SESSION 2:

PATHOGEN BIOLOGY AND GENETICS

DETERMINATION OF *FUSARIUM GRAMINEARUM* CHEMOTYPES PREVALENT ON OAT, RYE HEADS, AND WHEAT ROOTS IN SOUTH DAKOTA

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INTRODUCTION

Fusarium graminearum Schwab (telomorph: *Gibberrella zeae* (Schwein.)) is the primary pathogen responsible for causing Fusarium head blight (FHB) or scab on barley, oat, rye, and wheat in the USA. In addition to FHB, the fungus has also been reported to be responsible for crown rot on wheat. The disease limits small grain production by impacting yield due to poor grain filling and reduced seed test weight. The fungus contaminates grains with mycotoxins (trichothecenes) during and after the infection process and further reduces monetary value. *Fusarium graminearum* mainly produce two types of trichothecenes; Deoxynivalenol (DON) and Nivalenol (NIV). DON producing isolates were further grouped into 3ADON and 15ADON based on where they were acetylated (Miller et al. 1991). Further, it was reported that 15ADON and 3ADON populations were most prevalent in the USA and Asia, respectively. Mycotoxins contaminated grains are of health risk to both humans and animals. The FDA has set 1ppm and 5-10 ppm DON as the maximum contamination limit in the food products and feed products suitable for human and animal consumption, respectively.

Knowledge of pathogenic variation in virulence and/or aggressiveness is important to the success of any program for disease resistance breeding and in the development of disease management strategies. Variation in the pathogen virulence and/or aggressiveness can be affected due to the broad host range, continuous selection pressure of host resistance, intensive use of fungicides with similar chemistry, and adverse environmental conditions. In recent years, a change in the *F. graminearum* population from 15ADON to 3ADON has been observed in the USA, especially in the northern Great Plains (Burlakoti et al. 2008; Gale, 2007), and in Canada (Ward et al. 2008). It has also been observed in some independent studies that the 3ADON population is more aggressive in disease development and mycotoxin production compared to the 15ADON population (Puri and Zhang, 2010; Ward et al. 2008; Ali et al. 2009). In South Dakota, wheat is the major small grain crop planted on around two millions acres in 2012. In addition to wheat, oat and rye are also grown in the wheat production area. All three crops are prone to FHB and could harbor diverse *F. graminearum* population in the state. In this study, we attempted to recover fungal isolates from FHB infected oat and rye heads, and wheat roots for chemotype characterization.

OBJECTIVES

1. Analyze *F. graminearum* isolates for their chemotypes recovered from oat, rye, and wheat roots in South Dakota

2. Determine if oat, rye, and wheat favors any one of the two 3ADON or 15ADON fungal population

MATERIALS AND METHODS

FHB Infected Wheat, Oat and Rye Samples and Recovery of Fusarium graminearum Isolates. One hundred and seventy-nine isolates were recovered from FHB infected heads of oat (n=37), rye (n=69)

and wheat (n=73). Oat and wheat root samples were collected from 11 and 21 commercial fields across the state in the 2012 and 2013 growing seasons, respectively. All 69 isolates from rye were recovered from 69 individual infected spikes collected from a single 4-acre field located at the SDSU Southeast Research Station near Beresford, South Dakota. To collect FHB infected oat head samples, twenty heads were randomly clipped from each field plot. Whereas, rye diseased spikes were collected from the plants located about 200 feet apart to increase the chances of capturing more diversity in the pathogen population. To recover *F. graminearum* isolates from wheat roots, 25-30 plants were randomly uprooted from each field plot. The roots were thoroughly washed under running tap water. Roots with crown portion were cut into small pieces 1- 2 cm long and then rinsed, and then surface disinfested with 5% bleach prior to fungal isolation. To obtain the fungal isolates from oat and rye, scabby grains were separated from the individual head of each sample and kept separate until plated. Ten scabby grains (tombstones) recovered from each head were plated on ½ PDA medium in 15 x 100 mm plastic petri dishes. Five scabby grains were placed on each plate. The plates were incubated under 12 hours of light light and 12 hours of dark for four days. The fungal colonies grown out of the plated grains were transferred individually onto new ½ PDA plates and grown for seven days. Similar methods were used to recover fungal isolates from wheat roots except roots segments were plated instead of scabby grains. The isolates identity was confirmed based on colony growth and spore morphology described by Leslie and Summerell (2006). In total, 351 isolates were recovered from the plated samples and stored in 15% glycerol at -80°C in the freezer.

