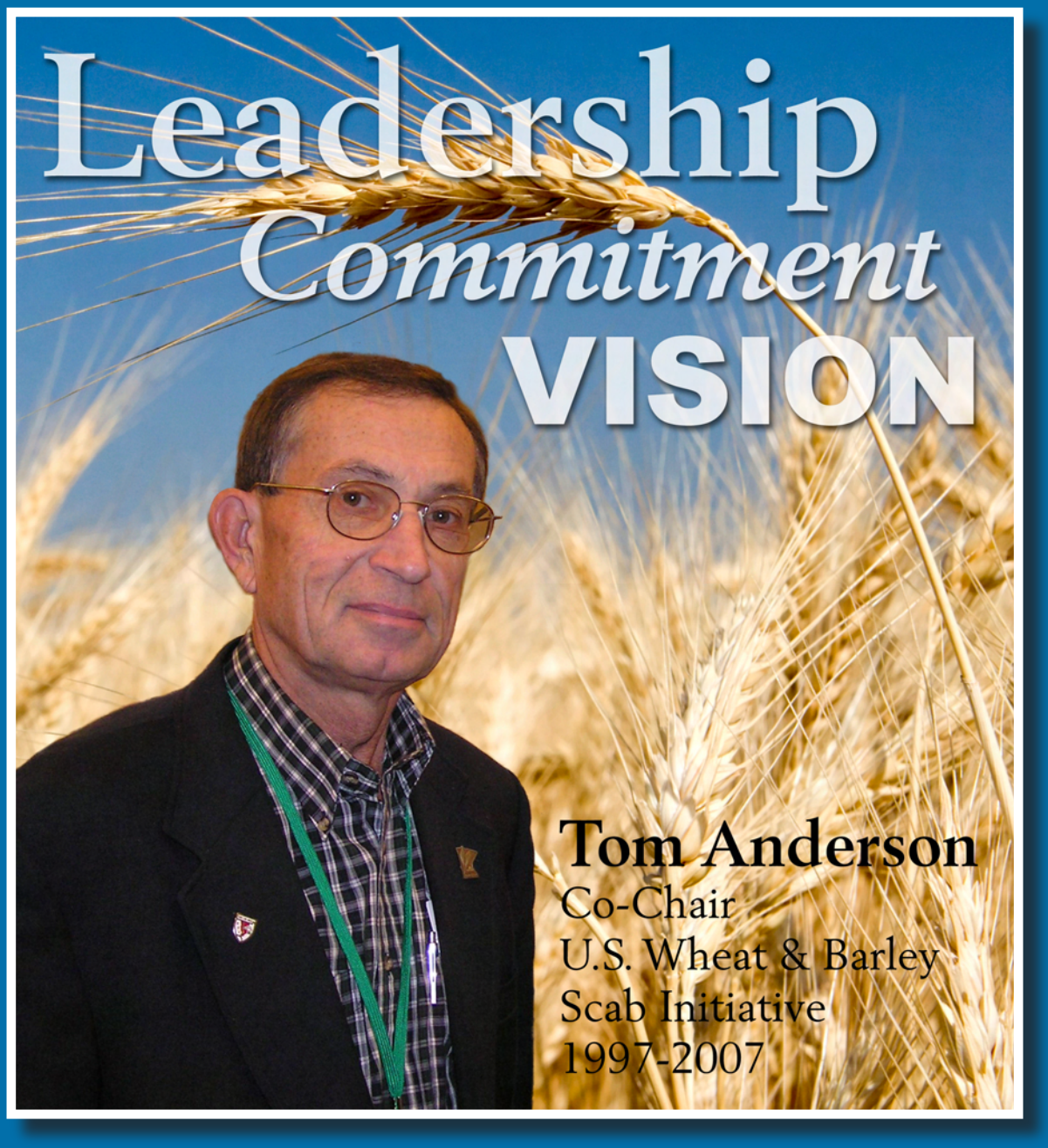

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Dedication

The 2007 National Fusarium Head Blight Forum and these Proceedings are dedicated to the memory of Thomas E. Anderson, who served as Co-Chair of the U. S. Wheat and Barley Scab Initiative from 1997 until his death in July 2007. His passion for research led Tom to become an activist in the arena of agricultural research, and he served on many commodity and research boards during the last twenty years of his life. As Co-Chair of the Initiative, Tom's steady hand, great sense of humor and penchant for asking hard questions earned him the respect, friendship and admiration of the entire USWBSI community.

The tenth annual National FHB Forum coincides with the implementation of the USWBSI Action Plan, and a rededication of our efforts to develop and implement "*control measures that minimize the threat of Fusarium Head Blight (Scab) to the producers, processors, and consumers of wheat and barley*". As we undertake this huge task it is appropriate to remember and draw inspiration from the example of our colleague, friend and leader, Tom Anderson.

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KEYNOTE PRESENTATION

FUSARIUM HEAD BLIGHT OUTBREAK FREQUENCY UNDER A CHANGING CLIMATE ENVIRONMENT.

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EXECUTIVE SUMMARY

Fusarium Head Blight (FHB) is a re-emerging disease of increasing concern to wheat and other small grains with devastating impacts worldwide (Goswami & Kistler, 2004). In Brazil, FHB epidemics have become more frequent and often resulting in significant yield losses (Panisson et al., 2003). FHB constitutes a disease complex in which several fungal species may cause largely indistinguishable symptoms, although the predominant causal agent worldwide is the fungus *Gibberella zeae* (*Fusarium graminearum sensu stricto*, anamorph) (Goswami & Kistler, 2004). Previous reports in Brazil showed that *G. zeae* was the principal causal agent of FHB in wheat (Angelotti et al., 2006).

FHB is best known as a disease of flowering stage but evidences suggest that wheat may be susceptible at later stages of kernel development. In temperate climates, it has been reported that monoculture, reduced tillage, and maize-wheat rotations have greatly increased inoculum levels in soil (McMullen et al., 1997). In southern Brazil, inoculum is available the year round because of the abundant crop residues from other hosts, widespread no-till and the absence of freezing temperatures or dry seasons impairing fungal development (Fernandes, 1997).

Even though progresses have been made over time, disease control is still challenging. Most of cultivars in use do not possess desirable levels of resistance that could lead to a good genetic control. Breeding for wheat scab resistance is a difficult task but some progress has been achieved in the recent years (Mesterhazy et al. 2005). Although a range of fungicides have been identified with good activity against FHB pathogens, their efficacy is influenced by dose rate, application timing and spray quality for adequate spike coverage (Cromey et al., 2001). The strong dependence on environmental conditions and the relative narrow window of vulnerability to infection by the fungus makes FHB a suitable system for modeling and forecasting, which could lead to a rational and more effective disease control.

In the last few decades, modeling of plant diseases, especially computer modeling, has expanded very quickly and played an important role in integrated disease management. The recent advances in computer hardware and information technologies have assisted in this development, bringing operational advantages to speed up development and application of computer models representing complex processes. Modularity and generic are terms that describe the new and widely accepted methodology to surpass the complexities in the development and re-use of models. Our group has been applying novel software engineering techniques towards the development of linked crop-disease generic simulation models. These techniques revealed to be appropriate and robust to guide in the development of plant disease simulation models. In addition, combining a suite of technologies proved to be possible to use existing knowledge legacy.

Critical epidemiological knowledge of a plant disease is of fundamental importance for developing disease models. Epidemiological aspects of FHB have been studied in southern Brazil since early 1980's in both field and controlled environment. Climatic conditions are most suitable for the disease in that region and moderate to severe FHB epidemics show a periodical occurrence. Studies for developing an FHB model initiated by our group since late 1990s and the result-

ing models has improved along the years. It has been built based on existing knowledge on disease cycle components and a series of local studies to empirically model host development and inoculum availability. The FHB model has been validated against observed epidemic data from Brazil and well explained the variation in FHB severity under distinct conditions (Del Ponte et al., 2005). The model was further coupled onto a wheat growth and development simulation model. The wheat-disease model has been validated with regards to prediction of wheat phenological stages and FHB severity observed in Passo Fundo location, Brazil.

In this presentation, two applications of the model using an integrated systems approach for the development of a web-based information technology platform will be illustrated. First, the potential effect of climate changing/variability in a selected location in the main wheat regions of the Argentinean, Brazilian and Uruguayan Pampas was studied using the wheat-disease model**.** Using daily weather data from a 50-year period and assuming non-limiting inoculum conditions as input into the wheat-FHB model, our results showed that climate at Passo Fundo, Brazil, for example, was very favorable for FHB outbreaks after 1990 following a period of less favorableness for epidemics in the 1970s and 1980s, when outbreaks were very sporadic. Outbreaks were more frequent in El Niño than in La Niña years, especially for later planting dates in a crop year. These results suggest that, considering a fixed amount of inoculum, a changing climate (especially wetter) was associated with a higher frequency of outbreaks especially for the later plantings after the 1990s. Earlier sowing and use of early maturing varieties with a shorter reproductive period would be good strategies for Brazilian growers to avoid more favorable environment later in a season.

Second, the web-based platform has also the capability to forecast infection risks of FHB near real-time during the season. The system integrates hourly weather data collected in a network of weather stations and 5-days hourly forecast weather data generated by computer models. The user interacts with the system by selecting the nearest location and the crop heading date. The model then calculates risk of infections and warns the user whenever a risk level of concern is achieved, based on both actual and forecast weather. A user-friendly mapping interface is under development and weather data from other locations are being integrated into the system in order to generate regional risk maps, besides the site-specific predictions already available.

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SESSION 1:

FOOD SAFETY, TOXICOLOGY AND UTILIZATION OF MYCOTOXIN-CONTAMINATED GRAIN

Chairperson: Jim Pestka

QUANTIFICATION OF THE *TRI*5 GENE, EXPRESSION AND DEOXYNIVALENOL PRODUCTION DURING THE MALTING OF BARLEY.

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ABSTRACT

Barley quality and safety is affected by *Fusarium* both in the field and during post-harvest processes. *Fusarium* strains can survive, grow and produce mycotoxins during malting. We evaluated percentage of *Fusarium* infection (FI), and deoxynivalenol (DON) concentration in three, raw barley samples (high quality, naturally-infected, *F. graminearum* inoculated barley) during various stages of malting. We also applied real-time PCR and real-time reverse transcriptase PCR (real-time RT-PCR) methods to quantify the *Tri*5 DNA concentration and expression respectively in the barley samples. We observed that FI significantly ($P < 0.05$) increased during the germination stage of malting in all the barley samples. Temperatures of 49°C and higher during kilning reduced the FI in high quality barley samples, but for inoculated samples more than 60°C during kilning was needed to reduce *Fusarium* infection. The average *Tri*5 DNA was found to be in the range of 0 to 3.9 ng/50 mg, 0.06 to 109.79 ng/50 mg and 3.38 to 397.55 ng/50 mg in malted high quality, inoculated and infected barley samples respectively. Strong gene expression (*Tri*5) in naturally infected barley samples was found during 3rd day of germination, however very low amounts of gene expression were observed in high quality and inoculated barley samples during malting. Deoxynivalenol was found to be present even at high kilning temperatures as DON is heat stable. The average DON concentration was found to be in the range of 0 to 0.13 µg/g, 0 to1.09µg/g and 1.53 to 45.86 µg/g during malting in high quality, inoculated and infected barley samples respectively. Overall, the last two days of germination and initial stages of kilning were peak processing stages for FI, *Tri*5 gene production, *Tri*5 gene expression and DON production.

HIGH-THROUGHPUT HOMOGENIZATION OF GRAIN SAMPLES FOR DON TESTING.

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ABSTRACT

Concerns about deoxynivalenol (DON) continue to mount, and there is a growing need to develop new tools and techniques to enhance the speed, capacity, and uniformity of DON testing services in the United States. Tens of thousands of wheat and barley samples associated with USWBSI research projects are processed by DON testing labs every year. Many of these samples consist of 100 g kernel lots, and they must be cleaned, milled, and sieved before DON is extracted and quantified. The processing of a high number of grain samples in such a manner is extremely laborious and costly. We developed a rapid and affordable high-throughput homogenization protocol for DON testing that can homogenize twelve grain samples weighing from 0.1 to 2.5 g in as little as ten seconds. A Biospec MiniBeadBeater-96 ™operating at 2100 oscillations per minute was used to homogenize grain samples in individual 7 mL HDPE vials containing 13.7 mm chrome balls. Grain samples were homogenized into a fine flour of nearly uniform particle size, and DON extractions were conducted in the same vials that were used for the homogenization of the samples. The extraction solvent containing DON was passed through a clean-up column, and a measured fraction of the flow-through was dried down using a nitrogen evaporator at 55C. DON samples were derivatized using TMSI and quantified using a GC/MS operating in a SIM/SIM scan mode for target and reference ions of DON. Over 800 grain samples originating from single inoculated or non-inoculated (control) wheat spikes from southern uniform FHB greenhouse trials in AR, NC, and VA were processed for DON testing using this new methodology. High-throughput homogenization protocols may assist in providing affordable and timely DON testing services for USWBSI-associated research projects in the future.

ANALYSIS OF DEOXYNIVALENOL, MASKED DEOXYNIVALENOL AND *FUSARIUM GRAMINEARUM* PIGMENT IN GRAIN CULTURES USING A NEW LC-UV/MS METHOD.

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ABSTRACT

The presence of the mycotoxin deoxynivalenol (DON, 3,7,15-trihydroxy-12,13-epoxytrichothec-9-ene-8-one) in grains presents a food safety risk. Also, DON may conjugate with sugars resulting in masked mycotoxins such as deoxynivalenol-3-glucoside (DON-3-glucoside) which may be metabolized *in vivo* to DON thus increasing the risk. Such masked mycotoxins and the potentially toxic *Fusarium* pigment are not routinely analyzed in grains. To promote continued understanding of the presence of masked mycotoxins in grains and their coexistence with DON, we analyzed rice and wheat culture samples inoculated with different *Fusarium* strains, using a new liquid chromatography (LC) - mass spectrometry (MS) method. We also quantified the *Fusarium* pigment. Grain samples cultured for 14 days were extracted by centrifugation with methanol:methylene chloride (50/50, v/v) followed by cleanup through C18 columns. Elution solvents included methanol, water, acetonitrile and acetic acid before analysis using LC-UV/MS. An isocratic mobile phase (70% methanol) was used. The DON average in rice culture was 15.2 (± 41.0) mg/kg while DON-3-glucoside averaged 5.4 (± 8.6) mg/kg in rice. Neither DON nor DON-3-glucoside were observed in wheat culture samples. The pigment averaged 142.2 ±256.3 mg/kg in wheat and 7.1±126 mg/kg in rice culture samples. Therefore, we report here how analytical tools such as this new LC-UV/MS method can be used to quantify masked and parent mycotoxins in rice plus potentially toxic pigment in rice and wheat culture for risk assessment studies. The coexistence of DON with DON-3-glucoside in grain cultures such as rice spiked with *Fusarium graminearum* emphasizes the conjugation of DON to form masked mycotoxins hence the need to regularly analyze grains for both parent and masked mycotoxins. Such studies can be optimized to further explore ways of producing and isolating masked mycotoxins in culture since standard masked mycotoxins are not commercially available. Potentially toxic pigments can also be studied further.

PRODUCTION AND PURIFICATION OF DEOXYNIVALENOL FROM RICE CULTURE AND ANALYSIS USING LIQUID CHROMATOGRAPHY-ULTRAVIOLET-MASS SPECTROMETRY.

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ABSTRACT

Mycotoxins such as trichothecenes represented by deoxynivalenol (DON) present a major global food safety problem with associated economic implications. Thus toxicological, analytical and detoxification studies relevant to industry and regulatory agencies are priority. Such studies require relatively large quantities of pure mycotoxins such as DON. Here we present a new method were we produced and purified (96-99%) large quantities of DON (403 μg/g) from rice (85g) inoculated with *Fusarium graminearum* ($3x10^7$ spores per ml) and incubated for 4 days at 30 °C. Purification was achieved using a combination of silica gel (32 g), alumina (7.2 g) and celite (4.8 g) in a glass column. This followed extraction by high speed centrifugation using methanol:methylene chloride (50/50, v/v). High recovery rates (100 ± 9.9%; CV=0.1) were also recorded. Elution of the mycotoxin was done using methanol, acetonitrile, water and acetic acid (60:30:10:0.1, v/v). Analysis of DON was done using thin layer chromatography and liquid chromatography connected to ultraviolet and mass spectrometer detectors. These findings present a new method to culture and purify DON in bulk using relatively cheaper clean up column followed by analysis using chromatographic and mass spectrometric techniques. Such findings also support toxicological, analytical and detoxification studies pertaining DON.

DEOXYNIVALENOL MEASUREMENT: SOURCES OF ERROR AND SAMPLING RECOMMENDATIONS.

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ABSTRACT

For any type of analysis there is measurement error that is composed of various factors such as sampling, sample preparation, and the analytical instrument itself. In the determination of deoxynivalenol (DON) by gas chromatography (GC), sample preparation steps include grinding, extraction, clean-up and derivatization. Factors influencing derivatization are probably most critical, and have greater influence than instrument variability. Inter-laboratory check samples of ground wheat and barley are periodically analyzed by the USWBSI funded laboratories as a means of assuring consistency of results. The coefficients of variation (CV) on these analyses typically range from 5 to 15%, which is considered acceptable for analytical work in the mg/kg (ppm) range. As an example, the expected analytical range in DON results with a CV of 10% for a sample at 1.00 mg/kg would be 0.89- 1.11 mg/kg. However, it must be remembered that much of the variability observed in DON levels in grain is related to the biology of the disease, rather than the chemical analysis. This follows as DON accumulation in grain results from a complex host-pathogen interaction which is subject to environmental variability. The production of DON, like visible symptoms of FHB, varies greatly from spikelet to spikelet, spike to spike, and environment to environment. Grain sampling greatly affects the accuracy of DON analysis, and the responsibility of providing a representative sample rests with the individual researcher. Sampling considerations include the collection of a representative samples from the experimental unit (plot, field), and then the reduction of this material to a representative sub-sample with a sample divider. The sample provided to the DON analysis laboratories should be no more than 100 g, and ideally around 20 g. This follows, as the grinding of 10,000 100g samples, as opposed to 20 g samples, requires an additional 21 days of labor. USWBSI recommendations on the sampling of grain for DON are posted at http://www.scabusa.org/pdfs/ptt/researchers_grain-sampling-protocols.pdf

DEVELOPMENT OF A MULTIPLEX REAL-TIME PCR ASSAY FOR RAPID DETECTION AND QUANTIFICATION OF *FUSARIUM* SPP. IN BARLEY.

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ABSTRACT

The persistence of trichothecenes in *Fusarium*-infected stored grains and in processed food poses a great risk to human health and animals. The ability to rapidly detect *Fusarium* species and monitor their distribution in collected wheat and barley grains across the state of ND is important due to the significant number of grain samples and the differences in the toxicity of these secondary metabolites. This can be accomplished rapidly using a polymerase chain reaction (PCR)-based detection of FHB-associated *Fusarium* species. Our objective is to develop a multiplex real-time PCR assay to identify and quantify pathogenic *Fusarium* species based on primer pairs derived from Intergenic Spacer (IGS) of rDNA unit sequences. The selected primers for species-specific detection showed amplification products of 123, 418, 462, 293 and 186 bp using positive controls (template DNA) which were derived from *F. graminearum* (NRRL R-6574), *F. avenaceum* (FRC# R-04608), *F. poae* (FRC# T-0487) *and F. sporotrichioides* (FRC# T-0348), respectively. Multiplexing (3 to 4-species) resulted in amplifications for species-specific detection using naturally-infected malting barley, Robust, with 0 and 2 ppm DON levels. Five picograms of fungal DNA were found to be enough to obtain a visible amplification product. For reliability, the multiplex real-time PCR assay will also test several isolates of each *Fusarium* spp. This high-throughput assay will help screen the malting barley samples and accurately assess the distribution of *Fusarium* spp. which are predominant in the region. Malting barley collections in 2005-2006 from 3 ND districts (and 1 MN district) will be utilized for future assays.

OZONE AS AN ANTIMYCOTIC AGENT IN MALTING BARLEY.

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ABSTRACT

Fusarium spp. are known producers of important mycotoxins such as trichothecenes. The persistence of trichothecenes in infected stored barley grains and in processed food poses a great risk to human health and concern in the malting and brewing industry. At present, the only available effective control is testing and diversion or dilution. The effectiveness of ozone as an insecticidal fumigant in stored grains has been reported previously. The objective of this project is to evaluate the efficacy of gaseous ozone as an antifungal and antimycotoxin treatment for barley. Preliminary tests by gaseous ozone treatment (GOT) of pure *Fusarium graminearum* (FRC# R-06574) culture at 26 mg/g O_3 for 90 min. on the broth surface showed a detrimental effect on the morphological structure (non-branching and breakage of hyphae) and a 30% decrease in fungal biomass. The fungus was not totally eliminated probably due to the presence of sugar and nutrients in the broth allowing recovery and slow growth. In the present study, an extended treatment of 120 min at 26 mg/g O_3 were tested on five *Fusarium* spp. (*F. graminearum, F. culmorum, F. poae, F. sporotrichioides* and *F. avenaceum*) separately grown in PD broth. The same treatment was applied to malting barley before steeping and after 2 or 8 hrs steeping through a submerged gas sparger. We report on the effect of ozone on the growth and survival (FS) of five *Fusarium* spp., germinative energy (GE) and DON in malting barley.

ECONOMIC PERSPECTIVES OF GROWERS FACING THE CHALLENGES OF FHB AND DON.

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ABSTRACT

The economic problem of Fusarium Head Blight (FHB) and DON is extremely complex, yet can provide insight into how to focus research and development to prevent these conditions. We start with the guiding question: How can the economic perspective of wheat and barley growers help us decide how we should focus our research and initiatives? This talk describes four categories of economic consideration: 1) the sources of uncertainty and variability in wheat and barley production, FHB, and DON; 2) the cost-effectiveness of various control methods to reduce FHB and DON; 3) benefit-cost analysis of adopting FHB/DON control methods; and 4) the tradeoffs for growers planning to plant wheat or barley compared with competing crops such as corn.

SEX DIFFERENCES IN APPARENT ADAPTATION TO IMMUNOTOXICITY OF DEOXYNIVALENOL.

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ABSTRACT

Deoxynivalenol (DON) is a mycotoxin produced by *Fusarium graminearum* and *F. culmorum* commonly found in grains. We hypothesized that DON was immunotoxic in BALB/c mice, suppressing peripheral blood lymphocytes at 1 ppm but not at lesser doses.

Groups of 10 female and 10 male BALB/c mice were fed DON at 0, 0.25, 0.5, 1, and 2 ppm over 14 days and 28 days. Peripheral blood and single spleen cells suspension were stained with fluorescently labeled antibodies for CD4, CD19, CD8a and CD11b leukocyte cell surface markers. Flow cytometry was used to detect leukocyte phenotypes.

In peripheral blood, the percentage of T cytotoxic and B cells were inhibited in both sexes of BALB/c mice after 14 days of DON exposure, and toxic dose of DON varied by sex, whereas exposure to DON over 28 days did not inhibit these lymphocytes, compared with the control diet. Dietary DON did not influence hematology in males but red blood cells (RBC) at 0.5 and 1 ppm DON and hemoglobin (Hb) at all DON doses were suppressed in female mice by dietary DON over 14 days. The inhibition of RBCs by DON disappeared after 28 days compared with the control diet. The percentage of monocytes (CD11b+) was decreased in peripheral blood (at doses of 0.5-2 ppm DON) and spleen (2 ppm DON) only in BALB/c female mice after 28 days compared with control diet.

These results indicate that BALB/c mice adapted to most signs of DON immunotoxicity and hematotoxicity after 28 days. At this time, the percentage of monocytes was decreased in peripheral blood and spleen by as little as 0.5 ppm DON in female mice, suggesting that female sex hormones potentiate DON immunotoxicity in BALB/c mice.

ACKNOWLEDGEMENT AND DISCLAIMER

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DOEHLERT MATRIX DESIGN FOR OPTIMIZATION OF THE DETERMINATION OF BOUND DEOXYNIVALENOL IN BARLEY GRAIN WITH TFA.

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ABSTRACT

Fusarium Head Blight (FHB) is an impediment to barley production in many regions of the world. Tricothecene toxins, associated with FHB infected grain, particularly deoxynivalenol (DON) pose a serious threat to human and animal health. Recent research has suggested that a portion of the DON present on grain is bound and escapes detection through conventional determination. The objective of this study was to optimize a method for determination of non-extractable DON in barley grain using TFA. A Doehlert matrix design was performed to determine the optimal conditions for time, temperature and TFA concentration. These conditions were treatment with 1.25 N TFA in 86:14 acetontrile:water for 54 min at 133°C. Clean-up, derivatization and determination of DON by GC-ECD was as normal. Treatment of the test sample resulted in release of an additional 58% DON under the optimized conditions, and an increase of 9 to 88% in a set of verification samples.

EFFECT OF ENZYME PRETREATMENTS ON THE DETERMINATION OF DEOXYNIVALENOL IN BARLEY.

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ABSTRACT

The impact of particle size and enzymatic treatment was determined on the quantification of deoxynivalenol (DON) in FHB-infected barley samples. Particle size significantly affected DON determination in eight of ten samples analyzed. Significance of the barley sample x particle size interaction demonstrated that samples did not respond uniformly to the different particle sizes in terms of DON. The fine-grind samples often yielded higher results than medium and coarse grinds. This trend was most pronounced in samples with higher DON content. Enzyme treatments involved either amylolytic (alpha-amylase/amyloglucosidase), proteolytic (papain) or cell wall degrading (cellulase/xylanase) enzymes. The interaction between barley sample and enzyme treatment was significant, meaning that samples did respond uniformly to the three treatments. Papain treatment resulted in significant increases (16 to 28%) in the amount of DON detected in five of the seven samples tested when compared to the untreated samples or enzyme controls. Treatment with cellulase/xylanase resulted in increased DON in three of the seven samples, while amylase/amyloglucosidase resulted in increased DON in only a single sample. The results strongly indicated that FHB infected barley samples can contain bound DON which might not be determined in the routine quantification, but can be released by proteolytic or cell wall degrading activity.

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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This material is based upon work supported by the U.S. Department of Agriculture. This is a cooperative project with the U.S. Wheat and Barley Scab Initiative. Any opinions, findings, conclusions, or recommendations expressed are those of the authors and do not necessarily reflect views of the U.S. Department of Agriculture.

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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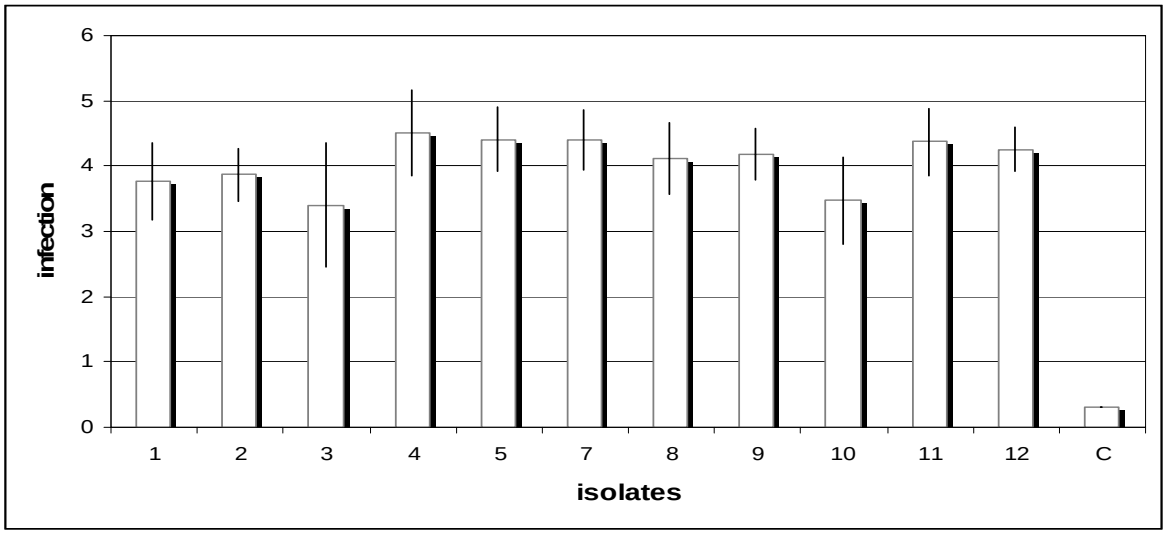

Figure 1. Degree of infection of seedlings of wheat cv.Torysa by a water control (C) and isolates 1-12 of *F .graminearum* Schwabe.

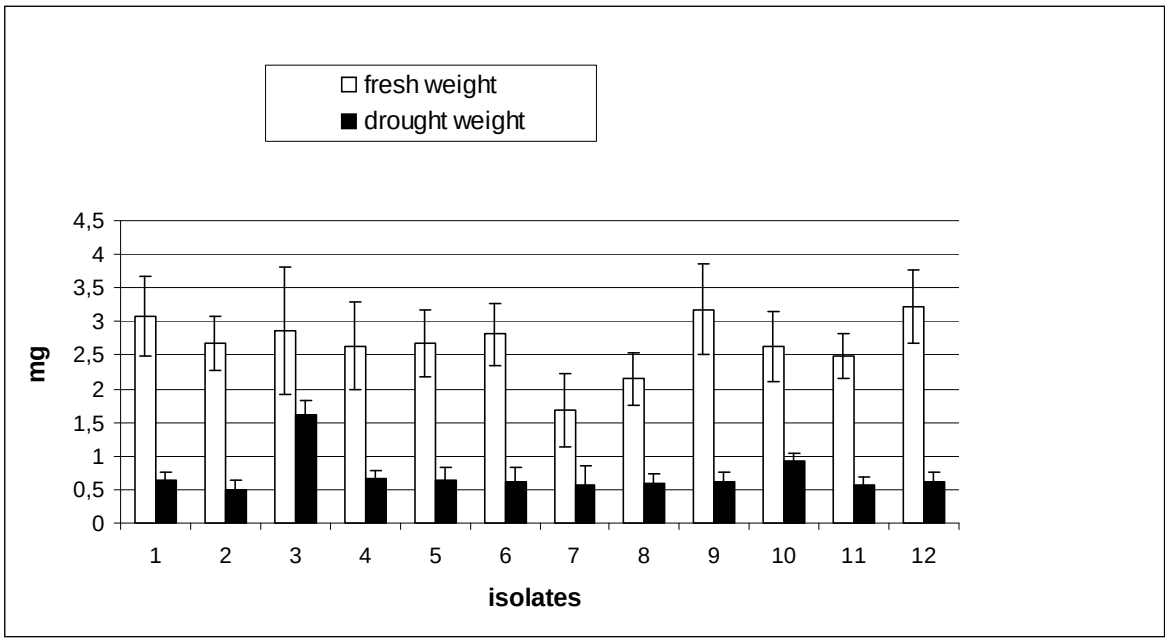

Figure 2. Fresh and dry weights of seedlings of cv. Torysa inoculated with *F. graminearum* isolates.

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.

SESSION 3:

GENE DISCOVERY AND ENGINEERING RESISTANCE

Chairperson: Blake Cooper

STUDIES ON BARLEY SPIKES TREATED WITH THE TRICHOTHECENE, DEOXYNIVALENOL: INSIGHT INTO BARLEY-*FUSARIUM GRAMINEARUM* INTERACTION.

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ABSTRACT

Fusarium head blight (FHB) of barley and wheat is a difficult disease to manage because of the complexity of the interactions. A serious problem associated with FHB is the accumulation of trichothecene mycotoxins such as deoxynivalenol (DON). Trichothecenes increase the virulence of the pathogen and reduce grain quality. A primary objective in our laboratory is to identify genes that reduce the impact of trichothecenes. Our laboratory has identified approximately 700 barley transcripts that respond to the invading pathogen and pathogen-derived trichothecenes. In an effort to further understand the barley-*F. graminearum* interaction, a subset of 54 genes encoding transcription factors, regulatory proteins, UDP-glucosyltransferases, cytochrome-P450s, and proteins participating in ubiquitination and cell death were selected and tested for their response to DON treatment compared to mock water inoculation at 1, 6 and 12 hours after inoculation (hai). Twenty-one transcripts showed a qualitative response and 28 transcripts showed quantitative response to DON treatment. Seven of the qualitatively responding genes responded by 1 hai, while 14 genes responded by 6 hai. All the quantitatively responding genes showed differential expression from 1 hai through 12 hai. To develop markers for mapping and other genetic studies, some of these genes were sequenced from barley mapping population parents and genotypes exhibiting FHB resistance and susceptibility. In separate experiments, the fate of DON *in planta* was tested. In barley spikes treated with DON, over 30.0% was converted to DON-3-O-glucoside. In addition, our preliminary experiments show a cell death-like phenotype on DON-treated barley leaves progressed in a distal manner, indicating that either DON or the signal transduction induced by DON traveled to the tip of the treated leaves.

EXPRESSION OF A TRUNCATED FORM OF RIBOSOMAL PROTEIN L3 IN TRANSGENIC WHEAT CONFERS RESISTANCE TO DEOXYNIVALENOL AND FUSARIUM HEAD BLIGHT.

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ABSTRACT

DON belongs to the group of trichothecene toxins, which target ribosomal protein L3 at the peptidyltransferase site of eukaryotic ribosomes and inhibit protein synthesis. The goal of our work is to identify mutations in L3 that confer resistance to DON and to determine if FHB resistance can be engineered in transgenic wheat plants by expressing DON resistant L3 genes. In previous studies, we have demonstrated that overexpression of a truncated form of yeast ribosomal protein L3 (L3Δ) in transgenic tobacco plants confers resistance to deoxynivalenol (DON). To determine if expression of the yeast L3Δ in transgenic wheat plants would provide resistance to FHB, the susceptible spring wheat cultivar, Bobwhite was transformed with the yeast L3Δ under the control of the barley floret-specific *Lem1* or the maize constitutive *Ubi1* promoter. Three homozygous *Lem1*::yeast L3Δ lines (771, 772 and 773) and two homozygous *Ubi1*::yeast L3Δ lines (8133 and 8153) were evaluated for resistance to FHB in greenhouse tests. The disease severity was reduced by 48-56% in four different transgenic wheat lines compared to the untransformed Bobwhite plants. The reduction in disease severity correlated well with the level of expression of L3Δ mRNA. These results demonstrated that transgenic wheat plants expressing the yeast L3Δ showed improved resistance to FHB over the untransformed Bobwhite plants. To determine if resistance to FHB would result in a reduction in DON levels, the mature kernels above and below the inoculated spikelets were analyzed for DON levels. There was a 63-76% reduction in DON levels in the four different FHB resistant transgenic lines. The DON levels in one transgenic line were lower than the DON levels in the resistant line, Alsen. These results provided evidence that resistance to DON correlates with resistance to FHB and results in reduced accumulation of DON in transgenic wheat plants. We have identified four more homozygouns lines containing *Lem1*::yeast L3Δ, eight more homozygous lines containing *Ubi1*::yeast L3 and four new homozygous lines containing *Lem1*::yeast L3, which will be evaluated for resistance to FHB. The wheat *RPL3A1* and *RPL3B3* genes were cloned and wheat expression vectors were constructed with the L3Δ versions of these genes. Point mutations that confer a high degree of resistance to DON were introduced into the wheat *RPL3A1*. We have generated transgenic wheat plants containing the DON resistant forms of the wheat L3 genes to determine if their expression will lead to a higher level of resistance to FHB and a greater reduction in DON accumulation.

INHIBITION OF *FUSARIUM GRAMINEARUM* GERMLING DEVELOPMENT CAUSED BY COMBINATORIALLY SELECTED DEFENSE PEPTIDES.

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ABSTRACT

To address the problem of head blight in wheat, we are applying combinatorial peptide techniques to identify molecules that serve as antagonists to developing germlings of *Fusarium graminearum*. In this methodology, we mixed phage-display libraries that display 8-mer random peptides with *F. graminearum* germlings derived from macroconidia. Phage clones with binding affinity for germlings were recovered and amplified in *E. coli*. After additional rounds of incubation and amplification, we have recovered numerous peptides with affinity for surface molecules of germlings. We have sequenced an initial collection of selected peptides and are now evaluating their abilities to inhibit germling growth and development. At completion of these phenotype screens, we will test candidate peptides for inhibitory function when displayed on recently developed scaffold proteins.

TRANSGENIC WHEAT EXPRESSING ANTIFUNGAL PLANT DEFENSIN MTDEF4 IS RESISTANT TO FUSARIUM HEAD BLIGHT (FHB).

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ABSTRACT

Plant defensin MtDef4 from *Medicago truncatula* is a potent inhibitor of *F. graminearum in vitr*o. Transgenic wheat lines expressing MtDef4 were generated using *Agrobacterium tumefaciens*-mediated transformation of spring wheat cultivar Bobwhite and a Chinese cultivar Xin Chun 9 (XC9). Single floret inoculation method was used to evaluate Type II resistance of these transgenics in the greenhouse. Of the two lines tested thus far, one Bobwhite transgenic line expressing MtDef4 has reduced FHB severity when compared to nontransgenic Bobwhite. The level of resistance in this line is similar to that of FHB resistant cultivar Alsen. Two more transgenic lines are being evaluated for Type II resistance in the greenhouse. The results of this study will be presented.

REDUCING DON POTENTIAL IN VIRGINIA HULLESS BARLEY LINES THROUGH GENETIC ENGINEERING.

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ABSTRACT

Hulless barley (HLSB) is a new and emerging crop in Virginia, and may be an important source of biofuels in the future. Dried distillers grains with solubles (DDGS), a byproduct of ethanol fermentation, are rapidly becoming one of the main sources of feed for domestic animals. Traditional ethanol production may concentrate trichothecene mycotoxins such as deoxynivalenol (DON) in DDGS, posing a significant threat to domestic animal health. Our work aims to genetically engineer Virginia HLSB lines with reduced DON potential and thus provide a safe supply of DDGS for animal feed. In 2006 and 2007, we determined the DON potential of 20 Virginia HLSB lines; a number of these lines demonstrated low levels of DON in both years. We generated callus from 17 HLSB lines, and five of the lines were selected for further tissue culturing analyses and genetic transformation. We amplified *TRI101*, a gene encoding a 3-O-acetyltransferase responsible for the conversion of DON to 3-acetyl-DON, from four different species of *Fusarium*. Preliminary expression studies using the yeast expression vector pYES2.1 suggested that these genes differ in their relative ability to reduce DON *in vitro*. We are currently developing an *Agrobacterium* transformation vector to move *TRI101* into five selected HLSB lines, and we plan to monitor potential decreases in DON in both raw grain and DDGS following ethanol production using our genetically-engineered lines. We are currently exploring the function of additional genes that may detoxify DON (e.g., orthologs of *TRI101* in *Arabidopsis*), and we hope to harness the potential of these genes to enhance food safety and security in the eastern U.S.

ENHANCING FUSARIUM HEAD BLIGHT RESISTANCE IN WHEAT BY MANIPULATING MECHANISMS CONTRIBUTING TO HOST RESISTANCE AND SUSCEPTIBILITY.

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ABSTRACT

Fusarium head blight (scab) caused by the fungal pathogen, *Fusarium graminearum* is a serious menace in wheat and barley, severely limiting crop productivity and quality. Previously, we had demonstrated that ectopic expression of the *Arabidopsis thaliana* At*NPR1* gene, which is a key regulator of salicylic acid (SA) signaling, enhanced FHB resistance in the hexaploid wheat cv. Bobwhite (Makandar *et al*. 2006). Similarly, FHB resistance is enhanced in transgenic At*NPR1* expressing durum cvs. Ben, Maier and Belzer. Three field trials to monitor the impact of At*NPR1* on FHB have been completed. Results of these trials will be presented.

Genetic studies in *Arabidopsis thaliana* demonstrate that SA has an important role in plant resistance against *F. graminearum*. Pretreatment with SA enhances FHB resistance in wheat, also. Furthermore, SA accumulation in spikes correlates with FHB resistance in wheat. SA levels increase >200% in the fungus inoculated and distal spikelets of the resistant cv. Sumai-3, within 24 h of point inoculation with *F. graminearum* macroconidia. In contrast, a similar increase in SA content was not observed in the cv. Bobwhite, suggesting that SA accumulation can be targeted to enhance FHB resistance. Indeed, resistance against *F. graminearum* is enhanced in *Arabidopsis* plants that constitutively express the At*PAD4* gene, which modulates SA synthesis and signaling. We have initiated experiments to ectopically express At*PAD4* from the maize Ubi1 promoter in transgenic wheat. In addition, we have generated transgenic wheat plants that express a salicylate hydroxylase encoded by the bacterial *nahG* gene, to further test the involvement of SA in wheat defense against *F. graminearum*.

In contrast to SA, our experiments in wheat and *Arabidopsis* indicate that jasmonic acid (JA) accumulation and the activation of JA signaling inversely correlates with resistance to *F. graminearum*, suggesting that JA or a related oxidized lipid (oxylipin) may be a susceptibility factor. Indeed, in *Arabidopsis* a lipoxygenase involved in oxylipin synthesis contributes to susceptibility to *F. graminearum*. Thus, oxylipin synthesis could provide another target to control FHB. In *Arabidopsis*, JA antagonizes SA accumulation. Similarly, JA accumulation could result in the suppression of SA accumulation in the spikes of FHB susceptible wheat cultivars. Alternatively, as was recently shown in maize (Gao *et al*., 2007), JA or a related oxylipin may contribute to fungal development, thereby contributing to susceptibility.

