SESSION 2:

PATHOGEN BIOLOGY AND GENETICS

Chairperson: Anne Desjardins

VIRULENCE OF *GIBBERELLA ZEAE* ON WHEAT FOLLOWING INDEPENDENT DISRUPTIONS OF TRICHOTHECENE BIOSYNTHETIC GENES.

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ABSTRACT

The plant-fungal interaction that occurs when *Fusarium graminearum* invades small grains such as wheat and barley is complicated and involves many interactions between the invading fungus and the plant host. Although trichothecene toxins are not required for the initial infection of wheat, they are involved in the progression of Fusarium Head Blight (FHB) disease. Mutants of *F. graminearum* (*Δtri5*) blocked in the first committed step in the trichothecene biosynthetic pathway do not produce deoxynivalenol (DON) or any other trichothecenes, and are reduced in virulence on wheat. In order to test if biosynthetic precursors of DON are sufficient for disease progression, we disrupted the coding region sequence of 5 genes (*FgTri1, FgTri3, FgTri8, FgTri11*, and *FgTri101*) to produce mutants blocked at various steps in the trichothecene biosynthetic pathway. The mutants were analyzed for production of trichothecenes in liquid media, in a rice solid medium, and *in planta*, and for ability to cause head blight on the FHB-susceptible wheat cultivar Wheaton. Disruption mutants of the esterase *FgTri8* did not show a significant reduction in virulence. However, *ΔFgTri8* mutants accumulated 3,15-diacetylDON in culture, while in the infected seed DON as well as 3,15 diacetylDON was detected. These results suggest that esterases in wheat can contribute to the deacetylation that produces DON following infection by strains that produce 3-acetylDON. Disruptions of four genes, *FgTri1, FgTri3, FgTri11*, and *FgTri101* blocked production of DON and led to the accumulation in culture of early pathway intermediates, such as isotrichodermol and its 3-acetylated derivative isotrichodermin, or calonectrin and its 3 and 15-deacetylated derivatives. Disruption mutants of *FgTri1, FgTri3,* and *FgTri101* were reduced in virulence. However, disruption mutants of the cytochrome P450 monooxygenase *FgTri11* retained wild-type virulence although they accumulated isotrichodermol and isotrichodermin in culture and in infected seed. In a previous study, both isotrichodermol and isotrichodermin were as phytotoxic as DON in an *Arabidopsis thaliana* bioassay. Together, these results suggest that some trichothecene early intermediates are as biologically active as DON, thus the earliest steps of the pathway should be high priority targets for trichothecene control.

METHODS FOR DETECTING CHROMOSOME REARRANGEMENTS IN *GIBBERELLA ZEAE*.

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ABSTRACT

Chromosome rearrangements between fungal strains may reduce fertility in sexual crosses through the production of genetically inviable recombinant progeny. Rearrangements can be important postzygotic reproductive barriers that contribute to the speciation process. The presence of chromosome rearrangements in crosses with *Gibberella zeae* was tested by counting asci with 8, 6, 4, or 2 viable ascospores. Counts were made by observing rosettes of asci extruded from crushed perithecia and by observing unordered ascospore tetrads ejected onto agar slabs from mature perithecia. The two methods gave similar results. Self-fertilized cultures served as controls and produced the normal eight ascospores per ascus in >98% of cases. Crosses with strains known to carry chromosome rearrangements produced significant frequencies of asci with 6, 4, or 2 ascospores, as expected. These results suggest that these methods will be useful to survey populations of *G. zeae* for chromosome rearrangements.

THE ROLE OF TRICHOTHECENE-CHEMOTYPES IN FUSARIUM HEAD BLIGHT DISEASE SPREAD AND TRICHOTHECENE ACCUMULATION IN WHEAT.

