SESSION 2:

PATHOGEN BIOLOGY AND GENETICS

Chairperson: Nancy Alexander

QUANTITATIVE EXPRESSION OF *FUSARIUM SPOROTRICHIOIDES* GENES IN THE PRESENCE OF XANTHOTOXIN.

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ABSTRACT

Xanthotoxin (8-methoxypsoralen) is a phototoxic furocoumarin that covalently binds to and crosslinks with DNA. It is also known to inhibit P450 oxygenases. To test the effect of xanthotoxin on gene expression in *Fusarium sporotrichioides*, we developed a reverse transcriptase, quantitative polymerase chain reaction (RTqPCR) method to measure the expression of genes involved in the biosynthetic pathway of trichothecenes. We found that xanthotoxin treatment of wild-type *F. sporotrichioides* blocked production of T-2 toxin and caused the accumulation of its hydrocarbon precursor, trichodiene. This suggested that *FsTri5*, the gene encoding trichodiene synthase, may be up-regulated and that *FsTri4*, a trichodiene oxygenase, may be down-regulated. However, our RTqPCR results showed that 1 and 5 h after xanthotoxin treatment, both *FsTri5* and *FsTri4* were upregulated while *FsTri101*, encoding the trichothecene C-3 transacetylase, was downregulated. When *FsTr4*$^{-}$ mutants that accumulate trichodiene in culture were treated with xanthotoxin, trichodiene accumulation increased. Although the FsTRI4 protein is non-functional in these mutants, the RTqPCR showed that *FsTri4* was transcribed and was up-regulated in the presence of xanthotoxin. These results suggest that xanthotoxin may be involved in the up-regulation of *FsTri5* expression, but that factors other than gene regulation account for the increased accumulation of trichodiene.

GENETIC DIVERSITY OF *FUSARIUM GRAMINEARUM* POPULATIONS FROM CEREAL AND NON-CEREAL HOSTS.

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ABSTRACT

Fusarium graminearum, a causal agent of Fusarium head blight (FHB) of wheat and barley, is of the most economically important pathogens of cereals worldwide. Although *F. graminearum* has been reported on non-cereal crops in the northern Great Plains, little is known about population structure of *F. graminearum* associated with non-cereal crops. We hypothesized that substantial genetic exchange has occurred between populations of *F. graminearum* across cereal and non-cereal hosts. In this study, we analyzed the genetic structure and the trichothecene diversity of four populations of *F. graminearum* collected from barley and wheat (cereals) and potato and sugar beet (non-cereals) hosts using ten variable number tandem repeat (VNTR) markers and primers designed from the genes involved in trichothecene biosynthesis. Both gene diversity (H = 0.449 to 0.616) and genotype diversity (GD = 0.984 to 0.998) were high, while estimates for linkage disequilibrium ($\bar{r}_d$ = 0.003 to 0.041) were low in *F. graminearum* populations, suggesting frequent recombination due to sexual reproduction. Our results further demonstrated that the deoxynivalenol (DON) genotype was the most frequently detected in the populations regardless of origin of host. The 3-acetyl (3-ADON) and 15-acetyl DON (15-ADON) genotypes were commonly found in both cereal and non-cereal populations, however, the 15-ADON genotype was predominant. In addition, low genetic differentiation (Fst = 0.043) and genetic distance (D= 0.144) was observed between the cereal and non-cereal populations. Sequence analysis of the representative isolates from four hosts confirmed that *F. graminearum* populations belonged to phylogenetic lineage 7, further supporting the hypothesis of a single interbreeding population in the United States.

IDENTIFY AND CHARACTERIZE GENES REGULATED BY THE *FMK1* MAP KINASE IN *FUSARIUM GRAMINEARUM*.

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ABSTRACT

Fusarium graminearum is a devastating pathogen of wheat, barley, and maize throughout the world. The *FMK1* gene encodes a well conserved MAP kinase that is essential for plant infection. To identify genes regulated by *PMK1*, in this study we conducted microarray experiments with the *fmk1* mutant using the *Fusarium graminearum* Affimetrix GeneChip. In comparison with the wild-type strain, a total of 333 and 155 genes were down- and up-regulated (≥2-fold), respectively, in the *fmk1* mutant. Functional classification of the probe sets revealed multiple processes were affected by the deletion of *FMK1*. Many of these genes were unique to *F. graminearum*. Forty four of them encoded putative transcription factors with DNA-binding motifs. We selected 12 genes with altered expression levels in the *pmk1* for verification by qRT-PCR. Four of the genes verified by qRT-PCR were functionally characterized. While two other genes appeared to be dispensable for growth and pathogenesis in *F. graminearum*, deletion of the *ATG8* homolog and a putative Zn2Cys6 transcription factor significantly reduced its virulence on flowering wheat heads. The ATG8 homolog in *Magnaporthe grisea* also was down-regulated in the *pmk1* mutant, suggesting that this MAP kinase pathway may have a regulatory role in autophagy. Our results also were useful to determine the transcription regulatory network controlled by this well conserved MAP kinase pathway for fungal development and pathogenesis.