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ings, 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.

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ENGINEERING BARLEY WITH *GASTRODIANIN* FOR IMPROVED RESISTANCE TO FUSARIUM HEAD BLIGHT.

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OBJECTIVES

Develop transgenic barley lines expressing the anti-fungal gene *gastrodianin* for resistance to Fusarium head blight (FHB).

INTRODUCTION

Control of Fusarium head blight (FHB) infection in barley remains difficult because of lack of genetic resistance. One strategy that has great potential to reduce FHB infection is introduction of anti-*Fusarium* genes into barley through genetic engineering. Unfortunately, engineering resistance has been slow since common pathogenesis-related (PR) proteins are not effective against *Fusarium graminearum*. Transgenic wheat over-expressing combinations of chitinases, glucanases, and thaumatin-like proteins (TLPs) had partial resistance to FHB in green house testing. However, the greenhouse results were not reproducible under field conditions and no resistance was observed (Anand *et al*. 2003). Apparently, genes known to specifically inhibit *F. graminearum* are required to give adequate protection from FHB. We have developed barley lines expressing anti-*Fusarium* gene *gastrodianin* for resistance to FHB.

Gastrodianin is an anti-fungal gene isolated from a traditional Chinese herb, *Gastrodia elata*. *G. elata* is devoid of chlorophyll and leads a parasitic life on the fungus *Armillaria mellea*. *A. mellea* hyphae usually infect the nutritive corms of *G. elata* but are digested in the cortical cells. The released nutrients are used by the host plant for growth and development. Expression of *gastrodianin* and other anti-fungal proteins protects the developing terminal corm from infection by *A. mellea* (Wang *et al*., 2007; Sa *et al*., 2003). *Gastrodianin* is a non-agglutinating, monomeric, mannose and chitin-binding lectin that belongs to the superfamily of monocot mannose-specific lectins (Liu *et al*., 2005; Wang *et al*., 2001). *Gastrodianin* effectively inhibits hyphal growth of pathogenic and saprophytic fungi including *Gibberella zeae, Armillaria mellea, , Rhizoctonia solani, Trichoderma viride* and *Valsa ambiens in vitro* (Wang *et al*., 2001). *In vivo* studies have also demonstrated the importance of *gastrodianin* in fighting pathogens. In transgenic tobacco, *gastrodianin* reduces root diseases caused by fungal pathogens *Rhizoctonia solani* and *Phytophthora nicotianae* (Cox *et al*., 2006). In cotton, field tests showed that transgenic plants expressing *gastrodianin* are resistant to another fungal pathogen *Verticillium* wilt (Wang *et al*., 2004). *Gastrodianin* maintains inhibitory properties at fluctuating temperatures (Wang *et al*., 2001, Xu *et al*., 1998). This stability and its inhibitory effects on *G. zeae* makes *gastrodianin* protein an attractive candidate for engineering resistance to fungal diseases.

MATERIALS AND METHODS

Expression vectors

Plasmids used for transformation are shown in *Fig*. 1. There are four *gastrodianin* genes in *G. elata* differing only by 3 to 4 nucleotides (Wang *et al*., 1999). In this study the variant VGM was used (GeneBank Accession AJ277785). *Gastrodianin* has 513 nucleotides and encodes a polypeptide with 171 amino acids. The coding region was amplified by PCR from a binary vector generously provided by the Plant Biology Institute, University of Ghent, Belgium. The PCR fragment was digested with restriction enzymes (*Eco*RV and *Pst*I) and fused to a *Lem2* promoter (Abebe *et*

al., 2005). The resulting fragment was ligated to pLem2gfp (Abebe *et al*, 2005) to get pLem2VGM2 (Fig. 1). Plasmid pLem2VGM2 also contains *gfp* under the control of the *Lem2* promoter, making visual screening of transformed plants easier.

Transformation of barley

Barley plants (*Hordeum vulgare* cv. Golden Promise) were transformed as described in Wang and Lemaux (1994) with minor modification. Immature kernels (approximately 14 days post-anthesis) were surface sterilized with 70% ethanol (v/v) and 20% chlorox (v/v). After three washes with sterile water, embryos were cut in half longitudinally and placed on callus induction medium (CIM), scutellum side down. After 3–5 days of incubation, embryos were bombarded with gold particles (0.6 µm) coated with an equimolar amounts of plasmids pLem2VGM2 and pAHC25 (contains the *bar*) using the He-driven PDS 1000 (Bio-Rad). The herbicide bialaphos was used to select transgenic calli and plantlets.

Characterization of transgenic plants

Integration of *gastrodianin* into the genome of transgenic barley was verified by PCR. Genomic DNA was isolated from wild type and transgenic barley plants using CTAB (Murray and Thompson, 1980). PCR was performed using 100 ng of genomic DNA, along with upstream and downstream VGM primers.

RESULTS AND DISCUSSION

We have recovered plants from 16 transformation events. Plants from four events were sterile, plants from two events were lost to fungal contamination and the remaining ten plants produced seeds. At least two plants were regenerated from each transformation event. Recovery of T_0 plants from tissue culture was significantly improved by visual screening of *gfp* expression. Both *gfp* and *gastrodianin* were placed in the same pLem2VGM2 plasmid (Fig. 1). By screening for *gfp* expression we were able to indirectly select plants incorporating *gasrodianin* in their genome. The *lem2* gene promoter directs tissue-specific expression (Abebe *et al.*, 2005). Visual screening of transgenic plants was performed by inspecting tissue-specific fluorescence of the GFP protein in the spike and auricle.

The sterile plants were bushy in appearance (had many tillers) and had thin stems and spikes (Fig. 2). We are screening plants for accumulation of the *gastrodianin* protein using western blotting and ELISA. It will be interesting to see if the phenotypes observed in sterile plants are due to high accumulation of the *gastrodianin* protein, disruption of spike-specific genes or somaclonal variations introduced during tissue culture.

To verify integration of *gastrodianin* in the genome, we screened some transgenic plants expressing *gfp* by PCR. This showed that all the plants expressing *gfp* also had the expected 0.5 kb *gastrodianin* insert (Fig. 3). In the next phase of the study, resistance of transgenic plants to *F. graminearum* will be tested under greenhouse conditions using T_1 and T_2 generations.

AKNOWLEDGEMENT

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DISCLAIMER

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.

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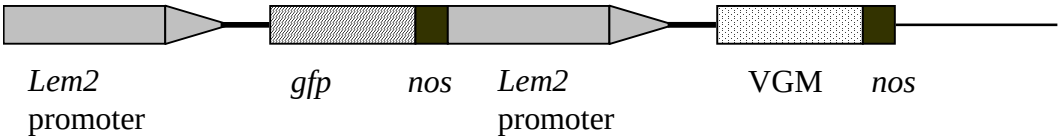
pLem2VGM2 (7.9 kb)



pAHC25 (9.7 kb)

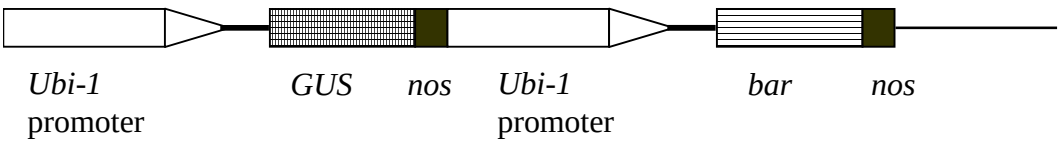


Fig.1. Map of plasmids used for transformation. Plasmid pLem2VGM2 contains *gfp* and *gastrodianin* (VGM) driven by the Lem2 promoter. Plasmid pAHC25 contains the *bar* gene for selection. It also contains the *GUS* reporter gene.

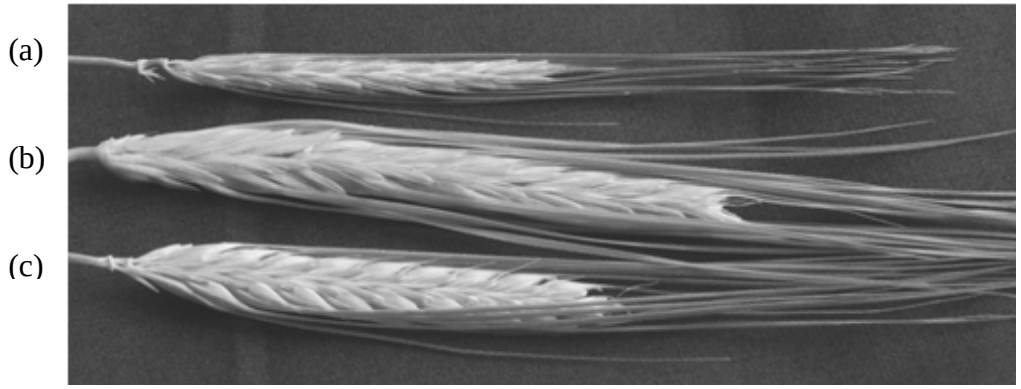


Fig.2. Phenotype of T_0 plants. Sterile T_0 plants (a) have very thin spikes compared to fertile T_0 (b) and no-transgenic (c) Golden promise plants.

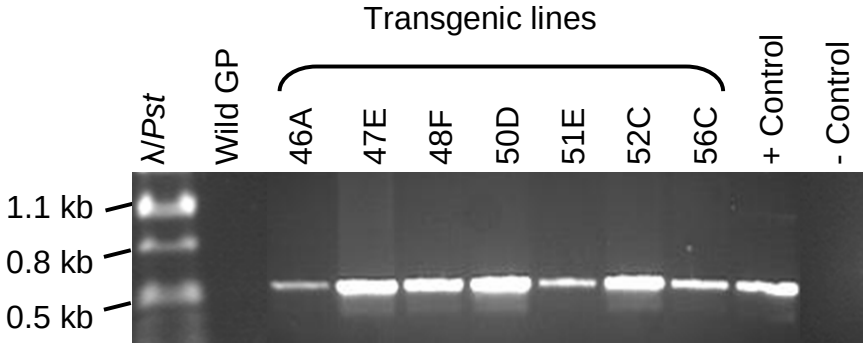


Fig.3. PCR showing integration of the *gastrodianin* gene in the genome of Golden Promise (GP) barley. Positive control (+) for the PCR was plasmid pLem2VGM2 DNA and negative control (-) was water.

GENES THAT CONFER RESISTANCE TO *FUSARIUM*.

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ABSTRACT

There is a pressing need for sources of germplasm or genes that are effective against *Fusarium graminearum*, the causal agent of Fusarium Head Blight (FHB) on wheat and barley. Since sources of resistance from wheat and barley are limited, we have developed a functional assay system to evaluate genes from other sources for their efficacy against FHB. The assay system is based on the plant *Physcomitrella patens*, which serves as a 'green yeast' for the rapid evaluation of novel genes. This plant, uniquely, allows the contribution of individual genes to be assessed through either the creation of targeted genes knockouts or through the introduction and overexpression of transgenes. Importantly, the wild type plant is fully susceptible to *F. graminearum* and highly sensitive to mycotoxins, including DON.

We have used this system to characterize genes that confer effective and robust resistance to FHB. The first set of genes acts through the plant programmed cell death pathway. Plants that contain knockouts for these genes are completely insensitive to DON and fully resistant to FHB. A similar level of FHB resistance can be conferred by overexpressing genes that suppress plant cell death. In these plants, FHB resistance is conferred by disabling a host susceptibility pathway (cell death) induced by mycotoxins.

A second set of genes confers FHB resistance through a pathway that is independent of cell death. These plants, which overexpress nuclease genes, are still sensitive to DON and other mycotoxins, yet display significant resistance to FHB. One explanation, indirectly supported by our studies, is that the overexpressed protein is itself directly antifungal. In these plants, FHB resistance is conferred by enhancing existing plant defense mechanisms.

A third set of genes that confer FHB resistance is associated with stress management, and in particular the response to reactive oxygen species (ROS), which are associated with the response to pathogen attack. Knockout and overexpressing lines for different genes associated with this response show enhanced resistance to both DON and to FHB but through a mechanism that acts downstream of the cell death pathway.

These results show that FHB resistance can be introduced by manipulating a variety of cellular targets. By combining these approaches it should be possible to introduce an enduring FHB resistance into wheat and barley plants. The efficacy of these FHB-resistance genes in wheat is currently being tested by the Scofield lab using a VIGS-based assay. This will provide an early indicator of likely performance in transgenic wheat.

ACKNOWLEDGEMENT AND DISCLAIMER

This material is based upon work supported by the U.S. Department of Agriculture, under Agreement No. 59-0790-6-063. 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 authors and do not necessarily reflect the view of the U.S. Department of Agriculture.

GENETIC STUDIES DEFINE DISTINCT PATHWAYS OF RESISTANCE TO FUSARIUM HEAD BLIGHT.

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ABSTRACT

We have used the *Physcomitrella patens* rapid assay system to characterize a number of genes for their ability to confer resistance to *Fusarium graminearum*, the causal agent of Fusarium head Blight (FHB). Using this approach we have screened several dozen genes for their ability to confer resistance to fungal mycotoxins and FHB. These studies have revealed that resistance to FHB can be achieved by manipulating multiple cellular pathways, including those involved in the regulation of programmed host cell death, the production and elimination of reactive oxygen species, the production of lytic enzymes and the expression of host defense responses.

These mutant plants show distinct patterns of susceptibility to FHB and to various *Fusarium*-derived mycotoxins, compared to wild type plants, which are fully susceptible to FHB and highly sensitive to DON and T-2 toxin. Plants that are mutated in the cell death pathway are highly resistant to FHB, and insensitive to DON and T-2 toxin. In contrast, plants that are mutated in the regulation of reactive oxygen species are highly resistant to FHB, insensitive to DON but partially sensitive to T-2 toxin. A further contrast is provided by plants that overexpress nuclease genes. These plants are resistant to FHB but fully sensitive to DON and T2-toxin. These results illustrate that different FHB-derived toxins target different cellular pathways, and suggest that robust resistance to FHB in the field may require the concerted manipulation of more than one cellular pathway.

Several of the genes we have manipulated are induced during the response to FHB inoculation. We tested whether these genes form part of a natural defense response by pre-treating plants with the defense response elicitor chitosan. Plants exposed to chitosan are highly resistant to subsequent inoculation with FHB. This indicates that *Physcomitrella* plants possess a natural and highly effective mechanism of induced FHB resistance. Presumably this response is suppressed during the interaction with *F. graminearum*. We will present data on this induced FHB-resistance response and discuss other approaches we have used to suppress virulence and enhance resistance to FHB in this system.

ACKNOWLEDGEMENT AND DISCLAIMER

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RAPID FUNCTIONAL IDENTIFICATION OF GENES CONTRIBUTING TO FHB RESISTANCE.

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ABSTRACT

This presentation will describe a new method being employed to rapidly identify genes that function in the Fusarium head blight (FHB) resistance mechanism of wheat. In this method, called virus-induced gene silencing (VIGS), genes thought to function in FHB resistance are switched-off, or silenced, and their role in FHB resistance is inferred if silencing results in resistant wheat plants becoming susceptible to FHB. This method utilizes the RNA virus, Barley stripe mosaic virus (BSMV), to activate RNA-mediated gene silencing in wheat. RNA-mediated gene silencing is an evolutionarily conserved defense mechanism in plants and animals that targets viral RNAs for sequence-specific degradation. In VIGS, the plant's RNA-based defense response is exploited to cause plant genes selected by the experimenter to be silenced by inserting a piece of the chosen plant gene into the viral RNA. In this way, the messenger RNA from the chosen plant gene is targeted for degradation, thus silencing the expression of the gene, as the plant defense mechanism works to degrade all the viral RNA. This approach has several important advantages: 1) As it is homology-dependent, it can simultaneously silence multiple copies of genes, which are almost always present in hexaploid wheat. Without this capability the expression of any closely related genes would prevent observation of the effects of silencing. 2) It is rapid; an experiment can be accomplished in as little as 2 months from identification of a candidate gene to observing the effect of its silencing. Examples of the utility of this important new method will be presented.

ENGINEERING RESISTANCE TO *FUSARIUM GRAMINEARUM* USING ANTIFUNGAL PLANT DEFENSINS.

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ABSTRACT

Small cysteine-rich plants defensins have potential as antifungal agents in transgenic crops. Two such defensins, MsDef1 and MtDef4, from *Medicago* spp., share only 41% amino acid sequence identity, but potently inhibit the growth of *Fusarium graminearum in vitro*. These two defensins exhibit different modes of antifungal action. Using the *Fusarium graminearum-Arabidopsis thaliana* pathosystem, we have found that over-expression of either MsDef1 or MtDef4 extracellularly or intracellularly (in the vacuole or endoplasmic reticulum) conferred strong resistance to this pathogen in transgenic *A. thaliana* plants. Transgenic plants exhibited reduced foliar symptoms and growth of fungal hyphae. Moreover, growth of the pathogen-challenged transgenic plants was similar to that of non-inoculated wild-type plants. Since *F. graminearum* colonizes host tissue by both inter- and intra-cellular growth, we will develop and test transgenic *A. thaliana* lines co-expressing extra- and intracellular defensins for more robust resistance to this pathogen. In parallel experiments, we have generated seven transgenic wheat lines over-expressing extracellular MtDef4. Of the two lines tested thus far, one line displayed improved FHB resistance when compared to non-transgenic Bobwhite. Furthermore, the level of resistance in this line was comparable to that of the disease resistant check, Alsen. No pleiotropic effects resulting from over-expression of defensins were observed in transgenic *A. thaliana* or wheat.

ENGINEERING SCAB RESISTANCE IN WHEAT WITH PLANT DEFENSE SIGNALING GENES.

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ABSTRACT

Fusarium graminearum is the principal causative agent of Fusarium Head Blight (FHB)/scab, a devastating disease of wheat and barley that severely limits crop productivity and grain quality. Our approach has been to utilize plant genes that regulate defense responses for enhancing FHB resistance in wheat. A plant-pathogen system consisting of *Arabidopsis thaliana* and *Fusarium graminearum* has been developed to identify genes involved in regulating plant defense against *F. graminearum*. The *Arabidopsis NPR1* (At*NPR1*) gene was one of the promising genes identified in this screen. NPR1 is a key regulator of salicylic acid (SA) signaling in plant defense. Our studies in *Arabidopsis* and wheat have indicated that SA is also an important regulator of defense against *F. graminearum*. Expression of At*NPR1* gene (At*NPR1*) was successfully engineered in the hexaploid wheat cultivar Bobwhite. In green house and growth chamber studies, At*NPR1* expression resulted in heightened FHB resistance in transgenic wheat. Furthermore, DON content was lower in the transgenic seeds. SA-regulated defense responses were turned on faster and to higher levels in the At*NPR1* expressing plants. Three field trials have been completed with At*NPR1* expressing transgenic Bobwhite plants. At*NPR1* expression has also been successfully engineered into the durum varieties Ben, Maier and Belzer. FHB evaluations of these transgenic plants are ongoing. Results on other promising genes identified in our *Arabidopsis-F. graminearum* screen and the status for engineering their expression in wheat will also be discussed.

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TRANSGENIC WHEAT WITH ENHANCED RESISTANCE TO FUSARIUM HEAD BLIGHT.

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ABSTRACT

We are developing and testing transgenic wheat for resistance to Fusarium Head Blight (FHB). We developed transgenic wheat carrying genes encoding chitinase, thaumatin-like protein 1 (tlp-1), ribosome-inactivating protein (RIP), lipid transfer protein (LTP), glutathione-S-transferase (GST), jasmonic acid inducible Myb transcription factor (JaMyb), germin-like protein1 (GLP1), and pathogenesis-related protein1 (PR1). Transgenic lines over-expressing these genes were generated using micro-projectile bombardment of the wheat cultivar 'Bobwhite'. Both single and combinations of transgenes were generated. We developed 4, 4, 2, 2, 1, and 4 lines carrying LTP, RIP, RIP/tlp-1, TRI101/tlp-1, TRI101/ß-1,3-glucanase, and tlp-1/ß-1,3-glucanase, respectively. In multiple greenhouse screens of these lines, we identified five lines (one RIP, two TRI 101/tlp-1, and two tlp-1/ß-1,3-glucanase) that exhibited statistically significant reductions in FHB severity compared to the non-transgenic controls ($p<0.05$). Combined with our previous greenhouse screens, we identified and evaluated 24 lines (seven chitinase, two RIP, two chitinase/tlp-1, one chitinase/RIP, six RIP/tlp-1, two TRI 101/tlp-1, two tlp-1/ß-1,3-glucanase, and two LTP) in field trials in 2005 and/or 2007. Three lines (two chitinase and one RIP) exhibited statistically significant reductions in FHB severity and very scabby kernels (VSK) compared to the non-transgenic control ($P<0.05$) in 2005 and 2007. In 2007, four lines (one TRI 101/tlp-1, two tlp-1/ß-1,3-glucanase, and one RIP) showed reduced FHB severity and five (two TRI 101/tlp-1, one tlp-1/ß-1,3-glucanase, two LTP) showed reduced VSK ($p<0.05$). We also crossed three transgenic wheat lines (two chitinase and one RIP), that exhibited statistically significant reductions in FHB severity in the field, to the type II resistant cv. Alsen. In addition, we developed 13, 10, 10, and 6 transgenic lines carrying GST, JaMyb, PR1, and GLP genes, respectively. Six lines (one GST, two JaMyb, and three GLP1) exhibited statistically significant reductions in FHB severity in compared to the non-transgenic Bobwhite in greenhouse screens ($p<0.05$).

COMPARATIVE ANALYSIS OF FHB QTLS IN THE MINI MANO/ FRONTANA AND FRONTANA/REMUS DH POPULATIONS.

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ABSTRACT

Fusarium head blight (FHB) is one of the most important diseases in the aspects of food safety and yield quality also. The most effective strategy for controlling FHB in wheat is through the development of resistant cultivars. This can be reached by analyzing QTLs, and using them in a marker-assisted selection.

Frontana is a Brazilian spring wheat cultivar that has small and medium effective QTLs. These types of QTLs are sensitive for the environmental factors and for the problems of heterogeneity.

220 DH lines from Frontana/Remus (IFA Tulln) /2005-2006/ and 110 DH lined of Mini Mano/Frontana (CRC Szeged) /2006-2007/ were inoculated with four *Fusarium* isolates of *F. graminearum* and *F. culmorum*. The Frontana/Remus population was developed traditionally, with about up to two weeks difference in flowering time and 60-70 cm differences in plant height. MM/Frontana was created by us so that too early and late DH lines were discarded and the remaining lines flowered within five days, so one inoculation time was enough to cover all genotypes and plant height differences were kept within 20-30 cm depending on season. The rest of the lines were discarded.

In the Frontana/Remus population QTLs were identified on the chromosomes 2D, 3A, 5A, 5B, 3B, 6B, 7A/ 7D. The most consequent markers were found on 5A and 5B chromosomes (BARC197 and GWM156), the others gave positive signal seldom and the LOD values were around 2 and 2,8. In the MM/Frontana population 2B, 3B, 5A, 5B and 7B gave positive signal. The LOD values on 5B chromosome were the highest (BARC115), between 2,76 and 5,32. Even so in both populations Frontana was the resistant parent not the same markers gave positive signal. It seems that the more homogeneous population increases the accuracy of the QTL analysis. An increased morphologic homogeneity is necessary to decrease „noise" in QTL analyses and increase preciosity. Until now no QTL were found that gave positive signs for all epidemic situations. Therefore, the conditions to perform consequent MAS to identify superior genotypes in Frontana descendants are not yet in sight.

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EXTRA- AND INTRACELLULAR TARGETING OF ANTIFUNGAL PLANT DEFENSINS IN TRANSGENIC *ARABIDOPSIS* FOR RESISTANCE TO *FUSARIUM GRAMINEARUM*.

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ABSTRACT

Recent studies have shown that *Arabidopsis thaliana*, a model host plant is susceptible to *F. graminearum*. Taking advantage of this foliar *Fusarium-Arabidopsis* pathosystem, we tested antifungal defensins, MsDef1 and MtDef4, from Medicago spp., for their ability to confer resistance to this pathogen. We generated chimeric defensin gene constructs that will result in over-expression of MsDef1 or MtDef4 either extra-cellularly or intra-cellularly (i.e., vacuole or endoplasmic reticulum) in transgenic *A. thaliana* ecotype Columbia (Col-0). Here, we demonstrate that constitutive overexpression of MsDef1 and MtDef4 confers strong resistance to *F.graminearum*. Transgenic Arabidopsis lines overexpressing MsDef1 or MtDef4 either extra-cellularly or intra-cellularly showed 59-68 % reduction in disease severity (DS) index as compared to that of the wild type plants (100%) and supported significantly less fungal growth as evaluated by trypan blue staining. Transgenic inoculated plants also bolted normally like the mock inoculated wild-type plants, whereas the inoculated wild-type plants showed much delayed bolting. Since *F. graminearum* has both biotrophic and necrotrophic life-cycles, we hypothesize that MsDef1 and MtDef4 co-expressed extra- and intra-cellularly will confer much higher level of resistance to FHB. Hence, transgenic *A. thaliana* lines co-expressing extra- and intracellular defensins will be tested for increased resistance to this pathogen.

SESSION 4:

FHB MANAGEMENT

Chairperson: Gary Bergstrom

EFFECTS OF WHEAT GENOTYPES AND INOCULATION TIMINGS ON FUSARIUM HEAD BLIGHT (FHB) SEVERITY AND DEOXYNEVALENOL (DON) PRODUCTION IN THE FIELD.

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ABSTRACT

Fusarium head blight (scab), caused primarily by *Fusarium graminearum* (teleomorph: *Gibberella zeae*), is an important disease of wheat and other cereals worldwide. The disease affects both yield and quality due to contamination of grains with various mycotoxins. Since 1993, the disease has caused billions of dollars loss to the wheat industry in the USA. Due to lack of effective resistant cultivars, FHB is managed through fungicide applications and cultural practices. New fungicides such as 'Proline' are effective in FHB management and DON reduction. It has been hypothesized that all wheat cultivars do not respond to fungicide applications in similar manner for DON production and yield increase. Research is in progress to make the FHB forecasting system more accurate. Information on wheat cultivars with various levels of resistance to FHB and their responses to the disease development are important parameters of accurate disease forecasting system.

The main objectives of this study were to determine the effects of three hard red spring wheat cultivars, Glenn (FHB resistant), Steel-ND (moderately susceptible) and Trooper (susceptible), and two inoculation timings on FHB development, and to examine the correlation between FHB severity and DON production under field conditions. Wheat cultivars were planted on May 4 and May 14, 2007 at North Dakota State University Experimental Station, Fargo. The experiment was planted as a split-split plot design with 3 replications. Planting date (early and late), wheat cultivars (Glenn, Steel-ND, and Trooper), and inoculation timing (no inoculation, inoculation at early flowering, and inoculation at mid flowering) were assigned in main plot, sub-plot, and sub-sub plot, respectively. Plants were spray-inoculated with *F. graminearum* (~100,000 spores/ml). Two hundred-twenty-five heads from each sub-plot were examined for FHB incidence and severity, and 20-40 heads with disease severity of 0%, 7-21%, 22-50%, 51-79%, and 80-100% in each sub-plot were tagged at dough stage (Feekes GS 11.2). Wheat ear heads with each disease severity category were collected separately to estimate DON, and correlation between FHB severity and DON production. The cultivars differed significantly in FHB severity, but not in disease incidence and DON production. The resistant wheat cultivar Glenn has the lowest severity (20.6%) while the susceptible cultivar Trooper has the highest disease severity (28.12%). Inoculation timings also had significant effect on FHB incidence, severity, and DON production. All three disease components incidence (12.75%), severity (41%), and DON (2.45 ppm) were higher when the cultivars were inoculated at mid flowering stage (GS 10.52). A positive correlation ($r = 0.98$) was observed between FHB severity and DON concentration in all three cultivars. As expected, the susceptible cultivar Trooper had higher DON concentration in all five disease severity categories (ranged from 1.06 to 75.68 ppm) as compared to Steel-ND (1.39 to 56.86 ppm) and Glenn (0.91 to 64.63 ppm). The samples with high DON concentration also had with high amount of 3-ADON. Our results indicate that infection at mid flowering growth stage is crucial in FHB incidence, severity, and DON production.

AEROBIOLOGY OF *GIBBERELLA ZEAE*: WHENCE COME THE SPORES FOR FUSARIUM HEAD BLIGHT?

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ABSTRACT

Gibberella zeae (*Fusarium graminearum sensu stricto*) is the principal causal agent in North America of Fusarium head blight (FHB) of wheat and barley and in several regions is the predominant causal agent of stalk rot and ear rot of corn. Research done primarily in New York over the past decade on the aerobiology, epidemiology, and population biology of *G. zeae* was summarized in terms of its implications for the regional management of FHB. The fungus survives between crop seasons as a saprophyte in infected crop debris, especially in corn stalks and small grain residues on which it sporulates (both conidia and ascospores) profusely during warm, moist conditions. Viable spores of *G. zeae* rely on atmospheric motion systems for transport to the florets of wheat and barley where they initiate FHB. Ascospore liberation into turbulent air currents is favored by spore release during daylight hours when peak discharge from perithecia on corn residues also occurs. Ascospore survival on the surface of wheat spikes is on the order of hours to days. Using unmanned aerial vehicles (UAVs), we documented the abundance of viable spores of *G. zeae* 60 m above the surface of the earth at all times of the day and night under a broad range of meteorological conditions. Viable spores were deposited across cereal fields and other landscape areas by gravitational settling mainly at random and predominantly at night. The temporal uncoupling of peak spore release and deposition suggests that inoculum in cereal fields may originate from distant as well as within-field sources. Genotypic diversity was extremely high in atmospheric populations of *G. zeae* collected in central New York over a four-year period. The predominant trichothecene mycotoxin genotype of *G. zeae* found in New York in both infected grain and in atmospheric populations is one that produces deoxynivalenol (DON) plus smaller amounts of 15-acetyl-DON. Our findings suggest that atmospheric populations of *G. zeae* are an abundant, well-mixed, and diverse source of inoculum for regional epidemics of FHB. Model computations with the atmospheric transport model HYSPLIT suggest that ascospores of *Gz* may be dispersed kilometer distances from area sources of inoculum in a matter of minutes. The ability to predict the regional transport of *Gz* from local inoculum sources may help refine risk models for FHB.

It is generally considered (but not proven) that airborne ascospores of *G. zeae*, constitute the principle inoculum for infection of wheat and barley florets. We provided evidence that viable ascospores are potentially transported at least kilometer distances from their site of discharge. Yet the conventional opinion among FHB researchers from Chester in 1890 to the present is that inoculum sources for FHB are mainly local and that long-distance dissemination of inoculum is of minor significance. For example, FHB risk forecasting models that predict local inoculum levels based on previous local weather are built, in part, on the assumption that local inoculum is derived exclusively or largely from nearby sources. This assumption awaits validation. Significant long-range dispersal would suggest that local management of overwintered inoculum (e.g., tillage, spraying of debris, etc.) may have negligible impact on the development of FHB in nearby cereal crops unless performed over extensive production areas. Long-range dispersal also implies that genotypes of *G. zeae* with novel toxin or virulence profiles could be rapidly disseminated across broad geographic regions. Published studies suggest that most rain-splash dispersal of spores to spikes occurs within 5 meters or less from inoculum sources on the

soil surface and that disease severity follows a similar gradient with distance from those inoculum sources. Various researchers have attempted to delineate spore dispersal gradients or disease gradients at linear distances from area sources of inoculum. Observations of 50% reduction in spore concentration or disease have ranged from 1 to 50 m distances from area inoculum sources with most studies indicating sharp gradients within 10 m of sources. In almost every study conducted, the background level of spores or disease has been at 50% or greater proportion of the level at the source area. Ascospores actively discharged from perithecia or even conidia caught in turbulent air may be deposited on local wheat spikes at a potentially much greater distance from debris than splash-dispersal. Spores may also escape the crop canopy, mix with spores over a wide area, and be transported in the atmosphere at least kilometer distances. Field survey-based studies of DON in grain have generally revealed that cereal cultivar and seasonal meteorological conditions were better quantitative predictors of toxin content than previous crop or tillage practice, strongly suggesting that regional inoculum plays a critical role in FHB epidemics. Based on several field studies with cereal debris level and crop sequence, within-field inoculum, where present, appears to be a significant source for local FHB, but regional inoculum appears to play an even greater role.

There are no reliable estimates of the relative contributions of within-field, local inocula to spike infection compared to other airborne sources. In New York and Virginia, we are utilizing a marked isolate, release-recapture experimental approach to assess relative contribution of localized clonal inoculum present in corn stalks to infection of wheat heads at varying distances from area sources of inoculum. Preliminary evidence from the first year of experimentation suggests that within-field sources of *G. zeae* provided a minor fraction of FHB inoculum compared to background atmospheric sources in a non-epidemic situation in New York and in a moderate epidemic situation in Virginia.

ACKNOWLEDGEMENTS AND DISCLAIMER

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2007 UNIFORM FUNGICIDE TRIALS ON SOFT WHITE WINTER WHEAT IN MICHIGAN.

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ABSTRACT

The objective of this project was to evaluate the effectiveness of commercially available and experimental fungicides for the control of Fusarium head blight (FHB) and reduction of deoxynivalenol (DON) in white winter wheat in Michigan. Four trial locations were planted in with Caledonia white winter wheat during 8 – 31 Oct 2006. Plots at the East Lansing and Clarksville locations were artificially inoculated with *F. graminearum* infested corn kernels at a rate of 0.2 oz/sq. ft. a week prior to heading. Irrigation was begun after inoculation was completed. Plots were irrigated four times /day for 20 minute intervals beginning the week prior to flowering until two weeks after flowering. The remaining two sites in Sandusky and Saginaw relied on natural inoculum and rainfall. All treatments were applied at early flowering (Feekes 10.5.1) at 25gpa and 40 psi using a CO_2-pressurized R&D tractor mounted spray boom with XR11003VS nozzles positioned forward and backward. Treatments consisted of: 1) Folicur (tebuconazole) 4 fl oz/a; 2) Proline (prothioconazole) 5 fl oz/a; 3) Caramba (metconazole) 13.5 fl oz/a; 4) Topguard (flutriafol) 14 fl oz/a; 5) Punch (flusiazole) 6 fl oz/a; 6) Proline 3 fl oz/a + Folicur 3 fl oz/a; and 7) untreated control.

Disease pressure (FHB and foliar diseases) in Michigan was generally very low in 2007. Plots were rated for foliar diseases 7 days after treatment and again at the soft dough stage (Feekes 11.2). Saginaw did not develop sufficient foliar disease for rating. FHB incidence, severity and index were rated at the soft dough stage. Only the Clarksville and East Lansing (both inoculated and irrigated) locations developed sufficient FHB for field ratings. Yield, test weight, percent moisture, *Fusarium* damaged kernels (FDK) and thousand grain weights were determined post harvest. Sub samples from each plot were sent to the University of Minnesota for DON analysis.

At the East Lansing location, FHB severity for all treatments was significantly lower (3.2-4.9%) than the control (21.9%). For FHB incidence, Punch (7.8%) was not significantly different from the control or other treatments, but other treatments (5.7-5.9%) were significantly lower than the control (21.9%). FHB index for all treatments (2.0-3.5%) was lower than the untreated control (21.9%). There were no significant differences in DON levels (1.1-1.9 ppm) among any treatments. Yields ranged from 72.1-87.9 bu/a. Proline + Folicur (87.9 bu/a) and Caramba (87.6 bu/a) were significantly higher than for Folicur alone (72.1 bu/a), but no treatments were significantly higher than the untreated control (73.7 bu/a). There were no significant differences among test weights or 1000 grain weights at the East Lansing location.

At the Clarksville location, there were no significant differences in FHB incidence (55.0-98.1%), severity (12.9-26.4%) or index (8.7-26.3%). There were no significant differences among treatments for test weight, 1000 grain weights, or yield (73.9-85.4 bu/a). Average DON levels ranged from 3.6 -10.1 ppm, but none was significantly different from the untreated control (6.8 ppm). All the treatments resulted in significantly less stagonospora and leaf rust than the untreated control. No phytotoxicity was observed in any of the treatments at any of the sites.

DURATION OF POST-FLOWERING MOISTURE AND INFECTION TIMING AFFECT ON FHB AND DON IN WHEAT.

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ABSTRACT

Our understanding of how environmental and host genetic influences interact to determine DON concentrations in small-grain spikes is incomplete. High levels of DON have sometimes been observed in the absence of abundant disease symptoms. This multi-year experiment explored the influences of post-flowering moisture duration, infection timing, and cultivar resistance differences on FHB and DON in winter wheat. The experiment had a split-plot design. Whole plots were four durations (0, 10, 20, or 30 days) of post-anthesis misting. Sub-plots were soft red winter wheat cultivars, of which one (2005) or two (2006 and 2007) were susceptible to FHB and six were moderately resistant. There were two plots of each cultivar under each duration of irrigation: one inoculated at anthesis with a backpack sprayer, and one in which individual funnel-isolated spikes were chosen at random and inoculated with a spray bottle at specific post-flowering intervals in order to study the effect of late infection. Inoculations utilized *F. graminearum* spore suspensions of 10^4 (2005) or 10^5 (2006 and 2007) macroconidia/ml. All treatments were replicated three times. In the backpack-inoculated plots, disease incidence and severity were assessed prior to the onset of senescence, and a DON time-course study was performed by collecting spike samples six times at 10-day intervals starting two weeks after flowering. Samples of all treatments were assayed for *Fusarium*-damaged kernels (FDK), percent infected kernels (using Komada's medium), and DON concentration. Assays of *F. graminearum* DNA by tissue type (kernel, rachis, or glume) were performed on a limited sample in 2005, using real-time PCR, and these assays are being conducted for all treatments in 2006 and 2007.

Preliminary results:

1) Under conditions conducive to disease (2006 and 2007), FHB incidence and severity and grain DON concentrations increased with increasing duration of post-flowering moisture (*P* d" 0.05). Cultivar grain DON rankings changed under longer moisture durations, suggesting that resistance to post-flowering moisture may be a distinct trait.

2) In 2006, spikes inoculated 10 days after flowering contained significantly less grain DON at harvest time than those inoculated at flowering ($P < 0.0001$). Spikes inoculated 20 days after flowering had still less harvest-time grain DON than those inoculated 10 days after flowering ($P < 0.0001$), and had the same level of grain DON as noninoculated spikes (*P* d" 0.48). Changes in DON rankings suggested that resistance to late infection may also be a distinct trait. (Data from 2007 not yet available.)

3) In 2006, for spikes inoculated 10 days after flowering, 0 and 10 days of post-flowering mist resulted in mean harvest-time grain DON levels of 0.6 and 1.2 ppm, respectively, while 20 and 30 days of post-flowering mist resulted in harvest-time grain DON levels of 2.0 and 2.4 ppm, respectively. At the same time, the percentage of FDK from spikes inoculated 10 days after flowering was significantly lower than that from spikes inoculated at flowering ($P < 0.0001$), and not significantly different from the FDK percentage from

noninoculated spikes ($P = 0.40$). Thus, late infections coupled with extended post-flowering moisture may be one scenario accounting for observations of visually healthy grain with excessive DON at harvest. (Data from 2007 not yet available.)

4) In 2005 and 2006, the time-course study results showed a significant decline in grain DON between mid-May and early June, which is normal harvest time. In 2006, DON progression was evaluated under varying durations of post-flowering moisture. Prolonged moisture delayed the DON decline, and raised the DON levels from which decline commenced, but DON levels continued dropping in those treatments during the three weeks after normal harvest time. This suggests that DON may actually be reduced by delaying harvest if DON levels are high early in grain-fill.

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DISCLAIMER

Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the author and do not necessarily reflect the view of the U.S. Department of Agriculture.

EFFECT OF POST INOCULATION MOISTURE ON DEOXYNIVALENOL ACCUMULATION IN *FUSARIUM GRAMINEARUM*-INFECTED WHEAT.

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OBJECTIVE

The objective of this study was to examine factors; including host genetics, pathogen aggressiveness and environmental moisture, affecting the production and accumulation of deoxynivalenol (DON) in wheat.

INTRODUCTION

Fusarium head blight (FHB) or scab of wheat and other cereals, primarily caused by *Fusarium graminearum*, has reemerged as a devastating disease in the United States. The disease causes yield losses both from reduced grain number and grain weight, including the formation of tombstones (shriveled kernels with a chalky appearance). FHB also affects quality of the grain through the production of a range of mycotoxins, among which DON is the most commonly present and that which is regulated in the grain trade. Though the production and accumulation of DON in infected grain is generally positively correlated to FHB severity, the correlation is not consistent and predicting the DON content of grain in commercial wheat and barley crops or breeding nurseries based on visual disease assessments prior to harvest is generally unreliable. The production and accumulation of DON in infected grain is not well understood and likely results from the complex interactions of host and pathogen genetics which is modified by the prevailing environmental conditions. The objective of this multi-year study was to identify factors affecting the production and accumulation of DON in wheat.

