SESSION 4:

PATHOGEN BIOLOGY AND GENETICS

Chairperson: Jin-Rong Xu

GIBBERRELLA ZEAE CHEMOTYPE DIVERSITY ON MODERATELY FHB RESISTANT WHEAT GENOTYPES IN SOUTH DAKOTA S. Ali^{*}, M. Eldakak, P. Gautam, K. Glover, J.S. Rohila, J. Gonzalez and W. Berzonsky

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INTRODUCTION

Gibberrella zeae (anamorph: Fusarium graminearum) causes Fusarium head blight (FHB) or scab on small grains worldwide, especially barley and wheat in the USA. The disease impacts wheat production by affecting both yield (poor grain filling and reduced seed test weight) and quality (contaminating grains with mycotoxins). Fungal isolates can be classified primarily into two groups based on the production of 8-Ketotrichothecenes deoxynivalenol (DON) and nivalenol (NIV). In the USA. The population that produces DON is predominant. deoxynivalenol producing isolates can be further grouped as one of two chemotypes, 3-acetyl-Deoxynivalenol (3ADON) or 15- acetyl-Deoxynivalenol (15ADON) based on their trichothecene profile. These mycotoxins are injurious to both human and animal health (Desjardins 2006). The FDA has set 1ppm the maximum contamination limit in the food products fit for human consumption (Schmale and Bergstrom 2003)

The pathogen virulence and aggressiveness pattern can be affected due to the continuous selection pressure of host resistance, excessive use of a single fungicide or fungicides with similar chemistry and adverse environmental conditions. A population shift in *G. zeae* (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). Several independent studies also indicated that the 3ADON population is more aggressive in scab development and DON production compared to the 15ADON population (Puri and Zhang, 2010; Ward et al. 2008; Ali et al. 2009). The reasons behind the population shift are still not fully understood but host resistance, excessive fungicides application, and change in weather conditions are thought to be the most likely suspects.

OBJECTIVES

- 1. Analyze *F. graminearum* isolates for their chemotypes recovered from hard red spring wheat genotypes in South Dakota
- 2. Determine if the host resistance favors 3ADON population over 15ADON population

MATERIALS AND METHODS

FHB samples collection and recovery of Fusarium graminearum isolates - Twenty-four FHB diseased head samples of 10 advanced breeding lines with moderate FHB resistance and two FHB susceptible cultivars 'Oxen' and 'Briggs' were collected from spring wheat breeding nurseries grown at SDSU experimental research Stations near Volga and Watertown in 2012 (Table 1). Five of 10 breeding lines had different genetic backgrounds but all had *Fhb1* gene as source of FHB resistance (Table 1). Ten diseased heads of each genotype were collected from each location. To recover fungal isolates, scabby grains (tombstones) were recovered from each sample. Five tombstones from each sample were randomly selected and plated on half strength potato dextrose agar (PDA) medium in 15 x 100 mm plastic petri plates. Five tombstones of each sample were plated on one plate. The fungal colonies grown out of the plated grains were transferred individually onto new 1/2 PDA plates. The identity of fungal isolates was determined based on their colony and spore morphology described in (Nelson et al. 1983). In total 93 *F. graminearum* isolates were recovered from all 24 collected samples. Ten isolates from each genotype (five/location) were recovered and stored in 15% glycerol at -80C in the freezer.

DNA Extraction and PCR-based Chemotyping -To recover DNA from the isolates, all 93 F. graminearum isolates were grown individually for 2 days on cellophane membrane placed on ¹/₂ PDA plates. Mycelia of each isolate were harvested by scraping the surface of cellophane membrane with a sterile spatula. DNA of all 93 isolates was extracted from the harvested mycelia using the protocol described in Liu et al., (2000). Trichothecene chemotype was determined for all the isolates using the trichothecene specific primers (3CON, 3NA, 3D15A, and 3D3A) (Starkey et al., 2007, Ward et al., 2002). PCR amplification was performed in a C-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 amplified products were run on 1.5% (wt/vol) agrose gels and scored with reference to 100 bp DNA ladder (New England Biolabs, USA). The PCR amplification produced bands of 610 and 243bp corresponding to the 15ADON and 3ADON chemotypes, respectively (Fig 1)