DIVERSITY IN *FUSARIUM GRAMINEARUM SENSU STRICTO* FROM THE U.S.: AN UPDATE.

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ABSTRACT

Efforts are ongoing to understand the population structure of *Fusarium graminearum sensu stricto* (Fg) in the U.S., its dynamics and its significance for small grain production. At previous FHB forums, we described the existence of genetically divergent populations of Fg in some regions of Minnesota and North Dakota (emergent populations) that were in the process of displacing the pre-existing population of Fg and that were also found to be more toxigenic, i.e. produced more deoxynivalenol (DON) on the susceptible variety Norm in greenhouse experiments. Recent population genetic analyses of 1,132 Fg strains from our 2006 collection indicated that the emergent populations are moving further south, as they were found to be present for the first time in South Dakota, at 3.5% of the total Fg population. Greenhouse experiments were conducted that assessed the toxigenic potential of these emergent populations on the commercially important cultivars Alsen, Knudson, Briggs, Freyr, Oklee and Granite that also represent various degrees of FHB susceptibility. Results from these experiments mirrored those from the initial experiments on Norm, i.e. substantially higher DON levels were obtained for all cultivars when inoculated with members of the emergent populations compared to when inoculated with member of the pre-existing Fg population. A second region that we also closely monitor is the southern United States. Previously, we reported that almost all Fg strains from Louisiana were nivalenol producers. Our 2007 collection from Louisiana originated from 17 commercial fields in three parishes. This collection was established to supplement Fg population information from nurseries. Very similar to population data from nurseries, nivalenol producers were predominant (79% of isolates). DON producers were mainly of a 3ADON trichothecene type (17% of isolates). Nivalenol producers also have been identified from Arkansas. From a limited sampling, 12% of isolates from Arkansas were nivalenol producers; among the DON producers the 15ADON trichothecene type was predominant (68% of isolates). Initial analyses of isolate genotypes established by using a PCR-RFLP marker system determined that overall the Fg population from Louisiana is genetically distinct from the Fg population that is commonly found in the Midwest.

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PHENOTYPIC AND MOLECULAR DIVERSITY OF *FUSARIUM GRAMINEARUM SENSU STRICTO* FROM THE U.S.

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ABSTRACT

Our long-term objectives are to accurately determine the composition and genetic structure of genetically coherent populations of FHB pathogens in the U.S., to evaluate their potential to change in composition and genetic structure over time and to determine the effect of such change on deployed host genotypes and/or other agricultural practices. To accomplish these goals we have established an extensive collection of *Fusarium graminearum sensu stricto* (Fg) strains from the U.S. gathered over eight years (1999-2007). Many of these strains originated from yearly disease surveys of the USDA-ARS Cereal Disease Laboratory, St. Paul, MN that cover routes of ca. 20,000 km of Midwestern and some Southern states. Other strains were contributed from collaborators or originated from directed sampling efforts of specific sites. To date, we have characterized about 7,500 U.S. Fg strains from 16 states using one or more markers or methods. Of particular usefulness is the molecular characterization of the trichothecene type. By using a multiplex PCR system developed by T. J. Ward (USDA-ARS, NCAUR, Peoria, IL) we can easily distinguish among the three trichothecene types of Fg, i.e. 15ADON, 3ADON or nivalenol (NIV). These trichothecene types accurately predict the specific chemotypes produced in host-pathogen interaction, i.e. 15ADON trichothecene type strains will produce [DON] > [15ADON] > [3ADON] and 3ADON trichothecene type strains will produce [DON] > [3ADON] > [15ADON], while the NIV trichothecene type will produce NIV. In the U.S. all three trichothecene types are present. While the NIV type of Fg has been identified from four states (LA, AR, MO, NC), it is currently common only in LA (ca. 80% of total strains) and AR (12% of strains). The likely presence of NIV in grain from these two states poses a problem insofar as NIV is currently not detected by commercial mycotoxin test kits. In addition to trichothecene type, we also have used a variety of molecular markers (RFLPs, VNTRs, PCR-RFLPs) to genotype strains. Genotyping allows us to further group strains into populations that are reproductively cohesive. Employing a population concept is important as each population may react to selective pressures in their own way. While most Midwestern states currently are populated by a genetically cohesive population of Fg with a predominant 15ADON trichothecene type, populations of Fg that are genetically distinct from this Midwestern 15ADON population have become very common in particular regions of MN and ND. These emerging populations are currently classified as the Upper Midwestern 3ADON population (UMW 3ADON) or as the Upper Midwestern 15ADON population (UMW 15ADON), depending on their trichothecene type. Members of both populations produce on average substantially more DON in greenhouse experiments on all cultivars tested compared to members of the Midwestern 15ADON population. Results of collections from 2006 indicate that the UMW 3ADON and UMW 15ADON populations are migrating further south and are now also present in South Dakota. Future proposed work will test the hypothesis of host genotype x pathogen chemotype/genotype interaction in field experiments.