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ABSTRACT

Three major strain-specific trichothecene-chemotypes have been identified in *F. graminearum*-infected crops in North America: 3-acetyldeoxynivalenol (3ADON), 15ADON and nivalenol (NIV). The emergence of the 3ADON- and NIV-chemotypes on the continent is a fairly recent phenomenon. In addition, strains with increased 15ADON production have recently been identified on the continent [*Ward et al. 2008. Fungal Genet Biol 45:473; Gale et al. 2007 Phytopathology 97:1434*]. In order to assess the potential impact of these new strains on North American wheat production, we are investigating the role of trichothecene-chemotype variation in FHB-spread and trichothecene-accumulation among susceptible and resistant wheat genotypes. The level of resistance in each of the wheat genotypes used had previously been established by point inoculation with a single isolate of a 15ADON-producer. In the current experiment, we used point inoculation with a composite of strains expressing either 15ADON, high-15ADON, 3ADON, or NIV chemotypes. Stable resistance or susceptibility to disease spread, as well as *Fusarium*-damaged kernel (FDK) scores, were observed in highly-resistant or highly-susceptible wheat genotypes. Chemotype-dependent interactions were observed in moderate or intermediate sources of resistance/susceptibility. Susceptibility to disease spread increased in wheat infected with either of the high DON-producers (3ADON and high-15ADON), and reduced in wheat infected with NIV-producers. Unexpectedly, while 3ADON-producers created as much, if not more, disease in wheat spikes as the high-15ADON-producers, FDK was as low as that caused by NIV-producers. The emergence of 3ADON-producers may imply a greater threat of FHB to North American farmers, although the severity of this impact in terms of grain quality and trichothecene contamination is uncertain. Trichothecene quantification is being performed on the collected kernels to shed some light on these discrepancies and to see if trichothecene accumulation in the grain is reflected in the FDK values. These studies will be followed by spray-inoculation experiments in order to assess the impact of trichothecene-chemotype on establishment of FHB in wheat.

LINKS BETWEEN POPULATION AFFILIATION AND TOXIGENIC POTENTIAL IN *FUSARIUM GRAMINEARUM*.

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ABSTRACT

A detailed understanding of the characteristics and dynamics of extant pathogen populations is necessary to evaluate the potential impact of pathogen diversity on specific management strategies, including the deployment of resistant varieties and chemical control strategies. Over the past seven years, several thousand members of the U.S. *Fusarium graminearum* pathogen population were genotyped using molecular markers. The observed variability could be organized into different genetic clusters or populations. Based on this initial molecular characterization and classification of isolates, an assessment of important phenotypic characteristics of the pathogen population could be pursued in a rational manner. We will present outcomes from four studies where we have utilized population affiliation information to inquire into the quantities of trichothecene toxins produced (toxigenic potential) of isolates or populations: 1. Influence of chemotype on the toxigenic potential of *F. graminearum* from the Southern U.S., where we show in greenhouse experiments that nivalenol-producing isolates overall produce much less toxin than DON-producing strains from the same region; 2. Discovery of *F. graminearum* strains that do not produce DON (or nivalenol), based on their multilocus genotypes that are not typical for known U.S. populations of *F. graminearum*; 3. Observation of differential toxigenic potential in greenhouse experiments that is correlated with population membership and that is cultivar-independent on wheat; 4. Observation of differential toxigenic potential in field experiments in wheat that may be indicative of population-synergistic effects, which were not observed in barley.

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COMBINATORIALLY-SELECTED ANTIMICROBIAL PEPTIDES PROVIDE NOVEL MEANS OF RESISTANCE TO FUSARIUM HEAD BLIGHT OF WHEAT.