MATERIALS AND METHODS

Experiments were conducted at the St. Paul Experimental Station of University of Minnesota in 2006 and 2007 as split-split plot designs, each with five replications. Main plots were days of mist-irrigation after inoculation (14, 21, 28 and 35 days after inoculation [DAI]), sub-plots were wheat genetics and sub-sub plots were individual *F. graminearum* isolates (49-3, B63A, Butte86-ADA11, 81-2, B45A) differing in their relative aggressiveness and a mock-inoculated (water) control. Two row plots (each 1.8 m long) of three wheat varieties; Alsen (moderately resistant, resistance source Sumai 3), 2375 (moderately resistant, unknown resistance source) and Wheaton (susceptible) were planted in mid-April each year of the study. All plots were inoculated at the anthesis (mid-June) and 3 days later with macroconidial inoculum (1×10^6 macroconidia ml^{-1}) using a CO_2-powered backpack sprayer dispensing inoculum at the rate of 30 ml per meter of row. Mist-irrigation was started immediately following the first inoculation. Disease was assessed visually 21 DAI by counting total infected spikelets in 20 arbitrarily selected heads in each plot (10 heads per plot row). Grain was harvested at maturity (late July), machine threshed and dried for 10 d at 95^0 C. The percentage of visually scabby kernels (VSK) analysis was assessed on a 25 g sub-sample of harvested grain following the procedures of Jones and Mirocha (1999). Following the assessment of VSK the sub-samples were analyzed for DON at the University of Minnesota's Mycotoxin Laboratory. Data were analyzed by ANOVA and LSD tests and correlations performed using SAS.

RESULTS AND DISCUSSION

In 2006, the average FHB severity was 22.3%, the average VSK was 4.9% and the average DON accumulation was 0.62 ppm. Overall, FHB severity, VSK and DON was higher in 2007 than 2006. In 2007, the average FHB severity was 37.5%, and the average VSK was 27.8% while the average DON concentration was 10.5 ppm. The effects of *F. graminearum*

isolate, wheat genetics and mist-irrigation were significant in both years of the study. While the isolate 49-3 generated the highest FHB severities, VSK and DON production in 2006, B63A had the lowest DON levels despite inciting high FHB severities and VSK. In 2007, isolates B63A and 49-3 produced the highest levels of DON and were associated also with higher FHB severities and VSK. Isolates 81-2 and Butte86-ADA11 generally were associated with lower FHB severities, VSK and DON.

In 2006 the severity of FHB and the percent VSK was significantly higher in Wheaton (42.5% and 11.5%, respectively) than the other two varieties (FHB severity < 15.5%, VSK < 2.9%). The DON concentration of Wheaton, across all isolates, was significantly higher (1.2 ppm) than for the other two wheat varieties tested (< 0.4 ppm).

FHB severity and VSK was significantly lower in the treatments receiving the least amount of mist-irrigation (14 DAI; FHB severity 19%; VSK 4%) than longer mist-irrigation treatments (FHB severity 22.6-25.4%: VSK > 5%). The DON concentration was however significantly lower in the longest mist-irrigation treatment (35 DAI; DON 0.5 ppm) than in treatments where the mist-irrigation was applied for shorter periods of time (DON 0.6 – 1 ppm). The Spearman's rank correlations of DON with FHB severity and VSK were 0.78 ($P < 0.0001$) and 0.85 ($P < 0.0001$), respectively.

Similarly, in 2007 Wheaton had significantly higher FHB severity (59%) VSK (53.87%) and DON (17.64 ppm), than the other wheat genotypes examined (FHB severity < 27.51%; VSK < 19.4%; DON < 7.45 ppm). The severity of FHB was highest in mist-irrigation treatments applying supplemental water for 28 DAI (40.3%) than the other mist-irrigation treatments (36.1-36.8%). VSK readings were significantly higher (37.7%) for the longest mist-irrigation treatment (35 DAI) than the others (19-33.2%). DON was significantly lower (7.95 ppm) in the 35 DAI mist-irrigation treatment compared to the other irrigation treatments (9.9-13.3 ppm). The Spearman's rank correlations of DON with FHB severity and VSK were 0.78 ($P < 0.0001$) and 0.78 ($P < 0.0001$), respectively.

Our results show that FHB severity, VSK and DON level increases in more susceptible wheat cultivars. It also varies with the fungal isolates aggressiveness with respect to disease and DON production. DON level increased with mist-irrigation applied till 28 DAI but was reduced in the 35 DAI irrigation treatments. These results are in concordance with several researches which reported a reduction in DON levels with the long irrigation treatments. Similarly, it has been reported that DON accumulation in *Fusarium*-infected tissues peaks approximately six weeks after infection and then declines prior to harvest. In our case, the peak DON level was observed between four and five weeks after inoculation. Since DON is water soluble, the decline in DON levels might have been accelerated by the mist-irrigation, perhaps from leaching of the DON. The observed increase in DON levels despite mist-irrigation until 28 DAI was likely due ongoing production of DON as the fungus continues to grow and infect new tissues under conditions favorable for the pathogen. Thus, any DON leached by mist-irrigation water before 28 DAI would likely have been replaced by that produced by the spreading fungus. As the plant begins senescence, growth of the fungus and the production of DON may be reduced or even stop, and thus the leaching effect of irrigation on reduction of DON was readily detectable. Based on our results it may be concluded that longer durations of wetting, from either mist-irrigation or rainfall, after infection will increase the severity of FHB and VSK and thus the damage to grain, although DON concentrations may be reduced.

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DISCLAIMER

Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the authors and do not necessarily reflect the view of the U.S. Department of Agriculture.

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PROSARO® – A NEW FUNGICIDE FOR CONTROL OF *FUSARIUM* AND MYCOTOXINS IN CEREALS.

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ABSTRACT

Fusarium head blight (FHB) and mycotoxins can be major challenges in cereal production. FHB is a disease caused by different *Fusarium* species. Under field conditions, all the predominant *Fusarium* species may produce mycotoxins with the exception of *M. nivale*. Quality and quantity of grain harvest are affected by FHB.

Combatting FHB is demanding because many factors may influence the severity of the infection. Agricultural practices (cultivars, cropping methods, crop rotation etc.) and environmental conditions are all contributing factors to any epidemic. Chemical control with *Fusarium* active compounds such as the triazole fungicides Prothioconazole and Tebuconazole contribute significantly to a reduction of FHB and mycotoxin production.

Suppression of the mycotoxins with these fungicides can reach values of higher than 70%. Success of the fungicide treatment is dependent on optimal application timing and spray coverage. In general, smaller droplet sizes provide more effective *Fusarium* control.

With the mixture of Tebuconazole & Prothioconazole – Prosaro ®- the application window can be broadened due to the combination of preventive and curative strengths of the two active ingredients involved. In addition to FHB control, Prosaro controls a broad range of fungal pathogens in cereals and thus contributes to higher yield.

ADDITION OF ADJUVANT TO IMPROVE COVERAGE AND FUNGICIDE EFFICACY ON BARLEY, LANGDON 2006

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INTRODUCTION

Most fungicide application technology studies have focused on maximizing deposition on the entire small grain head. Observations in the field would indicate that the Fusarium head blight (FHB) ascospore can infect the awn part of the grain head but generally will not move down the awn and infect the other parts of the spike or kernel. The awns are a very effective structure for collecting both ascospores that cause the initial infection for FHB and unfortunately the fungicide solutions we apply to protect against FHB. Most fungicides applied to control FHB have a localized systemic type of activity meaning that there is little translocation within the grain head. In addition, most of the translocation is in an upward and outward movement from the initial point of deposition. Studies have been initiated to determine if we can increase the amount of fungicide solution collected on the whole head and reduce the percentage of fungicide solution collected on the awns relative to the spike. This study reports results of those efforts. It includes evaluation of the addition of several adjuvants, suggested by adjuvant manufacturers, and two adjuvant compounds previously reported to significantly increase coverage on the other parts of the spike or kernel portion of the grain head.

MATERIALS AND METHODS

A study was initiated in 2006 at the North Dakota State University Langdon Research Extension Center, Langdon North Dakota. The objective of the study was to determine if an adjuvant included with fungicide could increase deposition on the grain head as a whole, the developing kernel portion of the spike and subsequently reduce deposition on the awn portion of the head decreasing FHB disease incidence and deoxynivalenol concentration in the harvest sample. The study was designed as a randomized complete block with five replicates. A block of barley was planted with a double-disk type drill, rows spaced 7-inches apart to cultivar 'Stellar' in mid May. After emergence and after application of weed control, the block was divided into plots 12 x 30 ft. Prosaro fungicide, prothioconazole, was applied at 3.25 fl oz/ acre which is one-half the rate recommended by manufacturer Bayer CropScience. The one-half rate was used with expectation that we could maximize and measure the beneficial effect of the adjuvant. A food grade dye, FD&C blue #1, was mixed with each of the fungicide solutions at a rate of 44 grams per acre. The dye was included as an indirect type measurement to determine differences in coverage on the parts of the grain head. After delineation of the plots, a *Fusarium* inoculum was hand-broadcast on each plot to encourage development of disease. Fungicides were applied with a tractor using a side-mounted spray boom. The tractor traveled 6 mph delivering the solution at 10 GPA and 40 psi with Spraying Systems XR8002 nozzles angled 30 degrees downward from horizontal and oriented to spray forward or the same direction of travel as the tractor. The spray system was equipped with a CO_2 type delivery system instead of a standard pump. After applying the treatments, a sample of 10 heads were collected from each plot, deposited in Ziploc type bags and placed on ice. The awns of each head were individually clipped from the kernels. The awns and the remainder of the heads were deposited in separate 250 ml Erlenmeyer type flasks and sealed with a rubber stopper. A solution of 80 ml 95% ethyl alcohol was added to each flask and shaken for three minutes with a Burrell wrist-type action shaker. A sub sample of the solution was placed in a cuvette

and placed in a Jenway photospectrometer to determine the absorbance of the solution. Each absorbance reading was indirectly used to determine differences in the amount of dye collected on the grain head parts. A whole head sample was the sum of the parts. After the fungicide was applied, a sprinkler irrigation system was installed to modify the environment as needed and encourage the development of disease to determine differences among treatments. North Dakota State University Extension recommended production practices for barley in Northeast North Dakota were followed. A visual estimation was made from 20 samples per plot collected 20 days after fungicide application to estimate the incidence (number of spikes infected) and field severity (number of FHB infected kernels per head divided by total kernels per individual spike) of FHB in each plot. A rotary mower removed the front and back five feet from each plot prior to harvest to minimize any chance of interference by drift from the tractor when stopping or starting. Each plot was harvested with a Hege plot combine and the grain sample cleaned and processed for yield, protein, plump, and test weight. A sub sample was ground and analyzed for deoxynivalenol (DON) by North Dakota State University. Data was analyzed with the general linear model (GLM) in SAS. Fisher's protected least significant differences (LSD) were used to compare means at the 95% probability level.

DISCUSSION AND RESULTS

The environment at the LREC was warm and dry both before and after fungicide application in 2006. Fusarium head blight developed later in the season and may have negated some of the beneficial effects of the adjuvants and the fungicide. The fungicide reduced FHB field severity over the untreated but no differences were measured among treatments (Table 1). Although DON levels were reduced by more than 50% from the untreated by WECO 6065 and AG 6470, they were not statistically significant. Several adjuvants increased the deposition on the whole head and there were differences recorded among adjuvants. The most notable was the adjuvant In-Place which is an encapsulating compound that could be used with an additional adjuvant to further increase deposition, distribution on the head, and fungicide efficacy. Also of note was the low deposition value of the Silkin adjuvant. Silkin is an organosilicate type adjuvant. The results may be a rate related effect and may be improved with the addition of another type adjuvant. Syl-Tac is also a silicon type adjuvant that includes a penetrator and performed considerably better. No differences were measured on the kernel portion of the spike. Significant correlations were determined between FHB incidence and yield, test weight and deposition on the awns, and most notably DON levels with deposition on the spike, awns, and whole head indicating that these efforts are focusing in the right areas. Significant negative correlations were measured between coverage parameters and DON levels.

Table 1. FHB incidence and field severity, yield, test weight, plump, coverage, and deoxynivalenol concentration (DON) by treatment, Langdon 2006.

Treatment/ Adjuvant	Adjuvant Rate	FHB Incidence (%)	FHB Field Severity (%)	Yield (bu/a)	Test Weight (lb/bu)	Plump (%)	Absorbance Spike	Absorbance Awns	Absorbance Whole	DON (ppm)
Syl-Tac	0.5% v/v	100	10.4	132.5	46.7	97	.114	.336	.450	1.84
Untreated		100	14.8	116.4	47.2	98	.054	.143	.197	1.80
AG06038	0.5% v/v	100	11.9	126.7	47.0	98	.130	.321	.451	1.64
no adjuvant		100	10.5	130.9	47.3	98	.116	.347	.463	1.62
WECO5036-7	0.25% v/v	99	10.6	117.9	47.6	98	.105	.296	.401	1.34
Triton X405	0.25% v/v	99	10.1	131.7	47.0	98	.123	.335	.458	1.20
Silkin	0.25 pint/100	100	10.0	127.6	47.3	98	.089	.281	.370	1.12
Preference	0.25% v/v	99	11.1	124.4	47.4	98	.108	.339	.448	1.10
Alfonic 1412-80	0.25% v/v	100	11.2	128.7	47.5	98	.108	.406	.514	1.08
In-Place	1/4 (adj./fung)	100	10.0	121.4	46.9	98	.137	.413	.551	1.02
AG 5004	8 fl oz/a	100	10.2	124.4	47.2	98	.121	.379	.500	1.02
Induce	0.125% v/v	100	10.7	127.2	47.4	98	.120	.322	.441	0.96
WECO6065	0.25% v/v	99	10.6	124.3	47.6	98	.084	.297	.381	0.86
AG06470	1%v/v	100	10.8	126.6	47.3	98	.139	.301	.441	0.76
LSD(0.05)		NS	2.1	NS	NS	NS	NS	.079	.103	NS
% C.V.		1	15	9	1	1	36	19	19	65
Pr>F		0.6947	0.0056	0.5656	0.1053	0.5381	0.1193	<0.0001	<0.0001	0.5012

Table 2. Pearson correlation coefficients for FHB incidence and field severity, yield, test weight, plump, coverage, and deoxynivalenol concentration (DON) Langdon, 2006.

	Incidence	Field Severity	Yield	Test Weight	Plump	Absorbance Spike	Absorbance Awns	Absorbance Whole	DON
Incidence	1.00	0.124	-0.255	-0.133	-0.153	-0.045	-0.067	-0.068	0.048
		0.308	0.033	0.272	0.204	0.714	0.584	0.545	0.692
Field Severity		1.00	-0.025	0.138	-0.162	-0.106	-0.212	-0.204	0.221
			0.840	0.253	0.181	0.381	0.078	0.090	0.066
Yield			1.00	-0.124	-0.275	0.113	0.234	0.234	0.111
				0.306	0.021	0.352	0.051	0.063	0.361
Test Weight				1.00	0.193	-0.106	0.143	0.072	-0.256
					0.109	0.381	0.239	0.5560	0.032
Plump					1.00	-0.185	-0.143	-0.180	-0.123
						0.125	0.239	0.137	0.310
Spike						1.00	0.448	0.719	-0.315
							<0.001	<0.001	0.008
Awns							1.00	0.943	-0.235
								<0.001	0.050
Whole								1.00	-0.300
									0.012
DON									1.00

ASSESSMENT OF AIR STREAM SPEED WITH TWO NOZZLE TYPES AS A TOOL TO IMPROVE DEPOSITION OF FUNGICIDE FOR CONTROL OF FHB IN WHEAT.

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OBJECTIVES

To determine most effective air stream speed using two contrasting nozzle types to maximize deposition on the grain spike and improve efficacy of fungicide on hard red spring wheat.

MATERIALS AND METHODS

A field was selected near Esmond, North Dakota that was previously cropped corn. The field was planted to 'Alsen' spring wheat in an east/west direction with an air seeder with tramlines every 80 feet. The study was arranged as a factorial (nozzle type x air stream speed) in a randomized complete block design laid out in four replicated blocks, split into plots 40 x 500 ft. to accommodate one spray boom and the grower's combine straight cut header. Plots were arranged in an east/west direction between tramlines and the plot length measured with a global positioning unit mounted on an all terrain vehicle after all herbicide applications had been completed. The sprayer was a Hardi-Twin (Hardi, Davenport, IA 58206) modified to accommodate the tramlines and to spray one half of the area between the trams, 40 feet width, beginning at the center of the tractor. The sprayer contained a diaphragm type pump and traditional flat fan hydraulic nozzles. The spray nozzles are mounted to direct the spray into the air stream which carried the spray solution to the grain. Before the field trial were completed, the spray booms were equipped with Teejet XR11003 and TT11003 nozzles (Spraying Systems Co, Wheaton, IL 60189)on each boom, respectively and calibrated. The nozzles were directed to spray forward from vertical at the maximum of 30 degrees forward. Both nozzles were calibrated at 40 psi to determine the output of the specific nozzles. The air stream speed was determined by setting the rpm on the fan at 1800, 2400, or 2900. The air stream velocities were about 23, 35 and 50 mph respectively, measured at one-inch from the air stream orifice. This was measured with a 'Kestrel' 2000 wind velocity meter (Niche Retail, Sylvan Lake, MI 48302). The spray drop size measurement application parameters were characterized by mounting two water sensitive papers (WSP) 1" vertically x 30" horizontally" side by side on a piece of flat iron at canopy height and spraying across the WSP. Each combination of the respective factors was measured. Volume median diameter (VMD) of the spray drops formed on the WSP, spray volume, and % area coverage was completed with a WRK 'Droplet Scan analyzer' Cabot, Arkansas.

The fungicide was applied at Feekes growth stage 10.51. The fungicide solution included Folicur (tebuconazole) fungicide 4 fl oz/acre + Induce adjuvant at 0.125% v/v and a food-grade tracer dye (FD & C Blue #1) added at 44grams/acre. Folicur is manufactured by Bayer CropScience and Induce by Helena Chemical Co. Immediately after the fungicide was applied, ten heads were sampled from each plot in each of three locations and placed in 250 ml Erlenmeyer flasks, sealed with a stopper, and placed on ice for transport to the laboratory to measure the relative volume of solution collected for each of the treatments. Barley production recommendations from the North Dakota State University Extension Service for northeast North Dakota were followed.

Eighty ml of 95% ethyl alcohol was added to each flask and shaken for three minutes with a wrist-action mechanical shaker (Burrell Scientific Instruments and

Laboratory Supplies, Model BT, Pittsburgh, Pennsylvania 15219). A sub sample of the wash solution was measured with a Jenway spectrophotometer (Jenway, Model 6300, Dunmow, Essex CM6 3LB England) and an absorbance of the tracer dye recorded to determine differences among application parameters. The absorbance reading quantifies differences in the amount of tracer dye deposited on the grain spike (a larger absorbance value is the result of more tracer dye in the solution).

A visual estimation of disease incidence (number of spikes infected) and field severity was made from 20 heads per plot at early dough stage. Field severity rating is the number of FHB infected kernels per head divided by total kernels per individual spike. All plots were harvested with a Caterpillar Lexion combine on 7 August and weighed with a weigh wagon and a grain sample collected. The yield was determined from the grain collected from the harvested plot. The grain sample was cleaned and processed to determine plump, test weight, and protein. A sub sample was ground and analyzed for the toxin deoxynivalenol (DON) by North Dakota State University. Data was analyzed with the general linear model (GLM) in SAS. Fischer's protected least significant differences (LSD) were used to compare means at the 5% probability level.

RESULTS

The VMD measured with water sensitive paper describes the relative drop size of the spray deposits from each of the nozzles. Half of the spray volume is in drops larger than this size in microns and half of the spray volume is in drops smaller than this value. The XR11003 nozzles had a smaller VMD than the TT11003 nozzle. Increasing air speed increased the VMD by spreading the drop over a larger area. Adding air as a spray solution carrier increased the area of coverage on the cards and increasing air stream speed increased area of coverage further.

Deposition (Absorbance) on the grain heads were the same with both nozzles when no air was used to assist in deposition. The XR11003 nozzle deposited more tracer dye than the TT11003 nozzle. The 2900 rpm air stream speed was statistically the same as no air but greater than the 1800 and 2400 air stream speed when averaged across both nozzle types indicating a benefit to the high speed air stream.

A characterization of the nozzles (F025_110) supplied with the Hardi-Twin and operating parameters traditionally used by the grower, indicated a reduction in VMD when nozzle pressure was increased and a large reduction in coverage of the cards when nozzle pressure was 90 psi. This indicates that the sprayer may provide better consistency of fungicide application due to increased deposition on the wheat head when operated with lower nozzle pressure and increased air stream speed.

The growing season was very dry during flowering and no measurable disease developed. Only the disease levels on the untreated plots were assessed, FHB incidence and field severity were found to be 6.3 and 0.5 percent, respectively. There were no differences among treatments in yield, test weight, plump, or deoxynivalenol concentration.

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The authors wish to acknowledge the cooperation of Bill and Louis Arnold, Esmond, ND. This material is based upon work supported by the U.S. Department of Agriculture, under Agreement No. 59-0790-3-079. This is a cooperative project with the U.S. Wheat & Barley Scab Initiative.

DISCLAIMER

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.

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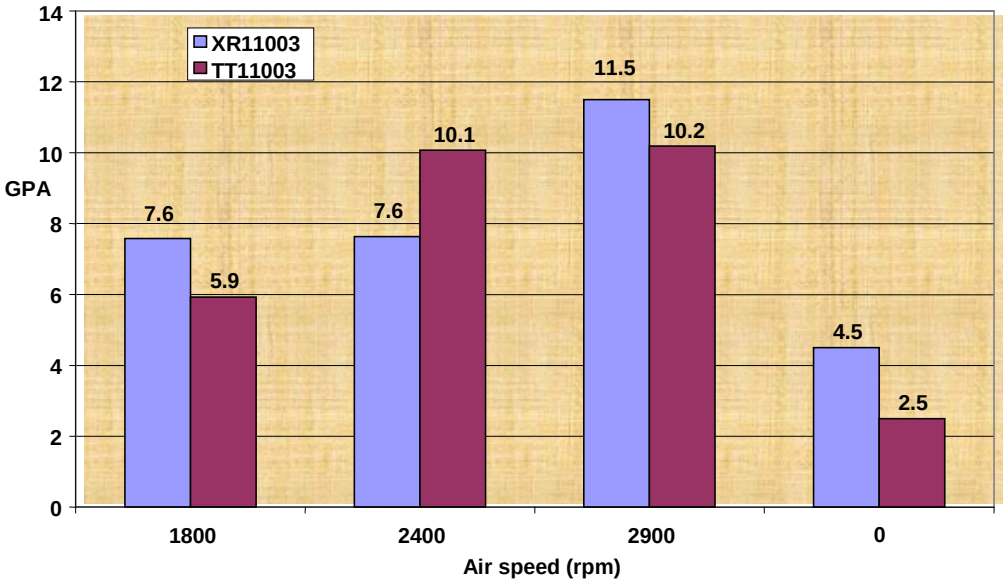

Figure 1. Volume Median Diameter (VMD) drop size produced using XR11003 and TT11003 nozzles at varying spray air delivery speeds.

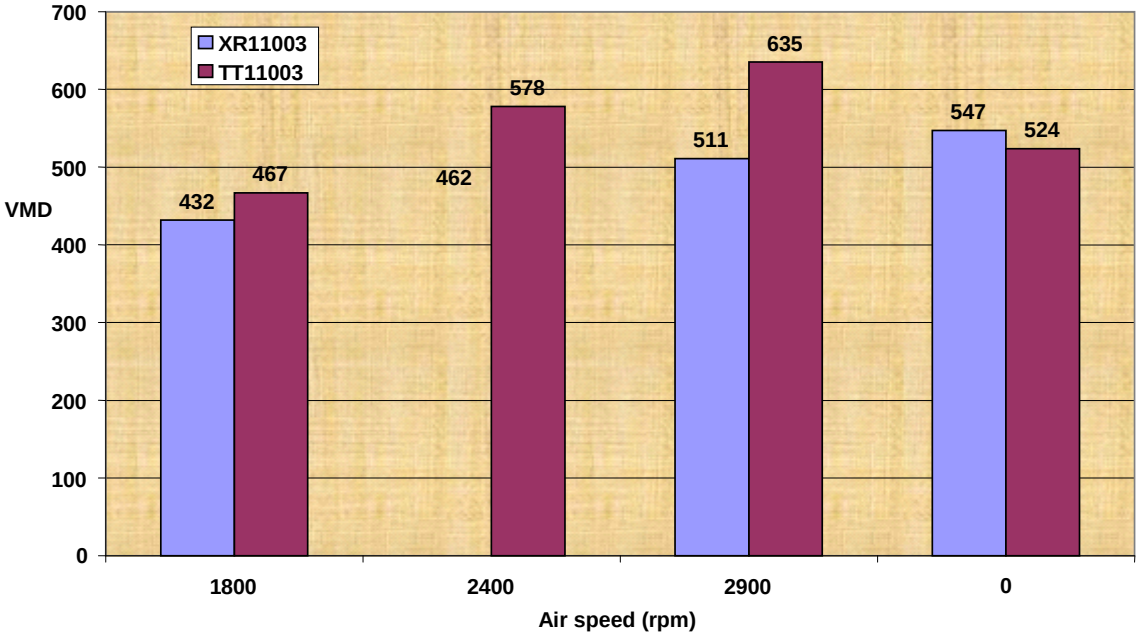

Figure 2. Estimated spray volume in GPA using XR11003 and TT11003 nozzles with varying spray air delivery speeds.

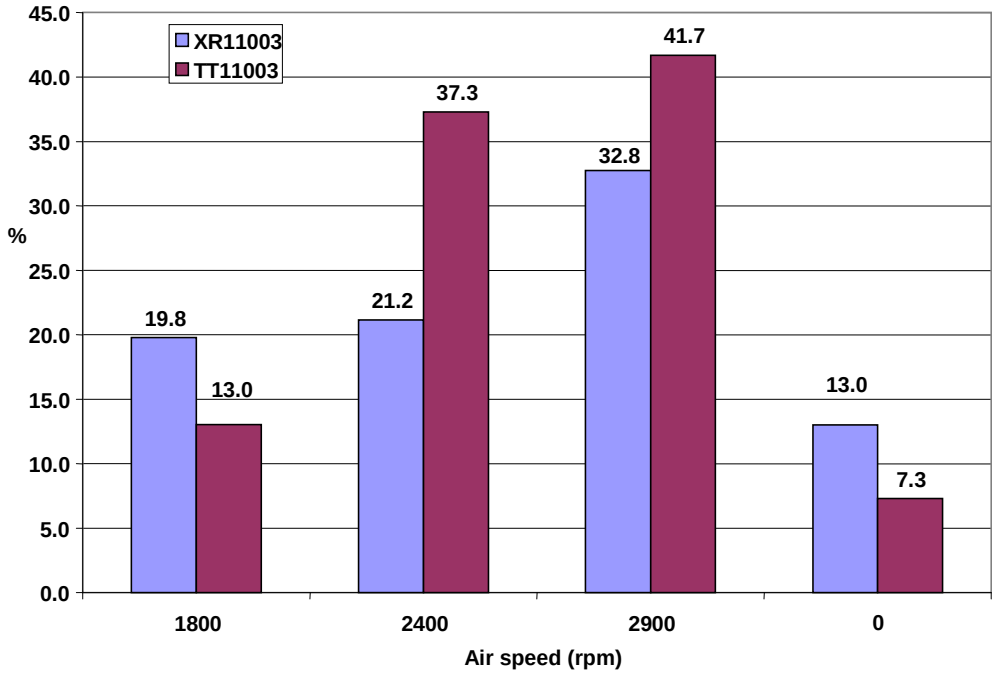

Figure 3. Relative percent area of water sensitive paper coverage using XR11003 and TT11003 nozzles with varying spray air delivery speeds.

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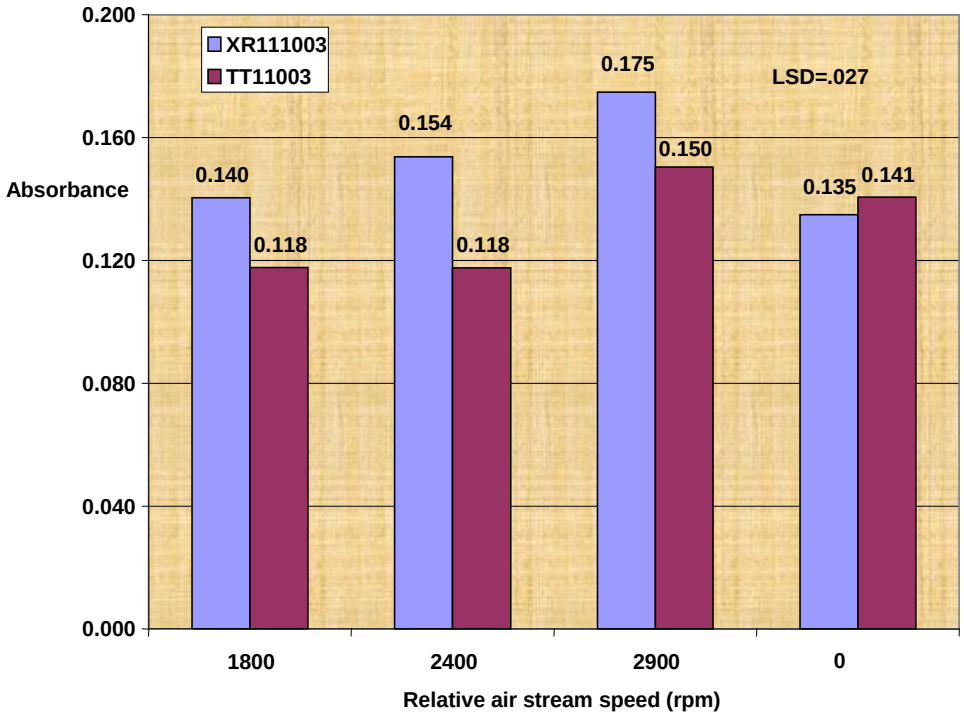

Figure 4. Relative deposition of spray on wheat heads using XR11003 and TT11003 nozzles in varying spray air delivery streams.

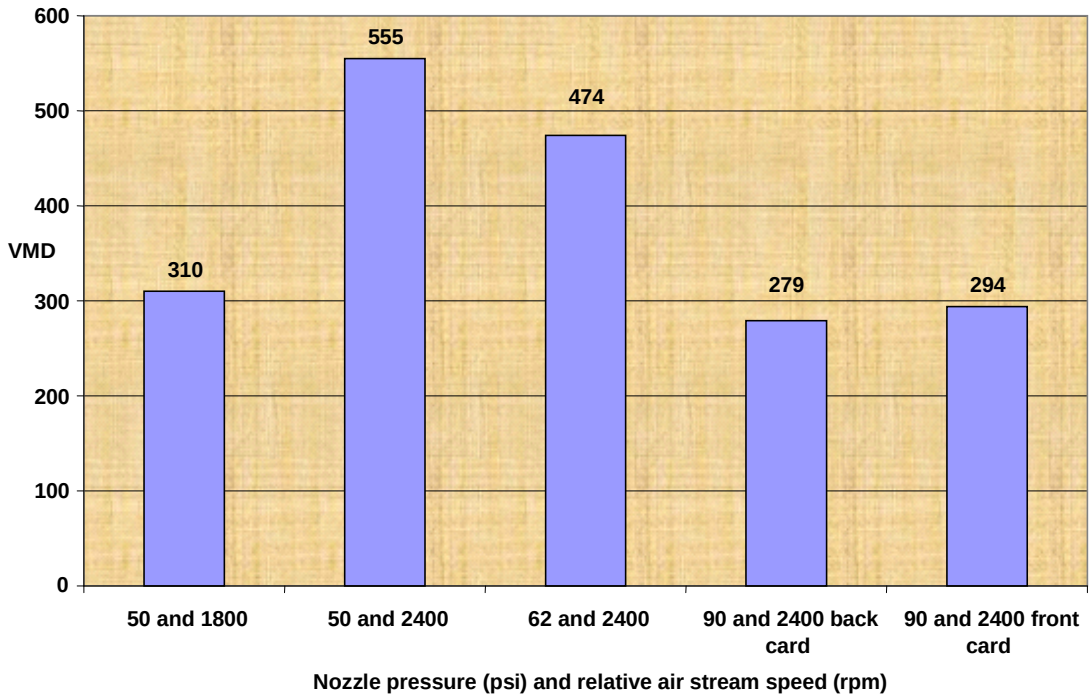

Figure 5. Volume Median Diameter (VMD) drop size measured with water sensitive paper using Hardi FO25_110 nozzles at varying pressures and spray air delivery speeds.

Table 1. Effects of fungicide on absorbance, yield, test weight and protein by nozzle and air stream speed, Esmond 2006.

Sources of Variation	Nozzle or Air Stream Speed	Absorbance	Yield (bu/a)	Test Weight (lb/bu)	Protein (%)
Nozzle		0.0444	0.1438	0.0867	0.1075
Air Stream Speed		0.0771	0.9207	0.6719	0.6693
Noz*Air		0.4275	0.7027	0.6749	0.8804
%C.V.		18	8	1	3
Nozzles averaged across air stream speeds					
	XR11003	.151	30.3	58.0	15.8
	TT11003	.132	28.8	57.4	16.1
$LSD_{(0.05)}$		.02	NS	00.6[Z]	NS
Air stream speeds averaged across nozzles					
	Fast	.163	29.0	57.4	16.1
	Medium	.136	29.5	57.7	16.0
	Slow	.129	29.2	57.7	15.8
	None	.138	29.7	57.9	15.9
$LSD_{(0.05)}$		.03[Z]	NS	NS	NS

[Z]Significant at 0.10 level.

CHARACTERIZING PARAMETERS OF AIR DELIVERY TYPE SPRAY SYSTEMS TO MAXIMIZE FUNGICIDE EFFICACY ON SMALL GRAIN.

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ABSTRACT

Several major manufacturers of ground application equipment (e.g. Hardi Spray Systems and Spray-Air Technologies Inc.), manufacture and sell sprayers that use an air stream to assist in delivering the spray solution to the plant canopy. These sprayers have been shown to offer several unique performance characteristics. First, the air stream minimizes spray drift by overpowering the ambient wind and carrying the smaller spray droplets to the target plant material. Second, the energy of the air stream tends to carry the small droplets (less than 200 microns) deeper into the plant canopy. Third, the turbulence of the air stream assists in more uniformly depositing the spray drops in the hard-to-reach areas of the canopy. The second and third characteristics would be important in controlling foliar diseases. The air stream, depending on velocity, also would be able to alter the orientation of the grain head and change potential deposition. Our objective is to characterize the effects of varying the speed of the air stream, drop sizes and application angles for improved fungicide efficiency to control Fusarium head blight on spring barley and hard red spring wheat (HRSW). The two studies were randomized complete block designs with factorial arrangements and replication. Factors included three drop sizes, three air speeds, and three spray angles. Prosaro fungicide and Induce adjuvant were applied at 6.5 fl. oz/acre and 0.125% v/v to control FHB.

RESULTS

Fungicide coverages were different among sprayer factor combinations on HRSW but not barley indicating the uniqueness of architecture of the individual crop. Fungicide applied with a 'large' fine drop at 60º angle had the lowest incidence and field severity on the HRSW. HRSW yield was greatest when a median air speed was used, 55.8 vs 52.4 and 51.3 bu/acre. On barley a smaller yield was measured when a coarse drop was used in combination with near vertical orientation and minimum air speed. Several sprayer configurations increased plump. The untreated control was included in the trials but was not included in the statistical calculations because it did not fit with the factorial arrangement.

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DISCLAIMER

EVALUATION OF FUNGICIDE FOR CONTROL OF FUSARIUM HEAD BLIGHT WITH AERIAL APPLICATION TECHNOLOGY.

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INTRODUCTION

Fusarium head blight (FHB) has been a major problem for cereal grain producers during the past decade. To combat this disease, growers have applied fungicide by both aerial and ground application. About 50% of the small grains acreage sprayed with fungicide in the Dakota-Minnesota region of the Great Plains is applied with spray planes. Aerial application has several advantages over ground application. The planes travel at speeds greater than 100 mph so large acreages can be sprayed in relatively short periods of time, the planes can make applications when surface conditions do not permit use of ground application equipment, and there is less damage to the crop due to tracking. Most aerial applications are applied at 3 to 7 GPA depending on the fungicide label. Applicators use a variety of nozzle types which often include deflectors to discharge spray perpendicular to the air stream. Spray discharge angles perpendicular to the air stream create a smaller drop size than nozzles directed parallel to the air stream. Faster travel speeds will decrease drop size and increasing liquid operating pressure will increase drop size. Spray volumes can be increased by increasing orifice size or by adding additional orifices along the spray boom. Aerial application spray drop size is determined by orifice size, nozzle orientation to the air stream, operating pressure and flying speed.

MATERIALS AND METHODS

An aerial application study was conducted near Esmond, North Dakota in 2006 to evaluate fungicide application for control of FHB on 'Tradition' cultivar barley. A site was selected on the Bill and Louis Arnold farm. The study team included Bill Arnold, farm operator, Dakota Aviation, Don Hutson-owner/pilot Grafton, ND, Vern Hofman and Scott Halley-North Dakota State University, Extension Engineer and Crop Protection Scientist, respectively. Several additional summer staff completed the team. The study was designed as a randomized complete block with four replicates. The plots were 150 ft wide (three application passes) by 450 to 850 ft long. Plots in blocks for replicate one and two were north/south and replicates three and four east/west. The treatments included Folicur 3.6 F (tebuconazole) fungicide (Bayer CropScience manufacturer) at 4 fl oz/acre applied with spray volumes of 3 or 7 GPA applied with a fine and a 'small' medium size drop and one volume of 5 GPA applied with a 'small' medium size drop (the 5 GPA treatment is a typical application standard of commercial aerial applicators). The applications were applied to heading barley (greater than 50% of main stem heads fully extended from the boot). The fungicide was applied with a fixed-wing Cessna Ag Truck aircraft equipped with CP-03 nozzles flying at 125 mph with an operating pressure of 40 psi. The different spray volumes were obtained by changing orifice size across the spray boom and the drop size adjustment was made by using the 30 or 90 degree deflector, large and smaller drop size, respectively. The treatments were applied on 30 June between 10:00 a.m. to 2:00 pm after the dew had dried from the plants. Wind conditions were WNW at speeds of 8.5 to 10.4 mph. This is a typical wind speed for the region at this time of year. The fungicide was applied with Induce adjuvant (Helena Chemical Co.) at 0.125% v/v and F D&C Blue #1 dye added at 44 grams per acre. The dye is a food grade type used in coloring food products. Water sensitive cards were placed on stands at grain head height in the center of each plot to replicate a head. The most commonly used method to evaluate spray technology is the use of water and oil sensitive paper (WSP Spraying Systems Co. ®, Wheaton, Illi-

nois 60189). Cards, 26 x76 mm, were placed at grain head height on stands (Panneton, 2002). One card was placed horizontal (Wolf and Caldwell, 2004). Applied stain size was determined with WRK DropletScan system (WRK, Cabot, Arkansas 72023) and presented as volume median diameter (VMD) which indicates that ½ of the spray volume is in drops smaller than this drop size and ½ of the spray volume is in drops larger than this size. The area of coverage is presented as percent of the card area analyzed.

Three 50 ft spray passes were made side by side (150 ft.) on each plot. All data were collected from the center of the plot. Additionally, three samples of ten heads were collected at 3 points across the center swath and placed in glass Erlenmeyer flasks for determination of head coverage of the spray solution. The collected heads were stored on ice until they could be measured for dye coverage. The spray coverage of the heads was determined by washing the dye from the heads by wrist action shaking for three minutes with 80 ml of 95% ethyl alcohol and determining the absorbance with a Jenway spectrophotometer (model 6300). Differences among treatments were determined by a visual assessment of FHB and foliar disease at mid dough growth stage by assessing twenty heads per plot and determining the incidence of the disease (present or not) and the severity of the individual head. The summation of the incidence times the severity of the twenty heads gave a field severity per plot. Foliar disease differences were determined by estimating the infected area on five leaves at two locations. The field was harvested on 5 August. One pass of the combine was made through the center of each plot with a Caterpillar Lexion combine with a straight cut header. The grain from the harvested area of each plot was measured with a weigh wagon and a sub sample saved to determine yield, test weight, protein, plump and deoxynivalenol (DON) from the processed grain sample. Data were analyzed with the general linear model (GLM) in SAS. Fisher's protected least significant differences (LSD) were used to compare means at the 5% probability level.

RESULTS AND DISCUSSION

The environmental conditions were in contrast in 2006 compared to 2005 when a duplicate trial was conducted. The crop in 2005 was devastated with FHB. In 2006 low relative humidity levels and little precipitation kept both FHB and foliar diseases from causing an economic loss. The limited available soil water also limited yields and reduced test weight and plump to levels so that malting barley standards were not met. No differences were determined among yield, test weight, protein and absorbance. Plump was increased 10% with a 3 GPA spray volume. The benefit was a result of increased amount of fungicide active ingredient collected on the spike th the larger fungicide concentration of the spray solution. Some fungicies extend the growing period of the plant before senescence and it is the authors' perspective that this may have occurred. A trend was established showing greater deposition with the finer type drop size. The awns of the barley are efficient collectors of fine drops and also spores. This trend is different from a typical application with ground equipment where a drop size of 300 to 350 microns will deposit in greater quantities than a fine drop size. The ASABE standard S-572 spray drop classification system for the two applications should have been about 240 and 300 microns. The WSP card showed a larger stain size than the reported limits of the technology. The differences show a drop size difference of about 50 microns between the two stain sizes and show one of the limitations of using WSP and field spray applications. The untreated in each replicate was not included in the statistical analysis because of the lack of fit with the factorial arrangement used to compare the mean volumes and drop sizes but is presented as a reference to overall fungicide efficacy.

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DISCLAIMER

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.

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Table 1. Yield, Test Weight, Plump, Protein, and Head Coverage (Absorbance) by Spray Volume and Drop Size Esmond 2006.