ACKNOWLEDGEMENTAND DISCLAIMER

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STRUCTURAL AND FUNCTIONAL STUDIES OF TRICHOTHECENE BIOSYNTHETIC ENZYMES: A NOVEL APPROACH TO COMBATING FUSARIUM HEAD BLIGHT.

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ABSTRACT

Fusarium Head Blight (FHB) is a plant disease with serious economic and health impacts. Although it has proved difficult to combat this disease, one strategy that has been examined is the introduction of an indigenous fungal protective gene into cereals such as wheat, barley and rice. Thus far the gene of choice has been *tri101* whose gene product catalyses the transfer of an acetyl group from acetyl Coenzyme A to the C3 hydroxyl moiety of several trichothecene mycotoxins. *In vitro* this has been shown to reduce the toxicity of the toxins by ~100 fold but has demonstrated limited resistance to FHB in transgenic cereal. In order to understand the molecular basis for the differences between *in vitro* and *in vivo* resistance the three-dimensional structures and kinetic properties of two TRI101 orthologs isolated from *Fusarium sporotrichioides* and *Fusarium graminearum* have been determined. The kinetic results reveal important differences in activity of these enzymes towards B-type trichothecenes such as deoxynivalenol. These differences in activity can be explained in part by the three dimensional structures for the ternary complexes for both these enzymes with Coenzyme A and trichothecene mycotoxins. The structural and kinetic results together emphasize that the choice of an enzymatic resistance gene in transgenic crop protection strategies must take into account the kinetic profile of the selected protein.

Examination of the trichothecene biosynthetic pathway suggest that other enzymes might provide a more suitable scaffold for engineering new degradative activities for improved resistance. Therefore, it is planned to continue the biochemical characterization and three-dimensional structure determination of the remaining enzymes in the biosynthetic pathways for deoxynivalenol, nivalenol, and T-2 toxin. To date the crystal structure for FsTRI3 both apo and in complex with 15-decalonectrin have been determined and the kinetics of this enzyme towards native substrate and final toxins evaluated. This structural information will be used to create new enzymes by directed evolution utilizing a yeast selection system to detect new activities that degrade or inactivate the toxins.

FUNCTIONS OF THE SEX PHEROMONES OF *GIBBERELLA ZEAE*.

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ABSTRACT

In heterothallic ascomycete fungi, idiomorphic alleles at the *MAT* locus control two sex pheromone/receptor pairs that function in recognition and attraction of strains with opposite mating types. In the ascomycete *Gibberella zeae*, the *MAT* locus is rearranged such that both alleles are adjacent on the same chromosome. Strains of *G. zeae* are self-fertile, but they can outcross facultatively. Our objective was to determine if pheromones retain a role in sexual reproduction in this homothallic fungus. Putative pheromone precursor genes (*ppg1* and *ppg2*) and their corresponding pheromone receptor genes (*pre2* and *pre1*) were identified in the genomic sequence of *G. zeae* by sequence similarity and microsynteny with other ascomycetes. *ppg1*, a homolog of the *Saccharomyces* α-factor pheromone precursor gene, was expressed in germinating conidia and mature ascospores. Expression of *ppg2*, a homolog of the a-factor pheromone precursor gene, was not detected in any cells. *pre2* was expressed in all cells, but *pre1* was expressed weakly and only in mature ascospores. Deletion mutations *Δppg1* or *Δpre2* reduced fertility in self-fertilization tests. *Δppg1* reduced male fertility and *Δpre2* reduce female fertility in outcrossing tests. In contrast, *Δppg2* and *Δpre1* had no discernable effects on sexual function. *Δppg1*/*Δppg2* and *Δpre1*/*Δpre2* double mutants had the same phenotype as the *Δppg1* or *Δpre2* single mutants. Thus, one of the putative pheromone/receptor pairs (*ppg1*/*pre2*) enhances, but is not essential for, selfing and outcrossing in *G. zeae*, whereas no functional role was found for the other pair (*ppg2*/*pre1*).

ISOLATION OF TWO XYLANASE FROM *FUSARIUM GRAMINEARUM*.