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ABSTRACT

We are confronting the problem of head blight in wheat by using combinatorial peptide libraries to identify molecules that may contribute to novel forms of disease resistance. Blight-defense peptides are being selected for their ability to inhibit the development of *Fusarium graminearum.* To identify candidate defense peptides, we incubated phage-display libraries that express 12-mer random peptides with *F. graminearum* germlings. Phage clones that bound to the germling surfaces were recovered and amplified. After additional rounds of phage/peptide affinity screenings, we recovered numerous peptides with affinity for the germling surface. By *in vitro* assays, several affinity-selected phage clones have been discovered that inhibit *F. graminearum* germling development. These peptides have been placed into scaffold-display constructs for expression in yeast to assay their inhibitory function in the absence of the phage vector. Upon confirmation of the scaffold-peptide's inhibitory ability, we intend to deliver these constructs into plants. Additionally, the inhibitory peptides, along with peptide-sequence data collected from affinity screenings, will be used to further explore germling surface molecules important for hyphal tip growth.

UNDERSTANDING THE LIFE CYCLE OF *FUSARIUM GRAMINEARUM* AND ITS IMPACT ON DISEASE.

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ABSTRACT

We have been examining the series of life cycle events that take the scab pathogen from infected wheat back to the florets. These events include the production of mycotoxins (DON and zearalenone), the acquisition of carbon sources from the plant, the storage of lipids, the overwintering and production of perithecia, and ultimately dissemination of ascospores and conidia to flowering plants. Gene expression analysis indicates that lipid accumulation is an important step during colonization of wheat tissue. The stored resources are used for development of ascospores under some environmental conditions, or formation of conidia under other conditions. Expression of genes for mycotoxin biosynthesis can be predicted by the stage of infection and the nutritional status of the fungus. In this presentation we will frame our findings in the context of the disease cycle on wheat. Implications of these findings for forecasting and for disease control will be discussed.

THE POWER OF OMICS: ANALYZING GLOBAL EXPRESSION PROFILES TO REVEAL INFECTION MECHANISMS.

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ABSTRACT

Genomics and proteomics technology has revolutionized the study of how fungal pathogens attack their hosts. Identifying the infection mechanisms of a pathogen permits the development of targeted resistance strategies. We have used gene, protein, and metabolite expression profiling to examine the early infection process of *Fusarium graminearum* and its cereal hosts. We are especially interested in the secondary metabolites *F. graminearum* produces during infection. Cellular targets of these metabolites are under investigation using the extensive yeast genomic tools such as the deletion mutant collection. Our gene expression libraries have been mined to identify novel mycotoxin biosynthetic genes. The disruption of known and putative regulatory factors of these genes is underway to study the regulation of mycotoxin production. A shotgun proteomics approach was used to monitor protein expression changes under conditions conducive to mycotoxin production, revealing 130 *F. graminearum* proteins that exhibited significant changes in expression. Seventy-two proteins were significantly up-regulated relative to their level at the initial phase of the time course and this group included predicted secreted proteins, cellular transport proteins, homologs of other fungal virulence proteins, and many conserved hypothetical proteins. We are currently disrupting several genes encoding proteins identified in this study to explore function and contribute to our search for mechanisms of host invasion and novel antifungal targets.

USING NATURAL VARIATION TO CHARACTERIZE VIRULENCE: THE TRI13 STORY.

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ABSTRACT

The *Fusarium graminearum* population in Nepal was characterized for population structure, toxin production and virulence on both wheat and maize. AFLP analyses divided the population into four distinct groups. All strains in AFLP 1 produced 4-deoxynivalenol (DON), while nivalenol (NIV) was produced by all strains in AFLP 2. The remaining two groups, AFLP 3 & 4, were variable with some strains producing NIV and others DON. The pattern suggests that: 1. *F. graminearum* can be highly variable within a small geographic area, and 2. different AFLP lineages can coexist within an area and remain distinct. Trichothecene levels were lower generally for NIV producers compared to DON producers. There was a trend for DON producers to be more virulent than NIV producers. This pattern was evident even for strains in AFLP 3. Within this group, pairs of strains that were genetically similar but differed in toxin type were compared. On average, DON producers caused 20% more disease than NIV producers. The pattern of toxin accumulation suggests different patterns of toxin accumulation and virulence in NIV versus DON producers. NIV producers caused more disease per unit of toxin accumulation, while DON producers accumulated more toxin and ultimately caused more disease.