Spray Volume	Drop Size	Yield (bu/ac)	Test Weight (lb/bu)	Plump (%)	Protein (%)	Absorbance
untreated		66.3	45.1	48.9	11.8	0.057
		Spray volume averaged across drop sizes				
3		62.0	45.8	58.3	11.8	0.255
7		64.8	45.4	52.8	12.7	0.263
LSD (0.05)		NS	NS	5.3	NS	NS
		Drop size averaged across spray volumes				
	Fine	62.3	45.6	55.3	11.9	0.289
	Medium	66.6	45.6	55.9	11.9	0.229
3	Fine	58.5	46.0	58.6	11.8	0.286
	Medium	65.4	45.5	58.0	11.9	0.225
7	Fine	61.9	45.2	51.9	12.1	0.292
	Medium	67.7	45.6	53.8	12.0	0.235
Sources of variation						
Rep		0.0474	<0.0001	0.0019	0.1765	0.0431
Volume		0.8153	0.1592	0.0430	0.3871	0.8911
Drop Size		0.2256	0.8025	0.7976	0.9459	0.3390
Vol*Drop		0.6634	0.1146	0.6099	0.5453	0.9735
%C.V.		10	1	9	3	45

Table 2. Volume Median Diameter (VMD), GPA, and Coverage determined Spray Solution Collected on Horizontal Placed Water Sensitive Cards, Esmond 2006.

Spray Volume	Drop Size	VMD	GPA	Coverage (%)
3	Fine	360	4.2	10.1
3	Medium	404	2.0	4.7
5	Medium	370	4.9	12.0
7	Fine	401	7.5	17.8
7	Medium	451	4.7	11.9
Untreated		200	0.07	0.2

RELATIONSHIPS BETWEEN YIELD, GRAIN QUALITY VARIABLES, AND FUSARIUM HEAD BLIGHT INTENSITY IN WINTER WHEAT.

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ABSTRACT

Fusarium head blight (FHB) of wheat, caused by *Fusarium graminearum*, can cause significant losses resulting from yield reduction, kernel damage, and presence of deoxynivalenol (DON), an important mycotoxin with serious food safety implications. In 2007, two experiments were conducted to identify relationships between (i) yield, grain quality variables, and FHB intensity and (ii) visual assessments of FHB and DON. In the first experiment, three winter wheat varieties (Jagalene, Harry and 2137) were planted on two planting dates, 5 and 27 October 2006. Plots were inoculated with conidia and ascospores of *F. graminearum* (1×10^5 spores/ml) at early and mid anthesis, or were not inoculated. Experimental design was a split-split-plot in randomized complete blocks with three replications. Planting date was the main plot, variety the subplot, and inoculation timing the sub-subplot. FHB severity was determined 21 and 25 days after inoculation on 20 heads in each of five arbitrarily selected locations in each plot. There was a significant positive correlation ($0.48 \leq r \leq 0.76$, $P \leq 0.05$) between FHB incidence and FHB severity in each variety (N = 18), first planting date (N = 27), and all varieties and planting dates combined (N = 54). Correlation between FHB index and yield was negative but not significant at $P = 0.05$. Correlation between FHB index and 1000 kernel weight was significant for the first planting date ($r = 0.45$, $P = 0.0191$) but positive, contrary to what as was expected. Correlation between FHB index and *Fusarium* damaged kernels (FDK) was significant for the first planting date ($r = -0.47$, $P = 0.0132$) but negative, contrary to what was expected. Correlation between FHB index and DON was not significant at $P = 0.05$. Correlation between FDK and 1000 kernel weight was not significant at $P = 0.05$ for Jagalene and Harry, but was significant for 2137 ($r = -0.47$, $P = 0.0507$), first planting date ($r = -0.65$, $P = 0.0002$), second planting date ($r = -0.63$, $P = 0.0005$), and all varieties and planting dates combined ($r = -0.64$, $P < 0.0001$). Correlation between FDK and DON was not significant at $P = 0.05$, as was correlation between FDK and yield. In the second experiment, two varieties (Harry and 2137) were planted on 9 October 2006. Plots were inoculated at early anthesis as described above. Varieties were arranged in randomized complete blocks with three replications. In mid June 2007, 20 heads were randomly tagged in each of 11 disease severity categories in each plot: 0, 5, 10, 15, 20, 25, 30, 35, 40, 45, and 50%. There was a significant positive correlation between FHB severity in the 11 severity categories and DON for both Harry ($r = 0.74$. $P = 0.0092$) and 2137 ($r = 0.70$, $P = 0.0157$). However, DON levels were higher in Harry than in 2137. The results from this study indicate that (i) relationships between yield, grain quality variables, and FHB intensity may not be clear cut, (ii) there is a positive association between DON levels and FHB severity, and (iii) wheat varieties differ in the levels of DON they accumulate.

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This material is based upon work supported by the U.S. Department of Agriculture, under Agreement No. 59-0790-7-080. 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.

OUTCOMES OF USING INTEGRATED FHB MANAGEMENT STRATEGIES ON MALTING BARLEY CULTIVARS AND GERMPLASM IN MINNESOTA.

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ABSTRACT

The objective of our trial was to determine grain and malt quality responses from four commercially-available malting cultivars and four advanced malting germplasm lines following exposure to four Fusarium head blight (FHB) disease management strategies. The experiment was planted on 9 May 2007 into soybean residue and was situated in a commercial production field within Marshall County, in northwest Minnesota. Barley entries included Tradition, a 2004 Busch Agricultural Resources, Inc. (BARI) release; Legacy, a 2000 BARI release; Drummond, a 2000 North Dakota State University (NDSU) release; B2218 and B2513, BARI advanced germplasm lines; ND20448, NDSU advanced germplasm line; and M122, University of Minnesota advanced germplasm line. Treatments were replicated four times and exposed to one of four fungicide strategies (Table 1). The test area was neither misted nor inoculated.

Environmental conditions did not support normal plant growth or stand establishment. Frequent rain events caused soil saturation for an extended period of time prior to Feekes growth stage (FGS) 10.5 (early heading). This resulted in plant stress which caused severe plant stunting, tiller abortion, and low yield (entry means for yield ranged from 21 bu/a to 37 bu/a). Split-plot analyses from PROC GLM in SAS were conducted where fungicide treatment represents whole plots and entry represents subplots. FHB symptoms were assessed at approximately FGS 14. Barley entry and fungicide treatment were significant for FHB incidence, while barley entry was also significant for FHB severity and FHB index ($P<0.05$). While FHB symptom expression was relatively low (entry means for FHB index ranged from 0.2% to 1.8%), resistant germplasm lines (B2218, M122, and B2513) had significantly lower index values than current commercial cultivars (Legacy, Tradition, and Robust). Deoxynivalenol (DON) levels in grain were miniscule to below detectable limits with an overall test mean of 0.1 ppm. The nontreated control fungicide treatment was not different from tebuconazole (4 fl. oz./a), but had significantly higher DON levels compared to either rate of Prosaro ($P<0.05$).

Three replicates of grain samples from treatments #1 and #4 (Table 1) were micro-malted in the BARI Seed Research Quality Lab located at Fort Collins, CO. Resulting malt was analyzed for alpha amylase, beta glucan, diastatic power, free amine nitrogen, percent fine extract, predicted extract, malt protein, wort protein, and turbidity. Differences between barley entries were significant across all quality traits ($P<0.05$). Responses of malt to fungicide were significant for alpha amylase ($P=0.03$). Treatment #4 resulted in larger levels (75.0) of alpha amylase than the nontreated control (69.7). There were no significant fungicide*entry interactions at $P<0.05$.

Data produced from a single growing season in northwest Minnesota indicate that interactions between fungicide treatment and barley entries generally did not influence FHB disease symptoms, grain yield, or kernel and malt quality traits. However, additional data is needed from a typical growing season before further conclusions can be drawn.

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The authors would like to thank Busch Agricultural Resources, Inc. for supporting this research; Bayer CropScience for supplying fungicide products; UM and NDSU breeders for providing germplasm; Busch Agricultural Resources, Inc. Seed Research Quality Lab and the University of Minnesota Mycotoxin Lab for providing malt quality analyses and DON results, respectively.

Table 1. Fusarium head blight disease management strategies tested on eight malting barley entries near Warren in the northwest Minnesota Red River Valley. Fungicide applications were made at Feekes growth stage 10.5 (early heading).

Trt	Product	Active ingredient	Rate* (fl. oz./a)
1	Nontreated control...		
2	Folicur.....	tebuconazole	4.0
3	Prosaro.....	tebuconazole and prothioconazole	6.5
4	Prosaro.....	tebuconazole and prothioconazole	8.2

*Treatments 2 through 4 included 0.125% Induce, a nonionic surfactant.

UNDERSTANDING PRACTICAL OUTCOMES FROM IMPLEMENTING FHB MANAGEMENT STRATEGIES ON SPRING WHEAT.

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ABSTRACT

The objective of our trial was to determine grain yield and quality responses, as well as economic outcomes from 13 hard red spring wheat cultivars when exposed to six different disease management strategies. This research represents Minnesota's participation in the multi-state, multi-year integrated disease management cooperative research which is meant to identify the most practical means in managing Fusarium head blight (FHB) across states and wheat classes.

The test included four replicates at each of two experiment locations. Planted into soybean residue, a site was located near Oklee in northwest Minnesota and another was near Fergus Falls in west central Minnesota. The Oklee site was planted on 27 April 2007 and the Fergus Falls site on 2 May 2007. Spring wheat cultivars included Ada, Alsen, Banton, Bigg Red, Briggs, Freyr, Glenn, Knudson, Oklee, Samson, Steele-ND, Ulen, and Walworth which were exposed to one of six disease management strategies (Table 1). The test areas were neither misted nor inoculated.

Environmental conditions varied substantially between locations. Split spilt-plot analyses using PROC GLM in SAS were made where 'location' represented the whole plot factor, 'fungicide' the subplot factor, and 'cultivar' the sub-subplot factor. Transformations were conducted on data identified with non-normal distributions. Fergus Falls had lower test weights, kernel protein, and FHB index ratings than the Oklee site ($P<0.05$). Deoxynivalenol (DON) levels in grain were miniscule to below detectable limits at Fergus Falls (d"0.13 ppm). Oklee location DON results are not yet available. Cultivar and disease management strategy were both significant for net revenue, yield, test weight, protein, and FHB incidence, while FHB severity and FHB index were significant for cultivar ($P<0.05$). Knudson (77.8 bu/a), Samson (77.6 bu/a), and Steele-ND (74.9 bu/a) had the largest yields, while Bigg Red (62.4 bu/a) and Alsen (63.3 bu/a) had the smallest. Cultivars Samson, Ulen, Steele-ND, and Oklee had the highest ratings for FHB incidence, FHB severity, and FHB index while Bigg Red, Alsen, Glenn, and Knudson had the lowest. Knudson ($611.27/a), Samson ($608.26/a), and Steele-ND ($591.48/a) had the greatest net return while Bigg Red ($482.89/a) and Alsen ($497.13/a) returned the least ($P<0.05$). Across all cultivars, yield, protein, and test weight were significantly increased with disease management strategies #3, #4, and #5, compared with strategy #1 (Table 1). Strategy #4 resulted in the largest net return and strategies #1, #2, #5, #6 the least returns ($P<0.05$).

Disease-associated limitations to yield were offset by timely fungicide application. Cultivars known for susceptibility to disease responded well to the growing environment, producing excellent yields of high quality grain. Fungicide application increased net returns compared with no fungicide even during a year of relatively low disease pressure. Economically-speaking, spring wheat growers in the Minnesota Red River Valley who benefited the most during 2007 grew cultivars that were moderately susceptible to FHB.

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Table 1. Disease management strategies tested on 13 cultivars of hard red spring wheat at two locations in the Minnesota Red River Valley.

			Application	
Strategy	Product	Active ingredient	Rate*	Timing**
1	Nontreated control..			
2	Dividend Extreme..	difenoconazole and mefenoxam	3 fl. oz./100 lbs.	Seed applied preplant
3	Headline.....	pyraclostrobin	3 fl. oz./a	FGS 2
	Folicur/Proline.....	tebuconazole & prothioconazole	3 + 3 fl. oz./a	FGS 10.51
4	Dividend Extreme..	difenoconazole & mefenoxam	3 fl. oz./100 lbs.	Seed applied
	Headline.....	pyraclostrobin	3 fl. oz./a	FGS 2
	Folicur/Proline.....	tebuconazole/prothioconazole	3 + 3 fl. oz./a	FGS 10.51
5	Dividend Extreme..	difenoconazole & mefenoxam	3 fl. oz./100 lbs.	Seed applied
	Folicur/Proline.....	tebuconazole & prothioconazole	3 + 3 fl. oz./a	FGS 10.51
6	Folicur/Proline.....	tebuconazole & prothioconazole	3 + 3 fl. oz./a	FGS 10.51

*Treatments 3 through 6 included 0.125% Induce, a nonionic surfactant.

** Feekes growth stage (FGS) 2 = 4 to 5 leaf, and FGS 10.51 = early anthesis.

CONTRIBUTION OF WITHIN-FIELD INOCULUM SOURCES TO FUSARIUM HEAD BLIGHT IN WHEAT.

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ABSTRACT

Knowledge of the relative contribution of within-field inoculum sources of *Gibberella zeae* to infection of local wheat and barley is important for developing and/or excluding strategies for managing Fusarium head blight (FHB). Our research is based on the hypothesis that spores of *G. zeae* that are deposited on wheat spikes and that result in Fusarium head blight come primarily from well-mixed, atmospheric populations in an area. Our experimental objective was to determine the relative contribution of within-field, clonal inoculum sources of *G. zeae* to FHB in susceptible wheat cultivars. In 2007, corn stalks and corn kernels infested with clonal, fingerprinted isolates of *G. zeae* containing rare alleles (relative to background populations) were released in replicated 1 m diameter circular plots in single wheat fields in New York and Virginia. We collected mature wheat spikes at the inoculum source, at a radius of 10 feet from the source, at a radius of 20 feet from the source, and in more distant parts of each field. We used amplified fragment length polymorphisms (AFLPs) to genotype isolates recovered from these spikes and to determine the contribution of released isolates to FHB at various distances from those sources. Since our inoculum sources contained clonal isolates that have unique AFLP haplotypes, we were able to observe these clones in a mixed/diverse background population containing numerous AFLP haplotypes. Nearly 500 isolates of *G. zeae* were collected and single-spored from NY and VA. Preliminary AFLP data from the first year of experimentation suggests that within-field sources of *G. zeae* provided a minor fraction of FHB inoculum compared to background atmospheric sources in a non-epidemic environment in New York and in a moderate epidemic environment in Virginia.

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TIME OF FLOWERING IN WHEAT FOR MANAGING FUSARIUM HEAD BLIGHT.

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ABSTRACT

Efficacy of management is increasingly being timed based on crop developmental stage and consideration of crop developmental physiology. In the case of Fusarium head blight, it has often been managed by one pesticide application timed to the developmental stage of anthesis (i.e., flowering). However, flowering is controlled by the interaction of genotype, environment, and management and can occur over an extended period of time, confounding when to make the application. The objective of this talk is to discuss wheat development to provide information for improving the current management practice and exploring alternative management options. The presentation will first present a brief overview of wheat development and highlight when the various yield components are being formed. Wheat development follows a few general principles beginning with development being an orderly and predictable process. The genetics provides the "blueprint" for the orderly sequence of events leading to flowering. Temperature, reflecting thermal time, is then used to predict when flowering will occur. Sources of variation in flowering time are identified including a) within a shoot, b) among shoots on a plant, c) among plants within small areas/plots, and d) across landscapes. Other sources of variation exist among genotypes and variable planting/emergence dates. Management options to reduce the period of flowering are discussed, along with the risks of doing so

DIFFERENTIAL EFFECTS OF INFECTION TIMING ON FUSARIUM HEAD BLIGHT AND ON DON AND DON DERIVATIVES IN THREE SPRING GRAINS.

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ABSTRACT

The effect of infection timing on the development of Fusarium head blight (FHB) and DON and DON derivatives was evaluated under a controlled greenhouse environment. Test plants were susceptible and resistant or moderately resistant lines of three spring grains - hard red spring wheat (HRSW) ('Grandin' and 'Glenn'), durum wheat ('Munich' and 'Divide'), and six-rowed spring barley ('Robust' and ND20448 [a line from R. Horsley's breeding program]). Infection timings included head half-emerged (Feekes 10.3), full head emergence (Feekes 10.5), anthesis in wheat (Feekes 10.51), and kernel watery ripe (Feekes 10.54), or dual infections at the two later growth stages. Infections were initiated by atomizing a mixture of four isolates of *Fusarium graminearum*, at 20,000 spores/ml, 20 ml/pot, with a DeVilbiss atomizer, followed by 24 hours of misting. FHB incidence and severity were determined at 21-25 days after inoculation. At maturity, kernels were hand threshed for subsequent mycotoxin analysis. DON, 3ADON, 15ADON and nivalenol (NIV) analyses were done using gas chromatography and electron capture techniques. FHB indices [(incidence x severity)/100] and mycotoxin levels (ppm) indicated that differential responses to single infection timings occurred among spring grain classes: a) in barley, values of these parameters were highest with infection at the watery ripe stage; b) in HRSW, at anthesis; and 3) in durum, about equally high at anthesis or watery ripe stage infections. In all three crops, infections at head half-emerged resulted in the lowest FHB severities and DON levels. The dual infections at the two latter growth stages, generally resulted in the highest FHB index and DON values in all grain classes. DON, 15ADON and 3ADON accumulations were highly correlated with FHB index in all spring grain classes. 15ADON and 3ADON levels also were highly correlated with DON levels. 15ADON was more frequently recovered and at higher ppm than 3ADON (highest average 15ADON was 4.5 ppm in barley, vs 1.1 ppm 3ADON in barley). 3ADON generally was detected only when the average DON levels were high: 22 ppm in barley, 45 ppm in HRSW and 37 ppm in durum, with average 3ADON levels well under 1.0 ppm. Resistant lines generally had much lower DON levels than susceptible cultivars, across all infection timings. 3ADON was not detected in the resistant HRSW or the moderately resistant durum cultivars. NIV was not detected in any of the grain classes.

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EFFECTS OF FUNGICIDE TIMING ON FUSARIUM HEAD BLIGHT AND ON DON AND DON DERIVATIVES IN THREE SPRING GRAINS.

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ABSTRACT

The effects of fungicide timing on the reduction of Fusarium head blight (FHB) and mycotoxins were evaluated under a controlled greenhouse environment. Fungicides were tested on susceptible and moderately resistant to resistant lines of three spring grains - hard red spring wheat (HRSW), durum wheat, and six-rowed spring barley. Fungicide timings included head half-emerged (Feekes 10.3), full head emergence (Feekes 10.5), anthesis in wheat (Feekes 10.51), and kernel watery ripe (Feekes 10.54) or a dual treatment at full head emergence in barley or anthesis in wheat, followed by treatment at kernel watery ripe. Fungicide was applied with a greenhouse track sprayer with XR8001 forward and backward flat fan nozzles, 18 gpa, at appropriate growth stages. Treatments were either Prosaro (tebuconazole + prothioconazole) at 6.5 fl oz/A, or Proline (prothioconazole) at 5 fl oz/A. Fungicide treatments were applied 4 hours after infection initiations. Infections were initiated by atomizing a mixture of four isolates of *Fusarium graminearum*, at 20,000 spores/ml, 20 ml/pot, with a DeVilbiss atomizer, followed by 24 hours of misting. FHB incidence and severity were determined at 21-25 days after treatment. At plant maturity, kernels were hand threshed for subsequent mycotoxin analysis. DON, 3ADON, 15ADON and nivalenol (NIV) analyses were done using gas chromatography and electron capture techniques. FHB indices [(incidence x severity)/100] and DON values (ppm) were significantly reduced by fungicide treatments in all grain classes and cultivars. In the most susceptible lines of all 3 grain classes, a single fungicide treatment, applied at optimal growth stage for infection, resulted in 90-98% reductions of FHB indices and DON levels (example: 24.4 ppm DON in durum wheat infected at anthesis, vs 0.56 ppm DON with fungicide treatment added at anthesis). With the dual timings of application, FHB indices and DON levels were reduced by 86 to 97%. Similar percent reductions were observed in the more resistant lines, but overall FHB and DON levels were lower in the more resistant lines. Fungicide treatment at any of the tested timings in HRSW and durum resulted in zero detection of 15ADON and 3ADON. In barley, fungicide treatment resulted in 100% reduction of 3ADON, and 91-94.4% reduction of 15A DON. These fungicide treatments were very effective in reducing FHB, DON and DON derivatives under greenhouse conditions.

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EXPERIENCES IN REDUCING DISEASE AND DON THROUGH COMPONENTS OF FHB MANAGEMENT.

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ABSTRACT

Few would argue that a favorable climate during vulnerable crop growth stages often is the key factor resulting in severe Fusarium head blight (FHB). However, as unfavorable weather is hard to avoid, researchers and producers have looked for implementable strategies for managing FHB. Champeil et al., 2004 (*Fusarium head blight: epidemiological origin of the effects of cultural practices on head blight attacks and the production of mycotoxins by Fusarium in wheat grains. Plant Science 166:1389-1415)* provided an extensive review of studies of cultural practices that may affect FHB severity and mycotoxin production, and more recent papers also have been published. Key strategies that have been extensively researched include: crop rotation, tolerant cultivars, and fungicide use.

From 2003-2005, various regions in the US had severe FHB outbreaks, and individual FHB management strategies used alone did not necessarily reduce disease severity and DON to levels required by the grain industry. Several 2005 research trials in eastern ND provided quantitative evidence that a combination of crop rotation, variety choice, and fungicide treatment reduced FHB severity and DON levels in an additive manner, ie 10 ppm DON levels in spring wheat with no strategy; 5 ppm DON with soybean rotation added; 2.0 ppm with soybean + resistant variety; and 1.2 ppm with soybean + resistant variety + fungicide.

Members of the management group of the US Wheat and Barley Scab Initiative (USWBSI) met in 2006 and decided to implement studies, across multiple states and grain classes, to quantify the value of additive strategies for FHB and DON management. These cropping systems studies were to be done under natural field conditions and the objectives were to:

1) demonstrate that integrated management is the most effective means of reducing losses to FHB/DON; and

2) increase grower adoption of integrated strategies by demonstration of their effectiveness in a wide range of environments.

Funded USWBSI cropping system studies were in place in 2007, a year in which some locations again had FHB. The Forum's presentation for this abstract will provide examples of 2007 results from these studies. Dr. Pierce Paul has statistically analyzed results from these cropping system studies, and his poster will be presented at the 2007 FHB Forum. Others also may be presenting their individual state's data. ND results will be published in the 2007 *Proceedings of the 5th Canadian Fusarium Head Blight Workshop, Winnipeg. Nov. 27-30*.

ACKNOWLEDGEMENT AND DISCLAIMER

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COMPARISON OF FUNGICIDES AND NOZZLE TYPES AGAINST FHB IN WHEAT AT FARM APPLICATION.

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ABSTRACT

In 2006 the Turbo FloodJet and the TeeJet XR nozzles were compared in three winter wheat cultivars Petur (MR), Kapos (MS) and Miska (S). In 2007 the AIC TeeJet and Turbo TeeJet Duo nozzles were added to have a wider spectrum of comparison. All fungicides were run at 250 L/ha water and 8 km/hr speed. 17 m wide boom was used, on both sides mounted with a different nozzle type. A plot was 7 m wide and 300 m long. Technologies were evaluated across cultivars and fungicides, the fungicides were rated across cultivars and technologies. In both years natural epidemic occurred. In cv Miska, the most susceptible genotype, about 30 infected heads/m^2 were recorded.

In 2006 eight, in 2008 10 different fungicides were used:

Prospect (200 g/L carbendazim, 80 g/L propiconazole) 1.5 L/ha;
Falcon 460 EC (167 g/L tebuconazole 250 g/L spiroxamine 43 g/L triadimenol) 0.8 L/ha;
Prosaro (125 g/L prothioconazole, 125 g/L tebuconazole) 1.0 L/ha;
Tango Star (84 g/L epoxyconazole, 250 g/L fenpropimorph) 1.0 L/ha;
Eminent 125 SL (125 g/L tetraconazole) 1.0 L/ha;
Amistar Xtra (200 g/L azoxystrobin, 80 g/L ciproconazole) 1.0 L/ha;
Coronet (Nativo) (200 g/L tebuconazole és 100 g trifloxystrobin) 1.0 L/ha;
Artea 330 EC (250 g/L propiconazole, 80 g/L ciproconazole) 1.0 L/ha; and
Juwel (125 g/L epoxyconazole, 125 g/L krezoxim-metil) 1.0 L/ha.

In 2006 the mean efficacy of the TeeJet XR nozzle across fungicides was 44 %. The TurboFloodJet nozzles gave 58.60 % reduction of the natural head infection. At the Turbo FloodJet nozzle the lowest efficacy was measured for Eminent (16 %) and 91.5 % for Prosaro. At the traditional nozzles 14.42 and 79 % were the corresponding values. For DON the mean efficacy for TeeJet XR nozzles was 51 %, the reduction for Turbo FloodJet 65 %. In 2007 four nozzles were compared, the two nozzles types from 2006 were supplemented by AIC TeeJet and the Turbo TeeJet Duo nozzles as the Turbo FloodJet nozzles need very uniform soil level to keep the boom constantly at 20-30 cm above stand. This is not always at hand. The data for the FHB data showed a 60.7 % efficacy for AIC TeeJet and 62.8 % for TeeJet XR across fungicides. The Turbo TeeJet Duo reached 70.2 % and the Turbo FloodJet finished at 79.2 %. Prosaro was again the best with 95 % efficacy at the Turbo FloodJet nozzle type. The results provide several important conclusions. The traditional nozzles can reach with the best fungicides up to 70 % reduction when technology, timing is optimal. The difference is very large between fungicides, in this test 42 % for Eminent and 92 for Prosaro across technologies. We believe therefore that the successful chemical control needs both better technology and better fungicides.

ACKNOWLEDGEMENTS

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FIELD AND LABORATORY STUDIES TO MONITOR CELL POPULATIONS, LIPOPEPTIDES AND LIPOPEPTIDE GENES OF *BACILLUS* 1BA, A BIOCONTROL AGENT. ACTIVE AGAINST FUSARIUM HEAD BLIGHT.

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ABSTRACT

Fusarium graminearum causes Fusarium Head Blight (FHB) on wheat, barley and other small grains. Biocontrol agents (BCAs), such as certain *Bacillus* sp., can be used to control FHB and/or reduce deoxynivalenol (DON) levels in grain. Population studies of the BCAs on inoculated grain heads show how BCA numbers fluctuate over time, and if there are differences in BCA populations between wheat and barley. In previous work to count populations of *Bacillus* 1BA in the field, we found that this bacterium is thermotolerant and salt tolerant, able to grow on Tryptic Soy Agar (TSA) at elevated salt and temperature conditions that inhibit most micrflora native to the grain heads. Field plot treatments in 2007 were 1BA by itself; and 1BA + Prosaro (a fungicide) + Induce NIS (nonionic surfactant). Population counts were done over a 20 day period on both the wheat and barley. Endospore formation was examined by pasteurizing the samples at 85°C for 10 minutes, then plating. 1BA was isolated using TSA containing 10% NaCl, with incubation at 50°C for 48 hours. Controls for wheat and barley that did not receive 1BA inoculation gave low cell counts throughout the experiment, no higher than about 3.5 X 10^2 CFU/g fresh plant weight. For wheat, in the treatment with 1BA alone, vegetative cell counts peaked around day 10, then declined sharply, followed by a second increase in numbers by day 20, with a concurrent increase in endospore numbers. Compared to the treatment with 1BA alone, in the wheat treatment combining 1BA with Prosaro and Induce NIS, the population peaks shifted in time. Peak numbers of vegetative cells occurred at about day 6 then declined, with a smaller second peak on day 17. As vegetative numbers of 1BA in this treatment declined, endospore numbers increased. Numbers of 1BA on inoculated barley were much lower than on wheat, not being much different from the uninoculated control and not fluctuating much over time. It appears that 1BA, originally isolated from wheat material, is much better able to colonize and grow on grain heads of wheat than barley. The biocontrol effect of 1BA may be due to its production of lipopeptides including surfactin. Attempts to directly detect presence of *Bacillus* lipopeptides in extracts from inoculated grain heads were not successful, but efforts to detect these molecules directly on inoculated plant material will continue. Direct or indirect detection of surfactin or other lipopeptide genes/products on inoculated grain heads will be checked by methods including PCR. As a step leading to this, DNA of 1BA was isolated, then PCR was performed using primers for the surfactin production genetic locus (sfp). Good yield of PCR product was observed on an agarose gel, verifying that the primers and PCR method may be useful in detecting lipopeptide genes on inoculated grain heads.

EFFECTS OF SOLAR RADIATION ON THE VIABILITY OF *GIBBERELLA ZEAE* ASCOSPORES.

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ABSTRACT

Ascospores of *Gibberella zeae* are considered to be an epidemiologically important type of inoculum for Fusarium head blight of wheat and barley. The objectives of this study were to evaluate the effects of solar radiation, temperature, and relative humidity on the survival of ascospores in the environment at Rock Springs, PA and Manhattan, KS. In each experiment, ascospores of *G. zeae* were collected by inverting cultures containing mature perithecia over glass cover slips for several hours. After deposition, the ascospores were placed on glass Petri dishes and exposed to solar radiation or shaded conditions for predetermined lengths of time. The temperature of the exposed and shaded ascospores was kept constant by allowing the dishes to contact a circulating source of water. Total solar radiation, UV radiation, air temperature, relative humidity, and temperature of the water in the spore exposure apparatus were recorded during each experiment. Following exposure, the spores were washed from the cover glass, placed on water agar and incubated for 8-10 h. The percentage of germinating spores in five sub-samples of 100 ascospores was recorded for each exposure period, and germination was expressed as a ratio of the initial germination of ascospores for that experimental run. The preliminary results of these experiments indicate that the mean initial germination rate of the ascospores produced by isolates considered in this study was 55.6% with a standard deviation of nearly 20%. Regression analysis suggests that total solar radiation and the dose of UV radiation significantly impacted spore survival, but that temperature and relative humidity may also be important variables. The dose of solar radiation resulting in 100% ascospore mortality was 19.8 MJ/m^2 at the KS location, but was significantly greater at the PA location. Differences between the locations may be explained in part by differences in the isolate considered and range of temperatures experienced during the exposure periods.

MECHANISTIC SIMULATION MODELS FOR FUSARIUM HEAD BLIGHT AND DEOXYNIVALENOL.

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ABSTRACT

An empirical model with a single mathematical equation is commonly used as a part of disease forecasting systems. A web-based Fusarium Head Blight (FHB) forecasting tool (http://www.wheatscab.psu.edu/) is one of example of this approach to disease forecasting. These models are relatively easy to implement, and provide reasonably accurate forecasts in many cases. However, it may be difficult to derive biological interpretation from these empirical models. Other modeling approaches, such as mechanistic modeling, can be considered as an alternative. In this study, an object-oriented language STELLA (isee systems, Lebanon, NH) was utilized to create a mechanistic simulation models for FHB and deoxynivalenol (DON) based on the results of past studies on disease development and pathogen biology. Several candidate models have been developed with different scopes of interests, and one of candidate models will be discussed this presentation. This model estimates a distribution of *Fusarium* damaged kernels among heads of wheat and DON accumulation of the grain based on environmental conditions. Major steps in the disease cycle, such as perithecia development by *Gibberella zeae* and infection events were expressed as differential equations that use environmental conditions as input variables. These equations were connected in a logical manner, and using past weather data, a theoretical disease cycle of FHB was simulated over time. The model development process, preliminary results, as well as potential usage of this modeling approach as a tool for hypothesis testing for future studies will be discussed.

SPORE LOAD, DISEASE, AND DON: A FOUR YEAR VARIETY BY RESIDUE STUDY FOR FHB MANAGEMENT.

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OBJECTIVES

A four year field study was established near Brookings, SD in the years 2003 through 2006 to examine the impact of maize residues, host susceptibility and timing of planting on disease and mycotoxin accumulation in hard red spring wheat due to Fusarium head blight. These factors were also examined to determine their impact on inoculum concentrations on spikes from emergence through mid-milk stage. Finally, inoculum concentration was compared with DON in grain at harvest to determine if any consistent relationships existed.

INTRODUCTION

Fusarium head blight, caused primarily by *Gibberella zeae* (Schwein.) Petch (anamorph: *Fusarium graminearum* Schwabe) in the Northern Great Plains (including South Dakota), is usually the greatest threat to wheat quality and production in the humid areas of the region. The fungus survives and over-winters in plant tissue residues including small grain stems and roots as well as maize stalks and ear pieces (Sutton 1982). Recent research suggests that for South Dakota, ascospores and conidia of *G. zeae* are somewhat ubiquitous in the air, thought to be a result of extensive acreage of spore-bearing residues in the region coupled with extensive air mixing and medium to long-distance spore movement (Osborne 2006). Management of FHB in South Dakota involves the use of alternative rotations (wheat not to follow maize), fungicide application at flowering, and the use of resistant varieties to reduce risk. Over the past five years, several moderately resistant varieties have been released which are adapted to South Dakota growing conditions. In 2003, however, only one variety, 'Alsen', was both a recommended variety for SD and classified as 'moderately resistant' to FHB (Hall et al., 2003). This variety, along with 'Norm', a HRSW with high susceptibility to FHB, were used in this study to investigate the interaction of host resistance and 'local' maize residues in a region with abundant airborne inoculum.

METHODS

Field plots were established in Brookings, SD in 2003 through 2006. Each year, two planting dates (PD1 and PD2), timed 10-13 days apart, were utilized creating two identical studies upon which all treatments were applied and all measurements were collected. Planting date 1 represented the typical planting time for area wheat producers, whereas PD2 represented late-planted fields. In general, plots consisted of residue treatment (0, 30, and 80% soil coverage, by line-transect method) to generate corresponding low, medium and high levels of 'local' inoculum (local inoculum being defined as that produced from within the plot area, in contrast to inoculum produced outside the plot area). The medium level was discontinued after 2004. Sub-plots consisted of spring wheat varieties 'Alsen' and 'Norm'. Plot size varied slightly from year to year due to space restrictions; however, final plot disease measurements were collected from areas no smaller that 3.1m by 4.6m. In each year, whole-plots (residue treatments) were buffered on all sides by 8m of a tall wheat variety to mitigate inter-plot interference. The study was dependant on inoculum formed locally (i.e. within or beneath the crop canopy in plant tissues or residues), or externally (i.e. from adjacent fields with corn or small grain residue) and received no additional inoculum in the form of spore suspension or colonized grain (for ascospore spawn). No environmental modification was implemented to alter the conditions for disease development.

Within all sub-plots, a designated area was sampled daily after spike emergence by collecting five spikes per sub-plot for enumeration of spike-borne inoculum as described by Francl, et al. (1999). At three weeks post-flowering, disease assessments were performed on all sub-plots as described by Stack and McMullen, (1995) and included incidence and severity estimates on 100 spikes. Incidence is defined as proportion of 100 rated spikes exhibiting disease symptoms. Severity is the mean severity per infected spike. Disease index, often called 'field severity', is the product of incidence and severity and represents the overall 'amount' of disease in a given area. Harvest data collected included plot yield, test weight and moisture content. Harvested grain was sent to the NDSU Veterinary Toxicology Lab for assessment of mycotoxin concentration in grain following Tacke and Caspers (1996). A Burkard volumetric spore collector was placed for daily monitoring of airborne inoculum in the study area. Weather data was collected using a Campbell Scientific CR10X data logger and peripheral sensors. Parameters measured included temperatures and relative humidity in and above the crop canopy, wind, solar radiation, precipitation, soil temperature, soil wetness and leaf wetness estimations.

RESULTS AND DISCUSSION

The growing seasons 2003 through 2006 in eastern South Dakota were each distinct in terms of FHB levels on spring wheat across East-Central and Northeastern South Dakota. These differences were mirrored in the overall disease levels observed within this study each year, summarized in Table 1. Years within this study represented four distinct categories: very low disease (2006), low disease (2003), moderate disease (2005), and high disease (2004) seasons. The variation across years and PDs in this study established a range of environments that allowed for a more broad examination of treatment effects and disease parameters than if environments had been consistently favorable or unfavorable for disease over years.

Impact of Maize Residue Treatments on Spike-borne inoculum. The intermediate objective in placing maize residues in the study area was to establish three (or two) distinct levels of local inoculum intensity. Treatments were compared in each of eight year-PD environments to determine the effect of residue level on spike-borne inoculum for this study. Table 2 shows the average cfu's per day washed from heads in each treatment. Residue treatments significantly affected cumulative inoculum load within two of the environments for 'Alsen' (2004-PD1 and 2004-PD2), and only one for 'Norm' (2004-PD2). In each case there was a significantly higher level of inoculum on heads within the 80% residue treatment compared to 0% or 30% residue treatments. In all other environments, residue treatments had no significant effect on inoculum load. Therefore, it cannot be said that distinct levels of spike-borne inoculum resulted from maize residue treatments. The whole-plot area may have been too small to overcome problems of fetch (upwind distance from major confounding inoculum sources) and edge-effects. These problems could have led to inter-plot interference. The 2004 experiments give the best example of local inoculum dynamics under a high disease-pressure environment. In 2004, local inoculum apparently did contribute significantly to total inoculum concentration on spikes and therefore the influence of maize residue under the wheat canopy cannot be ignored. However, their influence under most environments is less obvious because of the relatively high level of 'external' inoculum entering the system concurrently.

Impact of Maize Residue, Variety, and Late Planting on FHB and DON. Residue treatments produced no significant effects on any of the visual disease estimates in any environment (data not shown), however DON was significantly increased by the 80% residue treatment for one environment (2004-PD1). Table 3 presents average DON concentration in grain for all treatments under each environment. There was also a trend toward higher DON in grain with increased maize residue for several environments, though it was not significant. These results mirror closely the average CFU/head data (Table 2). It is hypothesized that inoculum on spikes and final DON in grain may have a strong relationship. This is being investigated further.

The two varieties in the study exhibited different levels of disease and DON under all environments and treatments. As expected, 'Norm' was significantly higher

in FHB and DON than 'Alsen' in nearly all cases. The most noticeable and most significant differences were in DON estimates, where 'Norm' accumulated toxin to a much higher level than 'Alsen' under comparable environments (Table 3). As overall FHB disease pressure increased (greater inoculum, higher overall disease) across environments, the differences between 'Norm' and 'Alsen' for all response variables increased, indicating a strong variety by environment interaction, which is also represented in the analysis of variance (Table 4). This suggests that even some level of resistance in the host to FHB was made relatively more valuable as disease pressure increased due to environment.

As mentioned, planting dates each year resulted in somewhat unique environments. The effect of late planting can be seen in the higher levels of FHB each year of this study and in the analysis of variance (Table 4). Though the effect of late planting could be inverted under certain environmental conditions such as a dramatic drought or cool period at or just prior to anthesis for a late planted crop, in general, late plantings will experience higher temperatures and greater stress on plants at anthesis.

Based on the higher degree of variability in disease and DON across environments than within any one environment, the overall influence of the weather component of the system was probably the largest factor in any of the disease estimates or in toxin accumulation. The environmental variability coupled with the earlier mentioned confounding effects on inoculum load (interplot or 'external' inoculum interference) probably masked the relatively subtle effects of the residue treatments. Thus, for this set of experiments, maize residue on the soil surface was not a good predictor of disease development risk. This is contrary to the generally accepted etiological models of FHB which place high risk on corn and small grains residue beneath a susceptible crop (Parry et al. 1995; McMullen et al. 1997; Sutton, 1982; Andersen, 1948; Bai and Shaner 1994; Pererya et al. 2004). The impact of maize residue in this set of studies was much less important than the impact of the environment on disease development. Apparently, non-local inoculum was a significant portion of total inoculum load in this study. Compared to plots in this study, residue-based inoculum in farm-scale fields would likely be more influential on disease development. Furthermore, a degree of host resistance will be made relatively more valuable under high disease pressure situations, potentially reducing disease and DON compared to more susceptible varieties. With varieties under development and in the first few years of production generally having higher levels of FHB resistance than in earlier decades, overall impact of FHB will likely begin to decline. The risk of severe epidemics such as in the Northern Great Plains in the early 1990's will be lessened by the widespread adoption of resistant varieties.

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Table 1. Mean amount (index[1]) of FHB (%) by variety and year across all residue treatments.

Year	Variety		Average of both varieties	Disease Category[2]
	'Alsen'	'Norm'		
2003	1.8	5.2	3.5	Low
2004	19.1	48.9	34.0	High
2005	7.0	17.4	12.2	Moderate
2006	0.6	1.5	1.1	Very Low

Differences in disease values were highly significant between varieties each year, and within each variety over years.

[1]index=disease incidence*severity; an indicator of disease level within a population.

Table 2. Average Daily Inoculum Load on Heads by Residue Treatment.