RESULTS AND DISCUSSION

Ninety-three of the 120 plated scabby grains produced *F. graminearum*. Twenty of the plated grains were infected with other *Fusarium* species, (i.e., *F. sporotrichioides*, F. avenaceum and F. equiseti). Recovery of *F. graminearum* from most plated samples seems to indicate that it is still the primary pathogen associated with FHB development in the state. The majority (94%) of isolates were grouped as 15ADON; whereas, 6% of the isolates were grouped as 3ADON (Fig. 1). 3ADON isolates were recovered from both FHB susceptible (n=2) and resistant (n=3) wheat

genotypes. The 2012 growing season was generally an FHB disease free year as it was hot and dry; however, occurrence of overnight dew periods provided some opportunity for FHB development on some heads in most of the plots in the breeding nurseries and in the commercial spring wheat field plots visited. The results of this study indicate that FHB moderately resistant cultivars may not have any potential role at least in favoring the recent fungal population shift from 15ADON to 3ADON in South Dakota. Also, the 15ADON population is still the predominant population in the state. However, the presence of a 3ADON population in the state and their higher aggressiveness than the 15ADON population in FHB development and DON production (Puri and Zhong 2010; Ali et al. 2009) warrant the use of 3ADON isolates for screening breeding material for FHB resistance to obtain durable resistant cultivars. More F. graminearum isolates, recovered from spring wheat and winter wheat FHB samples collected from breeding nurseries and commercial fields over multiple years, are under investigation to obtain a more complete picture of the fungal population chemotypes present in the state.

ACKNOWLEDGEMENT

The authors thank Richard Geppert and Manali Shirke for their help in samples collection and in the lab for DNA extraction, respectively. This research work was funded by the South Dakota Wheat Commission.

REFERENCES

Ali et al. 2009. Proceedings of the 2009 National Fusarium Head Blight Forum held at Orlando, Florida, USA from 7-9 December. Pp 19-21.

Burlakoti et al. 2008. Phytopathology 98:969-97

Desjardins, A. E. 2006. *Fusarium* mycotoxins chemistry, genetics and biology. The American Phytopathological Society press, St. Paul, Minnesota, USA

Gale et al. 2007. Phytopathology 97:1434-1439.

Liu et al. 2000. Journal of Clinical Microbiology 38:471

Nelson et al. 1983. The Pennsylvania State University Press. Pp226.

Puri, K, and S. Zhong 2010. Phytopathology 100:1007-1014

Schmale III, D. G. and G. C. Bergstrom 2003. The Plant

Health Instructor. DOI:10.1094/PHI-I-203-0612-01Starkey et al. 2007. Fungal Genet. Biol. 44: 1191-1204.Ward et al. 2008. Fungal Genetics and Biology 45:473-484.Ward et al. 2002. Natl. Acad. Sci. USA 99:9278-9283.



Fig1. Determination of chemotypes of *F. graminearum* isolates using the multiple PCR amplification method (Ward et al. 2008). The bands at 610bp and 243bp amplified from the *F. graminearum* isolates (42 to 56) correspond to the 15ADON and 3ADON chemotypes, respectively. M represents the 100bp DNA marker.

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Wheat	Pedigree	Number of F. graminearum
genotype		isolates tested
SD 4415	SD3934/SD4101/SD3934	3
SD 4417	SD3934/SD3948//Oxen	10
SD 4419*	SD3934/Granger//Briggs	10
SD 4420*	SD3934/Granger//Briggs	4
SD 4421	SD3934/Granger//Briggs	10
SD 4422	SD3934/Granger//Briggs	8
SD 4424	SD3934/Granger//Briggs	4
SD 4426	SD3934/Granger//Briggs	9
SD 4428	SD3934/SD3948//SD3944	10
SD 4429	SD3934/SD4102//Briggs	5
Briggs	BW114/BERGEN//SD3097	10
Oxen*	YW352/SBZ004A	10
*=3ADON isolates were recovered		

Table1. South Dakota hard red spring wheat genotypes and their pedigree from which *F*. *graminearum* isolates were recovered and analyzed for their chemotypes in 2012