PHYLOGENETIC RELATIONSHIPS OF FUSARIUM HEAD BLIGHT PATHOGENS FROM DIFFERENT SOURCES BASED ON *TRI101* GENE SEQUENCING DATA.

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ABSTRACT

A molecular data set from nucleotide sequences of trichothecene 3-*O*-acetyltransferase (*Tri101*) gene (1336 bp) was constructed for 58 isolates of Fusarium head blight (FHB) pathogen from Canada, Mexico, and Iran along with sequences of 7 representative isolates of *Fusarium graminearum* (*Fg*) clade sequenced in this study and 11 representatives of *Fg* clade species plus 1 *F. pseudograminearum* isolate as the outgroup available at NCBI. Cultures were grown and DNA extracted and sequenced according to published procedures. DNA sequences were processed and assembled using SOOMOS 0.6 and sequence multiple alignments were conducted using MEGA 4. Phylogenetic analysis was conducted using PAUP* 4.0 to characterize the genetic diversity and evolutionary relationships of the isolates. Maximum parsimony searches were conducted using 100 random sequence addition replicates and the tree bisection-reconnection method of branch swapping. Maximum parsimony analyses of the sequences identified 11 *Fg* clade species. All Canadian and Iranian isolates clustered with lineage 7 (=*F. graminearum*) of *Fg* clade (BP=91%). Mexican isolates fell into two different groups: 7 isolates clustered with lineage 3 (=*F. boothii*) (BP=100%), and 8 isolates formed a new monophyletic group (BP=100%) which is different from any of the 11 known lineages (species) in the *Fg* clade. Sequencing data from the present study and from NCBI supported isolates of *F. asiaticum*, *F. acasiae-mearnsii*, and *F. cortaderiae* as being single species (BP=99%, 99%, and 98%, respectively) but there was insufficient support for *F. meridionale*, *F. austroamericanum*, *F. brasilicum*, *F. mesoamericanum*, *F. gerlachii*, and *F. vorosii* isolates. Based in part on the results of this study, two isolates from each of Canada, Mexico, and Iran, with different characteristics, will be selected and used in the future in a host-pathogen interaction study.

COMPARATIVE GENE EXPRESSION ANALYSIS OF *FUSARIUM GRAMINEARUM* IN *TRITICUM AESTIVUM* AND *ORYZA SATIVA* SPP. JAPONICA.

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ABSTRACT

Negligible amounts of Type B trichothecenes accumulate in *Oryza sativa* spp. japonica infected with *Fusarium graminearum* relative to *Triticum aestivum* inoculated with an identical strain of the fungus. To identify differential fungal gene expression patterns that could be responsible for differences in toxin accumulation in these plants, analyses of expression were conducted during infection of *O. sativa* or *T. aestivum* using *F. graminearum* Affymetrix GeneChips. Gene expression profiles were generated for time points 48, 96, and 192 hours after inoculation (hai) of plants. Profile analyses revealed a subset of genes (236) expressed only in *T. aestivum*. Classification of these genes using MIPs FunCat categories showed 110 of these genes fell into the Unclassified category. Five of these genes encode InterPRO predicted zinc-finger transcription factors and are being targeted for functional analysis via gene knock-out mutagenesis. Sixty-three genes fell into the Metabolism category, the next highest representation among the remaining genes. An *in silico* search of non-coding upstream regions for regulatory sequences in all 236 genes revealed an enrichment of two nucleotide sequences: ACGTCA and CCCCGC. Differences in temporal patterns of global fungal gene expression were observed during infections of the different hosts. In *T. aestivum*, expression levels of all genes increased from time point to successive time point, whereas expression levels of genes in *O. sativa* remained relatively constant. These results were well correlated with symptoms observed on both plants. Onset of symptoms first occurred on *O. sativa* at 48 hai and slowly increased in severity over time. Symptoms were first observed on *T. aestivum* 72 hai and intensified continuously and more quickly than those observed on *O. sativa*.

