SESSION 2:

PATHOGEN BIOLOGY AND GENETICS

Chairperson: Ivan Rayment

INTERACTIONS BETWEEN *FUSARIUM GRAMINEARUM* AND THE HOST PLANT

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ABSTRACT

Fusarium graminearum infects and produces disease on 3 major crops in the US: barley, wheat and corn. In barley and wheat, the disease affects the development and quality of grain. In corn, the disease may affect kernels, but can also cause rot of the lower stalks. *F. graminearum* grows in each host uniquely, and each host responds differently to the fungus. Our research attempts understand the fungal relationship to each of these three hosts. All three hosts support the generation of perithecia in the crop residue. However, in wheat and barley, these structures are associated with specific cell types, whereas in corn, the specificity is not as clear. The three hosts have been shown by other researchers to have differential sensitivity to DON. This may dictate the way infection is initiated and colonization progresses. We will present our findings on perithecium production, colonization, and mycotoxin accumulation. Differences in fungal-host dynamics clearly affect disease manifestation and carryover from one year to the next.

CHEMOTYPES OF *FUSARIUM GRAMINEARUM* FROM WHEAT IN 2009 AND 2010 IN NORTH DAKOTA

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ABSTRACT

Fusarium graminearum, the major causal agent of Fusarium head blight on cereals in the USA northern Great Plains, consists of three chemotypes, 3ADON, 15ADON, and NIV. Of these three chemotypes, 3ADON and 15ADON are commonly observed in North Dakota, and increase of 3ADON isolates in the *F. graminearum* population has been reported in recent years. Also, the 3ADON population has been shown to produce higher DON and disease severity in spring wheat cultivars. To monitor the prevalence of the two chemotype isolates in North Dakota, we collected FHB infected wheat samples from 12 and 28 counties across ND during 2009 and 2010, respectively. A total of 436 isolates (90 isolates from 2009 and 346 isolates from 2010) were obtained from these samples and DNA was extracted from the 2-3 days old mycelia of each isolate for PCR-based chemotyping using the trichothecene specific multiplex primers (3CON, 3NA, 3D15A, and 3D3A). Among those isolates collected in 2009, 91% (n= 82) and 9% (= 8) were of 15ADON and 3ADON chemotypes, respectively, and the eight 3ADON isolates were all from samples collected in Ransom County in northeast ND. Among the 346 isolates collected in 2010, 55.8% (n=193) and 44.2% (n=153) belonged to the 15ADON and 3ADON chemotypes, respectively. No 3ADON isolates were recovered from the samples collected from the three counties (Dickey, Richland, Sargent) located in the southeast corner of North Dakota in both years. However, more 3ADON isolates were recovered from the samples collected from most of the northern counties of the state. This study indicated that the 15ADON isolates were still predominant in general in North Dakota although 3ADON isolates were more common than the 15ADON isolates recovered from the samples collected in the northern counties of the state. The occurrence of more 3ADON isolates in the northern counties of North Dakota might be due to the cooler weather conditions during the growing season. Population genetics and aggressiveness of the isolates are still under investigation.

ACKNOWLEDGEMENT AND DISCLAIMER

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FUSARIUM HEAD BLIGHT INTENSITY, MYCOTOXIN LEVELS AND CHEMOTYPES OF *FUSARIUM GRAMINEARUM* SPECIES COMPLEX IN INDIVIDUAL FIELDS FROM PARANÁ STATE, BRAZIL

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ABSTRACT

Fusarium head blight (FHB) in Brazil is caused by members of the *Fusarium graminearum* species complex (Fg complex) that posses either a deoxynivalenol (DON) or nivalenol (NIV) chemotype. The relationship between disease intensity, mycotoxin levels and fungal chemotypes has not been determined at the field level, especially in Paraná State. The objective of this study was to determine the within-field distribution of Fg complex chemotypes, FHB intensity and trichotecenes (DON and NIV) and zearalenone (ZON) contamination in three commercial fields across the central and central-southern regions of Parana State, Brazil, during the 2011 growing season. At each field, during the soft dough grain state, twenty sampling points were randomly defined and three symptomatic spikes were taken to the laboratory. Strains were isolated (one per spike), purified and its DNA extracted with a cetyltrimethylammonium bromide (2% CTAB) method from mycelia of pure cultures growing in potato-dextrose broth. All isolates were identified to species level using a Fg16F/R primer sets. The Fg chemotypes were determined by a multiplex PCR assay that amplifies portions of Tri3 and Tri12 genes. For FHB ratings, four sampling points were defined in a single field and 100 spikes were randomly harvested. FHB incidence and severity and percentage of *Fusarium*-damaged kernels (FDK) of symptomatic spikes were estimated visually. In a subsample of kernels, DON, NIV and ZON were determined using a liquid chromatography–mass spectrometry method. Results showed that both 15-ADON and NIV co-occurred in the field with a slightly higher prevalence of 15-ADON (55 to 57%) over NIV (40.8 to 42.5%). Identification to species using TEF-based sequencing confirmed previous studies by showing that NIV-producers are *F. meriodionale* and 15-ADON are *F. graminearum sensu stricto*. FHB incidence and severity ranged from 12.8 to 29.0% and from 1.8 to 15.9%, respectively, across the three fields; FDK incidence ranged from 6.6 to 18.9%. The mean DON and ZON content in grains ranged from 33.3 to 1,427.2 μg/kg, and from 0 to 49.7 μg/kg, respectively. NIV was not detected in the samples. A higher correlation among the variables was found between FHB severity and DON levels. This study adds to the current knowledge by showing that mixed populations that produced either DON or NIV co-occur in wheat fields of Paraná State, although only DON has been determined by the current methods. Further investigations need to focus on the toxigenic potential of the populations and factors driving toxin production in the field.