THE RELATIVE EXPRESSION OF *TRI5* GENE DURING WHEAT-*FUSARIUM GRAMINEARUM* COLONIZATION C.C. Amarasinghe and W.G.D. Fernando*

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ABSTRACT

TRI5 is the key gene in the deoxynivalenol (DON) biosynthesis pathway. It encodes for the enzyme trichodiene synthase that catalyses the first step in DON biosynthesis. The objective of this study is to examine the level of expression of TRI5 in resistant and susceptible wheat cultivars after inoculating with Fusarium graminearum 3-ADON and 15-ADON isolates. In this study the two wheat cultivars Glenn (rated moderately resistant to FHB) and Roblin (rated highly susceptible to FHB) were grown in the greenhouse and were inoculated with ON-06-39, Q-06-32 (3-ADON) and Q-06-10, ON-06-05 (15-ADON) isolates. The level of expression of TRI5 gene was evaluated at 0, 6, 12, 24, 48, 72 hrs and 7 days after inoculation. The relative expression of TRI5 gene was analysed in comparison with the fungal GAPDH house-keeping gene. The expression of TRI5 gene was initiated at 72 hrs after inoculation and no significant expression was observed before this time period. The level of expression was higher in 7 dai than 72 hai. In this study relative expression of TRI5 was higher in the cv. Roblin than in cv. Glenn in most of the treatments. It is expected to have a higher expression of TRI5 gene in highly susceptible cv. Roblin as it showed the highest spread of the pathogen. Also at 7 days, 3-ADON isolates showed higher level of expression than 15-ADON isolates except for one treatment. In several treatments, TRI5 gene expression differed among the two isolates within the same chemotype group confirming the isolate variation during plant-pathogen interaction. Findings from this study would help in understanding the cross-talk between Wheat-F. graminearum during colonization. Further work is being carried out to examine the expression of other genes in DON biosynthetic pathway.

PROFILING TRICHOTHECENE GENOTYPES OF FUSARIUM GRAMINEARUM ISOLATED FROM CORN, WHEAT AND POTATOES IN EASTERN CANADA R.R. Burlakoti^{1*}, L. Tamburic-Illincic², V. Limay-Rios², R.D. Peters³ and P. Burlakoti²

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ABSTRACT

Fusarium head blight (FHB) and Gibberella ear rot (GER) are economically important diseases of wheat and corn in Eastern Canada and cause millions of dollars in losses. Fusarium graminearum is the principal causal agent of both diseases. In addition to huge yield losses, the fungus is responsible for quality degradation and mycotoxin contamination in grain. The fungus is also reported as a storage rot pathogen of potatoes in the United States and Canada. Several studies were previously carried out on population structure and mycotoxin diversity of F. graminearum infecting Canadian wheat. However, there are limited studies on the diversity of F. graminearum populations collected from corn and potatoes in Canada. The purpose of the present study was to understand the influence of host, cultivars, geography, and weather variables on the diversity of trichothecene profiles of F. graminearum isolates in Ontario and eastern Canada. F. graminearum isolates were recovered from grain samples of popularly grown wheat cultivars and corn hybrids from diverse geographic locations of south western and central Ontario in 2010 and 2011. As well, Fusarium graminearum was isolated from potato samples collected from Manitoba, Quebec, New Brunswick, and Prince Edward Island (PEI) during national potato surveys. A total of 298 single spore isolates of F. graminearum were recovered: 54 isolates from wheat, 227 isolates from corn and 17 isolates from potatoes. All isolates were characterized to the species level. Out of 298 F. graminearum isolates, 176 isolates (117 from corn, 49 from wheat and 10 from potatoes) were genotyped using TRI3- and TRI-12 based molecular markers. All the tested F. graminearum isolates were DON genotypes. Only 3% of isolates from corn and 2% of isolates from wheat were 3-ADON genotypes, and rest of isolates were 15-ADON genotypes. Interestingly, all the F. graminearum isolates from potatoes were 3-ADON genotypes. Molecular genotyping of more isolates is in progress. The ability of representative isolates to produce DON, 3-ADON and 15-ADON will be assessed with chemical analysis by growing the fungal isolate in rice culture. This study will provide base-line data on 3-ADON and 15-ADON profiles of F. graminearum isolates of corn and potatoes. The outcome will provide information to screen wheat, corn, and potato germplasms against FHB, GER, and Fusarium rot, respectively.