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THE TRANSCRIPTIONAL REGULATOR *TRI*6 PLAYS A MULTIFUNCTIONAL ROLE ASSOCIATED WITH VIRULENCE IN *FUSARIUM GRAMINEARUM*.

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ABSTRACT

Tri6 which encodes for a C_2H_2 zinc finger protein positively regulates the trichothecene pathway genes in *F. sporotrichioides* (Proctor *et al.* 1995). RNA-mediated silencing of *Tri6* suppressed mycotoxin production in *F. graminearum* (McDonald *et al.* 2005). Here we report the phenotypic, exo-proteomic and transcriptomic characterization of *Tri6* mutant in *Fusarium graminearum*. Targeted disruption of *Tri6* failed to *in vitro* synthesize 15-aceytyldeoxynivalenol, a derivative of the mycotoxin deoxynivalenol (DON). Further, infection on a *Fusarium* susceptible variety of wheat was restricted to the inoculated site. Exo-proteomic evaluation of *ΔTri6* and wild-type strains revealed that disruption of the *Tri6* gene is associated with effects on secretion. The secreted proteins that are affected have been previously implicated in pathogen virulence. One such protein *Tri*8, located within the trichothecene cluster, is a member of a lip5 class of secreted lipases, and is associated with virulence in *Candida albicans*. Targeted disruption of *Tri*8 in *F. graminearum* resulted in drastic reduction in the virulence of the pathogen. Finally, whole gene expression profiling by Illumina- Solexa technology confirmed that *Tri6*, in addition to regulating secretion also affects transcription of genes involved in sequestering nutrition for pathogen growth.

REFERENCES

Proctor, R.H., Hohn, T.M., McCormick, S.P., and A.E. Desjardins. (1995). *Tri6* encodes an unusual zinc finger protein involved in regulation of trichothecene biosynthesis in *Fusarium sporotrichioides*. Appl. Environ. Microbiol. *61*, 1923-1930.

McDonald, T., Brown, D., Keller, N.P., and Hammond, T.M. (2005). RNA silencing of mycotoxin production in *Aspergillus* and *Fusarium* species. MPMI *18*: 539-545.

THE *CID1* CYCLIN C-LIKE GENE IS IMPORTANT FOR PLANT INFECTION AND DON PRODUCTION.

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ABSTRACT

Fusarium head blight is an extremely destructive disease on wheat and barley. Losses are due to reduction in yield and contamination of infected grain with mycotoxins. Although structural genes for deoxynivalenol (DON) biosynthesis have been well characterized, fungal regulatory genes and plant factors controlling trichothecene synthesis in infested wheat kernels are not well understood. In this study, we functionally characterized a cyclin C-like gene *CID1* in *F. graminearum*. *CID1* is homologous to *SSN8* of *Saccharomyces cerevisiae* and the *FCC1* gene of *F. verticilloides*, which is required for regulating fumonisin production on infected corn kernels. Complementing all defect phenotype in fcc1 mutant of *F.verticilloids* with *CID1* gene showed this gene really conserved in filamentous fungi. In *F. graminearum*, the *cid1* mutant was enhanced in the production of a reddish pigment on V8 agar plates and liquid cultures. In infection assays with flowering wheat heads and corn stalks, the *cid1* deletion mutant was significantly reduced in virulence. Only a very low amount of DON and 15ADON was detected in wheat kernels colonized by the mutant. The expression level of the trichodiene synthase gene *TRI5* was reduced in the *cid1* mutant. Re-introduction of the wild-type *CID1* allele into the *cid1* mutant complemented all its defects. These data suggest that *CID1* may function as a regulatory factor in DON synthesis.