GENOME SEQUENCING OF *FUSARIUM PSEUDOGRAMINEARUM* REVEALS A HORIZONTALLY ACQUIRED AMIDOHYDROLASE INVOLVED IN VIRULENCE

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ABSTRACT

Fusarium crown rot disease is a chronic problem in wheat and barley in arid environments such as Australia and there are currently no fully resistant wheat cultivars. In Australia, *F. pseudograminearum* is the pathogen predominantly associated with crown rot although related *Fusarium* species such as *F. graminearum* and *F. culmorum* have the ability to cause crown rot in some regions. These three *Fusarium* species can also cause globally important head blight disease of wheat. To increase our understanding of factors affecting pathogen virulence, we have sequenced the genome of an *F. pseudograminearum* isolate and compared it to the publically available genome sequence of *F. graminearum*. Despite overall sequence conservations, striking differences have also been observed between the genomes of these two *Fusaria*, including the presence of completely novel secondary metabolite gene clusters. Most strikingly we also identified a gene encoding an amidohydrolase that appears to have been acquired by horizontal gene transfer. This gene has a clear orthologue in the genome of the wheat pathogen *Phaeosphaeria nodorum* but not in any other fungal genome and the next closest sequence matches are from bacteria. Deletion of this gene from *F. pseudograminearum* resulted in a reduction in virulence on barley but not wheat. Population surveys suggest the gene has been present in both *F. pseudograminearum* and *Phaeosphaeria* lineages for a long time and could have been independently acquired by both species, possibly from bacteria. Its presence in these two unrelated pathogens but not in any other fungal species suggests a role for this gene in a common pathogenesis mechanism that targets an important defence pathway in cereals.

VEGETATIVE COMPATIBILITY – A NATIVE FUNGAL MECHANISM FOR INDUCING DEATH IN *G. ZEAE*

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ABSTRACT

Vegetative compatibility/incompatibility (vic) is a fungal version of self/nonself recognition and interaction that is known in many ascomycete fungi that results from two different alleles at a single locus being combined in the same cell, usually a heterokaryon, and collectively triggering apoptotic cell death. Genes responsible for the *vic* interaction are neither similar to one another nor necessarily evolutionarily conserved. Thus, individual *vic* loci may have this function in only a relatively few species. New anti-fungal control measures that rely on the ability to trigger apoptotic cell death on demand could result from an understanding of the mechanism used to trigger apoptotic cell death in response to the presence of *vic* heteroalleles in a common cytoplasm. At present we are mapping functional *vic* loci of *F. graminearum* onto an existing genetic map so that they can be localized in the physical genomic sequence and cloned. *vic* loci cannot be placed directly on a physical map since they are not evolutionarily conserved and easily identified by searching the published genome sequence, so they must first be mapped by their function. We have made a cross in which multiple *vic* loci are segregating and will select progeny that all have the same set of alleles at the *vic* loci. ORFs in the identified regions of the physical sequence will be screened for their ability to confer a vegetative incompatibility phenotype, with ORFs near or containing HET or WD0 domains in these regions tested first. Hypotheses regarding the mode of action for triggering cell death can be formulated once at least two alleles have been sequenced for several loci. Cloned *vic* alleles could be transformed into wheat with the goal of inducing apoptosis in *G. zeae* cells that penetrated beyond the surface of the wheat seed or seed head.

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EFFECT OF FUSARIUM GRAMINEARUM CHEMOTYPES ISOLATED FROM CANADA ON SYMPTOMS, DAMAGED KERNELS AND MYCOTOXINS IN WINTER WHEAT GRAIN