	Inoculum load (cfu) per head per day (average)[1]							
	2003		2004		2005		2006	
'ALSEN'	[2]PD 1	PD 2	PD 1	PD 2	PD 1	PD 2	PD 1	PD 2
0% Residue	[3]--	--	388[a]	1408[a]	529[a]	579[a]	29[a]	84[a]
30% Residue	--	--	380[a]	1523[a]	--	--	--	--
80% Residue	--	--	493[b]	2133[b]	475[a]	662[a]	39[a]	43[a]

	Inoculum load (cfu) per head per day (average)							
	2003		2004		2005		2006	
'NORM'	PD 1	PD 2	PD 1	PD 2	PD 1	PD 2	PD 1	PD 2
0% Residue	88[a]	167[a]	472[a]	1654[a]	639[a]	754[a]	34[a]	52[a]
30% Residue	102[a]	282[a]	355[a]	1686[a]	--	--	--	--
80% Residue	146[a]	200[a]	534[a]	1990[b]	693[a]	781[a]	38[a]	39[a]

[1] letters after mean values indicated significant differences among the means (within an environment only)
[2] PD=planting date
[3] 'Alsen' was not sampled in 2003, per collaborators protocol.

Table 3. Average DON In Grain for each environment

	DON in grain, ppm (average)[1]							
	2003		2004		2005		2006	
'ALSEN'	[2]PD 1	PD 2	PD 1	PD 2	PD 1	PD 2	PD 1	PD 2
0% Residue	0.25	0.25	0.53	3.75	0.25	0.66	0.25	0.25
30% Residue	0.25	0.25	0.59	4.95	--	--	--	--
80% Residue	0.25	0.25	1.53	7.38	0.51	1.00	0.25	0.25

	DON in grain, ppm (average)							
	2003		2004		2005		2006	
'NORM'	PD 1	PD 2	PD 1	PD 2	PD 1	PD 2	PD 1	PD 2
0% Residue	0.60	0.78	4.43	17.18	1.75	2.15	0.25	0.25
30% Residue	0.98	0.87	4.63	17.30	--	--	--	--
80% Residue	0.40	1.28	7.93	18.23	2.05	2.43	0.25	0.25

[1] limit of detection = 0.5ppm, samples below detection limits were assigned the value 0.25ppm for calculations
[2] PD=planting date

No significant differences were detected within an environment among residue levels for the same variety except for 2004-PD1 (80% treatment yielded higher levels of DON in grain for both 'Alsen' and 'Norm'.

In all environments except 2006-PD1 and PD2, 'Norm' contained significantly higher DON in grain than 'Alsen'

Table 4. ANOVA for Disease Parameters and Toxin Levels in Grain across environments (2003-2005 only).

[1]Analysis of Variance

	[2]F-values			
source of variation	DON	INC	SEV	INDX
planting-date(year)	294.12***	5.24**	20.99***	19.78***
variety	389.43***	36.18***	109.19***	125.91***
planting-date(year)*variety	60.38***	1.6	15.06***	11.91***

[1]PROC Mixed, SAS 9.1, SAS Inc. Cary, NC
[2]Prob.>F values indicated by asterisk: *<0.05; **<0.01; ***<0.001

SPORE LOAD, DISEASE, AND DON: AN INOCULUM GRADIENT STUDY USING SISTER WHEAT LINES.

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ABSTRACT

A greenhouse study was conducted utilizing a range of *Gibberella zeae* inoculum concentrations applied to differentially Fusarium head blight (FHB)-susceptible sister lines of hard red spring wheat. The study was designed to evaluate the relationship between spore concentration on wheat spikes and deoxynivalenol (DON) in grain. Prior observations from several years of field research indicated that high inoculum density on spikes often was associated with high levels of DON in grain, even when disease levels were not well correlated with either DON or inoculum level. The present study utilized aqueous inoculum treatments at seven concentrations from 100 to 100,000 cfu/ml plus a control treatment applied to two sister wheat lines, SD3851 and SD3854. The lines are similar in agronomic characteristics but differ in susceptibility to FHB. Both lines were derived from the same population however line SD3851 possesses resistance conferred by the *Fhb1* QTL while SD3854 does not. Inoculated spikes were incubated for 72 hours under 100% RH, then left under ambient GH conditions for 12 additional days. At 15 days post-inoculation, disease assessments were completed and sub-samples of either whole spikes or grain only were ground for mycotoxin analysis. As expected, line SD3851 had less disease and accumulated less DON than SD3854. Both lines tended to accumulate higher levels of DON as inoculum concentration increased, but the susceptible line showed a greater response in both grain and whole-head sub-samples. Whole-head samples contained 4 to 10 times as much DON as grain-only samples, suggesting that chaff tissues might serve as a source of DON which could move to grain under certain environmental conditions. Furthermore, the FHB-susceptible line accumulated about twice as much DON as the FHB-resistant line over the range of inoculum densities comparable to field levels in SD (approximately 125 to 1250 cfu/spike). This further supports the idea that the *Fhb1* QTL or any type of host resistance is a crucial part of the USWBSI-stated mission for developing "...control measures that minimize the threat of Fusarium head blight (scab), including the reduction of mycotoxins...". This study also lends support to the idea that inoculum incident on spikes may be a useful predictor of DON in grain when accompanied by information about host resistance.

A QUANTITATIVE SYNTHESIS OF THE RELATIVE EFFICACY OF TRIAZOLE-BASED FUNGICIDES FOR FHB AND DON CONTROL IN WHEAT.

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ABSTRACT

Fungicide efficacy against FHB and DON in wheat has been highly inconsistent. Of the classes of fungicides most widely tested, triazoles have been the most effective; however, even among triazoles, the results have been highly variable. A recent quantitative synthesis of the results from over 100 Uniform Fungicide Trials (UFTs), showed that the efficacy of 38.7% tebuconazole (Folicur 3.6F), the longstanding industry standard for FHB and DON control, varied among individual studies and was generally higher in spring wheat than winter wheat. Besides tebuconazole, other triazole-based fungicides have been tested against FHB and DON, with some showing numerically (if not always statistically) superior efficacy relative to tebuconazole. One such fungicide is the recently-registered 41% prothioconazole (Proline 480 SC). In some individual studies, when used as a solo active ingredient or in combination with tebuconazole (either as a premix or a tank mix), this product contributed to significantly greater reduction in FHB and DON than tebuconazole alone. In other studies, however, the differences in efficacy were merely numerical. Similar results were observed in comparisons between metconazole and tebuconazole, suggesting that study- or environment-specific factors were probably influencing the performance of these fungicides. Using data collected from 10 years of UFTs, a multivariate meta-analysis was performed to evaluate the overall and relative efficacy of triazole-based fungicides against FHB and DON, and to determine whether efficacy was consistent across wheat types. Propiconazole (PROP), prothioconazole (PROT), tebuconazole (TEBU), metconazole (METC) and prothioconazole+tebuconazole (PROT+TEBU) fungicides were applied at flowering and disease index and DON concentration were quantified. Based on percent control (*C*), all fungicides led to a reduction in FHB and DON relative to the untreated check. PROT+TEBU was the most effective product against index, with an overall mean *C* of 52%, followed by METC (50%), PROT (48%), TEBU (40%), and PROP (32%). For DON, METC was the most effective, with a mean *C* of 45%; PROT+TEBU and PROT were of equal efficacy, with a *C* of 42%; whereas TEBU and PROP were the least effective, with mean *Cs* of 23 and 12%, respectively. In general, fungicide efficacy was higher in spring wheat than in winter wheat studies. When considering the efficacy of PROP+TEBU, METC, PROT and PROP relative to TEBU, that is, using TEBU as the reference for comparison in the meta-analysis instead of the untreated check, all products, with the exception of PROP, were significantly more effective than TEBU against both index and DON. Using the estimated mean efficacy of each fungicide against IND and DON and the estimated between-study variability in efficacy form the meta-analysis, the probability of each fungicide achieving > 50 (p_{50}) percent control in a new (single), randomly-selected study (conducted in a way similar to the UFTs) was estimated. For spring wheat, METC had the highest p_{50} values for both index (0.64) and DON (0.56), followed closely by TEBU+PROT and PROT. For winter wheat, TEBU+PROT had the highest p_{50} value for index (0.42), followed by PROT (0.36),

and METC (0.31), whereas for DON, PROT, TEBU+PROT, and METC had comparable p_{50} values (0.31, 0.27, and 0.26).

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This material is based upon work supported by the U.S. Department of Agriculture, under Agreement No. 59-0790-4-112. 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.

AN INTEGRATED APPROACH TO MANAGING FHB AND DON IN WHEAT: UNIFORM TRIALS 2007.

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OBJECTIVES

1) Evaluate the integrated effects of multiple strategies for FHB and DON management under a range of environmental conditions; and 2) increase grower adoption of multiple strategies by demonstrating that integrated management is the most effective means of reducing losses due to FHB/DON.

INTRODUCTION

Fusarium Head Blight (FHB) and the associated toxin (deoxynevalenol, DON) produced by its causal agent, *Fusarium graminearum*, continues to be a concern in every sector of the wheat and barley industries. Through years of research funded by the US Wheat and Barley Scab Initiative (USWBSI), several chemical, biological, and cultural management approaches have been evaluated and shown to contribute to FHB and DON reduction. However, when used individually, none of these approaches have been fully effective against FHB and DON. The effects of fungicide application, genetic resistance, and residue management (through crop rotation or tillage) are highly variable and strongly influenced by the environment. Under favorable weather conditions, moderately resistant varieties may become infected and DON contamination may exceed critical threshold levels. In the case of fungicides, efficacy varies from one trial to another, with overall mean percent control between 40 and 60% for index and between 30 and 50% for DON (for the most effective fungicides). Fungicides are generally most effective at reducing FHB and DON under moderate disease pressure and when applied to moderately resistant varieties than to susceptible varieties. In 2006, members of the then CBCC RAC of the UWBSI met with researcher and established protocols for conducting integrated FHB management trials. In 2007, these trials were implemented for the first time across multiple states and grain classes. The results of these trials are summarized herein.

MATERIALS AND METHODS

Field experiments were conducted to investigate the integrated effects of multiple management strategies on FHB and DON accumulation in wheat under natural conditions. The standard experimental design was a split plot with 3 to 6 replicate blocks. Wheat variety and fungicide application served as the whole-plot and sub-plot factors, respectively. In some individual trials, biological control agents and cropping sequence were used as additional treatment factors.

Plot dimensions and planting and cropping practices varied somewhat from trial to trial (see individual trial reports for details). In general, between three and six locally adapted and commonly cultivated varieties, with a range of susceptibility to FHB, were planted. There were two adjacent plots of each variety in each block. Sub-plot treatments were established by applying Proline + Folicur (as a tank mix of 3 fl. oz of each) or Prosaro (6.5 fl. oz/A) to one plot of each variety at the flowering date (Feekes' growth stage 10.5.1) of

the variety and leaving the other plot untreated. A non-ionic surfactant was added to the treatment at a rate of 0.125% v/v, and applications were made using CO_2-pressurized sprayers, equipped with Twinjet XR8002 nozzles or paired XR8001 nozzles, mounted at an angle (30 or 60°) forward and backward.

In each plot, percent FHB incidence (INC), diseased-head severity (SEV), index (IND; also known as field or plot severity), and *Fusarium*-damaged kernels (FDK) were measured. Plots were harvested and yield and test weight determined. Milled grain samples from each plot were sent to one of the USWBSI-funded DON Testing Laboratories for DON analysis.

Analysis of variance (linear mixed model) was used to evaluate the effects of variety, fungicide and their interaction on FHB intensity and DON content at each location. Percent control of FHB and DON was estimated for each treatment and treatment combination by using the level of disease and DON in the untreated plot of the most susceptible variety as the reference.

RESULTS AND DISCUSSION

A total of 15 trials were conducted in eight states (Table 1). FHB intensity and DON varied from one location to another, with some trials having zero or nominal disease development and DON contamination.

Kansas – Plots in this trial were artificially inoculated with *F. graminearum*-infested corn kernels and mist-irrigated to enhance disease development. As a result, FHB intensity and DON contamination were high, with mean index and DON ranging from 2 to 95% and 7.5 to 30.2 ppm, respectively. The effects of fungicide, variety, and their interaction on FHB index were statistically significant ($P < 0.05$). For DON, only the main effect of fungicide was statistically significant. Averaged across the three varieties, mean index was 43% in Prosaro-treated plots compared to 70% in the untreated check. For the three varieties evaluated (Harry, Jagalene, and Pioneer 2137) treated plots had significantly lower levels of disease than untreated plots. Untreated plots of Jagalene had the highest level of disease, with a mean index of 87.5%, whereas treated plot of Harry had the lowest level of disease with a mean index of 6.7% (Table 2). For DON, Prosaro-treated plots had significantly lower mean DON (16.7 ppm) than untreated plots (20.14), averaged across varieties. Among the varieties, mean DON content was highest in Jagalene and lowest in Pioneer 2137, however, this difference was only numerical (Table 2).

Kentucky – Due to dry conditions, FHB intensity and DON contamination were very low in this trial. Mean index and DON ranged from 0.03 to 2% and 0.05 to 1.3 ppm, respectively. For index, the main effects of fungicide and variety were statistically significant, whereas for DON, only the main effect of variety was statistically significant.

Missouri – Two trials were conducted in Missouri to evaluate fungicide and variety effects on FHB and DON. In the first (MO1), plots were planted no-till into corn residue and in the second (MO2), no-till into soybean residue. In MO1, mean FHB and DON levels ranged from 0.12 to 38% and 0.25 to 5.6 ppm, respectively, whereas in MO2, the corresponding ranges were 0 to 8, and 0.25 to 2, respectively. For index, all main and interaction effects were statistically significant in MO1 and only variety and variety x fungicide effects were significant in MO2. In both trials, the effects of fungicide and variety x fungicide interaction on DON were not statistically significant. In MO1, averaged across varieties, mean index in Proline 3+3-treated plots was 7.1% compared to 11.7% in the untreated check. Among the varieties, averaged across fungicide treatments, Elkhart has the highest level of disease (23.8%), followed by Pioneer 25R47 (18.2%), whereas Bess had the lowest mean level of disease (0.32%). Overall, the highest and lowest levels of disease occurred in untreated plots of Elkhart and treated plots of Bess, respectively (Table 2).

Nebraska – Mean FHB index ranged from 2.8 to 47.7% in this trial, with very similar mean levels of disease occurring in fungicide-treated and untreated plots, averaged across varieties. Among the varieties, Pioneer 2137 had the highest mean index (17.5%), followed by Jagalene (16.8%), and Harry (12.8%). The main and interaction effects of variety and fungicide treatment on index were not statistically significant.

North Dakota - Six FHB integrated management trials were conducted in North Dakota - three HRWW, two HRSW and one Durum. Disease and DON levels were not reported for two of the winter wheat trials. For the Durum and one of the HRSW trials, previous crop was used as an additional treatment factor (along with fungicide and variety). For the other spring wheat trial and the third winter wheat trial, 20 different varieties were planted.

For the Durum wheat trial (ND_D), the three-way interaction effect of previous crop, variety and fungicide on index was not statistically significant. However, the main effects of variety and fungicide and the interaction effects of previous crop x variety and fungicide x variety were significant. Mean index (averaged across variety and cropping sequence) in Prosaro-treated plots (8.54%) was significantly lower than mean index in untreated plots (20.04%). Averaged across cropping sequence and fungicide treatment, Monroe and Divide had the highest and lowest mean levels of disease, respectively. Overall, untreated plots of Monroe planted following HRSW (as the previous crop) had the highest mean index (36.54%). For DON, only the main effects of fungicide and previous crop were statistically significant. The highest mean level of DON occurred in untreated plots of Monroe and Lebsock (3 ppm) planted after HRSW and the lowest mean level occurred in treated plots of Grenora (0.97 ppm) planted after canola.

CONCLUSIONS

Percent control was estimated for a few of the trials to evaluate the efficacy of individual treatments and treatment combinations against index and DON. The results for three trials with the highest levels of disease are presented in Table 2. Trials with nominal levels of disease and DON were not included in the table, because percent control tends to be highly variable at low index and DON levels. In general, moderately resistant variety x fungicide treatment combination resulted in the highest percent control. For the trials with cropping sequence as a treatment factor, none-host crop + moderately resistant variety + fungicide generally resulted in the highest percent control (Table 2).

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Table 1. States, principal investigator, institution, wheat class and number of FHB Integrated management trials conducted in 2007.

State	PI	Institution	Wheat class	No. trials
KS	Bill Bockus	Kansas State Univ.	HRWW	1
NE	Stephen Wegulo	Univ. of Nebraska - Lincoln	HRWW	1
MO	Laura Sweets	Univ. of Missouri	SRWW	2
KY	Don Hershman	Univ. of Kentucky	SRWW	1
ND	Marcia McMullen	North Dakota State Univ.	HRSW	6
	Joel Ransom		Durum	
	Scott Halley		HRWW	
	Kent McKay			
NY	Gary Bergstrom[a]	Cornell Univ.	SRWW	2
SD	Kay Ruden[a]	South Dakota State Univ.	HRSW	1
OH	Pierce Paul[a]	Ohio State Univ.	SRWW	1

[a] Fusarium head blight did not develop.

Table 2. Mean FHB index and DON and estimated percent control for different treatments and treatment combinations.

	Index					DON				
Trial	Previous Crop	Variety	Treatment	Mean Index (%)	Percent Control	Previous Crop	Variety	Treatment	Mean Index (%)	Percent Control
Kansas	…	Jagalene	Untreated	87.50	0.00	…	Jagalene	Untreated	20.08	0.00
	…		Prosaro	67.50	22.86	…		Prosaro	18.03	10.21
	…	Harry	Untreated	46.00	47.43	…	Harry	Untreated	22.53	-12.20
	…		Prosaro	6.66	92.39	…		Prosaro	14.68	26.89
	…	P2137	Untreated	77.83	11.05	…	P2137	Untreated	17.82	11.25
	…		Prosaro	55.00	37.14	…		Prosaro	17.60	12.35
Missouri	…	Elkhart B	Untreated	29.38	0.00	…	…	…	…	…
MO1	…		Proline 3+3	18.23	37.95	…	…	…	…	…
	…	25R47 C	Untreated	23.68	19.40	…	…	…	…	…
	…		Proline 3+3	12.66	56.91	…	…	…	…	…
	…	25R54 D	Untreated	2.02	93.12	…	…	…	…	…
	…		Proline 3+3	1.92	93.46	…	…	…	…	…
	…	Roane E	Untreated	3.13	89.35	…	…	…	…	…
	…		Proline 3+3	2.66	90.95	…	…	…	…	…
	…	Bess A	Untreated	0.42	98.57	…	…	…	…	…
	…		Proline 3+3	0.22	99.25	…	…	…	…	…

Table 2. cont.

North Dakota ND_D	HRSW	Monroe	Untreated	36.54	0.00	HRSW	Monroe	Untreated	2.98	0.00
			Prosaro	9.79	73.21			Prosaro	1.40	53.02
		Lebsock	Untreated	20.42	44.12		Lebsock	Untreated	3.00	-0.67
			Prosaro	8.31	77.26			Prosaro	1.98	33.56
		Grenora	Untreated	12.41	66.04		Grenora	Untreated	2.80	6.04
			Prosaro	6.29	82.79			Prosaro	2.15	27.85
		Divide	Untreated	14.74	59.66		Divide	Untreated	2.32	22.15
			Prosaro	11.89	67.46			Prosaro	2.10	29.53
	Canola	Monroe	Untreated	25.22	30.98	Canola	Monroe	Untreated	2.25	24.50
			Prosaro	4.97	86.40			Prosaro	1.08	63.76
		Lebsock	Untreated	19.4	46.91		Lebsock	Untreated	2.05	31.21
			Prosaro	12.26	66.45			Prosaro	1.27	57.38
		Grenora	Untreated	23.9	34.59		Grenora	Untreated	2.00	32.89
			Prosaro	9.37	74.36			Prosaro	0.97	67.45
		Divide	Untreated	7.66	79.04		Divide	Untreated	1.93	35.23
			Prosaro	5.49	84.98			Prosaro	1.15	61.41

Trials with nominal levels of disease and DON were not included in the table.

FUNGICIDE EFFECTS ON FHB AND DON IN WHEAT ACROSS MULTIPLE LOCATIONS AND WHEAT CLASSES: UNIFORM FUNGICIDE TRIALS 2007.

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OBJECTIVE

Evaluate foliar fungicides for effectiveness against Fusarium head blight (FHB) and deoxynivalenol (DON) accumulation in wheat across multiple trials and different wheat classes.

INTRODUCTION

Fusarium head blight (FHB), caused predominantly by *Fusarium graminearum*, continues to impact every sector of the wheat and barley industries, causing substantial yield and quality losses. *F. graminearum* produces a mycotoxin called deoxynivalenol (DON) (among other toxins) which may accumulate to unacceptable levels in harvested grain. DON levels above 2 ppm may render grain and their by-products unfit for commercialization and consumption. Efforts to minimize the impact of FHB and DON have been based on the use of management strategies such as host resistance, crop rotation, tillage, and fungicide application. Through collaborative research involving scientists from multiple states, representing various wheat-growing regions, Uniform Fungicide Trials (UFTs) have been conducted annually since 1998 to evaluate fungicide efficacy against FHB and DON. The 2007 results from 23 UFTs across 6 states are presented herein.

MATERIALS AND METHODS

In general, each trial consisted of six core fungicide treatments and an untreated control in a randomized complete block design, with four replicate blocks (one trial had 3, two had 5, and another had 8 blocks). The core treatments were:

- Non-treated control;
- Folicur at 4.0 fl oz/A;
- Prosaro at 6.5 fl oz/A;
- Caramba at 13.5 fl oz/A;
- Topguard at 14 fl oz/A;
  Proline at 5 fl oz/A;
- Tilt at 4 fl oz/a;

Other treatments evaluated in separate individual trials were Proline at 3 fl. oz + at Folicur 3 fl. oz; Caramba at 10 fl. oz/A; Caramba at 8.2 fl. oz/A; Punch at 6 fl. oz/A; Proline at 3 fl. oz/A; Topguard at 10 fl. oz/A; Stratego at 10 fl. oz/A; Quadris 8 fl. oz/A; Dithane at 2 fl. oz/A; Quilt at 14 fl. oz/A; Folicur at 2 fl. oz/A + Topguard at 8 fl. oz/A; Headline at 8 fl. oz/A; and Folicur at 2 fl. oz/A. All treatments were applied at Feekes 10.5.1. A non-ionic surfactant was added to each treatment at a rate of 0.125% v/v, and applications were made using CO_2-pressurized sprayers, equipped with Twinjet XR8002 nozzles or paired XR8001 nozzles, mounted at an angle (30 or 60^o) forward and backward.

Planting and crop production practices varied somewhat from trial to trial. See individual trial reports for details. Most plots were planted with a susceptible cultivar. To enhance disease development, plots were either planted into corn or wheat residue and/or artifi-

cially inoculated with *F. graminearum*-infested kernels. Many plots were mist-irrigated as a means of enhancing production of, and infection by fungal inocula. In each plot of each trial, percent FHB incidence (INC), diseased-head severity (SEV), index (IND; also known as plot or field severity), and *Fusarium*-damaged kernels (FDK) were measured as previously described (McMullen, et al., 1999). DON accumulation was measured at one of the USWBSI-funded DON Testing Laboratories.

Each trial was analyzed separately using a mixed effect model in PROC MIXED of SAS to determine treatment effect on FHB, DON, yield (bu/ac) and test weight (lb/bu). Linear contrasts were used to make pair-wise comparisons between treatment means or means across groups of treatments. Studies with zero or nominal levels of disease and DON were not analyzed.

RESULTS AND DISCUSSION

Weather conditions in both winter wheat and spring wheat areas were generally unfavorable (hot and dry during flowering) for FHB development. In addition, adverse weather conditions (floods in some areas and cold temperatures in others) caused plots to be lost and the results to be highly variable in some trials. Consequently, non-irrigated trials and a few irrigated trials had nominal disease development. Mean and maximum FHB index across all replicates of the untreated check plots ranged from 0 to 26.28 and 0 to 55.00%, respectively (Table 1). In 15 of the 23 trials, mean index in the untreated check was less than 1% and less than 2% in 18 of the 23 trials. FHB intensity was highest in the Clarksville and East Lansing trials, with mean index of 26.3 and 21.9%, respectively.

In three (Urbana, IL, East Lansing, MI, and Fargo, ND) of the five trials with mean index in the untreated check greater than 2%, fungicide treatment had a significant ($P < 0.05$) effect on FHB index (Table 1). Based on pair-wise differences between each treatment and the check, Caramba at 13.6 fl.oz/A was the most effective treatment in the Urbana trial; Proline at 3 fl.oz/A was the most effective treatment in the East Lansing trial, and Proline at 5 fl.oz/A was the most effective treatment in the Fargo trial (Table 1). The corresponding percent controls (Hershman and Milus, 2003, Paul et al 2007) resulting from these treatments in their respective trials were 97, 91, and 81%. Although the Clarksville trial had the highest level of disease and the greatest difference in mean index between the Topguard treatment (14 fl. oz/A) and the check (17%), this difference was not statistically significant ($P = 1.00$). This was probably because of the high variability observed in this trial. Similar results were observed for other measures of FHB intensity (SEV, INC, and FDK). Since index is a direct function of INC and SEV (see Paul et al., 2005), only the results for IND are summarized herein.

DON content of the grain was reported in 13 of the 23 trials. In nine of these trials, DON levels in the untreated check were below 1 ppm. Trials conducted in Browntown, IL; Clarksville, MI; East Lansing, MI; and Langdon3, ND were the only trials with DON levels in the check close to or above 2 ppm (Table 2). In these trials, Prosaro at 6.5 fl.oz/A, Punch at 6 fl.oz/A, Proline at 5 fl. oz/A, and Caramba at 14 fl. oz/A, respectively, were the most effective treatments. However, the difference in mean DON between fungicide-treated plots and the untreated check was not significantly different from zero in the Clarksville and East Lansing trials (Table 2). Punch resulted in a 47% reduction in DON relative to the check in the Clarksville trial; however, the mean level of DON in Punch-treated plots still exceeded the critical threshold level of 2 ppm.

CONCLUSION

In summary, in the trials with some level of disease, fungicide treatments reduced FHB intensity and DON accumulation relative to the untreated check (based mainly on data from four locations). Fungicide efficacy varied among the trials, with percent control ranging from 67 to 97% for index and 41 to 60% for DON. However, since the overall levels of disease and DON were very low, these results should be interpreted with caution. Paul et al. (2007a, 2007b) showed that FHB and DON responses to fungicide treatments were most variable at low levels of disease (index < 2%) and DON (< 1 ppm) than at intermediate or high levels. In general, the overall levels of disease and DON in 2007

were too low for us to make broad conclusions regarding the treatments evaluated.

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Table 1. Fungicide effect on Fusarium head blight index – 2007 UFT.

Trial		Wheat Type	Most effective Treatment[a]				Index (%) Check	
State/PI	Location		Treat	IND (%)	Percent Control	*P*	Mean	Max
IL/Adee	Browntown	W	…	…	…	…	0.00	0.00
	Monmouth	W	…	…	…	…	1.23	3.11
IL/Bradley	Urbana	W	Caramba 13.5 fl.oz	0.17	97	0.006	5.90	12.73
LA/Padgett	Crowley 1	W	…	…	…	…	1.20	1.44
	Crowley 2	W	…	…	…	…	0.34	0.55
	Macon Ridge 1	W	Prosaro 6.5 fl oz	0.51	78	0.189	2.37	3.60
MI/Brown-Rytlewski	Clarksville	W	Topguard 14 floz	8.73	67	0.100	26.28	55.00
	East Lansing	W	Proline 3 fl. oz	2.00	91	<0.01	21.90	32.50
	Saginaw	W	…	…	…	…	0.00	0.00
	Sandusy	W	…	…	…	…	0.00	0.00
MO/Sweets	Columbia 1	W	…	…	…	…	1.00	1.60
	Columbia 2	W	…	…	…	…	0.48	0.80
ND/McMullen	Fargo	S	Proline 5 fl. oz	0.55	81	<0.01	2.85	3.30
	Langdon 1	S	…	…	…	…	0.40	0.50
	Langdon 2	S	…	…	…	…	0.20	0.50
	Langdon 3	S/D	…	…	…	…	0.75	1.00
SD/Draper	Brookings 1	S	…	…	…	…	0.73	1.32
	Brookings 2	S	…	…	…	…	0.74	1.94
	Groton 1	S	…	…	…	…	0.00	0.00
	Groton 2	S	…	…	…	…	0.29	1.00
	Watertown 1	S	…	…	…	…	0.00	0.00
	Watertown 2	S	…	…	…	…	0.00	0.00

[a] Treat = the most effective treatment (s) within each trial based on the pair-wise difference between mean index for each treatment and the check; IND (%) = mean index across plots receiving the most effective treatment; *P* = level of significance from *t* test of the difference between mean IND across plots receiving the most effective treatment and the untreated check ($P < 0.05$ ➔ significant different). All tests of significance were done using arcsine-transformed IND. … = Trials with zero or nominal levels of disease.

Table 2. Fungicide effect on DON – 2007 UFT.

Trial		Wheat Type	Most effective Treatment[a]				Index (%) Check	
State/PI	Location		Treat	DON (ppm)	% Reduction	*P*	Mean	Max
IL/Adee	Browntown	W	Prosaro 6.5 fl.oz	0.76	60	<0.01	1.91	3.50
	Monmouth	W	Topguard 14 fl.oz	0.17	58	0.16	0.40	0.67
IL/Bradley	Urbana	W	Prosaro 6.5 fl.oz	0.21	52	0.02	0.43	0.62
MI/Brown-Rytlewski	Clarksville	W	Punch 6 fl.oz	3.60	47	0.15	6.80	8.40
	East Lansing	W	Proline 5 fl.oz	1.10	41	0.17	1.85	2.30
	Saginaw	W	…	…	…	…	0.00	0.00
	Sandusy	W	…	…	…	…	0.05	0.10
ND/McMullen	Fargo	S	Proline 5 fl oz	0.38	58	0.02	0.90	1.10
	Langdon 1	S	…	...	…	…	0.83	0.90
	Langdon 2	S	…	…	…	…	0.17	0.50
	Langdon 3	S/D	Caramba 13.5 fl.oz	1.40	53	0.01	2.97	3.80

[a]DON data were not available for some trials or available but equally low (below 1 ppm) for all treatments.
[b]Treat = the most effective treatment within each trial based on the pair-wise difference between mean DON for each treatment and the check; DON (ppm = mean DON across plots receiving the most effective treatment; % reduction = percent reduction in DON; *P* value = level of significance from *t* test of the difference between mean DON across plots receiving the most effective treatment and the untreated check ($P < 0.05$ ➔ significant difference). All tests of significance were done using log-transformed.
… = Trials with zero or nominal levels of DON.

INFLUENCE OF SRWW, HRSW, AND HRWW VARIETIES ON THE RELATIONSHIP BETWEEN FHB AND DON.

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ABSTRACT

The relationship between visual estimates of Fusarium head blight (FHB) intensity and deoxynivalenol (DON) content of wheat is of interest to both researchers and producers because visual symptoms often are used as an indication of DON contamination of grain. In general, there is a significant positive relationship between FHB and DON, however, this relationship may vary among studies, and in some instances, fairly high levels of DON may accumulate in the absence of visual symptoms of FHB, or conversely, relatively high levels of visual symptoms may be associated with disproportionately low levels of DON contamination. The association between FHB and DON may be influenced by weather conditions, fungicide treatment, pathogen aggressiveness and DON producing ability, and variety resistance to FHB and DON. Field experiments were conducted in Nebraska, North Dakota and Ohio to evaluate the influence of variety resistance on the relationship between FHB and DON. At each location, locally adapted varieties with different levels of resistance to FHB (based on visual symptoms) were planted in a randomized complete block design, with three replicate blocks. The varieties evaluated were HRWW varieties Harry and Pioneer 2137, in Nebraska; HRSW varieties Trooper, Steel-ND, and Glenn, in North Dakota; and SRWW varieties Cooper, Hopewell, and Truman, in Ohio. Plots were inoculated at anthesis, and in each plot of each variety, diseased spikes in different severity categories were tagged. Tagged spikes were hand-harvested, cleaned, and a sample of grain from each disease category was analyzed for DON. DON content varied among varieties in each disease category in the three wheat classes. In all cases, DON generally increased with increase in disease intensity. Of the two HRWW varieties evaluated, Pioneer 2137 had lower mean DON contamination than Harry at all FHB severity levels. Among the SRWW varieties, Hopewell had the highest and Truman the lowest mean levels of DON in all disease categories. In general, Cooper, the susceptible SRWW variety, had DON content comparable to that of Truman, the moderately resistant SRWW variety. Among the spring wheat varieties, Trooper had higher mean DON content than Glenn and Steel-ND at all severity levels. Between Glenn, the moderately resistant HRSW variety, and Steel-ND, the moderately susceptible HRSW variety, the levels of DON contamination were similar in most cases.

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DONCAST: SEVEN YEARS OF PREDICTING DON INWHEAT ON A COMMERCIAL SCALE.

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ABSTRACT

Accurate predictions of mycotoxins in harvested grain are useful to help prevent entry of toxins into the food chain. DONcast is an empirical model for predicting mycotoxins in mature wheat grain, mainly as a decision support tool. To our knowledge, DONcast is the only mycotoxin prediction tool that is published and deployed commercially in the world; it was deployed in Ontario (Canada) in 2000, Uruguay (South America) in 2002, and has been undergoing a validation/calibration process in France since 2004. DONcast is attractive to the industry because: 1) prediction accuracies of over 85% have been demonstrated across diverse environments for making decisions on whether or not to apply a fungicide at heading, and 2) of the efficient platform for hosting the model and access to accurate input variables (mainly weather) that result in the following outputs: a) field- or site-specific DON predictions (Site-Specific DONcast – SSD), or b) regional-scaled (map format) outputs, all of which are conveniently managed through Weather INnovations Incorporated or WIN www.weatherinnovations.com (Chatham, ON, Canada). However, after seven years of commercial deployment, users need constant reminders on the limitations of both versions, learn how to interpret predictions toward management decisions, and be warned about unrealistic expectations of model-based predictions especially when they are derived from unrealistic weather or agronomic (or lack thereof) inputs. It has been well documented by others that FHB infection and DON accumulation is highly responsive to weather (mainly around heading), varietal susceptibility, and to the management of crop residue on the soil surface; therefore, these inputs should not be ignored. We will demonstrate that the accuracy of predictions on a regional-scale (i.e., map format) may be less than acceptable because input variables such as weather, wheat variety, crop rotation, and tillage effects tend to be over-generalized; all of these inputs are used in the Site-Specific Calculator. Although prediction maps produced on a regional scale are useful for establishing warnings or trends and are popular amongst growers, the most accurate predictions are derived from input variables that are both accurate and representative of individual fields. Both the input variable database and deployment of these predictions on a field-scale effort are enormous, considering the database is updated daily and the platform must be easily accessible to agribusiness and growers through the internet. The platforms and experiences that have evolved over seven years of commercial use will be presented in more detail.

EFFECTS OF FUNGICIDES ON FHB CONTROL AND YIELD OF WINTER WHEAT CULTIVARS IN NORTH DAKOTA.

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ABSTRACT

Research was conducted during three years (2005-2007) to determine the benefits of applying registered fungicides on a range of adapted winter wheat cultivars in North Dakota (ND). Experiments consisted of a factorial combination of fungicides and cultivars laid out in a RCBD with a split plot arrangement. The fungicide treatments served as the main plots and consisted of no fungicide or applying tebuconazole in 2005-06 or tebuconazole and prothioconazole in 2007 at early flowering. Cultivars served as the subplots and consisted of 12-18 cultivars commonly grown in ND or cultivars recently released by breeding programs in the region. Fungicide consistently improved yield and grain quality. Yield increases were associated with the control of leaf spots and especially leaf rust, and in two environments with the control of FHB. Fungicides were profitable when applied to the most disease resistant cultivars when disease pressure was high, but were not beneficial when disease pressure was low. Most of the added value from the use of fungicides resulted from increases in grain yield, but in environments with significant disease pressure, improved test weight also contributed to an increase in the crop's value. Reductions in DON levels did not improve the grain's value in the years that it was measured as they were below the threshold where discounts apply. In the least fungicide-responsive environment the return from fungicides did not exceed the cost of the application when applied to the most disease resistant cultivars as they did not produce a yield improvement. In the environment with the greatest disease pressure, however, the most disease resistant types tended to be the ones with the greatest return from fungicides. This indicates the potential value of using disease prediction models if resistant cultivars are used. With the more susceptible cultivars, fungicides should probably always be applied in eastern ND. The value of using resistant varieties, even when planning to use fungicides, was illustrated in the high disease pressure years; the combination of resistance variety and fungicide resulted in the highest yield and generally the greatest return to the fungicide and the highest overall returns.

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EFFECTS OF FUSARIUM HEAD BLIGHT ON YIELD AND QUALITY PARAMETERS OF WINTER WHEAT.

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OBJECTIVES

To assess Fusarium Head Blight impact on winter wheat yield reduction, deoxynivalenol (DON) accumulation, sinking of DON content after grading, milling and baking. These parameters are discussed both from the view of susceptible and medium resistant varieties and by application of different fungicide treatment.

INTRODUCTION

Food safety is nowadays priority for cereal producers and grain-processing industry. Fusarium head blight causes severe yield losses and decreases baking and food quality (Mesterházy, 2003). The most frequent species in Europe are now *F. graminearum* and *F. culmorum* (Logrieco et Bottalico, 2001; Mesterházy, 2003), both of which produce mycotoxins (Joffé 1986, Abramson 1998, Chelkowski 1998). The basic toxins are deoxynivalenol (DON), zearalenone and nivalenol (Logrieco et al., 2003). These substances are highly heat and chemically stable. They may be entering the food chain by direct consumption of contaminated foodstuffs, implicitly through feedstuffs and consequently through animal products.

Most of registered winter wheat varieties are middle or high susceptible to FHB. The results of many researches show us that it is difficult to reach high resistance level and simultaneously high yield and necessary food quality (Mesterházy, 2003).

MATERIALS AND METHODS

We used six winter wheat varieties differed into 2 groups: a) tolerant group (with medium resistant varieties – Sakura, Simila, Petrus), b) susceptible group (Darwin, Mladka, Sulamit). The project was sown in 3 replications and 4 various fungicide treatments: 1) control – without artificial infection and fungicidal treatment, 2) infection – with artificial *Fusarium* infection, without fungicide, 3) infection + fungicide, 4) infection + targeted fungicide. Variant infection + fungicide was sprayed with Tango Super (1l/ha, active substances epoxiconazole 84g/ha and fenpropimorph 250g/ha) in growing stage DC 37 – 39. In the variant infection + targeted fungicide was used Tango Super fungicide in DC 37-39 and targeted fungicide Caramba 24 hours before *Fusarium* infection (1l/ha, active substance metconazole 60g/ha). The experiment was based by small parcel sowing machine type Hege. Final parcel area was $10m^2$.

Inoculum with spore concentrations of 6-7 x 10^6 spores/ml was prepared and each parcel was infected with 1 liter of inoculum. Infections run up in full flowering period according to each variety term. Symptomatic evaluation was carried in 21st day after the infection. The experiment was harvested by plot harvester. The grain was analyzed; mycotoxins assessment in grain, flour, bread and bran was determined immunochemically using ELISA.

RESULTS AND DISCUSSION

Symptomatic evaluation **-** The results are average of three years (2005-2007). Head blight symptoms were evaluated on a 1-9 scale (9 - without symptoms, 1 - 100% disease development). Tolerant varieties have with strong infectious pressure significantly lower occurrence of pathogen then susceptible varieties. The difference between infection and non-targeted fungicide is not significant, while targeted fungicide lead to the less presence of symptoms (the evaluation was about 1 point better).

Yield reduction – Targeted treatment was significantly effective in susceptible varieties, which increased their yield about 16% compared to infection variant. Targeted treatment was less significant in tolerant varieties; their yield was higher about 3%. In the susceptible varieties was the lowest yield reduction in targeted treatment (16%), the highest yield reduction was in untreated variant (32%). Yield reduction in tolerant varieties was 12% by targeted treatment, 15% by infection. These results clearly advert to importance of variety tolerence. The active fungicide protection is questionable. Fungicide must be used preventively before symptoms appearance respectively in the right time. ¨

DON content – In the chart 3 is deoxynivalenol content by 4 fungicide treatments. European Commission devised the limits for DON 1.25 ppm for raw wheat and 0.5 ppm for bread. Tolerant varieties contain about 2/3 less DON than susceptible ones. Targeted fungicide treatment takes positive effect both in tolerant and susceptible varieties and reduces the DON content about more than 50%.

Grain processing and DON content – Figure 4 represent infection variant of 4 varieties, 2 susceptible (Darwin, Sulamit) and 2 medium resistant (Sakura, Simila). Once again is perceived significantly lower DON content in resistant varieties. It is possible to lower DON content just by grading, and that was between 30 – 50%. 70 – 80% of DON proceeds from grain to flour. Due to the high heat stability is the DON occurrence in bread approximately the same as in flour.

Development of tolerant varieties is the most effective protection against FHB infection and mycotoxin accumulation. Targeted fungicidal treatment highly influences mycotoxin accumulation and yield in susceptible varieties. However the application date in this work was accurately determined (24 hours after infection), estimation of the application time is doubtful in practice. Non-targeted fungicidal treatment is not explicit. Grading on the 2,2mm sieve causes reduction of the DON content up to 50%. Further manipulation as milling or baking has not so significant influence and major part of DON proceeds to the bread.