DNA Extraction and PCR Assay. DNA of all 169 *F. graminearum* isolates was recovered by growing isolates individually for 2-3 days on cellophane membrane placed on ½ PDA. The fungal mycelia of the isolates were collected by scrapping the cellophane membrane surface using a flamed spatula. DNA was isolated from the collected mycelia by following the protocol developed by Liu et al. (2000). The isolates chemotypes were determined by using the tricothecenes specific 3CON, 3NA, 3D15A, and 3D3A primers (Starkey et al., 2007, Ward et al., 2002). PCR reaction was conducted in a S-1000 thermal cycler (BioRad, USA) using amplification steps of 94°C for 2 min, followed by 32 cycles of 94°C for 30s, 52°C for 30s and 72°C for 1 min with final extension of 72°C for 5 min. The PCR products were electrophoresed on 1.5% agrose gels and scored with reference to 100bp DNA ladder (New England Biolabs, USA). The PCR amplification produced bands of 243bp and 610bp corresponding to the 3ADON and 15ADON chemotypes, respectively (Figure 1).

RESULTS AND DISCUSSION

All plated scabby grains of oat and rye produced *F. graminearum*. In addition to *F. graminearum*, some of the plated grains also developed *F. sporotrichioides*, *F. acuminatum*, and *F. equiseti*. High-level recovery of *F. graminearun* from the plated samples indicates that this is still the major pathogen for FHB development on small grains in the region. The majority of the isolates from wheat roots (89%) and oat heads (91%) were grouped as 15ADON; whereas 3ADON isolates were recovered from both wheat (11%) and oat (9%) in low numbers (Table 1). All isolates recovered from rye produced 15ADON. Results indicate that the fungal population prevalent on wheat roots and oat harbor both chemotypes but 15ADON population is still the most prevalent chemotype within the sample collection area. The fungal population with 15ADON chemotype from rye could be expected because the isolates were collected from a single field plot. Recovery of 15ADON and 3ADON isolates from wheat roots samples could be expected as both chemotypes were recovered from wheat head samples of FHB resistant and susceptible cultivars in South Dakota (Ali et al. 2012). The results of this preliminary study also indicate lack of any specific trend of the fungal chemotypes prevalence on oat, rye and wheat. To obtain a complete inventory of *F. graminearum* population chemotypes and their trend of survival in the state, more isolates from all three hosts

collected from multiple locations in multiple years are under investigation. Occurrence of 3ADON population, low in intensity but more aggressive than 15ADON population in DON production and FHB development, warrant to use this in screening small grains germplasm for resistance to FHB in South Dakota.

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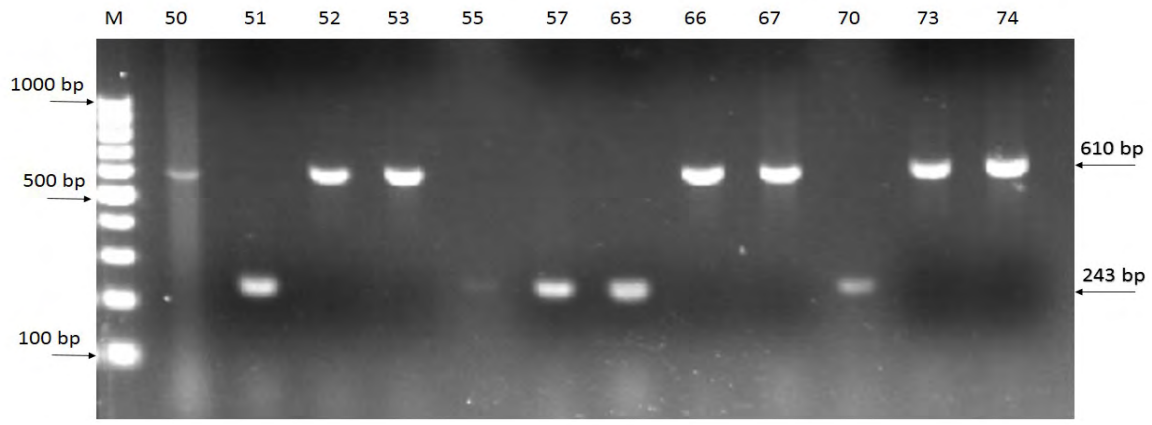

Figure 1. PCR amplification method (Ward et al. 2008). The bands at 610bp and 243bp amplified from the *F. graminearum* isolates (50 to 74) correspond to the 15ADON and 3ADON chemotypes, respectively. M represents the 100bp DNA marker.

Table 1. *Fusarium graminearum* chemotypes recovered from oat, rye and wheat in South Dakota.