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ABSTRACT

Fusarium head blight (FHB), caused by *Fusarium graminearum* (teleomorph *Gibberella zeae*), results in severe yield losses and crop quality reductions in wheat and barley, and is the predominant species of the FHB complex in North American. In addition to the toxins produced by the pathogen, cell wall degrading enzymes secreted by the pathogen may be involved in pathogenesis. The objective of this project was to purify and characterize xylanase(s) from *F. graminearum*. The in-vitro production of xylanase(s) by *F. graminearum* was obtained from cultures grown at 25°C for 5 days on modified synthetic media agar supplemented with sterile wheat bran. Xylanase activity was extracted by soaking one plate of the wheat bran agar in 100 ml of 100 mM sodium acetate buffer pH 4.5. Two xylanases have been purified 52- and 40- fold by a combination of ion-exchange, gel filtration, HPLC ion-exchange and HPLC hydrophobic interaction chromatography. The two xylanases were separated by the first ion-exchange step, and were then processed individually through subsequent steps. The purity and the relative molecular weights of the xylanases was estimated by SDS-PAGE to be 20 and 40 KDa, respectively. Only a single band was observed for each enzyme. The two xylanases were identified by trypsin digestion followed by LC-MS/MS as the gene products of FG03624 and FG06445. In the mass spectrometer, the high molecular weight xylanase, FG06445, 87% of the sequence was observed while for the low molecular weight xylanase, FG03624, 62% of the sequence was identified. After removal of the predicted signal sequence, the predicted molecular masses and iso-electric points were 22 and 38 KDa, and pH 9.2 and 8.5 for FG03624 and FG06445, respectively.

SPORE DEVELOPMENT AND TRICHOTHECENE MUTANTS OF *FUSARIUM GRAMINEARUM*.

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ABSTRACT

To understand trichothecene accumulation and the infection cycle of the head blight pathogen *F. graminearum sensu stricto*, fungal gene expression profiles were monitored during germination of ascospores and during plant infection. A total of 328 genes were determined to be specifically expressed in ascospores. Among genes highly up-regulated in ascospores was one most closely related to *FoStuA* of *F. oxysporum* and *StuA* in *Aspergillus*. Mutants deleted for this gene in *F. graminearum* (*FgStuA*) are greatly decreased in sporulation and produce no perithecia. Unlike *FoStuA* mutants in *F. oxysporum*, *FgStuA* mutants are greatly reduced in pathogenicity. Reduced pathogenicity may be due to decreased levels of trichothecene toxins, which in the mutant are <1% the levels of wildtype. Levels of transcripts corresponding to *Tri5*, but not other genes involved trichothecene biosynthesis, were extremely diminished in the *FgStuA* mutant. Thus both sporulation and trichothecene synthesis may be regulated under the control of *StuA*.

We are also developing isogenic lines of *F. graminearum* that differ only at the toxin biosynthesis cluster, in order to understand how DON and the chemical profile of trichothecene derivatives (trichothecene chemotype) influences fungal pathogenicity. The trichothecene biosynthetic gene cluster has been completely deleted from both a deoxynivalenol (DON) and a nivalenol producing strain of *F. graminearum* and will be replaced with the cluster from a different chemotype. Five separate genes from the cluster also have been individually deleted. Biological and regulatory characteristics of the mutant strains will be discussed.

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STRUCTURAL AND FUNCTIONAL STUDIES OF TRICHOTHECENE 3-O-ACETYLTRANSFERASE: PROGRESS TOWARDS DEVELOPMENT OF AN IMPROVED ENZYME FOR CONTROLLING FHB.

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ABSTRACT

Biological control of Fusarium Head Blight (FHB) is a difficult and complex problem. One strategy that has been examined is the introduction of an indigenous fungal protective gene into cereals such as wheat, barley and rice. Thus far the gene of choice has been *tri101* whose gene product catalyses the transfer of an acetyl group from acetyl Coenzyme A to the C3 hydroxyl moiety of several trichothecene mycotoxins. *In vitro,* this has been shown to reduce the toxicity of the toxins by ~100 fold but has demonstrated limited resistance to FHB in transgenic cereal. The reasons for this lack of success are unclear. Thus, a study to investigate the chemical framework that underlies the trichothecene biosynthetic pathway has been initiated with the goal of understanding the molecular basis for the differences between the *in vitro* and *in vivo* resistance. To this end the three-dimensional structures and kinetic properties of two TRI101 orthologs isolated from *Fusarium sporotrichioides* and *Fusarium graminearum* have been determined. The kinetic results reveal important differences in activity of these enzymes towards B-type trichothecenes such as deoxynivalenol. These differences in activity can be explained in part by the three dimensional structures for the ternary complexes for both these enzymes with Coenzyme A and trichothecene mycotoxins. The structural and kinetic results together emphasize that the choice of an enzymatic resistance gene in transgenic crop protection strategies must take into account the kinetic profile of the selected protein.

The structural and functional studies now suggest that the enzymatic activity, stability, and solubility of Tri101 can be improved quite readily by protein engineering. This represents an exciting opportunity to utilize the fundamental knowledge of a pathogen's biosynthetic pathway to modify the biochemical and biophysical characteristics an enzyme so that it can provide improved protection against FHB.

TRICHOTHECENE CHEMOTYPES OF ISOLATES OF *GIBBERELLA ZEAE* RECOVERED FROM WHEAT IN ARGENTINA.