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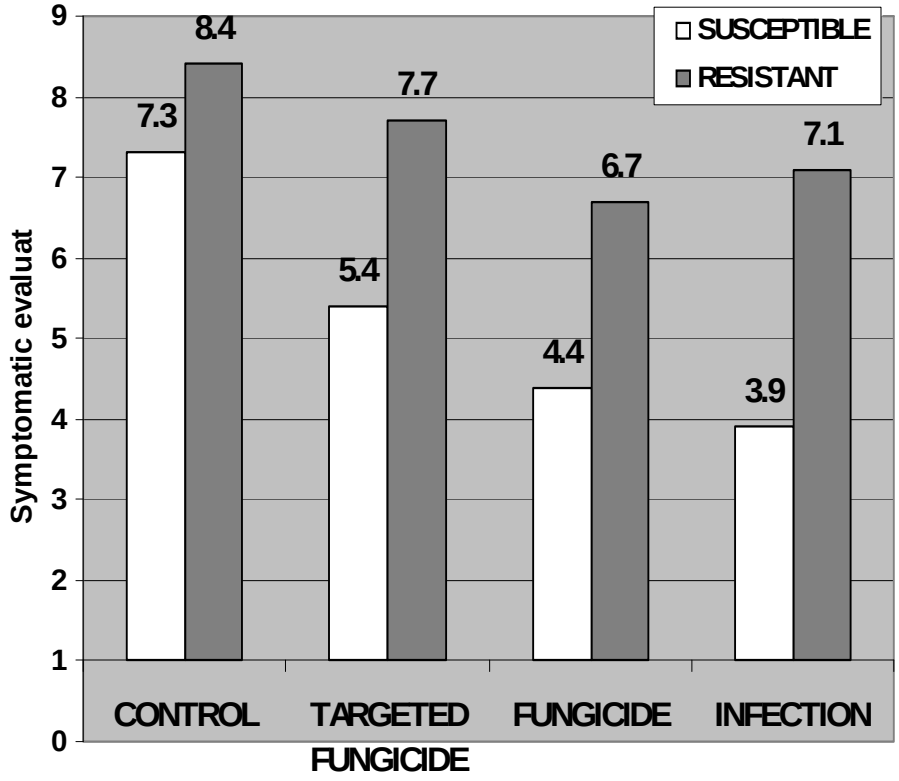

Fig. 1: Symptomatic Evaluation (2005-2007)

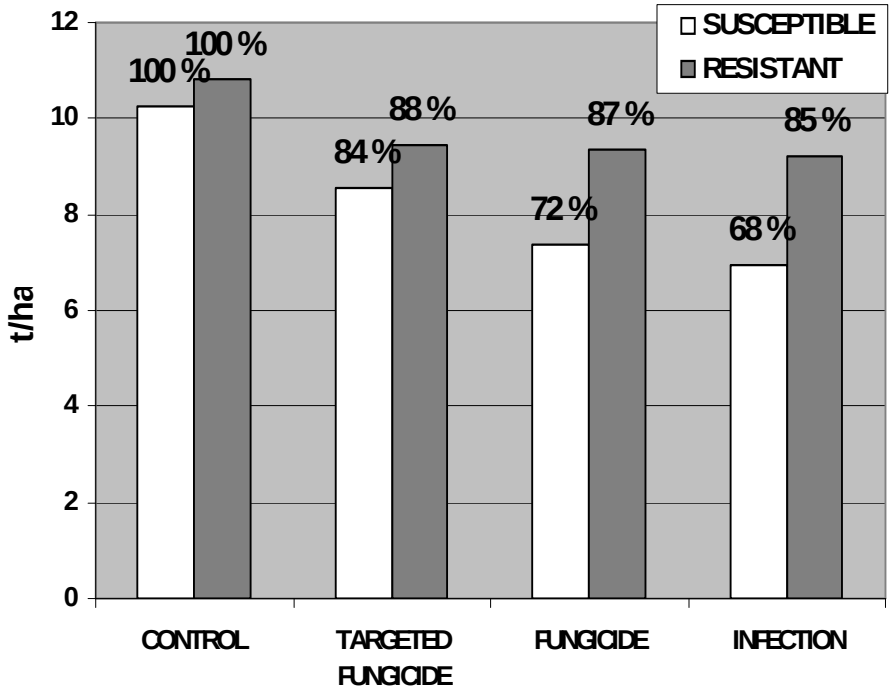

Fig. 2: Yield in Different Treatment (2005-2007)

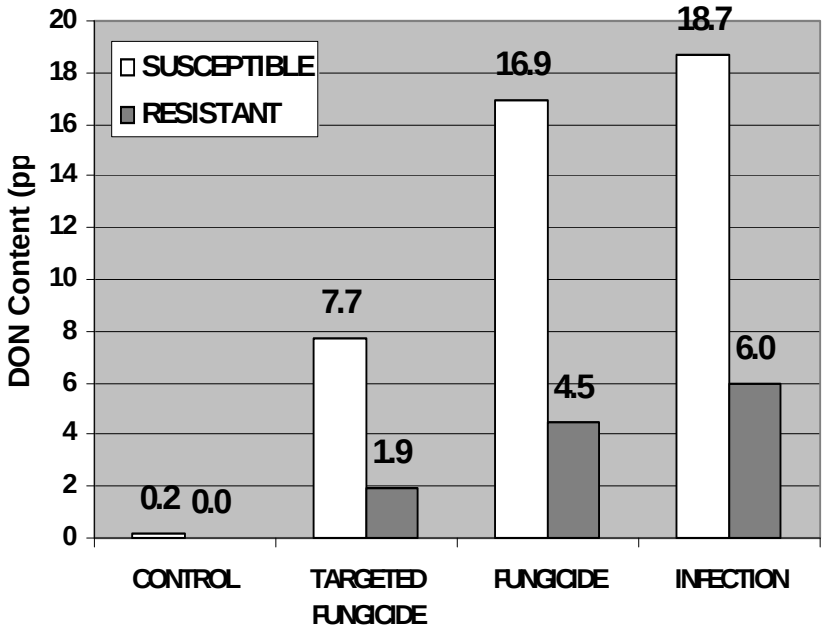

Fig. 3: DON Content (2005-2006)

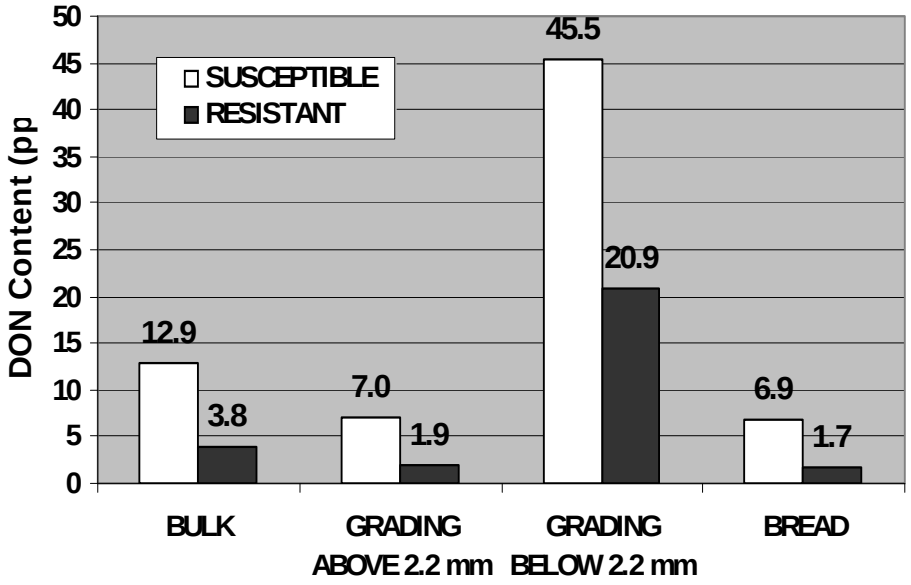

Fig. 4: Grain Processing (2005-2006)

2007 UNIFORM FUNGICIDE PERFORMANCE TRIALS FOR THE SUPPRESSION OF FUSARIUM HEAD BLIGHT IN SOUTH DAKOTA.

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ABSTRACT

Fusarium head blight (FHB – scab) has been a serious concern for wheat and barley producers in South Dakota for ten years and a serious epidemic impacted the state's wheat and barley crop in 2005. The objective of this study was to continue to evaluate the efficacy of various fungicides and fungicide combinations for the suppression of Fusarium head blight and other wheat diseases. Two hard red spring wheat cultivars, 'Briggs' and 'Forge', were planted at three South Dakota locations (Brookings, Groton, and South Shore/ Watertown) and Robust barley was planted at Brookings. Studies at two of these sites were conducted under ambient conditions. At the Brookings site, both the barley and the spring wheat trials received supplemental mist irrigation. Trial treatments from the Uniform Fungicide Trial treatments list for the suppression of FHB included an untreated check, Folicur (tebuconazole) applied at 4.0 fl oz/A, Prosaro (a premix of prothioconazole and tebuconazole) applied at 6.5 fl oz/A, Caramba (metconazole) applied at 13.5 fl oz/A, Topguard (flutriafol) applied at 14 fl oz/A, Proline (prothioconazole) applied at 5 fl oz/A and Tilt (propiconazole) applied at 4 fl oz/ A. All treatments included Induce, a non-ionic surfactant, applied at 0.125% v/v. Spring wheat trials were planted in a factorial randomized complete block design with six replications. The barley trial included four replications. Trial treatments were applied at anthesis (Feekes growth stage 10.51). The spring wheat and barley plots at the Brookings location were inoculated by spreading *Fusarium graminearum* (isolate Fg4) inoculated corn (*Zea mays*) grain throughout the field and providing overhead mist irrigation applied from 6:00 pm until 8:00 am each day for two weeks following anthesis. Other sites had natural inoculum from corn stalk residue and natural moisture conditions. Twenty-one days following treatment, plots were evaluated for leaf diseases, FHB incidence, FHB head severity, and FHB field severity. Samples were collected for *Fusarium* damaged kernels (FDK), deoxynivalenol (DON), grain yield, and test weight. Spring wheat under dryland conditions at South Shore/Watertown and Groton had negligible FHB. No significant differences resulted from the barley trial. On spring wheat in the Brookings trial, Prosaro, Caramba and Proline significantly reduced FHB Incidence, FHB Severity and FHB Index. All products except Folicur significantly increased grain yields with increases ranging from 35-42%. Total leaf disease pressure was very significant, as was leaf rust pressure which occurred late in the season. Control of the leaf diseases likely had a larger effect on yield than FHB control. Data is not yet available for DON.

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2007 UNIFORM TRIALS FOR THE PERFORMANCE OF BIOLOGICAL CONTROL AGENTS IN THE SUPPRESSION OF FUSARIUM HEAD BLIGHT IN SOUTH DAKOTA.

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ABSTRACT

Fusarium head blight (FHB – scab) has been a serious concern for wheat and barley producers in South Dakota for ten years and was very severe in parts of SD in 2005. The objective of this study was to continue to evaluate the efficacy of various fungicides and fungicide combinations for the suppression of Fusarium head blight and other wheat diseases under SD conditions. Briggs hard red spring wheat and Robust barley were planted at Brookings, South Dakota. Trial treatments included an untreated check; Prosaro (a premix of prothioconazole and tebuconazole) applied at 6.5 fl oz/A; TrigoCor 1448 (*Bacillus* sp.) from Cornell University, Ithaca, NY; and TrigoCor 1448 + Prosaro coapplied; 1BA (*Bacillus subtilus*) from South Dakota State University, Brookings, SD; 1BA + Prosaro coapplied, C3 (*Lysobacter enzymogenes*) from University of Nebraska, Lincoln, NE; C3 + Prosaro coapplied. The treatments were applied at anthesis. Plots were inoculated by spreading *Fusarium graminearum* (isolate Fg4) inoculated corn (*Zea mays*) grain throughout the field and providing overhead mist irrigation applied from 6:00 pm until 8:00 am each day for two weeks following anthesis. Twenty-one days following treatment, plots were evaluated for FHB incidence, FHB head severity, and FHB field severity. Plots were harvested for yield and test weight and samples were collected for *Fusarium* damaged kernels (FDK) and deoxynivalenol (DON).

Even with amending the environment in 2007, significant drought severely limited disease development. In the preliminary analysis, the assessments of FHB Severity and FHB Index disease components indicate a possible treatment effect in the barley study.

In the wheat study, all treatments showed a non significant increase in yield as compared to the untreated check. There were no differences among the treatments for the components of FHB.

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CHARACTERIZATION OF DON ACCUMULATION IN SRWW CULTIVARS WITH DIFFERENT LEVELS OF TYPE II RESISTANCE TO FHB.

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ABSTRACT

Under favorable conditions, deoxynivalenol (DON), a mycotoxin produced by *Fusarium graminearum*, may accumulate to unacceptable levels in harvested grain, making the grain and their by-products unfit for commercialization and consumption. The use of cultivars with resistance to FHB and DON is a widely recommended management approach for reducing the impact of this disease. However, resistance to *F. graminearum* is complex, with several different types (I, II, III, IV and V) reported, but not completely characterized. While it is clear that there is a positive association between FHB development and DON accumulation, the association between Type II (resistance to disease spread within the spike) and Type III resistance (resistance to DON accumulation) is unclear. It is quite possible for cultivars with similar levels of resistance to FHB to have different levels of resistance to DON accumulation. Some speculate that differential accumulation of DON among cultivars may be the result of differential fungal colonization of grain or the ability of some cultivars to detoxify DON. To quantitatively characterize the associations among FHB, fungal colonization, and DON accumulation in SRWW cultivars with different levels of Type II resistance to FHB, inoculated field trials were conducted at the Ohio Agricultural Research and Development Center, Wooster, during the 2007 growing season. Two experiments (1 and 2) were established, with three wheat cultivars (Cooper, susceptible; Hopewell, moderate susceptible; and Truman, moderately resistant) planted in a randomized complete block design with three replicate blocks. Plots were spray inoculated at early anthesis with a spore suspension (10^5 spores/ml) containing an equal proportion macroconidia and ascospores of *F. graminearum*/*G. zeae*. Approximately 35 days after inoculation, 20 wheat spikes in each of 11 severity categories were tagged. At maturity, spikes in each category were harvested and prepared for DON and PCR analyses. *F. graminearum* genomic DNA was extracted from each sample and a SYBR green-based real time polymerase chain reaction (RT-PCR) assay used to quantify fungal biomass. DON content was quantified by GC-MS at the USWBSI-sponsored laboratory at the University of Minnesota. Results from an analysis of covariance showed that DON content (ppm) increased with increasing FHB severity in all three cultivars. However, the rate of change in DON with change in severity (the regression slope) was greater for the susceptible cultivars than the resistant cultivar. The magnitude of the difference in DON content at a given level of severity among the cultivars was generally higher at high severity than at low severity. Contrastingly, estimated slopes for relationships between fungal biomass (log-transformed ng/mg) and DON (ppm) were similar for the three cultivars, suggesting similarity in DON accumulation with fungal colonization among the cultivars. However, the heights of the regression lines for the fungal biomass/DON relationships differed among the cultivars, indicating that for a similar level of fungal colonization, DON accumulation differed among the cultivars. Further research is in progress to evaluate these associations under different environmental conditions in an attempt to learn more about possible mechanisms involve in resistance to FHB and DON in wheat.

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This material is based upon work supported by the U.S. Department of Agriculture, under Agreement No. 59-0790-4-112. 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.

CONTRIBUTION OF LOCAL INOCULUM SOURCES TO REGIONAL ATMOSPHERIC POPULATIONS OF *GIBBERELLA ZEAE*.

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ABSTRACT

Decreases in tillage may have contributed to recent epidemics of FHB by increasing the amount of regional atmospheric inoculum available for infection. Where a large, regional source of atmospheric inoculum exists, crop rotation or tillage practices may not effectively reduce the risk of FHB in individual fields. An increased understanding of the contribution of local inoculum sources of *Gibberella zeae* (*Gz*) to regional atmospheric populations of the pathogen may aid in developing and/or excluding strategies for managing FHB. In 2007, we conducted 35 sampling flights with unmanned aerial vehicles (UAVs) 100 m above a large clonal inoculum source of *Gz* established at Virginia Tech's Kentland Farm. The UAVs were programmed to fly an orbital pattern such that one leg of the sampling path of UAV flew directly over the inoculum source during each of the passes. Our first flight was on 25 March at 11:30 am, and our last flight was on 24 May at 10:30 pm. We collected over 100 isolates of *Fusarium* spp. during these flights. All of the isolates were single-spored, grown in liquid culture, and suspended in 20% glycerol for cryogenic storage. We have tentatively identified a large portion of these isolates as *Gz*, and we will be conducting amplified fragment length polymorphisms (AFLPs) on these isolates in the coming months to unambiguously determine the percentage of the clonal isolate of *Gz* in our atmospheric collections. A series of runs with the atmospheric transport model HYSPLIT suggested that our inoculum source of *Gz* was transported at least a kilometer away from the ground surface within an hour. Our work continues to (1) elucidate the contribution of a local inoculum sources to atmospheric populations of the pathogen, and (2) develop and test a robust long-distance transport model for FHB forecasting/risk assessment. The ability to predict the regional transport of *Gz* from local inoculum sources may help refine risk models for FHB.

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ENVIRONMENTAL FACTORS INFLUENCING FHB SEVERITY AND DON IN BARLEY.

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ABSTRACT

We are investigating the relationship between environmental factors, crop stage, and barley genotype with Fusarium head blight (FHB) and DON accumulation in the grain. This project is associated with the established spring and winter wheat FHB-modeling efforts and aims to produce the information required to either validate one of the current FHB models for use in barley, or generate unique models.

Varieties of regionally adapted barley of both 2- and 6-row types were planted at multiple locations in the Northern Great Plains during the 2005-7 growing seasons. At least three varieties were common to each location. Plots were un-irrigated, a minimum of 1.5m x 4.6m in size, and replicated four times in a RCBD. Additional varieties were planted based upon availability and local producer preference. Crop stage was monitored regularly throughout the season and the date at which each plot was at Feekes 10.5 stage was noted. No additional inoculum was introduced into the plots. The incidence and severity of FHB was recorded on a minimum of 50 heads per plot at the soft-dough stage (approximately 21 days after heading). Environmental variables consisting of temperature, relative humidity, and precipitation were recorded by an on-site, or nearby, weather station.

Over the past three seasons, we have successfully collected data for 27 of the 38 locations planted. Unsuccessful locations were generally the result of extreme weather-related situations (e.g. floods) that resulted in crop destruction. The remaining locations provide a range in disease intensity, severity, and final deoxynivalenol concentration that we have used to identify weather variables, both simple and complex, that were associated with high FHB/DON situations in barley. For example, the average hourly temperature and relative humidity in the 10 days prior to full head emergence were both significantly correlated with final disease severity, but not DON content. In the available dataset, measurements of humidity after heading (e.g. vapor point depression) were the only factors associated with final DON concentration. From these results, we hypothesize that different environmental factors may be impacting this pathosystem in various ways and the development of a single model for both disease and DON prediction is unlikely.

DIFFERENTIAL SENSITIVITY TO TRIAZOLE-BASED FUNGICIDES AMONG ISOLATES OF *FUSARIUM GRAMINEARUM*.

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ABSTRACT

Samples of Fusarium head blight infected wheat spikes were collected from wheat fields across the state of Ohio. All isolates of *Fusarium graminearum* were identified based on colony and spore morphology. A subset of isolates from different Ohio counties was tested in vitro for sensitivity to Proline (41% prothioconazole) and Folicur (38.7% tebuconazole). Five isolates obtained from conventional wheat fields in Wood, Wayne, Shelby, Van Wert, and Delaware counties and a sixth from an organic wheat field in Wayne County were compared. The fungicides were dissolved in deionized water to achieve stock solutions with the proper concentration of active ingredient and added to PDA. Commercial grade prothioconazole and tebuconazole were evaluated at concentrations of 0.001, 0.01, 0.1 and 1.0 µg/ml and non-amended PDA was used as the control. A 5-mm-diameter plug from the edge of a fully colonized plate was transferred to the center of each plate for each concentration to be evaluated. Colony diameter was measured in three places once every 24 hours for seven consecutive days. The percent growth relative to growth on the control was calculated as the average colony diameter (at each concentration) minus 5 mm (diameter for the PDA plug) divided by the average colony diameter on the non-amended media, multiplied by 100. Sensitivity varied between Proline and Folicur and among isolates of *F. graminearum*. At concentrations of 0.1 and 1 µg/ml of active ingredient, isolates were generally more sensitive to Proline than Folicur, exhibiting slower and more restricted growth on Proline-amended media than on Folicur-amended media. The sensitivity profile of the isolates was similar for the two fungicides; the same isolates that exhibited the highest and lowest sensitivity to Folicur also exhibited the highest and lowest sensitivity to Proline. With the exception of isolate OHSHE6613, all isolates showed some level of growth on Folicur-amended media at all tested concentration. Conversely, with the exception of OHWAY1619, none of the isolates grew on Proline-amended media at 1.0 µg/ml. Research is in progress to conduct a more comprehensive evaluation of sensitivity of isolates from different wheat-growing regions to Folicur, Proline and other triazole-based fungicides.

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A METHOD FOR QUANTIFYING TRICHOTHECENES AND ERGOSTEROL IN SINGLE WHEAT FLORETS USING GAS CHROMATOGRAPHY WITH ELECTRON CAPTURE DETECTION.

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ABSTRACT

The relationship between fungal biomass and mycotoxin accumulation during Fusarium Head Blight infections is not completely understood. The purpose of this research is to develop a method to quantify deoxynivalenol and its acetylated derivatives as well as fungal biomass within single wheat florets. Ergosterol, a sterol unique to fungal cell walls, was used to estimate fungal biomass. Gas chromatography with electron capture detection (GC-ECD) and derivatization with hepta-fluorobutyric anhydride (HFBA) were chosen due to their sensitivity. Gas chromatography with mass spectroscopy was subsequently used to confirm these derivatization and detection methods. The extraction solvent used was acetonitrile-water (84:16). Wheat floret extracts were then cleaned through a charcoal alumina column. While previous work has shown GC-ECD to quantify HFBA-trichothecene derivatives, to our knowledge, no studies have used these protocols to also detect ergosterol. This method may be used to study FHB infection patterns within single wheat spikes and the extent of fungal colonization and toxin accumulation within single kernels with varying symptoms.

INFLUENCE OF INFECTION-TIMING ON FUSARIUM HEAD BLIGHT SEVERITY, WHEAT KERNEL DAMAGE AND DEOXYNIVALENOL ACCUMULATION DURING A 2007 FIELD STUDY.

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ABSTRACT

Previous field studies conducted in 2006 have shown that *Fusarium graminearum* infections during the grain-fill stages of wheat development may lead to kernels with low disease intensity yet significant levels (>2ppm) of deoxynivalenol (DON). Interestingly, infections during both flowering and grain-fill resulted in lower disease severity than when infections occurred during flowering alone. In the most susceptible cultivar a decrease in DON was also observed. The goal of the 2007 study was to gather additional data on infection-timing patterns. Three winter wheat cultivars were used in this field study: Hopewell (susceptible), Truman (moderately resistant) and Valor (moderately resistant). The experiment was a split-plot design with infection-timing treatment as the main effect and cultivar as the sub-plot. Four misting treatments were used to facilitate infection: ambient (no supplemental moisture), misting during flowering, misting during grain-fill and misting during flowering and grain-fill. Misting chambers and moveable greenhouses were used to supplement and prevent moisture respectively. All plots were spray inoculated with a mixture of four DON-producing *F. graminearum* isolates at anthesis and late milk stages. Misting treatments commenced immediately following inoculations and lasted four consecutive nights. Disease incidence and severity were measured in the field during dough stages. Following harvest, yield, kernel damage and DON accumulation were also assessed. Overall, disease intensity and DON levels were low, likely due to dry weather and low humidity during the growing season. In Hopewell, the amount of disease severity and percent kernel damage did not differ between ambient and misting during grain-fill treatments. However, the grain from the misting during grain-fill treatment contained significantly (*P* d•0.05) higher DON than that grown under ambient conditions. Also in Hopewell, disease severity and DON were significantly (*P* d•0.05) less under the misting during flowering and grain-fill treatment than in grain grown under the misting during flowering only treatment. This increased moisture – low disease and DON pattern warrants further study. Results from 2007 suggest late infections during grain-fill lead to grain with low disease intensity yet kernels with greater than 1ppm DON. Despite low disease pressure the data gathered in 2007 corroborates with overall infection-timing patterns observed in 2006.

CONTROL OF *FUSARIUM* INOCULUM PRODUCTION IN CORN RESIDUE BY MECHANICAL, BIOLOGICAL, AND CHEMICAL TREATMENTS.

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OBJECTIVES

I. Determine the effects of chopping of field corn residue on the saprophytic growth and sporulation of G*ibberella zeae* in the residue and the development of Fusarium head blight (FHB) in the following wheat crop.

II. Evaluate commercially available fungicides and biological agents as spring applications on the residue to disrupt the sporulation of *G. zeae* in the residue and reduce the development of FHB in wheat.

INTRODUCTION

Host resistance and fungicides individually can reduce head infection by *G. zeae* (=*Fusarium graminearum*) resulting in partial control reduction of FHB and deoxynivalenol (DON) formation. More complete control, however, will require the integration of these strategies with methods that reduce inoculum production in residue from previous crops. In this study, we examined mechanical, biological and chemical strategies to affect inoculum production. The mechanical strategy involved chopping of residue in the fall. By providing greater surface area for entry of saprophytic organisms and for contact with moisture and soil, chopping could hasten the decomposition of the residue and thus restrict growth of the pathogen. Some residue-colonizing microbes also might be antagonistic to the pathogen and, thus, might be effective in displacing the pathogen from the tissue or preventing its sporulation. The biological and chemical strategies involve application of biocontrol agents and fungicides, respectively, to the residue prior to flowering. Research with fungi, *Microsphaeropsis* sp. and *Trichoderma* sp., showed promise in using them to reduce pathogen growth and perithesia production in residue (Bujold and Paulitz, 2001; Fernandez, 1992; Gilbert and Fernando, 2004). Application of fungicides to residue has been investigated on a very limited basis. Tebuconazole impaired decomposition rate and eliminated *F. graminearum* in residues soaked in the fungicide (Yi et al. 2002), while captan applied to surface residue reduced numbers of fungi (including *Fusarium* spp.) and slowed residue decomposition (Beare et al., 1993). Thus, it appears that fungicides might directly inhibit the growth of the pathogen in residue but could also have a negative effect on colonization by competitive, decomposing microbes. In the limited studies on the treatment of residue with biological and chemical agents, fall applications were more effective in inhibiting pathogen growth than spring treatments. In this study, we examined whether spring treatments would exert sufficient impact on pathogen spore production in infested residue to reduce FHB and DON.

MATERIALS AND METHODS

Experiments were conducted in two University of Nebraska experiment station sites, ARDC near Mead and Haskell Agricultural Laboratory near Concord, to test the effects of fall mechanical treatments and spring biological and chemical treatments. Each experiment had a split-plot design with 'residue type' (chopped, unchopped, no residue) being the primary factor and 'spray treatment' (biocontrol products, fungicides, no treatment) the split factor. At each site, hard red winter wheat 'Overley' was block planted in fall 2006 into 4-acre fields having a previous soybean crop. Residue from BT corn in a neighboring field was chopped using a bush hog mower or left intact. The chopped and whole residue was left to decompose in place until early March, 2007. At that time, unchopped

corn debris was cut close to the ground with a sickle mower and residue of each type was collected and spread into 10' X 20' plots within the wheat field. Approximately 14 Kg (31 lb.) of residue was introduced per plot. There were three blocks containing one strip for each residue type. Each strip had six plots separated by 30'-wide buffer zones of wheat. Each plot within a strip was assigned one of six spray treatments which included three chemicals: Headline, Dithane DF, Prosaro; two biologicals: Serenade (*Bacillus subtilis* strain QST713) from AgraQuest, T-22 (*Trichoderma harzianum* strain KRL-AG2) produced by BioWorks; and a distilled water control. Spray treatments were applied to debris on the soil surface once at early stem extension stage (Feekes 6-8). Each material was applied at manufacturer's label rate for foliar applications in 20 gal water per acre using a CO_2-pressurized backpack sprayer with nozzles configured for spraying herbicides.

Residue samples were collected from plots containing chopped and unchopped residue plots at anthesis (Feekes 10.5) for determination of numbers of *G. zeae* ascospores. Samples were weighed and then washed in a standard amount of water with Tween. Ascospore concentration in each wash was determined by counting with a hemacytometer. Field data collected were FHB incidence and severity. DON content, yield of kernels, and percentage of *Fusarium* disease kernels (FDK) were measured after harvest. All data was subjected to ANOVA for split-plot design and Fisher's LSD was used for means separation.

RESULTS

There was a higher number of ascospores detected on chopped residue collected from Mead at anthesis than on whole residue (1.95×10^3 per g residue vs. 5.42×10^3 per g, respectively; $P = 0.012$). At Concord, there was no significant difference in ascospore numbers between residue types, with fewer than 2×10^3 per g being counted.

There was sufficient rain at both sites to cause moderate disease incidence, but disease severity was moderate at Mead and low at Concord. The effects of residue type and spring spray treatments on disease parameters differed between the two experiments. At Concord, there were significant spray treatment effects for FHB incidence and index, with Prosaro being the only treatment to have lower levels than the control (Table 1). Test weight was significantly higher in plots in which residue was sprayed with Headline as compared to the control. When data for each residue type was analyzed across spray treatments, the no-residue control had the lowest DON level and the highest yield measurements, but it also had the highest disease incidence. At Mead, there were significant residue by spray treatment interactions for disease incidence and index, but except for a decrease in disease index in chopped residue by Prosaro, none of the spray treatments reduced disease measurements in any residue types compared to the respective control (Table 2). Instead, Serenade and Headline increased disease incidence and index. Disease incidence, DON level and % FDK, averaged across spray treatments, were significantly higher in the whole residue and chopped residue plots than in the no-residue plots. In addition, presence of residue significantly decreased yield compared to no residue.

CONCLUSIONS

The results with Prosaro suggest there is some promise to the strategy of applying fungicides to residue in the spring to reduce FHB development in the wheat crop. The strategy by itself provided on low levels of disease control and DON reduction, so it would need to be integrated with host resistance and/or fungicide treatments applied to flowering heads. Given that Prosaro might be widely applied to flowering heads, it would be not be desirable to use the same product to treat residue as well because of increased the risks of selecting for resistant pathogen populations. Therefore, it is necessary to evaluate a larger selection of fungicides with different modes of action than Prosaro specifically for use as residue treatments. In this respect, biocontrol agents theoretically would be good candidates. While the results with the two biological products in this study were not encouraging, there are other commercial agents available for future testing.

The increased disease development and decreased yields observed in the presence of residue as com-

pared to no residue were agreement with other reports (Dill-Macky and Jones, 2000). While disease levels in chopped residue tended to be lower than in the whole residue, the differences largely were not significant. This could be related to chopping having only a small influence on sporulation, as suggested by the ascospore counts. Another explanation is that differences could have been masked by substantial aerial inoculum entering the experiment plots, as evidenced by the moderate levels of disease occurring in the no-residue plots. The wet weather experienced in eastern Nebraska during the experiments was atypical. In more typical drier years, the regional inoculum load might be lower and, thus, diminution of residue by chopping might exert a greater effect in reducing inoculum in a given field.

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DISCLAIMER

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 authors and do not necessarily reflect the view of the U.S. Department of Agriculture.

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Table 1. Results from 2007 residue management trial at Concord, Nebraska (Haskell Ag. Lab.)

Residue type	Spray treatment	Incid. (%)	Sev. (%)	Index (%)	DON (ppm)	FDK (%)	Yield (K)	200seed wt (g)
None	None	53	27	15	1.4	4.3	2.5	6.0
	Dithane DF	49	25	12	1.8	3.8	3.1	6.7
	Headline	46	22	10	1.8	2.7	3.0	6.5
	Prosaro	41	23	10	1.2	3.5	2.7	6.8
	Serenade	44	23	10	2.8	4.7	3.0	6.0
	T22	44	25	11	2.4	3.8	3.1	6.3
Whole	None	42	29	12	2.6	5.8	2.2	5.9
	Dithane DF	35	29	10	2.5	3.5	2.6	6.0
	Headline	41	24	10	2.8	4.7	2.5	6.4
	Prosaro	30	15	4	1.9	4.2	2.4	5.9
	Serenade	49	28	14	3.5	4.3	1.9	5.3
	T22	49	27	13	1.9	4.3	2.4	5.7
Chopped	None	47	28	14	2.4	4.5	2.2	6.0
	Dithane DF	33	26	9	1.8	4.3	2.2	6.0
	Headline	37	28	10	2.3	6.3	2.9	6.6
	Prosaro	33	26	8	1.4	3.3	3.1	6.3
	Serenade	43	30	13	2.3	5.2	2.5	6.0
	T22	36	21	8	2.2	5.7	1.6	5.3
P residue type X spray trt.		NS	NS	NS	NS	NS	NS	NS
None	Means across	46 a*	24	11	1.9 b	3.8	2.9 a	6.4 a
Whole	spray	41 ab	25	11	2.5 a	4.5	2.3 b	5.9 b
Chopped	treatments	38 b	26	10	2.1 ab	4.9	2.4 b	6.0 b
P residue type		0.013	NS	NS	0.050	NS	0.014	0.002
Mean	None	47 a	28	13 a	2.1 bc	4.9	2.3	6.0 bc
across	Dithane DF	39 ab	26	10 ab	2.0 bc	3.9	2.6	6.2 ab
residue	Headline	42 ab	25	10 ab	2.3 ab	4.6	2.8	6.5 a
types	Prosaro	35 b	21	7 b	1.5 c	3.7	2.7	6.3 ab
	Serenade	45 a	27	12 a	2.9 a	4.7	2.4	5.8 c
	T22	43 ab	24	11 ab	2.2 abc	4.6	2.4	5.8 c
P spray treatment		0.034	NS	0.050	0.018	NS	NS	0.001

* Letters denote means separation at P = 0.05.

Table 2. Results from 2007 residue management trial at Mead, Nebraska (ARDC).

Residue type	Spray treatment	Incid. (%)	Sev.(%)	Index (%)	DON (ppm)	FDK (%)	Yield (K)	200seed wt (g)
None	None	38 d*	29	11 e	5.3	11.0	2.7	6.3
	Dithane DF	50 bcd	53	26 abcde	5.4	9.0	3.1	6.8
	Headline	42 cd	39	17 cde	4.8	10.3	3.0	6.8
	Prosaro	42 cd	37	16 de	5.2	9.8	2.4	6.8
	Serenade	61 abc	62	38 abc	6.2	9.7	2.4	5.3
	T22	53 bcd	48	25 abcde	6.1	11.0	3.0	6.2
Whole	None	45 cd	35	16 cde	8.4	14.5	2.4	5.9
	Dithane DF	59 abc	57	34 abcd	8.0	13.8	1.8	6.4
	Headline	73 a	57	43 a	10.8	17.8	2.3	6.1
	Prosaro	52 bcd	44	25 abcde	10.0	14.0	1.6	5.9
	Serenade	67 ab	58	40 ab	9.7	11.5	1.9	5.8
	T22	53 bcd	43	23 abcde	12.0	14.3	2.4	5.8
Chopped	None	68 ab	59	40 ab	8.9	14.2	2.3	6.0
	Dithane DF	53 bcd	43	25 abcde	8.9	12.0	2.7	6.2
	Headline	49 bcd	40	20 bcde	7.7	14.1	2.5	6.2
	Prosaro	51 bcd	33	18 cde	7.0	12.5	2.6	6.4
	Serenade	58 abc	52	30 abcde	9.7	15.5	2.4	6.2
	T22	52 bcd	50	27 abcde	7.8	12.2	2.1	6.0
P residue type X spray trt.		0.024	NS	0.050	NS	NS	NS	NS
None	Means across	47 b	45	22	5.5 b	10.2 b	2.8 a	6.4
Whole	spray	58 a	49	30	9.8 a	14.3 a	2.1 c	6.0
Chopped	treatments	55 a	46	27	8.4 a	13.4 a	2.4 b	6.2
P residue type		0.008	NS	0.087	<0.001	0.006	<0.001	NS
Mean	None	50 b	42	23	7.5	13.2	2.5	6.0 ab
across	Dithane DF	54 ab	51	28	7.5	11.6	2.5	6.5 a
residue	Headline	55 ab	45	27	7.8	14.2	2.6	6.3 a
types	Prosaro	48 b	37	20	7.4	12.1	2.2	6.4 a
	Serenade	62 a	57	36	8.5	12.2	2.2	5.8 b
	T22	52 b	47	26	8.6	12.5	2.5	6.0 ab
P spray treatment		0.085	0.06	0.064	NS	NS	NS	0.039

* Letters denote means separation at P = 0.05.

EFFECTS OF SPRAY APPLICATION METHODS ON BIOCONTROL AGENT VIABILITY.

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OBJECTIVE

Determine the effects of commercial ground spray application systems on viability of representative biological control agents.

INTRODUCTION

All microorganisms used for biological control are sensitive to environmental extremes and thus, environmental conditions occurring during the application process could affect the viability of the agents and, consequently, affect disease control efficacy. Bacteria and yeast have been evaluated as biological agents in field trials for efficacy in controlling Fusarium head blight and reducing deoxynivalenol levels. Field tests conducted thus far utilized CO_2-pressurized backpack systems with care taken to avoid subjecting biological materials to temperature extremes. There is no information available as to how biological agents would respond to "real-world" conditions occurring during operation of commercial spray equipment. Under such conditions, accumulation of heat from sunlight or pump motors and sheer forces within the pumps, filters, nozzles and other systems mechanisms could possibly be injurious to biological agents.

MATERIALS AND METHODS

Three microorganisms were tested, each representing a different microorganism group: *Bacillus* sp. 1BA (Draper et al., 2001) representing spore-forming, gram-positive bacteria; *Lysobacter enzymogenes* C3 (Jochum and Yuen, 2006) representing gram-negative, non-spore-forming bacteria; and *Cryptococcus aureus* OH71-4 (Khan et al., 2004) representing epiphytic yeasts. Spontaneous mutants of the bacterial strains resistant to the drug rifampicin were used so that they could be detected on 10% tryptic soy agar (TSA) medium amended with the drug and a fungicide. The yeast OH71-4 was detected on 10% TSA amended with antibacterial drugs. Quantification of the agents in cell suspensions was done by dilution plating. Cultures of bacterial strains C3 and 1BA in chitin broth and nutrient broth, respectively, and yeast strain OH71-4 in a proprietary frozen concentrate (provided by D. Schisler, NCAUR) were used in two experiments. One hypothesis tested in the experiments was that heat accumulation in the biocontrol agent suspensions will reduce organism viability. Another hypothesis was that each stage of a spray application (i.e., agitation of cell suspension in tank, pumping of cell suspensions through the spray line, and discharge of cell suspension as droplets through nozzles) also will affect biocontrol agent viability.

Experiment 1 was conducted at North Dakota State University research facilities at Langdon using a customized spray system with a 10-gal. tank, shortened lines, and a single nozzle. These changes were made to accommodate small liquid volumes. In addition, ports were added to the line between the pump and the filter and between the filter and nozzle to allow collection of liquid samples. Otherwise all parts were standard as would be used on conventional spray systems, including a cast iron gear drive centrifugal pump (HYPRO Model 9006C-O) and XR8002 nozzle. The

bacterial cultures and yeast formulation were diluted with water in the tank to 4 gallons. The pump was operated at standard PTO speed (540 rpm) and manifold pressure was maintained at 40 psi. The contents of the tank were continuously agitated by recirculation (8 gal/min). At 10-minute intervals, temperature within the tank liquid was measured, and samples of liquid were collected from the tank and the two sampling ports. Liquid was then emitted from the nozzle and a sample of the spray collected.

Experiment 2 was conducted at the UNL Agronomy Research Farm using an unmodified commercial spray system having a piston pump (Ace model F-1), a 115-gal capacity tank, and the same nozzle type as in experiment 1. The biological materials were diluted to 50 gal with tap water. The suspensions were sprayed continuously out three nozzles under 40 psi pressure. As in experiment 1, temperature and tank liquid samples were collected at 10-minute intervals. Spray samples were collected from each of the nozzles as well.

RESULTS AND CONCLUSIONS

During experiment 1, temperatures in each of the biocontrol suspensions re-circulating in the tank rose rapidly due to transfer of heat from the pump (Fig. 1). Within 30-40 minutes, temperatures exceeded 50^{o}C, which is injurious or lethal to most microorganisms. The heat accumulation was likely the primary factor leading to loss of viability of the biocontrol agents in the liquid (Fig. 2). In this respect, the three organisms exhibited widely different responses to similar rises in temperature. There was little difference in numbers of live cells between samples taken at various points in the spray system and samples collected from the tank (Fig. 3), indicating that passage of the cell suspensions through individual parts (pump, filter, and nozzle) had negligible effects on organism viability and did not account for the drops in population within the tank. When the biocontrol agents were sprayed through commercial equipment in experiment 2, temperatures in the liquid remained stable at favorable levels (Fig. 4). Because ambient temperatures and sunlight conditions were similar between experiments, the lower liquid temperatures recorded in experiment 2 can be attributed to the large liquid volume accumulating heat from the pump at a much slower rate. Consequently, there were no appreciable changes in biocontrol populations within the tank over time (Fig. 5). In addition, biocontrol agent population levels sprayed out of the nozzles were not significantly different from those measured in the tank (Fig. 6), thus confirming that the various mechanisms in a conventional spray system collectively have little effect on biocontrol agent viability. We conclude that when biocontrol agents eventually become available for application to cereals, they will be compatible in the most part with existing equipment used for ground applications of fungicides. Heat accumulation in the liquid may reduce biocontrol agent numbers, but this would most likely occur as tanks are close to being empty.