Host plant	Isolates chemotyped	3ADON	15ADON
Oat[a]	37	3	34
Rye[a]	69	0	69
Wheat[b]	73	8	65

a = isolates were recovered from FHB infected heads; b = Isolates were recovered from roots

BIOPROSPECTING FOR DON DEGRADING ENZYMES AND MICROORGANISMS

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ABTRACT

New strategies are needed to mitigate deoxynivalenol (DON) contamination in wheat and barley. Here, we present preliminary data from a series of experiments to discover and employ unique DON degrading enzymes and microorganisms. Enzymes for DON transformation were identified using a systematic algorithm to identify possible enzyme-catalyzed reactions based on the functional groups present in DON and confirmed by molecular docking studies of DON-enzyme interactions. Nine candidate genes (five epoxide hydrolases and five cycloisomerases) were selected and cloned. These sequences will serve as templates for further engineering of enzymes to alter substrate specificity and enhance catalytic efficiency by combinatorial cloning. Environmental samples collected in Virginia in 2013 completely eliminated 100 ppm DON following growth in a minimal medium containing DON as a sole carbon source. Microorganisms from these environmental samples are in the process of being isolated and characterized. This research will offer new strategies for detoxifying DON in wheat and barley products.

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QUANTITATIVE DETECTION OF TOXIGENIC *FUSARIUM* SPECIES AND TRICHOTHECENE GENOTYPES IN WHEAT FROM WESTERN CANADA IN 2011–12

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ABSTRACT

In 2011 and 2012, a total of 198 producer samples of Canada western red spring (CWRS), Canada western red winter (CWRW), Canada western soft white spring (CWSWS) and Canada western amber durum (CWAD) wheat from across the Canadian Prairies were analyzed for various *Fusarium* species and contamination by trichothecenes. Detection and quantification of toxigenic species, namely *Fusarium avenaceum*, *F. graminearum*, *F. poae* and *F. sporotrichioides*, was performed on DNA extracts from several grams of ground grain using validated real-time PCR assays and species-specific TaqMan probes. For the determination and quantification of deoxynivalenol (DON) genotypes, a qPCR assay was developed based on TRI8 (C-3 / C-15 esterase) of the *Fusarium* trichothecene gene cluster. The multiplex TaqMan assay allowed us to quantify 15-acetyldeoxynivalenol (15ADON) and 3-acetyldeoxynivalenol (3ADON) genotypes simultaneously. Validation of the assay was based on a number of *Fusarium* species including producers of trichothecenes type A and/or B.

The predominant species on wheat in 2011 and 2012 was *Fusarium graminearum*. Only in some areas of Alberta and south-western Saskatchewan, *F. avenaceum* was more frequently detected. *Fusarium poae* was detected in a number of wheat samples at trace level, but found in higher quantities only on CWAD and CWSWS from eastern Saskatchewan. Occasionally, *F. sporotrichioides* was detected on CWRS samples from south-western Manitoba and south-eastern Saskatchewan. DNA concentration of *F. graminearum* was highest on CWRW (2011) and on CWSWS (2012) grown in Manitoba and south-western Saskatchewan. In most of the wheat samples, DNA concentration of *F. graminearum* correlated with the percentage (by weight) of *Fusarium* damaged kernels (FDK) and concentration of deoxynivalenol (DON). In 2011, the frequency of the 3ADON genotype on CWRS and CWRW was above 50% in samples grown in Manitoba and eastern Saskatchewan. Only on CWAD grown in southern Alberta and Saskatchewan, the 15ADON genotype was more dominant. The 15ADON genotype was also predominant in CWSWS from Alberta. CWSWS samples from the other provinces showed mean frequencies of the 3ADON genotype ranging from 32% to 89% in 2012. There was no correlation between the concentration of DON and the predominant chemotype, as measured by DNA quantities of the two trichothecene genotypes, in naturally infected wheat samples.

FREQUENCIES OF 3-ADON AND 15-ADON *FUSARIUM GRAMINEARUM* FROM CORN STUBBLE, ATMOSPHERE, AND WHEAT HEADS IN THREE AGRICULTURAL REGIONS IN NEW YORK IN 2013