COMPARATIVE GENOMICS REVEALS NEW INSIGHTS INTO THE EVOLUTION OF *FUSARIUM* PATHOGENESIS IN WHEAT Donald M. Gardiner, Kemal Kazan^{*} and John M. Manners

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ABSTRACT

Fusarium head blight and crown rot are globally important fungal diseases of cereal crops such as wheat and barley. In Australia, these diseases are caused by two related *Fusarium* pathogens: *Fusarium* graminearum and *F. pseudograminearum*. Our earlier work on the inducers of trichothecene (DON) biosynthesis in *F. graminearum* has led to the identification of a variety of amine compounds that induce the fungal *TRI5* gene involved in DON biosynthesis in *F. graminearum* (Gardiner et al., 2009). We also found the activation of wheat genes encoding polyamine biosynthetic enzymes in infected heads prior to detectable DON accumulation (Gardiner et al., 2010), suggesting that the pathogen exploits this host stress response as a signal for production of trichothecene mycotoxins.

More recently, we have undertaken a comparative genomics approach to help understand how *Fusarium* pathogens cause disease on cereals. For this, we have sequenced the genome *F. pseudograminearum* using next generation sequencing technologies. As expected, the *F. pseudograminearum* genome was highly similar to the previously sequenced genome of *F. graminearum*. Interestingly, however, comparison of the predicted proteins encoded by the *F. pseudograminearum* genome to those from a range of other cereal and non-cereal pathogens revealed genes that have orthologues only in certain cereal pathogens. The fungal deletion mutants for two of these genes showed reduced virulence on wheat, indicating the importance of these genes for fungal virulence (Gardiner et al., 2012). Therefore, the comparative genomics approach appears to be useful for not only studying the evolution of *Fusarium* pathogenesis but also for better understanding of infection strategies used by these pathogens. This latter knowledge may lead to the development of new crop protection strategies.

REFERENCES

Gardiner DM, Kazan K, Manners JM. (2009) Nutrient profiling reveals potent inducers of trichothecene biosynthesis in *Fusarium graminearum*. Fungal Genet. Biol. 46:604-613.

Gardiner DM, Kazan K, Praud S, Torney FJ, Rusu A, Manners JM. (2010) Early activation of wheat polyamine biosynthesis during Fusarium head blight implicates putrescine as an inducer of trichothecene mycotoxin production. BMC Plant Biol. 10:289.

Gardiner DM, McDonald MC, Covarelli L, Solomon PS, Rusu AG, Marshall M, Kazan K, Chakraborty S, McDonald BA, Manners JM. (2012). Comparative pathogenomics reveals horizontally acquired novel virulence genes in fungi infecting cereal hosts. PLoS Pathog. 8:e1002952.

RNA-SEQ REVEALED GENE EXPRESSION DIFFERENCES BETWEEN 3ADON AND 15ADON POPULATIONS OF *FUSARIUM GRAMINEARUM IN VITRO* AND *IN PLANTA* K.D. Krishna¹, C. Yan² and S. Zhong^{1*}