In experiment 1, the high recirculation rate relative to the small liquid volume resulted in foaming of the liquids in the tank. Foaming was particularly a problem with the culture of *Bacillus* 1BA, resulting in difficulties in maintaining stable manifold pressure. While it is unknown if foaming would affect the viability of the organisms in the liquid, foaming could hasten the degradation of antifungal proteins and antibiotics excreted into broth culture by the bacteria and, thus, could potentially reduce biocontrol effectiveness. In experiment 2, when the biocontrol materials were diluted into much larger water volumes, foaming was not a problem for the bacterium C3 and the yeast. Addition of the an antifoaming agent Biospumex 36K to the suspension of 1BA arrested foaming. Therefore, the use a nontoxic antifoaming agent is recommended as an adjuvant for those biocontrol materials for which foaming may be an issue.

ACKNOWLEDGEMENTS

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DISCLAIMER

Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the authors and do not necessarily reflect the view of the U.S. Department of Agriculture.

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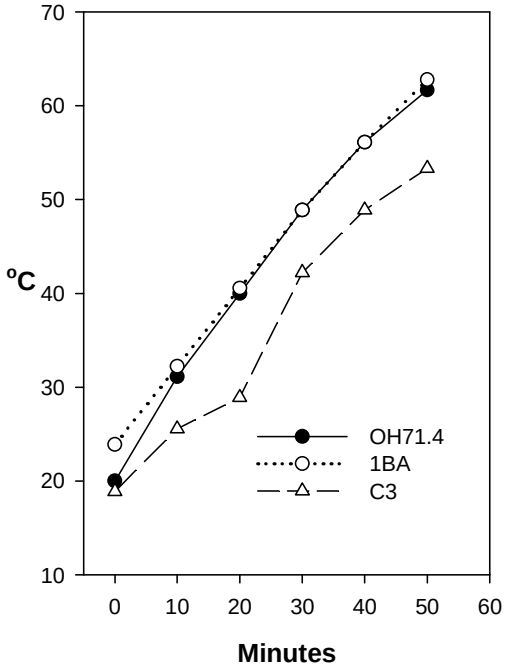

Fig. 1 Expt 1 - Temperatures in biocontrol agent suspensions in tank.

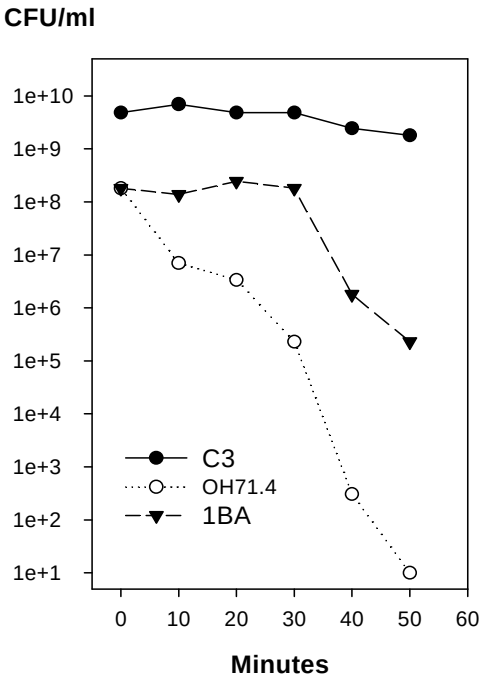

Fig. 2 Expt 1 - Biocontrol agent populations in suspensions in tank.

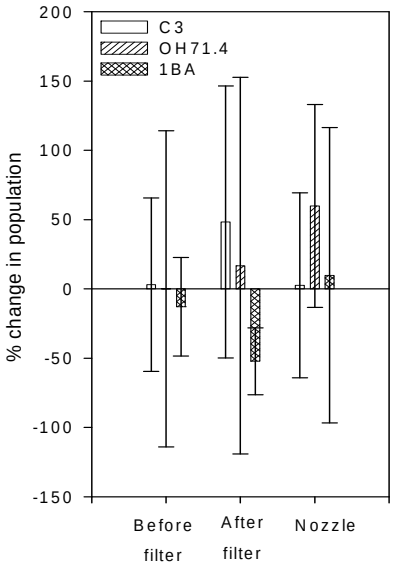

Fig. 3 Expt 1 – Changes in biocontrol agent populations at various points in spray system relative to tank populations. Standard deviations shown.

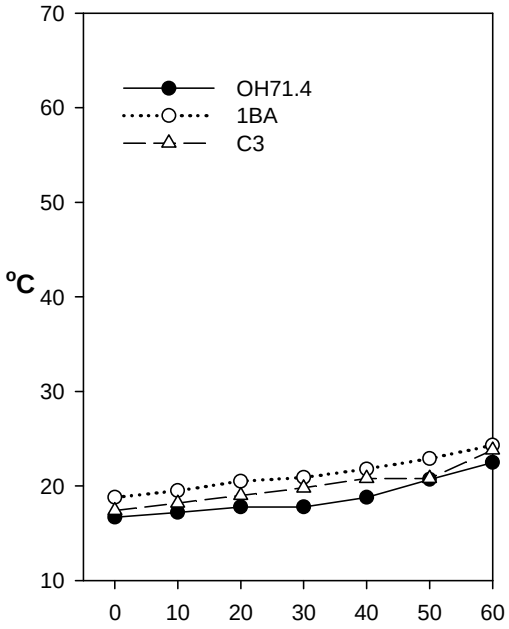

Fig. 4 Expt 2 - Temperatures in biocontrol agent suspensions in tank.

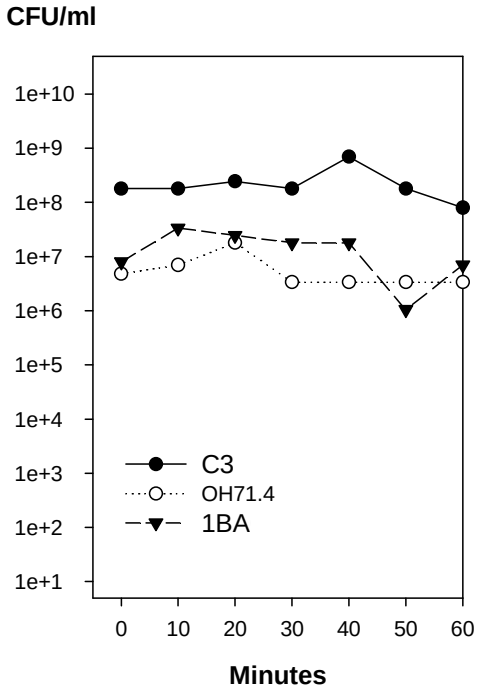

Fig. 5 Expt 2 - Biocontrol agent populations in suspensions in tank.

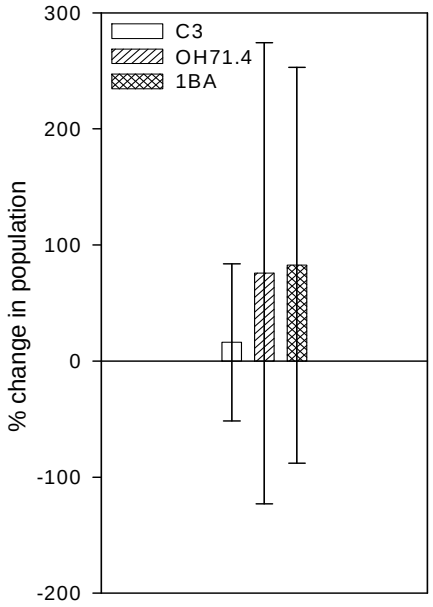

Fig. 6 Expt 2 – Changes in biocontrol agent populations at nozzles relative to tank populations. Standard deviations shown.

RESULTS FROM THE 2007 STANDARDIZED EVALUATION OF BIOLOGICAL AGENTS FOR THE CONTROL OF FUSARIUM HEAD BLIGHT ON WHEAT AND BARLEY.

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OBJECTIVE

To evaluate, using standardized methodology, a set of biological control agents applied alone and in combination with a fungicide for effectiveness in managing Fusarium head blight (FHB) and deoxynivalenol (DON) in wheat and barley across a range of environmental conditions.

INTRODUCTION

Some of the biological agents reported to have potential for controlling FHB are bacterial strains *Bacillus subtilis* TrigoCor 1448 (Stockwell et al., 2001) and *Bacillus* sp. 1BA (Draper et al., 2001), and *Lysobacter enzymogenes* strain C3 (Jochum et al., 2006). Each strain has shown efficacy in some field tests when evaluated separately (Stockwell et al., 2001; Jochum et al., 2006; Yuen and Jochum, 2004). In 2004 through 2006, they were directly compared for efficacy as part of the USWBSI-funded program for standardized evaluation of biological agents. Because combinations of biological control agents and fungicides were reported to be more effective in controlling FHB than the microorganisms or fungicides alone (DaLuz et al., 2003; Khan et al., 2004; Yuen and Jochum, 2004), standardized evaluations in 2005 and 2006, also compared these bacterial strains in combination with the fungicide tebuconazole. In the three years of testing, however, results were inconclusive as to the effectiveness of the treatments across a range of environmental conditions and crop genotypes (Yuen et al, 2004; Yuen et al., 2005; Yuen et al., 2006) due to low disease pressure in most or all test sites. Uniform fungicide trials in 2006 (Paul et al., 2006) showed increased yield and reduction of DON by a fungicide formulation Prosaro 421 SC that combines tebuconazole and prothioconazole. Therefore, trials in 2007 were designed to test the efficacy of the three biological agents, alone in combination with Prosaro, for the control of FHB and DON.

MATERIALS AND METHODS

Six trials were conducted across three states on barley and a range of wheat market classes (Table 1). In each trial, three bacterial biological agents (Table 2) were tested alone or in tank mix with the fungicide Prosaro 421 SC (6.5 fl oz/A). There also was a treatment of Prosaro alone and a non-treated control. A broth culture of each organism was provided by the originating laboratory and sent to the researcher in each location. The pre-application population of each agent in the inoculum was determined by the local researcher using dilution plating. All treatment liquids were amended with 0.125% Induce (v/v). One application was made per treatment at early flowering (Feekes 10.51) in 20 gal/acre using CO2-pressurized sprayers (approximately 40 psi) equipped with flat-fan nozzles oriented forward and backward. The size and number of replicate plots varied among trials. Some of the trials were inoculated with *Fusarium graminearum* spore suspensions and or inoculated corn grain, with mist irrigation systems utilized to stimulate infection. In all trials, FHB incidence (% heads infected per plot), severity (% spikelets infected per diseased head), and index (% plot severity) were determined from at least 40 heads per plot around 3

weeks after anthesis. The incidence of *Fusarium*-damaged kernels (%FDK), as well as yield of seed and test weight, were determined after harvest. Samples from each plot were sent to the North Dakota State University Veterinary Diagnostic Laboratory, Fargo, ND for analysis of DON content. Analysis of variance was performed on results from each trial separately, with Duncan's multiple range test used for means separation. Data from Missouri and Nebraska trials were analyzed together using ProcMixed (SAS), with trials being treated as fixed variables and the LSD method used to separate LS means. Results from South Dakota were excluded because of low disease levels.

RESULTS AND DISCUSSION

Weather conditions in South Dakota were dry, preventing significant disease or DON production. Wet weather in Missouri and Nebraska resulted in higher FHB development. Low temperatures in Missouri occurring during heading, however, caused damage to wheat heads and kernels, particularly in cv. Elkhart, making assessments of FHB severity and FDK difficult.

The most effective biological agent applied alone was *L. enzymogenes* C3, reducing disease severity in two trials and disease index averaged across four trials (Tables 3A and 3B). No combination of a biocontrol agent with Prosaro 421 SC exhibited greater efficacy than the fungicide alone. None of the biocontrol agents alone increased yields over the control (data not shown). Prosaro 421 SC applied alone or in combination with a biological agent was effective in reducing FHB measures and DON levels in multiple trials (Tables 3A and 3B). The fungicide alone and in combinations with some biocontrol agents increase plot yields in the two Nebraska trials and in the Missouri with 'Roane' (data not shown).

The collective results from this year's multistate trials indicated *L. enzymogenes* C3 to the be the most effective biocontrol agent across a range of environments, but treatments with the bacterium are not as effective or as consistent as treatment with Prosaro 421 SC. No benefit was revealed in this study from combining biocontrol agents with Prosaro 421 SC, contrary to past studies with biocontrol agent-tebuconazole combinations. The difference in results is most likely related to the greater effectiveness of Prosaro 421 SC over tebuconazole. Therefore, it may be desirable to explore combinations of biocontrol agents with less efficacious fungicides as a means to broaden the selection of treatments that can be used to protect florets from *Fusarium* infection.

ACKNOWLEDGEMENTS

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DISCLAIMER

Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the authors and do not necessarily reflect the view of the U.S. Department of Agriculture.

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Table 1. 2007 uniform biological control trial locations, crop cultivars, and researchers

State (location)	Crop market class and cultivar	Researcher and Institution
MO	Soft red winter wheat 'Roane'	L. Sweets, University of Missouri
MO	Soft red winter wheat 'Elkhart'	L. Sweets, University of Missouri
NE -1 (Mead)	Hard red winter wheat '2137'	C. Jochum & G. Yuen, University of Nebraska
NE - 2 (Lincoln)	Hard red winter wheat '2137'	C. Jochum & G. Yuen, University of Nebraska
SD	Hard red spring wheat 'Briggs'	K. Ruden & B. Bleakley, South Dakota St. Univ.
SD	Six-rowed barley 'Robust'	K. Ruden & B. Bleakley, South Dakota St. Univ.

Table 2. Biological control agents tested in 2007 uniform trials.

Organism	Supplier
Bacillus sp.1BA	Bruce Bleakley, South Dakota State University
Bacillus subtilis TrigoCor 1448	Gary Bergstrom, Cornell University
Lysobacter enzymogenes C3	Gary Yuen, University of Nebraska

Table 3A. 2007 results across six uniform biocontrol trials denoted by state and crop

Treatment		MO 'Roane'	MO 'Elkhart'	NE-1 2137	NE-2 2137	SD Briggs	SD Barley	LS mean
INCIDENCE (% heads infected)								
Control		12 a*	15 c	95 a	68 a	2	57	59 a
Prosaro		7 cd	22 abc	58 b	47 c	1	42	40 b
1BA		9 bc	18 bc	87 a	70 a	3	62	58 a
1BA + Prosaro		6 d	24 abc	65 b	52 bc	2	65	44 b
TrigoCor 1448		10 ab	22 abc	92 a	69 a	1	90	60 a
TrigoCor 1448 + Prosaro		7 cd	30 a	62 b	47 c	1	50	43 b
C3		12 a	26 ab	84 a	62 ab	1	79	56 a
C3 + Prosaro		7 cd	16 c	70 b	58 abc	1	84	46 b
	P	<0.001	0.045	<0.001	<0.001	0.758	0.174	<0.0001
SEVERITY (% spikelets infected)								
Control		16 a	32	38.0 a	29 b	45	5	31 a
Prosaro		6 c	32	15.6 e	28 b	17	5	21 cd
1BA		12 ab	23	33.0 ab	28 b	13	5	27 bc
1BA + Prosaro		7 bc	36	20.0 de	28 b	12	5	23 bcd
TrigoCor 1448		11 ab	26	35.8 a	28 b	38	7	28 ab
TrigoCor 1448 + Prosaro		9 bc	34	22.1 cde	36 a	22	5	26 abc
C3		10 bc	28	28.1 bc	28 b	27	6	25 abcd
C3 + Prosaro		9 bc	19	24.7 cd	26 b	5	10	20 d
	P	0.017	0.175	<0.001	0.039	0.755	.0545	<0.0001

*Means separation (P=0.05) shown only when treatment effect was significant.

Table 3B. 2007 results across six uniform biocontrol trials denoted by state and crop.

Treatment		MO 'Roane'	MO 'Elkhart'	NE - 1 2137	NE - 2 2137	SD Wheat	SD Barley	LS Mean
INDEX (plot severity)								
Control		1.9 a	4	36 a	20 a	1.1	3 b	20 a
Prosaro		0.4 c	8	9 e	13 c	0.7	2 b	9 d
1BA		1.1 b	4	29 ab	20 a	0.6	3 b	18 ab
1BA + Prosaro		0.4 c	9	14 de	14 bc	0.3	3 b	11 d
TrigoCor 1448		1.2 b	7	33 a	19 ab	0.8	6 ab	19 ab
TrigoCor 1448 + Prosaro		0.6 bc	10	15 de	17 abc	0.3	2 b	12 cd
C3		1.1 b	8	24 bc	17 abc	0.5	5 ab	15 bc
C3 + Prosaro		0.7 bc	4	18 cd	15 bc	0.2	8 a	11 d
	P	0.001	0.133	<0.001	0.039	0.894	0.029	<0.0001
FDK (%)								
Control		10	16	18	15	1.2	ND#	10
Prosaro		7	20	10	9	0.5	ND	7
1BA		11	22	16	15	0.8	ND	11
1BA + Prosaro		9	18	13	12	0.5	ND	9
TrigoCor 1448		12	19	12	12	1.0	ND	10
TrigoCor 1448 + Prosaro		11	23	11	11	0.8	ND	10
C3		12	19	15	13	0.8	ND	10
C3 + Prosaro		5	17	14	14	0.8	ND	9
	P	0.164	0.232	0.150	0.130	0.517	---	0.121
DON (ppm)								
Control		<0.5	0.8 c	5.4 a	3.6 a	<0.5	<0.5	3.7 a†
Prosaro		<0.5	1.4 ab	2.6 c	2.3 b	<0.5	<0.5	2.2 b
1BA		<0.5	1.1 bc	4.9 a	3.4 a	<0.5	<0.5	3.5 a
1BA + Prosaro		<0.5	1.2 abc	2.9 c	2.7 ab	<0.5	<0.5	2.3 b
TrigoCor 1448		<0.5	1.0 bc	4.6 a	3.3 a	<0.5	<0.5	3.4 a
TrigoCor 1448 + Prosaro		<0.5	1.6 a	2.6 c	2.1 b	<0.5	<0.5	2.2 b
C3		<0.5	1.3 ab	4.2 ab	3.4 a	<0.5	<0.5	3.3 a
C3 + Prosaro		<0.5	1.0 bc	2.9 bc	2.3 b	<0.5	<0.5	2.2 b
	P	---	0.027	<0.001	0.003	---	---	<0.0001

*Means separation (P=0.05) shown only when treatment effect was significant.
#ND=no data.
†Data from MO 'Elkhart' and NE trials were used to calculate LS means for DON.

SESSION 5:

VARIETY DEVELOPMENT AND HOST RESISTANCE

Chairpersons: Gina Brown-Guedira and Mohamed Mergoum

AIR SEPARATION AND DIGITAL PHOTO ANALYSIS AS NOVEL METHODS TO MEASURE THE PERCENTAGE OF *FUSARIUM* DAMAGED KERNELS.

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ABSTRACT

One of the greatest problems in breeding for Fusarium head blight (FHB) resistance lies in the difficulty of assessing the disease. At the present time, researchers generally measure disease incidence and severity in the field, deoxynivalenol (DON) content and percentage *Fusarium* damaged kernels (FDK).

FDK is presently measured in two ways: i) visual comparison of samples with reference samples and ii) manual separation of diseased and healthy kernels. Visual comparison of samples is a quick way of assessing FDK but is arguably too subjective. On the other hand, manual separation could be less subjective but is highly time consuming. Furthermore in manual separation, due to the amount of work that it takes, only small samples (e.g. 100 kernels) can be evaluated. This may not be an adequately representative sample size. To improve the efficiency of FDK measurement we should look for a method that: (i) reduces subjectivity, (ii) reduces the amount of work and time required, and (iii) allows increased sample size. To achieve this, two new methods are being proposed: air separation and digital photo analysis.

Air separation methods have long been used in the seed industry for seed conditioning purposes and in seed labs to measure the proportion of different components of seed samples. An air separation machine was specifically developed from a Precision Machine head thresher and a Shop-Vac vacuum to separate scabby kernels from healthy ones. Once a sample is loaded into the machine air-driven elevation of the lighter portion of wheat (i.e. scabby seeds) occurs until it reaches the top of the column where is collected in a receptacle. The heavier portion of wheat (i.e. asymptomatic seeds) is suspended midair and does not reach the top of the column. Once the air is turned off, the asymptomatic seeds fall and are collected in the bottom of the column. Finally, both portions of the sample are weighed separately and FDK is calculated. Approximate time per sample is 90 seconds.

In the digital photo analysis method, samples are evaluated based in their color composition. Color histograms are generated from the digital photos of the samples by image editing software. Mean blue value appears to have a consistent correlation with the FHB damage of kernels.

The air separation and the photo analysis methods emerge as two prospective techniques to measure FDK in an efficient and objective way and, ultimately, appear as promising tools in the difficult endeavor of assessing FHB in scab breeding programs.

ACKNOWLEDGEMENT AND DISCLAIMER

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USE OF MAS FOR FHB RESISTANCE: IS IT WORKING FOR WHEAT BREEDERS?

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ABSTRACT

Marker-assisted selection (MAS) is most appropriate and efficient when 1) the gene/QTL under selection has a large contribution to the phenotype; and 2) diagnostic markers are available. MAS is especially attractive to wheat breeders needing to improve FHB resistance because of the difficulties posed by phenotypic selection, including multi-genic inheritance and the need to establish inoculated, misted nurseries. Although not a substitute for established phenotypic screening methods, MAS, when used in early generations (F_2-F_4) can effectively increase the frequency of resistant genotypes. The *Fhb1* QTL on chromosome 3BS is the best known and by far the most heavily selected QTL for FHB resistance. The presence of this QTL results in 20% or more reduction of disease symptoms, making it the most potent FHB QTL mapped to date. The SSR markers *Xbarc133* and *Xgwm493* are in a 5 cM interval that flank *Fhb1*. *Xbarc133* is less than 2 cM from *Fhb1* and can be effectively used as a stand-alone marker, but is not diagnostic in all genetic backgrounds. New STS markers based on the sequences of candidate genes should be more efficient than marker *Xbarc133*. The QTL on chromosome 5AS, *Qfhs.ifa-5A*, provides only Type I (initial infection) resistance, but is complementary to *Fhb1*. However, this QTL is in a centromeric region of 5AS, making it less accessible for fine mapping and development of diagnostic markers. We have had success using *Xbarc180* to track this QTL in our germplasm. From our surveys of germplasm in the U.S. spring wheat region and some soft red winter cultivars, we discovered numerous cases of highly resistant material not having *Fhb1*, so the presence of this gene cannot be inferred based on pedigree and high levels of resistance. Robust phenotypic screening is still essential to identify resistant cultivars. Survey results regarding the use of these and other markers for FHB resistance will be presented.

ACKNOWLEDGEMENT AND DISCLAIMER

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MARKER-ASSISTED TRANSFER OF 3BS QTL FOR FHB RESISTANCE INTO HARD WINTER WHEAT.

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ABSTRACT

Epidemics of Fusarium head blight (FHB) can significantly reduce wheat grain yield and quality in the central and northern Great Plains of the U.S.A. Use of resistant cultivars is the most effective measure to control the disease. However, most hard winter wheat (HWW) cultivars currently grown in this area are highly susceptible to FHB. In addition, the disease screening procedure is laborious, time consuming, and costly, the progress in breeding for resistant HWW cultivars has been relatively slow with conventional methods. We used high-throughput marker-assisted backcross method to successfully transfer the major quantitative trait locus (QTL) from Sumai 3 and its derivatives into locally adapted HWW with minor FHB-resistance QTL to develop marketable FHB resistant HWW cultivars and useful germplasm lines. Three crosses were made between Sumai 3 derived soft red wheat lines and three locally adapted hard winter wheat cultivars (Harding, Wesley and Trego). Harding and Wesley are red wheat cultivars from South Dakota and Nebraska, respectively, and Trego is a white wheat cultivar from Kansas. Using marker-assisted backcross, about 80 Bc_2F_2 plants homozygous for the 3BS QTL were selected from each backcross population based on closely linked markers to the 3BS QTL. All selected Bc_2F_3 lines were evaluated in the greenhouse for Type II resistance in the USDA Genotyping Center in the fall of 2006. The result indicated that most selected lines were either highly resistant or moderately resistant. These materials have also been planted in mist-irrigated fields for further selection of FHB resistance, winter hardiness, hard-textured grains, and other traits. Some lines with good FHB resistance and other desirable traits will be released as new germplasm or cultivars in the hard winter wheat growning region after further yield trials.

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GENETIC LINKAGE MAPPING WITH DART MARKERS TO DETECT SCAB RESISTANCE QTLS IN A 'SUMAI-3' DERIVED WHEAT POPULATION.

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ABSTRACT

Much effort has been invested in identification of molecular markers linked to quantitative trait loci (QTL) that confer Fusarium Head Blight (FHB) resistance, though levels significantly higher than those of 'Sumai-3' remain elusive. Additional resistance QTLs may exist which typically are overshadowed by partial resistance of susceptible parents used in development of mapping populations. The objectives of this research were: (1) to create a genetic linkage map using Diversity Array Technology (DArT) and Simple Sequence Repeat (SSR) markers and (2) to associate FHB resistance phenotypes with the markers. Our population was created by crossing Sumai-3 with the very susceptible 'Y1193-6' (a Tibetan accession with unknown pedigree). An $F_{2:6}$ recombinant inbred mapping population was developed. A framework map consisting of 65 polymorphic SSR markers has been used to place most of 352 DArT markers. Our report will include associations between markers and FHB resistance QTLs for disease incidence, severity, index, and FDK percentage values.

USING THE AFFYMETRIX ARRAY TO DISCOVER SINGLE NUCLEOTIDE POLYMORPHISMS IN WHEAT.

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ABSTRACT

Gene expression arrays have been used to discover single nucleotide polymorphisms (SNPs) in several crop species. This study was designed to explore the possibility of using the Affymetrix Wheat Genome Array for the discovery of SNPs in wheat. Complementary DNAs synthesized from the mRNA isolated from the seedlings of six wheat cultivars of diverse origins (Ning 7840, Clark, Jagger, Encruzilhada, Chinese Spring and Opata 85) were hybridized to Affymetrix Wheat Genome Arrays. Cluster analysis of array data selected a total of 396 genes/probe sets with a signal intensity of at least 200, p-value of <1e-10 and overall R^2 ratio >0.8 for SNP confirmation through DNA sequencing. Sequencing results confirmed that 87 probe sets had at least one SNP within the probe sequences. In addition, SNPs were also identified in 21 additional genes, but they were detected outside the probe sequences. A total of 387 SNPs were discovered from the 108 genes. One SNP was selected from each gene to design primers for SNP analysis in a mapping population using SNaPshot kit and only 62 primers were successfully designed for SNaPshot analysis. Forty-two SNP markers were further analyzed in 96 F_{8-12} recombinant inbred lines from the cross of Ning 7840/Clark and 25 markers were integrated into the existing SSR map of the population. The result shows that Affymetrix arrays can be used to discover SNP markers in wheat.

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ENHANCING HOST RESISTANCE TO FUSARIUM HEAD BLIGHT: PYRAMIDING GENES IN SPRING WHEAT.

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ABSTRACT

Spring wheat (*Triticum aestivum* L.) production in the Northern Great Plains is severely affected by Fusarium head blight (FHB). Damage due to FHB is characterized by bleached spikes with white shriveled kernels, which ultimately reduces grain yield, lowers test weight, and results in an accumulation of deoxynivalenol (DON) in the kernels. Type II resistance has been associated with genes on chromosome 3B of Sumai 3 and chromosome 3A of *Triticum turgidum* L. var. *dicoccoides*. The objective of this study was to evaluate and compare the FHB resistance of two lines. One line, designated Line 1 has the Sumai 3 source only and another line, designated Line 2 has both the Sumai 3 and *T. dicoccoides* sources. These lines were developed by crossing a synthetic hexaploid wheat having the *T. dicoccoides* resistance to Alsen, a hard red spring wheat with the Sumai 3 source of resistance. The F_1 hybrid was backcrossed twice to Alsen and then pollinated with maize to produce doubled-haploid lines. These Alsen backcross-derived doubled-haploid (BC_2F_1DH) lines were initially screened with the SSR markers *Xgwm533* and *Xgwm2* for detection of the 3B QTL of Sumai 3 and the 3A QTL of *T. dicoccoides*, respectively. Additional STS markers were later used to verify the presence of these QTL. Phenotypic evaluation of these lines was done in three greenhouse seasons. A 10 µl inoculum with 50,000 spores ml^{-1} was injected into a single floret in the middle of the spike at anthesis. FHB resistance was assessed by measuring disease severity at 7, 14, and 21 d after inoculation (dai), percent *Fusarium*-damaged kernels (FDK), and DON content of the grain. A combined ANOVA indicated a significant genotype by dai interaction for disease severity. When means among genotypes were compared at the same dai, no significant differences were observed at 7 dai. However, a significant increase in disease severity was noted at 14 and 21 dai. The disease severity of Line 1 and Line 2 was not significantly different from Alsen, but both lines exhibited significantly lower disease severity than the synthetic parent at 14 dai. At 21 dai, Line 1 exhibited significantly higher disease severity than either Line 2 or Alsen. When comparisons were made within the same genotype across different dai, Line 1 exhibited a significant increase in disease severity, progressing from 5% severity at 7 dai to 17% severity at 21 dai. However, Line 2 exhibited no significant change, progressing from 5% severity at 7 dai to 8% severity at 21 dai. Alsen and the synthetic wheat exhibited a significant change in disease severity, progressing from 5% (7 dai) to 13% (14 dai) and 5% (7 dai) to 33% (14 dai) severity, respectively. The percent FDK of Line 1 and Line 2 was not significantly different from Alsen, but the FDK of both lines was significantly lower than the synthetic wheat. There were no significant differences in DON content for either Line 1, Line 2, or Alsen across greenhouse seasons, but the synthetic wheat had a significantly higher DON content in one of the three greenhouse seasons. Line 1 and Line 2 exhibited Type II resistance in all evaluations, and although differences between them were not always significant, in some instances, the combined effect of the 3A and 3B QTL in Line 2 may have contributed to a lower expression of FHB severity, percent FDK and DON content.

QTL ASSOCIATED WITH REDUCED KERNEL DAMAGE AND RESISTANCE TO FUSARIUM HEAD BLIGHT IN WHEAT.

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ABSTRACT

Resistance to Fusarium head blight (FHB) is controlled by a number of genes, suggesting that the identification and accumulation of multiple resistance genes in a variety would result in increased resistance. Our objective in this study was to identify QTL associated with resistance to FHB, especially QTL associated with a decrease in percent *Fusarium* damaged kernels (FDK). A population of 269 recombinant inbred lines (RILs) was developed from a cross between Patton and IL94-1653, and evaluated for FHB resistance in a mist-irrigated FHB nursery in Urbana, IL in 2006 and 2007. The parent lines, Patton and IL94-1653, are moderately susceptible to FHB, and IL94-1653 appears to exhibit resistance to kernel damage. The RIL population was genotyped using SSR markers and QTL analysis was performed using MapQTL 4.0. Preliminary results identified a QTL on chromosome 4B associated with reduced percent FDK in both years. The markers associated with this region are gwm513, gwm495, and wmc47. Chromosome 4B was also associated with DON content in 2007 but not in 2006. In 2006, a QTL on 2B was associated with reduced percent FDK, severity, FHB index, and ISK index. This QTL was not significant in 2007. Further genotyping and QTL analysis is in progress to identify additional resistance QTL in this population.

RESISTANCE TO KERNEL DAMAGE CAUSED BY FUSARIUM HEAD BLIGHT IN AN RIL POPULATION.

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ABSTRACT

Fusarium head blight (FHB) infection of wheat results in *Fusarium* damaged kernels (FDK) that contain the mycotoxin deoxynivalenol (DON), reducing the value of the grain. By developing lines with resistance to kernel damage resulting in a low percent FDK, breeders may be able to reduce DON content in grain. A population of 269 recombinant inbred lines (RILs) was developed from a cross between two soft red winter wheat lines, IL94-1653 and Patton, with IL94-1653 thought to exhibit resistance to kernel damage. Both parents are moderately susceptible to FHB. The RIL population was evaluated for FHB resistance in the greenhouse in 2005 and 2006, and in field in 2006 and 2007. A wide range of FHB symptoms and DON content were observed in the RIL population. The RIL population also exhibited transgressive segregation for all measures of FHB resistance, including disease severity, percent FDK, and DON content. In the field, the correlation between percent FDK and DON content was positive, and varied by year from moderately low to medium ($r = 0.38$ in 2006; $r = 0.53$ in 2007). The ISK index, a combination of severity, incidence, and percent FDK measurements, gave a better correlation with DON content for both years ($r = 0.47$ in 2006 and $r = 0.64$ in 2007), with all correlation values significant at $p<0.0001$. However, identifying RILs with consistent resistance to kernel damage was difficult and seems to be influenced by environmental variation as well as by resistance to initial infection and resistance to spread of infection.

BARLEY CHROMOSOME 2(2H) BIN 10 FUSARIUM HEAD BLIGHT RESISTANCE QTL: MAPPING AND DEVELOPMENT OF ISOLINES.

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INTRODUCTION

Development of commercially acceptable cultivars with Fusarium Head Blight (FHB) resistance and good agronomic qualities is the goal of the barley SCAB project. One of the best FHB resistance quantitative trait loci (QTL) resides in the chromosome 2(2H) bin 10 region. Our contributions are focused on genetic and physical mapping of this region with the long-term goal of cloning the genes responsible for FHB resistance. To facilitate this, we have isolated recombinant lines with introgressed small chromosome 2(2H) bin 10 genomic segments in a susceptible genomic background. We have also developed 6-rowed recombinants in the resistant CI4196 genomic background. To further facilitate development of agronomically acceptable barley cultivars with FHB resistance, we have undertaken to modify the resistant line CI4196 by mutagenesis. Mutants with desirable traits such as semi-dwarf, early and 6-rowed are easily selected. These provide improved FHB resistant parent material that can be rapidly incorporated in breeding programs.

RESULTS AND DISCUSSION

Genetic and physical mapping

We have developed an extensive genetic map for the bins 8-10 region (markers ABC306-MWG882). This map includes 43 loci, 111 markers, and 423 bacterial artificial chromosome (BAC) clones identified in our laboratory (the Tim Close barley physical map database increases this number to over 800). The details of this map will be published elsewhere. Here we focus on the bin 10 region from MWG699 to MWG503. This region has relatively few markers and has been difficult to saturate, as it has been for others who have published maps for this region (Marcel et al., 2006; Stein et al., 2007). Nevertheless, there are nine loci with 20 markers and 125 BAC clones identified in our laboratory (Fig. 1). There are a total of 295 BAC clones identified for this region if one includes those in the Tim Close barley physical map database. Some of those BAC clones likely belong to different loci, but nevertheless the physical map is fairly saturated. Based on just the BAC clones identified in our laboratory (because we are more confident that these really belong in this region), we have identified nine BAC clone contigs. We can reasonably exclude the two BAC contigs associated with the MWG503 locus because that region is far from the FHB resistance QTL (Horsley et al., 2006). The most likely FHB resistance peak resides around marker BF265762A just below the Vrs1 locus (Fig. 1). This region is covered by a completed BAC contig that also includes the next downstream marker BI955972. The area between Vrs1 and BF265762A has been fine-mapped by Pourkheirandish et al. (2007), providing further probes for picking BAC clones to build a complete contig. This region should be sequenced as soon as possible.

Isoline development

We have been developing small introgressed CI4196 FHB resistant line chromosome 2(2H) bin 10 region fragments into a susceptible cv. Morex background in order to more accurately define the region responsible for FHB resistance (Fig. 2). We now have identified three homozygous recombinant lines from the A171 x Morex cross that integrate either the region directly below Vrs1 (07-83-11) or a slightly larger region (07-76 and 07-84). An additional five homozygous lines

were developed from the cross A80 x Morex (07-85-1; 07-87; 07-90; 07-91; 07-97). These lines contain the BG369629 to BG416977 CI4196 region, which we do not think is important in FHB resistance. They also contain various small fragments introgressed from the Vrs1 to BG343659 region that appears to be critical to FHB resistance. All of these lines are being phenotyped in China winter '07-'08. A number of less advanced recombinant lines have already been tested for FHB resistance in China and North Dakota. Among them, line 06-310-18 stands out as being reproducibly FHB resistant and about the same height as Foster. This line is six-rowed, but the spike is not very robust, probably due to the presence of the two-rowed *Int-c* allele. This allele will be replaced by the mutant *int-c* allele that we have isolated from CI4196 (see below).

Mutant selection and analysis

All of the agronomically important CI4196 mutants that were identified in the field 2006 and 2007 were confirmed as CI4196 by PCR (Boyd et al., 2006). The mutants isolated in 2006 were phenotyped in China winter '06-'07 (Table 1). The male sterile mutants show good FHB resistance. These should be useful to facilitate backcrossing and breeding efforts. Early mutants (desirable for breeding) were identified and confirmed, but only two (G07-83 and G07-105) showed FHB resistance levels comparable to CI4196. The higher FHB susceptibility may be due to longer exposure of the early-emerging spike to the disease (Nduulu, 2007), or it may be the product of other unintended mutations. Of the two semi-dwarf mutants tested, only G07-66 is about the same height as Foster with FHB resistance comparable to CI4196. This line may be suitable as an improved CI4196 parent for FHB resistance breeding. Another line of potential interest is lax spike (G07-52). This line maintains CI4196 FHB resistance and is not excessively tall. The lax spike trait may be useful in reducing the opportunities for FHB infection. The intermedium line isolated in 2006 is not very promising and may be just a distorted head mutant. Two much more promising apparently intermedium type mutants were isolated in 2007 (see below).

New mutants identified in the field 2007 from a gamma irradiated population and sent for FHB phenotyping in China winter '07-'08 include intermedium, early, premature ripening, semi-dwarf, dwarf, erectoides, anthocyanin-less, and glossy head. The two intermedium types are particularly interesting. They have reasonably good 6-rowed heads and are probably of the *int-c* type (based on phenotype). The *Vrs1* gene was sequenced from these mutants and appears to be identical to the CI4196 *Vrs1* gene. Thus, unless the mutation is in a regulatory region, they are not *Vrs1* mutants. However, they will be very useful for crossing with the 06-310-18 line described above to recover a true 6-rowed head in a mostly CI4196 line. We are also very much interested in the FHB response of the new semi-dwarf and early mutants. Since these come from a gamma irradiated population, we expect that the genome may not be as highly rearranged as in the mutants selected from fast neutron irradiated material in 2006.

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DISCLAIMER

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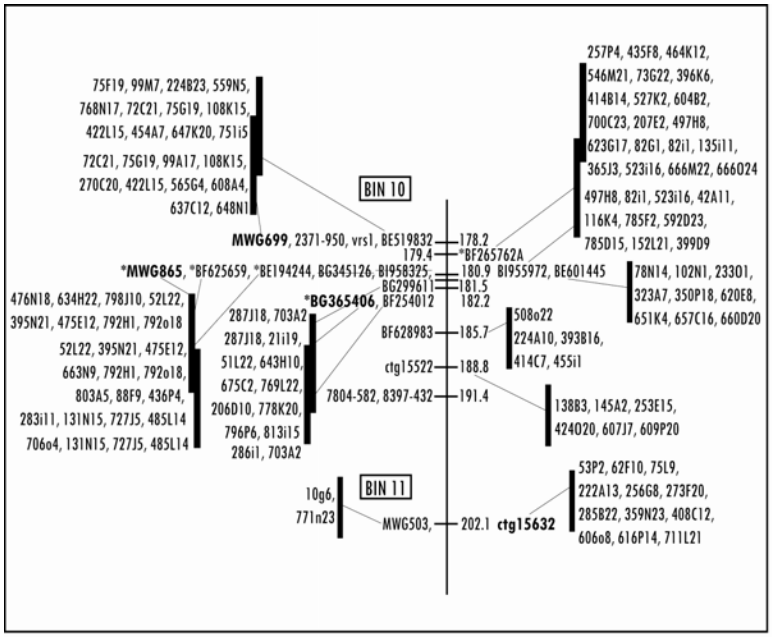

Fig. 1 Genetic map of Chr. 2(2H) bin 10 showing all markers currently mapped to the region as well as all BAC addresses picked by each marker listed next to the solid bars. Double solid bars indicate that the same contig (according to the Close database) is picked by different markers, creating a physical contig across the genetic region. Distances are in centimorgans based on a 94-line population. Bold = CAPs marker. Strikethrough = picked no BACs.

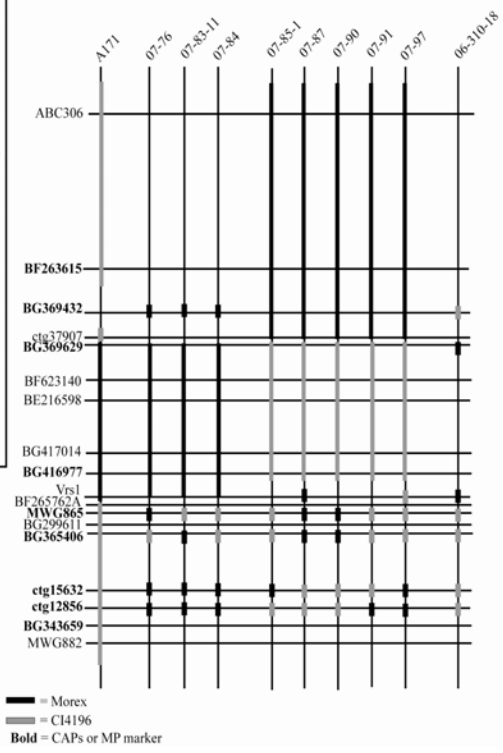

Fig. 2 Near-isogenic lines with introgression of CI4196 (gray) and Morex (black) in bin 10 (markers Vrs1 to ctg 15632). Markers in bold are either CAPs or multiplex (MP) PCR markers.