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ABSTRACT

Fusarium head blight (FHB) caused by *Fusarium graminearum* (Schwabe) is a serious wheat disease in North America and most cereal growing areas of the world. FHB results in economic loss due to decreased yield and quality, and the accumulation of mycotoxins such as deoxynivalenol (DON) in grain. The objective of this study was to investigate if there were differences in aggressiveness and mycotoxin production between Canadian *F. graminearum* strains (15-acetyl-DON (15DON) and 3-acetyl-DON (3DON)) in the moderately susceptible winter wheat 'Emmit'. In the fall of 2010, 'Emmit' was planted at Ridgetown Ontario in a randomized complete block design with four replications. One 15DON and one 3DON producing isolate was chosen from each of Nova Scotia, Quebec, Ontario and Manitoba for a total of eight isolates. At 50% anthesis, plots were inoculated with a backpack sprayer using each single isolate or a mixture of both isolates from each region for a total of twelve treatments, plus a control plot inoculated with water alone. To ensure spores were not transferred between experimental plots, guard plots were placed around each inoculated plot. We measured FHB symptoms by incidence (I), severity (S) combined into an FHB index (I X S/100), percentage of *Fusarium* damaged kernels (FDKs) using a monochromator near infrared reflectance spectrometer and DON, 15DON, 3DON content using a GC-MS system. Inoculated treatments resulted in significantly (P=0.05) higher field symptoms and FDKs compared to control. FHB indices 21 days after inoculation were highest after inoculation with 3DON *F. graminearum* isolates, followed by the mixture of isolates, 15DON isolates and control. In this moderately susceptible winter wheat cultivar 3-ADON *F. graminearum* isolates appear more aggressive in terms of field symptoms and when analyses are complete we expect to find higher levels of DON and its corresponding acetylated compound compared to mixture of both chemotypes and 15DONchemotypes.

DISCOVERY OF GENOME-WIDE VARIANTS IN THE *FUSARIUM GRAMINEARUM* CLADE FOR MAPPING AND EVOLUTIONARY ANALYSIS

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ABSTRACT

Next-generation sequencing technologies provide a rapid entry into the characterization of DNA variation within many species. Variable DNA markers, such as AFLP, are abundant in *F. graminearum* and have been used to characterize the relationship between distinct pathogen populations, but since the genome sequence of *F. graminearum* has been determined, sequencing-based markers provide an added layer of information that can reveal what parts of the genome and thus what genes even diverged populations still share in common. This information can lead to a more rapid identification of the functional genetic differences between populations that can affect pathogen management and strategies for developing host plant resistance. Here we present results from a next-generation sequencing strategy for the efficient discovery of thousands of sequence variants across a sample of 24 isolates from the *F. graminearum* clade. We describe how these variants are being used to highlight genome regions that are highly differentiated between members of this clade as well as describe population structure within *F. graminearum sensu stricto* samples and genetic recombination patterns among these samples. We also describe similar next-generation sequencing strategies that are expected to lead to development of markers efficiently targeted in larger samples for mapping and evolutionary studies of the *F. graminearum* clade species. Finally, we highlight a methodology to use such high-density, sequence-based markers to identify novel pathogen variants that rapidly spread in populations. These rapidly spreading variants represent pathogen adaptations such as overcoming host plant resistance, and mapping these variants to the gene level will provide candidates for pathogen control targets and a better understanding of the pathogen adaptation process.

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TIMING AND EVENTS OF *F. PSEUDOGRAMINEARUM* INFECTION OF WHEAT LEADING TO CROWN ROT

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ABSTRACT

Crown rot (CR) is an economically important disease of wheat in several countries around the world. One of the most common causal agents of CR around the world is *F. pseudograminearum*, however, little has been done to study the process by which *F. pseudograminearum* infects wheat stems leading to CR development. To characterise the infection process of *F. pseudograminearum*, histological and real-time quantitative polymerase chain reaction analyses were used to assess fungal colonisation following a time course from infection to plant maturity.

Seven distinct phases were identified: i) attachment of spores to leaf sheath surface, ii) detachment or break down of non-germinating spores, iii) colonisation of external leaf sheath surface by hyphae, iv) penetration of host tissues, possibly through stomatal openings, followed by a reduction of superficial hyphae, v) migration of fungi to basal stem tissue, vi) colonisation of stem tissue from the base, and vii) a large increase of fungal biomass in all colonised stem tissue towards maturity of host. Fungal DNA was detected from plant tissues well in advance of those tissue showing visible CR symptoms of stem discoloration at all-time points assessed during the experiment, however, fungal colonisation never exceeded 50% of the stem length.

These results indicate that CR disease development involves multiple phases of colonisation possibly leading to the pathogen to prepare for a saprophytic phase following pathogenic colonisation of tissue. They also indicate that CR infection is unlikely to lead to the development of Fusarium head blight leading to a contamination of mycotoxins in the grain.

MICROBIAL DETOXIFICATIONS OF DEOXYNIVALENOL (DON) AND THEIR POTENTIAL APPLICATIONS IN MITIGATING MYCOTOXIN CONTAMINATIONS

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ABSTRACT

It has been found that microbial communities of different ecological niches such as soils, plants and animal digesta are able to transform mycotoxins into their less or non-toxic forms. Bacterial strains capable of transforming DON under aerobic conditions into its isomer, 3-*epi*-DON, have been isolated from enhanced agricultural soil mixtures. Both in vitro and in vivo evaluations have indicated that this transformation results in significant toxicity reduction. In addition, several bacterial isolates, identified from different sources, are able to transform DON into its de-epoxy derivative DOM-1, a compound much less toxic than DON, under both aerobic and anaerobic conditions. These bacterial isolates are also capable of transforming certain other trichothecene mycotoxins into their less toxic forms. An animal trial has confirmed that pre-feeding treatment of DON contaminated feed with a DON transforming bacterial isolate can eliminate the adverse effects of DON on pig performance. Also, the identification of genes responsible for mycotoxin detoxification may result in applications for reducing mycotoxins in *Fusarium* infested cereals.