-	-	93.2 A	<0.5[#]	<0.5
	Water/water	3.4 C	-	-	4.6 C	-	-
'Wheaton'	WG-9/PH-1	64.8 A	11/20	1/20	91.7 A	12.1 B	2.8 B
	Water/PH-1	12.0 B	-	-	86.4 A	97.9 A	16.5 A
	WG-9/water	78.5 A	-	-	96.5 A	1.7 C	<0.5
	Water/water	8.0 B	-	-	7.5 B	-	-

[†] Letters denote significant differences at P=0.05 based on LSD test.
* Not determined.
[#] Values below detection level of 0.5 ppm were not used in statistical analysis.

Table 2. Results of point inoculations with Tox- WG-9 and Tox+ PH-1.

Cultivar	Inoculum	Percent infected spikelets 1 week after inoculation	% FDK	DON in diseased kernels (ppm)	DON in asymptomatic kernels (ppm)
'Bobwhite'	PH-1 only	38.4 A[†]	32.9 A	202.4 A	2.5 A
	WG9 + PH-1	53.9 A	46.7 A	119.8 A	0.5 B
	WG9 only	4.6 B	4.5 B	<0.5#	<0.5#
	Water	0.4 B	0.3 B	-*	-
'Wheaton'	PH-1 only	68.0 A	75.1 A	48.4 A	1.0 A
	WG9 + PH-1	56.7 A	81.1 A	18.7 B	0.3 B
	WG9 only	23.3 B	46.5 B	<0.5#	<0.5#
	Water	2.8 C	9.4 C	-	-

[†] Letters denote significant differences at P=0.05 based on LSD test.
[#] Values below detection level of 0.5 ppm were not used in statistical analysis.
* Not determined.