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ABSTRACT

Fusarium graminearum is the major causal agent of Fusarium head blight (FHB), a devastating disease of wheat and barley in North America and worldwide. The fungus produces several trichothecenes [Deoxynivalenol (DON) and its acetylated derivatives, 3-acetyldeoxynivalenol (3ADON) and 15-acetyldeoxynivalenol (15ADON) as well as nivalenol (NIV)], which are harmful to humans and animals. Recent studies showed that the 3ADON-producing isolates dramatically increased in the fungal population and were more aggressive and accumulated more DON in wheat grains than the prevalent 15ADON-producing isolates in North America. However, the genetic and molecular basis for the differences between the two populations is still unclear. In this study, we compared transcriptome of the 3ADON and 15ADON populations in vitro and in planta using the RNA-seq technology. The in vitro RNA samples were isolated from bulked mycelia of 10 3ADON-type isolates or 10 15ADONtype isolates grown on mung bean agar plates for five days. The in planta RNA samples were extracted from spikelets of the susceptible spring wheat cultivar 'Briggs', which were harvested at 48, 96 and 144 hours after inoculation (HAI) with equally mixed spore suspension (10⁵ spores/ml) of 10 isolates from each population. Two biological samples (replicates) were taken from each treatment or time point, and thus two (1×2) in vitro and six (3×2) in planta RNA samples were sequenced for each of the fungal populations using the Illumina HiSeq 2000 platform. Total numbers of reads generated from each sample (replicate) ranged from 26.4 to 49.5 million for the 3ADON population and 27.8 to 39.1 million for the 15ADON population. Over 80% of the sequence reads from each of the in vitro RNA samples (replicates) mapped to the reference genome sequence of F. graminearum (PH-1). However, the percentages of sequence reads mapped to the fungal genome ranged from 5.3 to 13.3% for the in planta RNA samples from inoculation with 3ADON isolates and 6.5-8.2% for the RNA samples from inoculation with 15ADON isolates. Pearson's Correlation Coefficient (PCC) between two biological replicates within each treatment was significantly high, ranging from 0.903 to 0.997 (p<0.0001). Comparative analyses of in planta versus in vitro gene expression profiles revealed 2,159, 1,981 and 2,095 genes up-regulated in 3ADON isolates, and 2,415, 2,059, 1,777 genes up-regulated in 15ADON isolates during infection at 48, 96 and 144 HAI, respectively. Of these genes, 633, 526 and 668 were up-regulated at the three time points, respectively, only in the 3ADON population. Among the 65 genes up-regulated at all the three time points, 29 were found to be in the category of unclassified proteins while 25 had functions related to protein synthesis (ribosome biogenesis and translation), amino acid metabolism (aspartate, threonine, tryptophan, and pyruvate family), DNA processing and degradation, polyketides metabolism, and peptide, antigen and GTP binding, and cation transport (H+, Na+, K+, Ca2+, NH4+ etc.). Gene expression profile comparison between the 3ADON and 15ADON population grown in-vitro identified a total of 479 genes up-regulated and 801 genes down-regulated in the 3ADON population. Of the 479 up-regulated genes in the 3ADON population, 21.2% and 8.95% of them are involved in functions for C-compound and carbohydrate metabolism, and for polysaccharide, respectively, although a majority of the genes (58.3%) encode for unclassified proteins. Pair-wide comparisons between the two fungal populations *in planta* revealed 484, 451 and 310 differently expressed genes, respectively, at the three time points. The 3ADON isolates had 186, 87, and 63 genes up-regulated, respectively, at the three time points *in planta*, compared to the 15ADON isolates. Although a majority of these genes (69.3%) are in the category of unclassified proteins, genes involved in C-compound and carbohydrate metabolism, non-vesicular cellular import, cellular transport, allantoin and allantoate transport, C-compound and carbohydrate metabolism, secondary metabolism, and detoxification were identified among the others. Our RNA-seq analyses provide a foundation for further understanding of the molecular mechanisms contributing to the higher aggressiveness and DON production of the recently emerged 3ADON population.

ACKNOWLEDGEMENT AND DISCLAIMER

This material is based upon work supported by the U.S. Department of Agriculture, under Agreement No. 59-0790-8-067. 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 author(s) and do not necessarily reflect the view of the U.S. Department of Agriculture.

EFFECT OF CLIMATE ON THE DISTRIBUTION OF *FUSARIUM* SPECIES CAUSING CROWN ROT OF WHEAT IN THE PACIFIC NORTHWEST OF THE U.S. Grant J. Poole¹, Richard W. Sailey², Carl Walker³, David Huggins⁴, Richard Rupp⁵, John Abatzoglou⁶, Kimberly Garland-Campbell^{7*} and Timothy C. Paulitz⁸