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ABSTRACT

Wheat production in Argentina covers about 6.24 million hectares. Production reached 15 million tons during the 2006 harvest season, ranking Argentina as the fourth largest exporter in the world. The main pathogen associated with Fusarium Head Blight (FHB) in Argentina is *Gibberella zeae* (Schwein.) Petch (anamorph *Fusarium graminearum* Schwabe), which reduces both grain quality and yield. Wheat grains infected with *G. zeae* often are contaminated with a Type B trichothecene, usually deoxynivalenol (DON) or nivalenol (NIV), that is toxic to humans and domesticated animals. Strains of *G. zeae* usually express one of three sets of trichothecene metabolites (chemotypes): (i) nivalenol and acetylated derivatives (NIV chemotype), (ii) deoxynivalenol and 3-acetyldeoxynivalenol (3-ADON chemotype), and (iii) deoxynivalenol and 15-acetyldeoxynivalenol (15-ADON chemotype). Other *Fusarium* isolates that can produce both deoxynivalenol and nivalenol (NIV/DON) have been described and can not be assigned to any of these three chemotypes. We used a multiplex PCR assay to identify the trichothecene chemotype of 123 strains of *G. zeae* lineage 7 (identified by AFLP) isolated from 3 localities (San Antonio de Areco, Alberti and Marcos Juarez) within the main Argentinean wheat production area. Most (> 92%) of the Argentinean isolates of *G. zeae* had the 15-ADON chemotype, with the remainder having the NIV/DON chemotype. We did not detect the NIV or the 3-ADON chemotypes. Results from the PCR assays were consistent with those obtained by chemical analyses for all strains that produced trichothecenes. Knowledge of the chemotypes present in the *G. zeae* population is important when conducting mycotoxin surveys, implementing breeding programs, and identifying new and emerging populations of this fungal pathogen.

TRICHOTHECENE MYCOTOXIN GENOTYPES OF *GIBBERELLA ZEAE* IN BRAZILIAN WHEAT.

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ABSTRACT

Fusarium head blight (FHB), caused by *Gibberella zeae*, is a disease of increasing concern to wheat production in Brazil. Infested grain may be contaminated with trichothecene mycotoxins such as deoxynivalenol (DON) and nivalenol (NIV), posing a significant threat to the health of humans and domestic animals. Little is known about the mycotoxin potential of strains of *G. zeae* in Brazil. We obtained 82 single-spored strains of *G. zeae* from infected kernel samples originating from twenty locations in southern Brazil. Polymerase chain reaction (PCR) assays were used to characterize trichothecene mycotoxin genotypes of *G. zeae* (genetic profiles associated with the production of DON, NIV, and two acetylated derivatives of DON) and to assist in the identification of strains to species. To identify strains of *G. zeae* that may produce DON and NIV, we amplified portions of *Tri5* and *Tri7*. To identify strains of *G. zeae* that produce 3-acetyl-deoxynivalenol (3-ADON) and 15-acetyl-deoxynivalenol (15-ADON), we amplified portions of *Tri3* and *Tri12*. Nearly all of the strains studied (76/82) were of the DON/15-ADON genotype. Six of the strains were of the NIV genotype. We did not observe the 3-ADON genotype in our samples. The NIV genotype was observed in multiple samples from the same field and was present in all three southern states of Brazil studied. This is the first detailed report of trichothecene mycotoxin genotypes of *G. zeae* in southern Brazil. Additional information is needed to better determine the relative impact of different trichothecene mycotoxins in Brazilian wheat, and to employ appropriate methodologies for detecting mycotoxin contamination in the future. We are currently expanding our assays to screen for trichothecene mycotoxin genotypes in other geographic regions of Brazil, across additional growing seasons, and in other hosts such as barley and oats.

POPULATION OF *FUSARIUM GRAMINEARUM* SCHWABE ASSOCIATED WITH HEAD AND SEEDLING BLIGHT IN SLOVAKIA.

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OBJECTIVES

To determine any significant differences among population isolates of *F. graminearum* from wheat in Slovakia in cultural and pathogenicity assays *in vitro* and *in vivo*.

INTRODUCTION

The growth of *Fusarium* species associated with Fusarium Head Blight (FHB) varies depending on agronomic characters and edaphic conditions (Bottalico and Perrone, 2002). We have identified fifteen *Fusarium* species during the ten years of our investigations in the Slovak Republic. The most commonly identified *Fusarium* species involved in FHB in wheat were *F. graminearum* Schwabe and *F. culmorum* (W.G.Smith) Sacc. (Šrobárová and Vašková,1987). Both species produce mycotoxins, such as deoxynivalenol (DON) and zearalenone (ZEN) that can reduce the quality of grain. A recent study we carried out demonstrated a drift in the populations from *F. culmorum* (W. G. Smith) Sacc. to *F. graminearum* Schwabe. Our hypothesis is that *F. graminearum* is a more aggressive species perhaps by producing more toxin as it invades the plant tissue, by adapting to climatic conditions better, or by having some other selective advantage over *F. culmorum*. Strains of *F. graminearum* harvested from infected wheat in Slovakia during the years 2000 and 2001 were a source for our study.

MATERIALS AND METHODS

Cultures: *Fusarium* spp. were isolated from the caryopses of wheat stands in Slovakia during 2000 and 2001.