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ABSTRACT

In North America, Fusarium head blight (FHB) in cereals is caused predominantly by *Fusarium graminearum* of the 15-acetyl(A)deoxynivalenol(DON) trichothecene genotype. However, recent population shifts have been documented in some northern areas in the United States and in Canada where 15-ADON isolates are being displaced by 3-ADON isolates, apparently driven by adaptive fitness of the latter. Previous surveys of symptomatic wheat heads revealed a predominance of 15-ADON isolates in the principal wheat producing areas of central and western New York. Still lacking is any information on trichothecene genotypes obtained from saprophytic, airborne, and pathogenic phases of the life cycle of *F. graminearum*. We conducted intensive sampling of isolates from a) overwintered corn stubble, b) lower atmosphere, and c) symptomatic wheat heads in three diverse agricultural locations in 2013. These locations were: 1) Aurora, Cayuga County, central NY, Central Plain – mostly flat with widespread corn and wheat production; 2) Belmont, Allegany County, southwestern NY, Allegany Plateau – hilly, forested region with crops grown in valleys; and 3) Willsboro, Essex County, northeastern NY, Champlain Valley – broad valley with scattered farms between Adirondack Mountains and Lake Champlain. Approximately 100 *F. graminearum* monosporic isolates each from corn stubble, lower atmosphere, and wheat heads were collected within a 10 square mile area in each geographic location. Polymerase chain reaction (PCR) assays were used to identify B-trichothecene genotypes, 3-ADON, 15-ADON, or nivalenol (NIV), based on amplification of portions of *Tri3* and *Tri12* genes. Of a total of 882 isolates analyzed statewide, 23% were of the 3-ADON genotype and 77% were of the 15-ADON genotype. No NIV isolates were found. No significant differences were found in the trichothecene genotype frequency of *F. graminearum* among the three collection niches in any location (Belmont, χ^2=3.236, P=0.198; Aurora, χ^2=0.145, P=0.930; Willsboro, χ^2=3.662, P=0.160). Overall frequency of the 3-ADON isolates differed (χ^2=179; P<0.001) across the locations, being lowest at Aurora (6%), somewhat higher at Belmont (12%), and highest at Willsboro (50%). At least as viewed by the frequency of trichothecene genotypes, local populations of *F. graminearum* seem to not be structured by the three niches analyzed. The predominance of 15-ADON isolates in Aurora and Belmont is consistent with wider surveys of winter wheat in western New York in 2007 and again in 2011. The equivalence of 3-ADON and 15-ADON isolates in Willsboro also is not surprising as frequencies of 3-ADON exceeding 30% have been observed in other areas of eastern New York and in Vermont. We are currently investigating landscape ecology-related factors that may affect the population structure of the FHB pathogen in New York.

FUNCTIONAL ANALYSIS OF TRANSCRIPTION FACTORS IN THE CEREAL HEAD BLIGHT FUNGUS, *FUSARIUM GRAMINERAM*

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ABSTRACT

Fusarium graminearum is an important plant pathogen that causes head blight of major cereal crops. The fungus produces mycotoxins that are harmful to animal and human. During the last 5 years, we constructed a mutant library of 657 putative transcription factors (TFs) through homologous recombination in *F. graminearum*, providing a resource for understanding gene regulation in the fungus. By screening these mutants in 17 phenotypic categories, we constructed a dataset of over 11,000 phenotypes. This study provides new insight into understanding multiple phenotypes caused by single TFs as well as regulation of gene expression at the transcription level in *F. graminearum*. Furthermore, our TF mutant library will be a valuable resource for fungal studies through the distribution of mutants and easy access to our phenotypic and genetic data.

SAYING GOOD-BYE TO *GIBBERELLA ZEAE*

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ABSTRACT

The names of fungal species are governed by the International Code of Botanical Nomenclature. The Code includes rules for proposing names, describing species, and providing material for type specimens, amongst others. Until the recent International Botanical Congress in Melbourne, it was permissible for fungi to have two names – one for the asexual state and the other for the sexual state. This convention originated because the two different stages were sometimes observed independently and could not necessarily be connected unless one stage could be identified as originating from material of the other. This convention has out-lived its practical usefulness and at the Melbourne Congress the exception to the Botanical Code that allowed dual names for fungi was removed, resulting in "One name, one fungus". In species with an asexual stage in the genus *Fusarium* the alternatives are to retain the name "*Fusarium*" with the inclusion of some related groups that had been excluded, or to no longer use the *Fusarium* name and to split the genus up into a number of pieces in which only the sexual stage names, *e.g.*, *Gibberella*, would be used. A proposal was made in a Letter to the Editor in *Phytopathology* (Geiser *et al.*, 2013) earlier this year that the name "*Fusarium*" be retained and that the names for the sexual stages should no longer be used. Other than *Gibberella zeae*, most sexual stage names for species with asexual stages in the genus *Fusarium* are neither well known nor widely used. Thus, the recommended future usage for the name of the fungus that is the major cause of scab in the United States will be *Fusarium graminearum* rather than *Gibberella zeae*.

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INTERACTIONS OF *FUSARIUM GRAMINEARUM* WITH BARLEY AND WHEAT

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ABSTRACT

As the causal agent of head blight of wheat and barley, *Fusarium graminearum* leads to crop losses by damaging kernels and contaminating grain with the mycotoxin deoxynivalenol. We are studying the interaction of *Fusarium* with wheat and barley during fungal ingress of the surface of barley and progression through the wheat head following infection. We are particularly focused on surface interaction of *F. graminearum* with barley. Histological analysis of barley varieties has been done to identify changes in the process of fungal penetration. In both barley and wheat, we are examining how expression patterns from both fungus and host variety shift during disease progression and resistance response.