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ABSTRACT

Fusarium crown rot, caused by multiple Fusarium spp. including F. culmorum and F. pseudograminearum, is one of the most widespread root and crown diseases of wheat in the Pacific Northwest (PNW) of the U.S. Fusarium crown rot (FCR) occurrence and distribution has been associated with temperature and precipitation. Our objectives were to characterize crown rot disease severity and distribution throughout the PNW by conducting a survey of 210 fields covering the diverse dryland wheat-producing areas of Washington and Oregon. We used a factor analysis statistical approach to determine the effects of climate and geography on species distribution and disease severity. Climatic variables of mean annual temperature (MAT), mean temperature in the coldest month (MTCM), mean temperature in the warmest month (MTWM), mean annual precipitation (MAP), elevation, soil type and cropping intensity were highly intercorrelated and used in a factor analysis. The factor analysis detected two latent factors that could be used as predictor variables in linear mixed models with repeated measures of FCR disease scores and in generalized linear mixed models for the presence/absence of Fusarium spp. Isolates of Fusarium spp. were obtained from 99% of 105 fields sampled in 2008 and 97% of 105 fields sampled in 2009. Results of the factor analysis showed that the distribution of F. pseudograminearum occurred in a greater frequency in areas on the PNW at lower elevations with lower moisture and higher temperatures, whereas F. culmorum occurred in greater frequency from areas at higher elevations with moderate to high moisture and cooler temperatures. The factor analysis approach allowed us to quantify the effects of several environmental and climate variables on disease and species occurence for Fusarium spp. in the PNW.

THE 3-ADON AND 15-ADON GENOTYPES OF *FUSARIUM GRAMINEARUM* IN NEW YORK ARE NOT DISCRIMINATED BY PATHOGENIC OR SEXUAL REPRODUCTIVE FITNESS P. Spolti^{1,2}, E.M. Del Ponte¹ and G.C. Bergstrom^{2*}

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ABSTRACT

Some recent studies in the northern U.S.A. and Canada have suggested that isolates of Fusarium graminearum sensu stricto with the 3-ADON trichothecene chemotype have greater pathogenic fitness on wheat than isolates of the 15-ADON chemotype, enough to displace 15-ADON chemotypes within local populations of the fungus. We tested the hypotheses that contemporary 3-ADON isolates are more fit pathogenically and reproductively than contemporary 15-ADON isolates in New York. A geographically diverse collection of F. graminearum sensu stricto was made from spikes of commercially produced wheat in New York in 2011, and the isolates were categorized for B-trichothecene genotype (corresponding to presumptive chemotypes) based on PCR amplification of the TRI3 and TRI12 genes. Trichothecene genotypes were recovered in the proportion of 15% 3-ADON isolates and 85% 15-ADON isolates, with no NIV isolates found. Twenty-five isolates each of 3-ADON and 15-ADON genotypes were selected arbitrarily for the fitness study. For the pathogenicity assay, 10 spikes of the susceptible wheat cultivar Norm were inoculated into the central spikelet for each isolate, and Fusarium head blight (FHB) severity (proportion of symptomatic spikelets) was recorded 10 days later. For the sexual reproduction assay, corn stalk segments (3cm x 2.5cm) were prepared, autoclaved and inoculated with each isolate, and incubated in moist chambers. Twenty-one days after inoculation the segments were evaluated for the percent segment area covered with perithecia into four classes (0 = no perithecia, 1 =1-10%, 2 = 11-30%; 3 = >30%). Following enumeration of perithecia, stalk segments were incubated an additional 48 h and the number of discharged ascospores was estimated based on the number of colonies counted on water-agar plates that were positioned 1.5 cm above two segments. Permutation tests were used to test the effect of isolates and orthogonal contrast to test the association of trichothecene genotype with the three variables of FHB severity, perithecia formed, and ascospores discharged. There was strong evidence (P < 0.0001) of the effect of isolate for the three variables analyzed but no evidence of difference between the two genotype groups (P>005). Thus the hypotheses that isolates of the 3-ADON genotype are more fit pathogenically and reproductively than those of the15-ADON genotype were rejected. The mean FHB severity was 41% (range 8-74%) and 40% (range 7-92%) for the 3-ADON and 15-ADON isolates, respectively. The number of isolates in classes 0, 1, 2 and 3 for the production of perithecia among isolates of 3-ADON was 1, 11, 9, 4, respectively, and similar to that observed among isolates of 15-ADON: 1, 10, 9, 5. The number of ascospores per plate ranged from 0 to 100 for the 3-ADON genotypes and from 0 to 97 for the 15-ADON genotypes with a mean of 38 and 34 ascospores per plate, respectively. These results suggest that, within the population of F. graminearum infecting wheat in New York, isolates with a 3-ADON genotype do not possess any obvious pathogenic or sexual reproductive advantage over 15-ADON isolates.