Culture conditions: The single-spore isolates from 2%- water agar (WA) were grown on potato-dextrose agar (PDA) from Difco Laboratories (Detroit, MI) using 40 grams in 1L distilled H_2O, pH 6 (± 0.2). Radial growth rates of all isolates were determined by measuring colony diameters of single conidial cultures on PDA in 90-mm-diameter Petri dishes. The colony growth and sporulation were measured each of three days. Cultures were incubated for 5 weeks in a 14-h photoperiod at 22°C by day and 15°C by night. Measurements were made on three replicate cultures that each originated from single conidia per each strain.

Pathogenicity tests: Strains were assayed for pathogenicity by inoculating seedlings of wheat cv. Torysa, a wheat cultivar that is moderately resistant to *Fusarium* infection (Pavlová and Šrobárová, 1998). Seeds were surface-sterilized with 1% sodium hypochlorite (diluted 5% commercial bleach) for 2 min and rinsed three times in sterile distilled water for 2 min. After rinsing with sterile water, the seeds were placed into Petri dishes (d = 90mm) on wet filters and kept in the dark for 2 days at 22 °C. The imbibed seeds were transferred into test jars (150 by 100 mm) containing 15 mL of solidified sterile 0.6% WA (ten uniform seedlings per jar). The jars were covered with sterile aluminum and incubated for 10 days with a 14-h photoperiod at 22°C by day and 15°C by night. For

each of the 12 *Fusarium* isolated, inoculum was prepared and seedlings were inoculated with 0.5 mL of a $1x10^5$spores/ml suspension and incubated for 10 additional days under the same conditions as those use for the initial growth of the seedlings. Controls were inoculated with 0.5 mL of sterile potato dextrose broth. Plants were rated visually for disease severity on a 0 to 5 scale reflecting the proportion of the root system with visual lesions as described in Wildermuth and McNamara (1994). Analysis of variance was performed and disease severity ratings were ranked. Fresh and dry weight (the seedlings were dried in an incubator at 105°C) were taken. Redascreen fast deoxynivalenol (DON) kit (R-Biopharm GmbH, Darmstadt, Germany) was used for semiquantity measurements of DON, according to the manufacturer's instructions.

RESULTS

Vegetative growth: After 3 days of incubation at constant temperature, all *F. graminearum* isolates had grown significantly on PDA. Radial growth rates for all isolates (Table 1) were similar at 22°C, ranging from a mean of 22 mm (#2 isolate) to 46 mm (isolates 7 and 12) by 72 h The greatest differences were seen on the fourth day when the average difference between the slowest growth and the fastest growth was 3.1 cm. By day 6, almost all the isolates had reached the edge of the plate (9.0 cm)

The pigmentation of the reverse side of the colony was usually carmine red for all the *F. graminearum* isolates while aerial mycelium was white to carmine red. No unusual colors or colony morphology were seen among the isolates. Perithecium were formed on WA in thirty to forty days except for two isolates, Michalovce #7 and Šariš #12, which did not form perithecium within the allotted time frame (Table 1).

Pathogenicity: Relative pathogenicity was examined under laboratory conditions for all 12 isolates. All strains were pathogenic to wheat seedlings, as indicated by disease severity rankings (Fig.1). The highest degree of infection (DI) was measured for #4, #5, #6, #10, and #11 isolates of *F. graminearum*, but all isolates showed a degree of 3 or more. The DI of the controls ranged from 0.2 to 0.3. The isolates of *F . graminearum* may be said to be strongly aggressive but were not significantly different from one another. Based on mean values, they were significantly more virulent than the water controls to the seedlings. Fresh weights and dried weights of the plants infected with the *Fusarium* isolates were compared to control plants (Figure 2). Plants infected with isolates #7 and #8 had the lowest fresh weight while #9 and #12 had the highest. Almost all of the plants had a similar dry weight, except those inoculated with isolate #3 while the lowest were #2 and #7.

Toxin levels: The highest levels of DON (Table1) were produced *in vitro* by isolates of *F. graminearum* #4 and #5. The lowest levels were produced by isolates #7 and #12.

DISCUSSION

Low levels of genetic differentiation among geographic regions yet high levels of genetic variation within populations have been reported for the sexually reproducing wheat pathogen *F. graminearum* (Dusabenyagasani et al., 1999; Miedaner et al., 2001; Leslie et al. 2007). Our data also suggests genetic variation among populations isolated from distinct regions of Slovakia. Traditionally, species differentiation has been based on morphological characteristics. As the interest in *Fusarium* has increased during the last two decades as a result of the increased devastation of Fusarium Head Blight (FHB) worldwide, more efforts have been extended on using molecular techniques to characterize the populations of *Fusarium.* Although it has been suggested that *F. graminearum* consists of at least 9 separate species (O'Donnell et al. 2004), it appears that there is only a single species within *F. graminearum* (Leslie et al. 2007). Within this species, there is genetic variation in morphology, pathogenicity, and gene sequence variation.

In pathogenicity tests on wheat seedlings, Miedaner et al. (2000, 2001) found a variation of aggressiveness among *F. graminearum* isolates. Our results show there is no precise correlation in fresh and dry weight of infected seedlings among the *Fusarium* isolates. Variation in aggressiveness is associated with

the genetic diversity of this species and is most likely due to the amount of toxin produced by the isolate (Goswami and Kistler (2005). There is a positive correlation between head blight and DON (Proctor et al. 1995) however, mutants unable to produce toxin are still able to initiate infection (Bai et al., 2001) which suggests that aggressiveness is correlated with the amount of toxin produced. The results presented in this study show that all of our isolates are capable of producing DON *in vitro*, however, there was no precise correlation between the amount of DON produced and the degree of infection.

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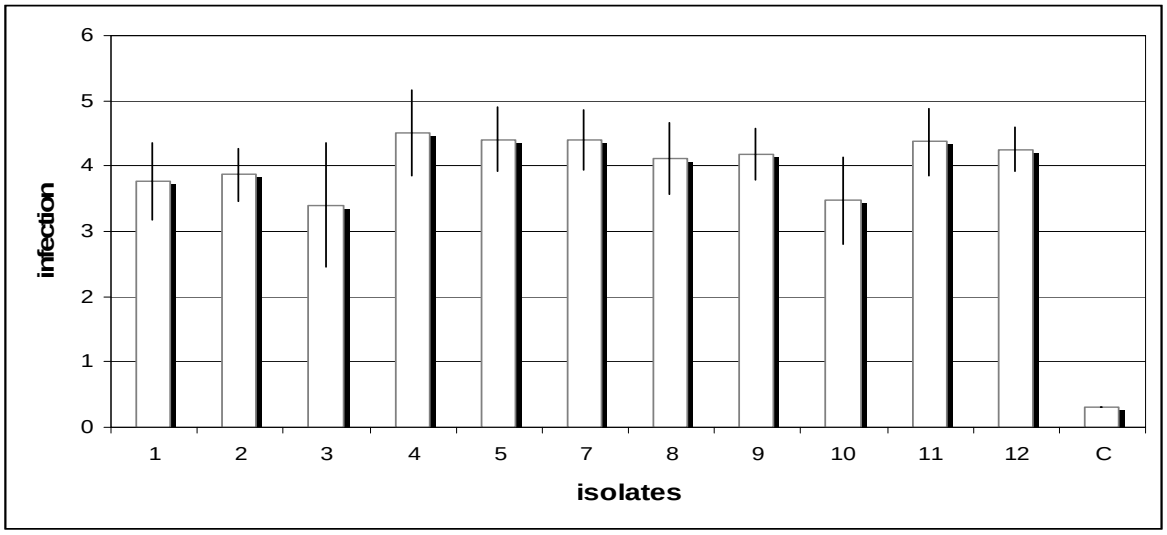

Figure 1. Degree of infection of seedlings of wheat cv.Torysa by a water control (C) and isolates 1-12 of *F .graminearum* Schwabe.

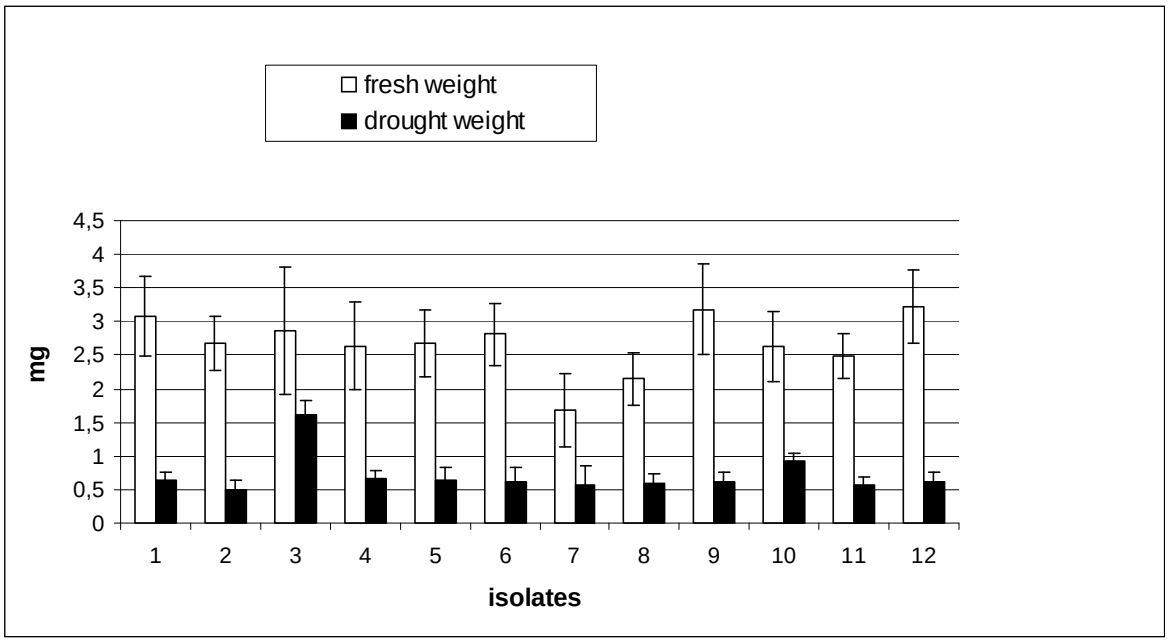

Figure 2. Fresh and dry weights of seedlings of cv. Torysa inoculated with *F. graminearum* isolates.