TOTAL DEOXYNIVALENOL (DON), 15-ADON AND 3-ADON DETECTED BY GC-MS IN WHEAT GRAIN AFTER INOCULATION WITH TWO *FUSARIUM GRAMINEARUM* CHEMOTYPES AND FUNGICIDE APPLICATION Lily Tamburic-Ilincic^{1*}, Chami Amarasinghe², Anita Brule-Babeli², Dilantha Fernando² and Jeannie Gilbert³

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ABSTRACT

Fusarium graminearum (Schwabe) is the principal cause of Fusarium head blight (FHB) in North America, one of the most serious diseases of wheat. Deoxynivalenol (DON) is the most important mycotoxin produced by *F. graminearum* (FG), but 15-acetyl DON (15-ADON) and 3-acetyl DON (3-ADON) analogs are also produced at low levels. The fungicides FOLICUR® (tebuconazole), PROLINE® (prothioconazole), PROSARO® (tebuconazole + prothioconazole) and CARAMBA® (metaconazole) are commonly used to control FHB in Canada. The objective of this study was to investigate the effect of the fungicides on DON, 15-ADON and 3-ADON levels after inoculation with 15-ADON and 3-ADON *F. graminearum* isolates in inoculated, misted wheat plots. Moderately resistant (MR) cultivars 'Alsen' and 'Glenn' were planted in Ridgetown, ON and Winnipeg, MB, respectively. The highly susceptible (HS) cultivar 'Roblin' was also planted at both locations. The cultivars were sprayed with the fungicides at 50% anthesis and inoculated individually with six *F. graminearum* isolates (both chemotypes) two days later. The harvested grain was analyzed for total DON, 15-ADON and 3-ADON using gas chromatography-mass spectrometry (GC-MS).

The highest level of total DON was recorded in 'Roblin' in MB without fungicide application and after inoculation with 3-ADON FG isolates (69.4 ppm in 2009 and 29.9 ppm in 2010). In addition, 1.4 ppm of 3-ADON (2% of total DON) and 0.5 ppm of 3-ADON (1.8% of total DON) were detected in the same samples in 2009 and 2010, respectively. In 2009, in addition to total DON, both analogs (15-ADON and 3-ADON) were detected in 'Roblin' in MB after inoculation with 15-ADON isolates of FG, while only 3-ADON was detected after inoculation with 3-ADON FG isolates. Neither analog was detected in wheat grain from Ontario because of low levels of total DON. Our results indicate that all four fungicides controlled FHB in spring wheat regardless of the *F. graminearum* chemotype inoculated. A higher levels of DON were detected in the HS cultivar compared to the MR cultivars, suggesting that planting wheat with increased level of resistance to FHB combined with fungicide application is the best strategy to lower DON levels in harvested grain.

THE IMPACT OF THE *FUSARIUM GRAMINEARUM* GENOME SEQUENCE ON THE QUEST FOR CONTROL OF HEAD BLIGHT Frances Trail^{*}

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ABSTRACT

In the last ten years, genome sequences have become available for hundreds of filamentous fungi, including mycotoxigenic *Fusarium* species *Fusarium graminearum*. The availability of genome sequences has stimulated and facilitated research on this important pathogen worldwide. The information has enhanced our ability to dissect the molecular basis of pathogenicity, explore the biosynthesis and regulation of mycotoxins, understand population structure, and elucidate elements of the life cycle. We have, in particular, used this information to understand the life cycle, including development and dispersal of ascospores, overwinter survival in crop residues and biosynthesis of mycotoxins during plant infection and colonization. The results of our research and that of others continue to reveal information on this important agricultural species that can be harnessed to reduce the impact of this mycotoxigenic fungus on grain crops.