LIFE CYCLE AND SURVIVAL OF *FUSARIUM GRAMINEARUM*.

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ABSTRACT

We have been studying the life cycle of *F. graminearum* in association with wheat. Each stage of the life cycle is intimately tied to the host life cycle. Infection occurs primarily through ascospores during host flowering. Following infection, the fungus must colonize the stalk and store lipids before the plant senesces. Stored lipids in hyphae are then used to fuel sexual development and spore production for the next disease cycle. In fungi, lipids are stored in vegetative hyphae and spores as lipid bodies. Lipid-filled hyphae produce perithecium initials in association with stomates along the stems and in association with silica cells at the notes. These initials go dormant and become competent to form perithecia during the final stages of grain maturation before harvest. After harvest, the dormant hyphae in the crop residue protect their resources by secreting antimicrobials. Consideration of these aspects of the life cycle of this pathogen will allow us to use controls such as fungicides and biological control agents in a more effective manner.

UPDATE ON THE LIFE CYCLE OF *FUSARIUM GRAMINEARUM*.

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ABSTRACT

We have focused our studies on 3 stages of the life cycle that may provide opportunities for introducing novel controls. The process of forcible ascospore discharge launches the primary inoculum of the head blight disease from crop residues. The fungus must heavily colonize the crop tissue and store lipids in order to survive the winter and produce perithecia. We have characterized the process of lipid accumulation and utilization in association with perithecium development in culture and leading up to perithecium development *in planta*. Lipid-filled hyphae must protect their resources in crop residues until they use them to generate perithecia. We will present our latest findings on each of these stages as they are particularly vulnerable and may present targets for new controls.

TRICHOTHECENE CHEMOTYPE COMPOSITION OF *FUSARIUM GRAMINEARUM* AND RELATED SPECIESIN FINLAND AND RUSSIA.

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ABSTRACT

Fusarium head blight (FHB) caused by *Fusarium graminearum* and related *Fusarium* species is an important fungal disease of cereals worldwide. FHB pathogens cause significant yield and quality losses and they pose a serious threat to food safety. *F. graminearum* isolates can be divided based on mycotoxin production into 3 main chemotypes (3ADON, 15ADON and NIV), which can be identified by SNP genotyping. These chemotypes are not species-specific. Isolates with the NIV chemotype are more toxigenic than those producing either 3ADON or 15ADON.

The species and chemotype composition of 286 single-spore isolates causing FHB collected between 1986-2006 in different parts of Russia, Finland, China and Germany was investigated using a multilocus genotyping assay (MLGT) including multiplex PCR with six primer pairs followed by allele-specific primer extension (ASPE) utilizing 38 species- and chemotype-specific probes. Hybridization and detection were performed using a Luminex 100 flow cytometer.

All *F. graminearum* isolates from Finland (15) and western Russian (23) possessed the 3ADON chemotype, while all isolates from southern Russia (43) except for one from barley and one from corn possessed the 15ADON chemotype. In other parts of Russia and northern China isolates with the 3ADON and 15ADON chemotype were both present. The only *F. graminearum* isolate with the NIV chemotype was from Germany. All (27) *F. culmorum* isolates (Finland and Russian Federation) possessed the 3ADON chemotype. In contrast, all six isolates of *F. cerealis* possessed the NIV chemotype. These results are in accordance with previous mycotoxin analyses of pure cultures of Finnish FHB isolates on rice and analyses of field samples. In Finland there were no differences in the *F. graminearum* chemotype composition between the years 1986-93 and 2001-2004, while in the Far East (85 isolates) the 3ADON chemotype frequency increased between the years 1998-2006. This apparent shift in trichothecene chemotype frequency is similar to recently observed shifts in FHB pathogen composition within North America.

Two Russian *F. graminearum* isolates, one from southern Russia and one from Siberia, produced a positive signal with a 3ADON and 15ADON MLGT probe from opposite ends of the trichothecene gene cluster, suggesting that it may reflect recombination between isolates with these two chemotypes. Six single-spored isolates from this isolate gave the same result. Twelve isolates (ten from Far East and two from Siberia) produced unusually low positive signals for the *F. graminearum* probes, but they were all clearly positive for the B-clade (species producing B type trichothecenes) and the *F. graminearum* species complex probes. These isolates likely harbor previously unrecognized variation at the probe sites and will be sequenced to confirm the species identification and to inform additional probe design.