HAPLOTYPING OF KNOWN FHB RESISTANCE QTL IN PACIFIC NORTHWEST WHEAT GENOTYPES.

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ABSTRACT

This study was conducted to discern the genetic variation of Fusarium head blight resistance in PNW wheat genotypes via haplotyping of fifteen SSR and STS markers associated with six QTL for FHB resistance previously identified in known resistance sources. A total of 15 markers on six chromosome regions (2D, 3A, 3BS, 5AS, and 6B) are being used in 94 PNW wheat genotypes which have no Sumai 3 related backgrounds. Based on the preliminary results derived from haplotyping of six markers, we identified some of the known major FHB QTL present in the adapted PNW cultivars/lines. The frequency of the known QTL on 3BS and 6BS were higher than expected. The known target alleles for marker STS3B-256 on 3BS was present in 30 lines and the one for WMC 152 on 6BS was present in 45 lines out of the 94 genotypes studied. Evaluation of field FHB resistance of these genotypes is needed and they will be done in the 2007-08 growing season. This study has the potential to identify novel and adapted sources of resistance through allele size comparisons of known SSR loci associated with QTL identified in known resistance sources. Identified cultivars/lines having good field FHB resistance and/or known FHB resistance QTL can then be grown in PNW region and used as adapted resistance sources in the PNW and Great Plains breeding programs.

VALIDATION OF SIX QTLS ASSOCIATED WITH FUSARIUM HEAD BLIGHT RESISTANCE IN ADAPTED SOFT RED WINTER WHEAT.

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ABSTRACT

This study was conducted to validate molecular markers linked to six FHB resistance QTL previously identified in different bi-parental populations using elite breeding lines incorporated FHB resistance to initial infection, spread, and DON accumulation in different genetic backgrounds. A total of 129 SSRs were characterized in the 145 breeding lines. Forty-four unrelated SSRs (4 SSRs per chromosome) were used in background selection and the remaining 85 SSRs were used in validation of target QTL. The 145 wheat lines were also evaluated in yield performance trials at two locations, Blacksburg and Warsaw, VA, and for type I, type II, and DON resistance in a scab nursery at Blacksburg, VA in 2005 and 2006. Molecular markers linked to scab resistance genes located on wheat chromosomes 2BS, 2DS, 3AS, 3BS, 5AS, and 6BS were confirmed and allelic effects of associated marker loci were analyzed. Adapted resistant lines with novel alleles different from known exotic sources were characterized. Renwood 3260 and its derived lines have good overall resistance and high yield potential. These lines have unique resistance with alleles differing from those of known resistance sources W14 and Sumai 3 at marker loci Gwm429, Gwm120, Gwm261, Barc133, and Gwm186 in the chromosome 2BS, 2DS, 3BS, and 5AS QTL regions. Ernie and its derived lines also have good overall resistance but didn't produce promising grain yields in Virginia. These lines have unique resistance comprised of the same resistant alleles as Renwood 3260 at loci Gwm429, Gwm120, and Gwm261 in 2BS and 2DS QTL regions. Both the Ernie and Renwood 3260 derivatives contain the same resistant alleles as donor parent W14 at loci Wmc264, Barc133, and Barc117 in 3AS, 3BS, and 5AS QTL regions. In addition, these lines have unique resistant alleles in their background at Gwm493 and Wmc152 in 3BS and 6BS QTL regions.This is the first study validating six FHB QTL in elite breeding lines. QTL-markers validated in the current study have been used widely in parental selection, gene pyramiding, and in postulating and selection of FHB resistance of progeny derived from such newly developed FHB resistant lines. This is also the first study evaluating the effects of allelic differences and genetic backgrounds on FHB resistance. Newly developed FHB resistant lines with unique QTL/allele combinations have been used as parental lines in most of eastern wheat breeding program. Some of these lines will be released as varieties and/or adapted germplasm. The newly developed FHB resistant lines and unique QTL/marker allele profiles identified in this study will set the stage for using MAS not only for FHB resistance but also in combining FHB resistance with other important agronomic traits.

DEVELOPMENT OF SCAB RESISTANT SOFT RED WINTER WHEAT GERMPLASM USING MARKER-ASSISTED SELECTION.

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ABSTRACT

Scab of wheat, caused by *Fusarium graminearum*, is a disease that periodically strikes the US mid-Atlantic region. Breeding for resistance is an effective measure of disease control. The objective of this study was to develop scab resistant soft red winter wheat germplasm adapted to the US mid-Atlantic region using marker-assisted selection. McCormick, a genotype adapted to the Mid-Atlantic region, was used in a backcross program with the Chinese variety Ning7840. An accelerated backcross scheme was developed to incorporate scab resistance QTLs found on chromosomes 3BS, 5A and 2DL in the Chinese variety Ning7840. Two rounds of backcrossing were completed using McCormick as the female parent. Progenies from the first round of backcrossing were selected for the presence of the Ning7840 scab resistance alleles at 3BS, 5A, and 2DL, and then for a high background of McCormick alleles. Two backcross progenies had over 60% McCormick background. Using these two selected BC1F1s, 400 BC2F1s were produced in a second round of backcrossing. Additionally, the two selected BC1F1s were crossed with a wheat line with stripe rust resistance (GA96229-3A41). 800 BC2F1 seeds were screened with molecular markers to identify those with Ning7840 alleles (on 3BS, 5A and 2DL) and most McCormick background. A single BC2F2s population derived from a selected BC2F1 plant was screened with 3BS, 5AS, and 2DL markers to select those homozygous for the resistant alleles. Additionally, we derived near-isogenic lines from this F2 population to identify the effect of each QTL on scab resistance, agronomic and quality traits. We plan to test some of the BC2F3s for scab resistance in the spring of 2008. We anticipate having a small amount of seed of selected BC2F4s, containing the Ning7840 alleles in the McCormick background, available for distribution to other soft red winter wheat breeders for crossing in the fall of 2008.

APPLYING SINGLE KERNEL SORTING TECHNOLOGY TO DEVELOPING SCAB RESISTANT LINES.

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ABSTRACT

We are using automated single-kernel near-infrared (SKNIR) spectroscopy instrumentation to sort Fusarium head blight (FHB) infected kernels from healthy kernels, and to sort segregating populations by hardness to enhance the development of scab resistant hard and soft wheat varieties. We sorted 3 replicates of 192 samples into a damaged fraction yielding an average of 61.3 ppm DON, and a healthy fraction yielding an average of 0.73 ppm DON. This collaborative work with Dr. Gene Milus and Peter Horevaj investigated the resistance of soft red winter wheat lines to DON and NIV chemotypes of *Fusarium graminearum*. In another study, we also sorted the soft portion of a hard x soft cross into FHB infected and healthy fractions, and likewise sorted the hard portion into FHB infected and healthy fractions. The hard x soft crosses were separated into the hard and soft portions in 2006 where the respective portions were inoculated and planted. The 2007 scabby and healthy fractions of the hard and soft lines will be planted this fall to determine if our sorting will result in populations with FHB resistance. This work is in cooperation with Dr. Anne McKendry and Dr. Stephen Baenziger. Another project that was done in cooperation with Dr. Stephen Wegulo, Julie Breathnach and Dr. Stephen Baenziger used the automated SKNIR system to rapidly assess lines for FHB resistance by running multiple samples and obtaining a count of infected and healthy kernels. We have done this for about 300 lines and the information is being used to select resistant lines for further developement.

TUNISIAN DURUM WHEAT AS NEW SOURCES OF RESISTANCE TO FUSARIUM HEAD BLIGHT.

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ABSTRACT

There are limited sources of resistance to FHB which mostly restricted to Chinese hexaploid genotypes like Sumai3 and Wangshuibai. Therefore it is necessary to use other sources of resistance to expand the number of genes that may be used in the gene pyramiding programs. The North Dakota Durum Wheat breeding program has identified four tetraploid wheat sources of resistance from Tunisia, which were selected among a large number of lines evaluated over 55 repeated FHB trials. Since their identification, these lines have been extensively used in the breeding program to derive resistant breeding lines. We used a collection of backcross derived advanced resistant lines, susceptible sibs, and parental lines to identify markers that are associated with these novel sources of resistance. In this study we used 184 BC1F6 and 189 BC1F7 lines derived from crossing of Tun7, Tun18, Tun34, Tun36 with durum cultivars 'Ben', 'Maier', 'Lebsock' and 'Mountrail' for association studies. As Tunisian lines pedigree shows no relation to Chinese genotypes, they probably carry different genes or alleles for resistance to FHB. We checked all the parents and RILs in the greenhouse in two seasons for type II resistance to FHB by single floret injection inoculation method. The data showed that the Tunisian lines have different amount of resistance varying from 18% to 10% infection rate through the spikes. The data also showed Maier may have some minor resistance genes because it showed a moderate resistance in our greenhouse study and the crosses between Maier and different Tunisians had more transgressive resistant progenies compared with the other crosses.

To accelerate the identification of markers associated to FHB resistance, we initially screened the parents. The amounts of recombination in wheat chromosome arms are low so we picked 10-14 SSR markers per chromosome which were roughly 10cm apart and cover the whole genome. Among the 179 SSR markers that we applied on the parents about 45% showed polymorphism for at least two parents and about 8 % showed polymorphism between the whole set of Tunisian lines and susceptible cultivars. The most polymorphism was found on chromosomes 5A and 3B and the least on chromosome 6A. About 22 SSR markers that had been mentioned in different articles to be linked to FHB resistant were also applied to the parents. Among them barc117 and gwm129 from chromosome 5A showed the same pattern in Tunisian lines but not the susceptible lines. We also did the Diversity array (DArT) marker analysis to have a more complete coverage of the whole genome and to find closer markers to the genes of interest. DArT analysis used 2300 markers which showed 25% polymorphism between the parents. About 8% of the polymorphic markers were present in all the Tunisian lines but not the susceptible cultivars. The cluster analysis of the polymorphic markers revealed three distinct groups. Tun7 was in a separate group far from the other two and all the other Tunisian lines fell in a separate group from susceptible cultivars. Our data shows Tun7 and Tun18 are potential candidates for new sources of resistance which will be discussed in detail in our presentation.

ACKNOWLEDGEMENTS AND DISCLAIMER

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RESPONDING TO FUSARIUM HEAD BLIGHT FOR THE NORTHERN ROCKY MOUNTAINS AND WESTERN GREAT PLAINS.

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OBJECTIVES

To improve the efficiency of individual breeding programs' development of FHB resistance and minimize the impacts of associated diseases in irrigated systems.

INTRODUCTION

Fusarium head blight is a perennial disease problem for irrigated acreage in northern Rocky Mountains and western Great Plains. Montana, alone, has 150,000 acres of irrigated spring wheat with an annual production of 9.8 million bushels amounting to $50 million annually. All of this acreage is potentially impacted by this disease. Neighboring states in the northern intermountain region (Idaho, Washington, Oregon and Wyoming) have an additional 390,000 irrigated acres, all of which may be affected by FHB. FHB tolerant varieties have been utilized by producers in the past years but there are significant deficiencies which discouraged their continued use. In particular, varieties had problems with lodging, ergot and black chaff. In addition, current FHB tolerant varieties lack sawfly resistance, which is important to the western Great Plains region. The purpose of this proposal is to develop new lines specifically adapted for these areas and to determine the suitability advance FHB tolerant materials from other states for high-yielding, irrigated production.

MATERIALS AND METHODS

FHB Nursery - Wheat scab conditions were optimized with sprinkler center pivot irrigation, continuous cropping of wheat, and wheat residue serving as inoculum of *F. pseudograminearum* and *F. graminearum*. Residue management involved a fall cultivation with irrigation to germinate volunteer seed with residue remaining on the soil surface. Spring cultivation was a minimum tillage following by the planting with a no-till drill. Nursery was planted with a no-till small plot drill and seed was treated with Raxil-MD. Individual plots were 3.5 x 20 ft, 4 replications, planted May 9, 2006 and 3.0 x 12 ft, 3 replications planted May 8, 2007. Pre-plant, top dress and fertigation at soft dough was applied for potential yield of 90-100 bu. MCPA and Discover broadleaf herbicide and Quilt fungicide for leafspots during seedling stage. Best management practice to minimize FHB consisted of a Folicur fungicide (14 oz/acre) applied at anthesis (Feekes 10.5) and irrigation discontinued for nine days, July 7 to July 16. Normal sprinkler irrigation was on a daily cycle, 0.3 in/day, with 4.5 in up to flowering and then resumed on alternate days with a 4.5 in through grain development. Harvest completed with a small plot combine and chaff air volume was minimized to avoid loss of light, scabby grain. Spring wheat entries, 15 in total, were selected based on commercially available variety or advanced lines. Hank was entered twice as a susceptible check and for evalution of the uniformity of FHB distribution in the nursery.

Disease Evaluation - Scabs heads were visually determined by the pre-mature head blight and dark tan of the peduncle at ripening and hard dough stage. DON mycotoxin in the grain was processed according to standard protocols and evaluated by the NDSU Veterinary Diagnostic Lab. Germination blotter test for viability of seed and ergot quantity on percentage weight was conducted by the MSU Seed Analysis Lab. Lodging of the variety was determined as a visual assessment for the plot area at harvest.

RESULTS AND CONCLUSIONS

FHB Effects & Agronomic performance **-** The overall grain deoxynivalenol (DON) concentration was 2.2 ppm for 2006 and 2007 MSU FHB nursery. "Hank" is highly susceptible to FHB with a grain DON of 8.8 ppm, yield loss of 37%, test weight of 54 lb/bu and a head scab incidence of 58%. Other varieties without Sumai3 gene had 0.6 to 2.0 ppm grain DON, including; Vida, Howard, Granite, Explorer (HWW), Choteau and Expresso. Tolerant varieties had less than 0.5 ppm grain DON, including; Kuntz, Volt, Freyr, Granite, Knudson, Alsen, Glenn, Kelby, and an experimental line MT0550 (Choteau/ND709-9). Tolerant varieties had a 7% incidence of symptomatic scab heads as compared to 33% among varieties lacking the Sumai3 gene. Overall, varieties with the Sumai3 gene yielded 67 bu/ac or 18% higher than varieties lacking tolerance to FHB. Grain test weights were 62.3 lb/bu in the tolerant varieties and 57.8 lb/bu in the susceptible varieties. Seed germination by a blotter test was below an acceptable "92% for foundation class" on those varieties lacking tolerance to FHB. Several of the short statured varieties are adapted to high production under irrigation, but susceptible to FHB, whereas the FHB tolerant varieties will lodge under these conditions. Ergot and bacterial black chaff susceptibility of varieties are a concern for irrigated wheat production in these regions.

Breeding Lines **-**We have used molecular markers to backcross the Sumai3 QTL into Choteau, a solid-stem variety with resistance to the wheat stem sawfly, and MT0249, a variety with long green leaf duration and short stature. Molecular marker GWM 533 was utilized for selection in Choteau lines, such as MT0550, and this line is expressing tolerance to FHB. A similar marker selection line, MT0551, was removed from consideration following poor FHB tolerance in the 2006 nursery BARC 133 was used when MT0249 was a recurrent parent and there are several shorter statured lines that are under evaluation for FHB tolerance. We will have sufficient seed of for single screening and seed increase rows in the MSU FHB nursery in 2008. An FHB resistant line similar to Choteau will find utility in the western Great Plains and the northern Rocky Mountains.

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Table 1. Effects of Fusarium head blight on performance of spring wheat varieties under sprinkler irrigation in Montana during 2006 and 2007.

VARIETY	FHB reaction	Grain Yield 2 yr aver	Grain DON 2 yr aver	Test Wt 2 yr aver	Scab Heads 2 yr aver	Germ Blot Test	Lodge 1 yr 2006	Ergot 1 yr 2006
		Bu/ac	ppm	lb/bu	%	%	%	% wt
EXPLORER		53.2	2.2	55.4	13.6	83.6	37	0.00
HANK		56.7	8.8	53.5	53.9	57.3	0	0.00
HANK		59.3	8.4	54.2	48.9	57.1	0	0.00
HOWARD		65.6	1.1	60.2	21.6	86.6	64	0.04
VIDA		69.8	1.4	57.7	18.1	83.4	50	0.00
GLENN	Sumai3	70.7	0.2	63.9	3.1	96.6	31	0.01
ALSEN	Sumai3	70.7	0.2	62.2	5.9	95.6	10	0.14
GRANITE	Tolerant	75.0	0.4	62.3	16.2	93.9	5	0.07
EXPRESSO		76.7	3.6	59.9	25.8	81.1	5	0.00
MT0550	Sumai3	77.8	0.3	61.9	4.8	94.4	49	0.02
CHOTEAU*		77.9	2.8	60.8	21.5	na	na	na
KELBY	Sumai3	80.8	0.5	61.8	16.6	92.6	24	0.02
KNUDSON	Sumai3	83.8	0.4	60.3	5.6	92.6	63	0.02
KUNTZ	Sumai3	85.9	0.4	61.3	11.9	94.9	16	0.01
VOLT	Tolerant	87.6	0.5	62.4	5.6	95.6	8	0.02
FREYR	Sumai3	87.6	0.3	61.5	6.3	91.3	78	0.03
Lsd P<0.05						3.5	22	Ns
C.V.%						2.8	49	
*Choteau 2007								

RESISTANT GERMPLASM FROM SUSCEPTIBLE PARENTS: AN EVOLUTIONARY APPROACH.

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ABSTRACT

The Chinese wheat line Sumai 3 is widely considered the standard for resistance to Fusarium Head Blight (FHB). Much research effort aims to introgress its FHB resistance into germplasm with better quality, agronomic traits, geographic adaptation and resistance to other diseases. As several, or perhaps many, genes are involved in expressing the trait, it has proved difficult to combine high FHB resistance with desirable agronomic and quality traits while advancing through cycles of crosses with elite, FHB-susceptible parents. Instead of aiming to transfer the complement of genes that condition FHB resistance in Sumai 3, we pursued an alternative approach of emulating in our desired germplasm the evolution of resistance that had occurred in Sumai 3 itself as it derived from moderately susceptible parents

Evolution within a population can be speeded by enhancing variation, selection and generation of fertile progeny among its individual members, and is more readily followed using small populations that can be carefully examined and advanced through as many as 4 generations per year (3 indoors and 1 field trial). After initial crosses with lines resistant to wheat streak mosaic virus (WSMV), we identified and selected in each generation of back-crossing regimes those individuals that appeared to combine vigorous growth under pressure from virus infection with the best resemblance to the recurrent cultivar parent. After the BC2 generation, lines that consistently performed well under pressure from WSMV infection were spray-inoculated with macroconidial suspensions of *Fusarium graminearum* and the most promising individuals selected for additional backcrossing and selection.

We had chosen a Canadian amber durum spring wheat cultivar, Strongfield, to apply this novel approach of 'speeded evolution', as the germplasm in this wheat class is highly susceptible to FHB and there are no well-characterized tetraploid wheat sources of resistance that might readily be introgressed. To date, we have generated lines equivalent to BC3F4-6, and with repeated backcrosses and selection, the lines have increasingly come to resemble Strongfield in all desirable agronomic traits. In 2007, BC3F4 and BC3F5 lines were evaluated in FHB nurseries. Families of lines were observed with excellent FHB reactions (similar to the most resistant hexaploids) that were consistent with results seen in predecessor generations selected in indoor tests. The 'speeded evolution' approach allows only small quantities of seed to advance from each generation of highly selected germplasm, precluding testing for quality until disease reactions and agronomic traits are consistently good. Initial assessments, however, of gluten index of selected lines of BC3F5 and BC3F6 seed harvested from 2007 FHB nursery plots indicate several of these FHB-resistant lines have acceptable quality.

RESISTANCE OF WINTER WHEAT LINES TO DEOXYNIVALENOL AND NIVALENOL CHEMOTYPES OF *FUSARIUM GRAMINEARUM*.

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OBJECTIVES

The objectives of this research were to determine if wheat lines selected for resistance to Fusarium head blight (FHB) caused by deoxynivalenol (DON) chemotypes also have resistance to the nivalenol (NIV) chemotypes and to determine which lines appear to have resistance to mycotoxin accumulation in the grain.

INTRODUCTION

Even after several decades of intensive research on FHB of wheat caused by *Fusarium graminearum*, mycotoxins produced by this pathogen still cause serious concerns for food and feed safety. The most prevalent mycotoxins produced by *F. graminearum* are DON and NIV. Strains that produce mainly DON (DON chemotypes) predominate in the U.S. (Chandler et al. 2003). However, strains that produce mainly NIV (NIV chemotypes) were found recently in Louisiana and Arkansas (Gale et al. 2005 and 2007), and NIV is ten times more toxic to humans than DON (Ueno and Ishii 1985). Developing resistant wheat cultivars is perceived to be the most effective means for managing FHB and reducing levels of mycotoxins in grain. The presence of both DON and NIV chemotypes in the Midsouth necessitates having resistance to both chemotypes in wheat cultivars adapted to this region.

MATERIALS AND METHODS

A susceptible check and 15 resistant winter wheat lines (Table 1) were grown in the greenhouse. Heads were inoculated at flowering with two DON and two NIV chemotypes of *F. graminearum*. Inoculum (20µl at 5.10^4 cfu/µl) was injected into one floret of each head, and plants were misted for 72 hours to promote infection. The number of infected florets per head was counted 21 days after inoculation, and the percentage of infected florets (%IF) was calculated. Heads were harvested at maturity and threshed by hand to retain all of the rachis tissue and grain. Grain and rachis tissue from heads within a pot were bulked, and each bulked grain sample was separated into "healthy" and "scabby" fractions by the USDA ARS Grain Marketing and Production Research Center at Manhattan, KS. Healthy and scabby grain and rachis tissue were ground and sent to the University of Minnesota for mycotoxin analyses using GC/MS.

The statistical model was a full factorial with 16 lines, two DON chemotypes, two NIV chemotypes, and three replications (pots). Analysis of %IF was based on three experiments, and analyses of toxin concentrations were based on two experiments because there was little variation for toxin concentration in the third experiment. Data were analyzed using JMP version 7.0. Data were transformed before analyses to achieve homogeneity of variances using the most appropriate transformation suggested by the software. Means were back-transformed for presentation of results.

RESULTS AND DISCUSSION

Percentage of infected florets (%IF). The two isolates within each chemotype caused similar levels of disease, and data were pooled by chemotype for analysis. The line ´ chemotype interaction was not significant (P=0.0713), indicating that lines ranked similarly for both chemotypes. The two DON isolates averaged 16.9% IF and caused significantly more disease (P<0.0001) than the two NIV isolates that averaged 11.9% IF. All resistant lines had significantly lower %IF than the susceptible check for both chemotypes, and lines Fg 368, ARGE97-1033-10-

2 and VA04W-433 had the lowest %IF (Table 2). Wheat lines with resistance to isolates of the DON chemotype were even more resistant to isolates of the NIV chemotype, and therefore selecting lines for resistance to the DON chemotype should also select for resistance to the NIV chemotype.

Toxin concentration in grain and rachis tissue. Averaged across 16 wheat lines in experiments 1 and 2, the two isolates of the NIV chemotype primarily produced NIV and little to no DON, 3-ADON, or 15-ADON, and the two isolates of the DON chemotype primarily produced DON and little to no NIV, 3-ADON, or 15-ADON in both grain and rachis tissue (Table 3). Therefore, all comparisons of toxins only considered NIV for the NIV chemotype and DON for the DON chemotype. The two isolates within each chemotype produced similar levels of mycotoxins (Table 3), and data were pooled by chemotype for analysis.

Averaged across 16 wheat lines in experiments 1 and 2, toxin concentrations for the two NIV and two DON isolates were 0.25 ppm NIV and 0.73 ppm DON, respectively, in the healthy grain fraction and 25.71 ppm NIV and 61.30 ppm DON, respectively, in the scabby grain fraction. These results indicate that the grain sorting procedure effectively sorted grain into healthy and scabby fractions.

There were significant wheat line ´ chemotype interactions for toxin concentration in grain ($P<0.0001$) and in rachis tissue ($P=0.02$). For each line, however, the NIV concentration was always less than the DON concentration (Table 2), indicating that the interactions were due only to the magnitude of the differences between DON and NIV concentrations. Lines ARGE97-1033-10-2 and VA04W-433 had the lowest concentrations of toxin in both grain and rachis tissue and were among the most resistant lines as measured by the percentage of infected florets (Table 2). Both lines have the cultivar 'Freedom' in their pedigree, and Freedom may have contributed genes for resistance.

This report is based on a preliminary analysis of the data. Additional statistical models and transformations will be evaluated to determine which are the most appropriate, and data for healthy and scabby grain fractions will be analyzed to determine if these data are useful for characterizing resistance to FHB and mycotoxin accumulation.

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DISCLAIMER

Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the authors and do not necessarily reflect the view of the U.S. Department of Agriculture.

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Table 1. Winter wheat lines used in this study, their pedigree and contributors.

Line	Pedigree	Contributor
ARGE97-1033-10-2	FREEDOM/CATBIRD	Milus
ARGE97-1042-4-5	MASON / CATBIRD	Milus
ARGE97-1047-4-2	P2643 / 3 NING 7840 // PARULA / VEERY # 6	Milus
ARGE97-1048-3-6	MASON // SHA 3 / CATBIRD	Milus
ARGE97-1064-13-5	MASON//FREEDOM/SUPER ZLATNO	Milus
VA04W-433	NING 7840/PION2684//96-54-244 (CK9803/FREEDOM)	Griffey
VA04W-628	ERNIE//NING7840/ERNIE	Griffey
ROANE	VA71-54-147(CI17449)/Coker68-15//IN65309C1-18-2-3-2	Griffey
AR97002-2-1	AR396-4-2/NING 8026	Bacon
BESS	MO 11769/MADISON	McKendry
NC03-11465	NING 7804/P2643//NC95-22426	Murphy
COKER 9835	Susceptible check	Check
SZ 13	Ringo Star / Nobeoka Bozu	Mesterházy
SZ 14	Ringo Star / Nobeoka Bozu	Mesterházy
Fg 365	Sgv / Nb / MM / Sum3	Mesterházy
Fg 368	Zu / Re / Nobeoka Bozu	Mesterházy

Table 2. Toxin concentrations in grain and rachis and percentage of infected florets for each winter wheat line.

Line	Toxin concentrations (mg/kg) [1]								Infected florets (%) [1]	
	grain[2]				rachis[3]					
	DON for DON chemotype		NIV for NIV chemotype		DON for DON chemotype		NIV for NIV chemotype			
ARGE97-1033-10-2	0.54	fg	0.37	cd	75.61	d	13.97	c	6.20	e
VA04W-433	0.65	fg	0.29	cd	73.84	d	21.44	bc	6.42	e
VA04W-628	1.39	fg	0.58	bcd	119.33	cd	26.94	bc	9.43	de
AR97002-2-1	1.72	defg	0.95	bcd	136.91	bcd	30.69	bc	10.00	de
ROANE	1.76	efg	0.25	d	165.66	bcd	24.54	bc	9.52	de
BESS	1.97	cdef	0.72	bcd	138.61	bcd	28.24	bc	8.31	de
Fg 368	2.88	cdef	0.82	cd	175.37	bc	28.03	bc	5.74	de
SZ 14	7.93	bcde	0.60	cd	226.49	bc	37.86	bc	10.60	cde
Fg 365	8.57	bcd	4.69	ab	238.08	b	56.29	b	13.24	bcd
NC03-11465	11.35	bcd	0.95	bcd	192.73	bc	36.01	bc	15.57	bc
SZ 13	11.81	bcde	0.66	cd	229.72	bc	45.82	bc	9.45	bcd
ARGE97-1064-13-5	13.19	bc	1.41	bcd	223.83	bc	30.82	bc	16.90	bc
ARGE97-1047-4-2	27.41	b	3.80	abc	201.90	bc	40.91	bc	17.65	b
ARGE97-1042-4-5	31.53	b	9.96	a	217.24	bc	53.69	b	20.46	b
ARGE97-1048-3-6	40.90	b	1.40	bcd	201.11	bc	23.53	bc	15.44	b
COKER 9835	178.05	a	12.18	a	438.73	a	172.01	a	48.13	a

[1] Values within a chemotype followed by the same letter are not significantly different by a Tukey's HSD test at P=0.05.

[2] Data were transformed for statistical analyses by the formula: Log(:Tox.Con. in grain) * 1.10101230397926 ; however, values represent actual back-transformed LS means for each line and variable.

[3] Data were transformed for statistical analyses by the formula: (:Name("TOX_Rachis (ppm)_F") ^ 0.4 - 1) / 0.0350989281248155 ; however, values represent actual back-transformed LS means for each line and variable.

Table 3. Toxin concentrations in grain and rachis for each isolate of *Fusarium graminearum* DON and NIV chemotypes (averaged across 16 wheat lines and two experiments).

		Toxin concentrations in harvested grain[1, 2]				Toxin concentrations in rachis[1, 3]			
Chemotype	Isolate	DON	NIV	3-ADON	15-ADON	DON	NIV	3-ADON	15-ADON
		mg/kg							
NIV	03-29	0.06a	1.93a	0.00a	0.00a	1.74a	40.10a	0.10a	0.00a
	03-112	0.08a	3.03a	0.00a	0.00a	0.24a	43.75a	0.01a	0.00a
DON	03-57	17.69a	0.07a	0.07a	0.00a	199.78a	0.51a	13.93a	0.59a
	03-113	25.01a	0.10a	0.11a	0.00a	182.11a	0.40a	13.67a	0.46a

[1] Values within a chemotype followed by the same letter are not significantly different by an Student's t – test at P=0.05.

[2] Data were transformed for statistical analyses by the formula: Log(:Tox.Con. in grain) * 1.10101230397926 ; however, values represent actual back-transformed LS means for each isolate and variable.

[3] Data were transformed for statistical analyses by the formula: (:Name("TOX_Rachis (ppm)_F") ^ 0.4 - 1) / 0.0350989281248155 ; however, values represent actual back-transformed LS means for each isolate and variable.

CURRENT STRATEGIES FOR BREEDING FUSARIUM HEAD BLIGHT RESISTANT WHEAT IN CANADA.

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ABSTRACT

Fusarium head blight (FHB) is a destructive fungal disease of wheat that annually results in yield and grade losses for producers as well as reduced feed and end-use quality for the wheat industry. Efforts to develop FHB resistant wheat cultivars can be roughly divided into three research areas: (1)Germplasm development; (2)Molecular breeding; and (3)Line development and evaluation. Non-Canadian sources of FHB resistance, from Brazil, China, CIMMYT, Germany, Japan and the USA, are being used to develop FHB resistant material suited to Canadian growing conditions and registration requirements. FHB screening in inoculated nurseries has been established across Canada permitting evaluation of germplasm and breeding materials in multiple environments. Novel germplasm is being developed through *in vitro* selection of wheat microspores for tricothecene resistance. Molecular breeding strategies have been used to develop new breeding materials that combine resistance genes from multiple sources in improved backgrounds. Fine mapping of genes *fhb*1 and *fhb*2 has facilitated screening for FHB resistance in parental, *in vitro* selected, backcrossed, and doubled haploid lines. High throughput screening technologies such as DNA extraction robotics and multi-channel capillary electrophoresis permit the screening of multiple markers. In our wheat breeding program, lines are regularly screened for FHB resistance loci on chromosomes 3BS, 5A, 6BS, and 2D. Haplotype analyses of breeding materials at these loci permit selection of FHB resistant lines for advancement and crossing. Future breeding efforts will focus on traits which reduce deoxynivalenol content, and the mapping and deployment of non-Asian FHB resistance.

EFFECTS OF AGRONOMIC AND MORPHOLOGICAL CHARACTERS ON FHB SEVERITY, DEOXYNIVALENOL AND ERGOSTEROL CONCENTRATIONS IN NEAR-ISOGENIC LINE PAIRS OF BARLEY.

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ABSTRACT

Fusarium head blight (FHB) is a devastating disease on barley. Several agronomic traits have been shown to be associated with lower FHB severity in barley. To evaluate the relationship between different agronomic and morphological traits and FHB levels, we examined FHB severity, deoxynivalenol (DON) and ergosterol concentrations in 20 pairs of near-isogenic lines (NILs) carrying the traits two-rowed/six-rowed, lax/dense, erect/normal, club/normal spike, hulled/hulless caryopsis, fertile/infertile lateral florets, and early maturity/late maturity. These lines were planted in FHB disease nurseries at Langdon, ND, Fargo, ND, St. Paul, MN, and Crookston, MN in 1995, 2000, 2005, and 2007. Inoculations were applied either by spreading *Fusarium graminearum* infected barley kernels over the nursery for consecutive weeks starting 10 days prior to spike emergence or by spraying macroconidia inoculum once after head emergence. The FHB severity (number of FHB infected kernels out of total number of kernels per spike) was evaluated on 10 or 20 randomly selected spikes in each replicate at the mid-dough stage of development. After harvest, the concentrations of DON and ergosterol were assessed in random 3 gram grain samples of each replicate. Differences between the means of NILs for levels of FHB severity, DON and ergosterol concentrations were analyzed for statistical significance using the paired t-test. The results will be presented in the poster. Overall, few statistically significant and consistent differences were observed for the scored disease parameters (FHB severity, DON and ergosterol concentrations) on the NIL pairs. However, in general loci controlling six-rowed and six-rowed like spike phenotypes exhibited more disease symptoms.

FUSARIUM HEAD BLIGHT (FHB) RESISTANCE INTO SOFT RED WINTER WHEAT AGS2000.

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ABSTRACT

Fusarium head blight (FHB) is a potential devastating disease in the southeast region in the United States where low temperature and misted weather occurs frequently during soft red winter wheat flowering. Releasing new cultivars resistant to FHB is the most effective option to minimize the chance of FHB incidence and reducing DON contamination. Crosses were made since 2001 between AGS2000 or its derivatives and FHB resistant donor VA01-461 to introduce the exotic resistant genes into our widely local adaptive genetic background. Twelve advanced lines, 941523-E21, 991109-6E8, 991109-6A7, 991371-6E12, 991371-6E13, 031454-DH7, 031454-DH31, 031307-DH6, 031307-DH14, 031354-DH30, 981621-5E34, 951306-2E13, derived from VA01W-461, which is a derivate of Sumai3, were evaluated in scab nursery and field in 2006 and 2007 for FHB resistance and agronomy performances with Ernie and Coker 9835 as resistant and susceptible control respectively under misted conditions in Griffin-Campus, Georgia. DNA markers, XGWM533, BARC133, XGWM493, STS3B-256 for QTL on 3BS; BARC117, XGWM156, BARC186, BARC56, for QTL on 5AS; BARC18, and BARC91 for QTL on 2BS were employed to genotype 12 new lines with the donor parent of VA01W-461. Here, we reported the results of DNA genotyping and performances of our elite lines. The scab resistance and QTLs in VA01-461 are discussed in this study.

CHARACTERIZATION OF RESISTANCE TO DEOXYNIVALENOL (DON) ACCUMULATION IN DIFFERENT WHEAT LINES.

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ABSTRACT

ANOVA results suggested highly significant differences among wheat genotypes for DON accumulation (F= 22.72, P< 0.0001). As expected, disease and genotype × disease interaction for resistance to DON accumulation were also significant: F=329.66 and 15.98, respectively, P<0.0001. In the combined analysis for DON accumulation: replicate, collection date, rep × genotype, collection date × genotype, and genotype × collection date × disease interaction effects were not significant: F= 0.166-0.382, P= 0.202-0.841. Significant variation for DON content was observed between 'healthy + diseased' seeds and 'healthy' seeds. But, no significant difference was observed in the healthy seeds between resistant and susceptible genotypes. However, for the DON content in the 'healthy + diseased' samples, the FHB-resistant or moderately resistant genotypes including 0128A1, INW0411, INW0412, Bess, Freedom, and Truman, exhibited lower DON accumulation than FHB-susceptible cultivars Patterson and Pioneer2545. Patterson and Pioneer2545 both are susceptible to FHB, but, on average, the DON content for Patterson (12.4 ppm) was only approximately half of that of Pioneer2545 (21.2 ppm).

DEVELOPMENT OF CIMMYT'S 11TH SCAB RESISTANCE SCREENING NURSERY.

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ABSTRACT

CIMMYT has regularly developed and distributed a Scab Resistant Screening Nursery (SRSN) over the past decade. These nurseries have consisted of the best scab resistant material identified through CIMMYT's FHB screening trials and have been distributed to interested programs around the world upon request. The most recent nursery distributed was the 10th SRSN, which was made available in 2006. Since that time CIMMYT's method for screening FHB has been modified for more effective identification of FHB resistant germplasm. These changes have included modifications in the location of the screening nursery, isolates used for inoculation, inoculation technique and misting technology. After two years of screening a range of materials using the modified methodologies, entries for the 11th SRSN have been identified. This nursery primarily includes the best FHB resistant advanced lines developed by the CIMMYT wheat breeding programs. The 11th SRSN will be available for distribution in 2008.

PRELIMINARY EXAMINATION OF THE INFLUENCE OF GRAIN COLOR IN FHB RESISTANCE.

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ABSTRACT

Breeding programs around the world have seen noteworthy improvements in FHB resistance in recent years. However, there is the perception that white wheat lags behind red wheat in the development of resistant varieties. In our study, we asked the question whether or not grain color itself is influencing the amount of disease development. Thirty-six genotypes from fourteen sibling groups originating from different breadwheat crosses were examined. Each sibling group was comprised of at least one red and one white sibling pair. In 2006 and 2007, genotypes were screened for FHB resistance in a four replication incomplete block design at CIMMYT headquarters, Mexico. Plots were spray inoculated at anthesis and three days following, and were rated for % severity and % incidence at thirty-one days post inoculation. Post harvest, DON levels of the 2006 samples were evaluated via ELISA. Preliminary results of this study will be shared.

FHB RESISTANCE AND DON CONTAMINATION IN VIRGINIA BARLEY.

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ABSTRACT

Knowledge of FHB resistance and DON contamination in Virginia barley is essential for providing growers and producers with new and improved commercial cultivars. In 2006 and 2007, we screened 12 hulless (HLS) and 19 hulled (HLD) lines of barley in inoculated, mist-irrigated field plots at Blacksburg, VA for FHB incidence, FHB severity, and DON contamination. In 2006 and 2007, FHB incidence ranged from 40% to 80% for HLS lines and from 30% to 90% for HLD lines, and FHB severity ranged from 4.0% to 12.5% for HLS lines and from 6.1% to 33.5% for HLD lines. In 2006, DON concentrations ranged from 0.1 to 2.0 ppm for HLS lines and from 0.5 to 11.5 ppm for HLD lines; in 2007, DON concentrations ranged from 0.2 to 2.4 ppm for HLS lines and from 0.1 to 3.3 ppm for HLD lines. In 2006, FHB incidence was correlated with DON for HLS lines ($r = 0.92$, $P < 0.001$) and HLD lines ($r = 0.48$, $P < 0.05$); in 2007, FHB incidence was not significantly correlated with DON for HLS lines ($r = 0.14$, $P = 0.6$) or HLD lines ($r = -0.16$, $P = 0.5$), but FHB development was generally low in 2007 in VA. DON concentrations in 100 g kernel lots were correlated among DON testing labs in MN (Dong), ND (Schwarz), and VA (Schmale) (range of r from 0.81 to 0.89, $P < 0.001$). HLS line VA01H-125 had the lowest level of DON contamination in both years (0.2 ppm in 2006 and 2007), and HLD line VA92-42-46 had relatively low levels of DON contamination in both years (1.0 ppm in 2006, 0.36 ppm in 2007). We are continuing to develop and test new cultivars of barley in VA for FHB resistance and reduced DON potential.

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META-ANALYSES OF QTL ASSOCIATED WITH FUSARIUM HEAD BLIGHT RESISTANCE.

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OBJECTIVES

The objective of this study is to estimate the CI's of 63 QTL associated with different types of FHB resistance using meta-analyses and align them onto the consensus ITMI maps to determine if different QTL on the same chromosomes from different studies overlap.

INTRODUCTION

Many QTL have been mapped on to chromosomes of resistant sources from China, Japan, Brazil, USA and various European countries. This paper reviewed 63 QTL from 23 studies including four QTL identified for type I FHB resistance (resistance to initial infection), 50 QTL for type II resistance (resistance to spread), six QTL for type III resistance (resistance to DON accumulation) and three QTL for type IV resistance (resistance to kernel damage). Among these studies, 10 chromosomes were identified that have more than two FHB QTL regions. Each QTL explained more than 10% of the phenotypic variation in the corresponding experiment was included in the analyses. Some QTL identified from the same resistance source mapped onto the same chromosome but were not linked to the same marker loci. Furthermore, some QTL from different sources mapped in proximity to each other on the same chromosome. Differences in marker orders across studies make it very difficult to select markers linked to respective QTL from individual studies.

Meta-analyses have been used to estimate the confidence intervals (CI) of identified QTL in plants and animals (Wise et al., 1999; Guo et al., 2006). It combines the information for each individual study to estimate QTL CI which can be aligned on the consensus map. This permits the QTL position to be compared to determine if they overlap. This may provide some distinguishable common flanking markers linked to different QTL that can be used more effectively in MAS breeding.

MATERIALS AND METHODS

Estimate CI of QTL - The following formulas were used to estimate the 95% CI of each QTL:

$CI = 530 / (N*R^2)$ for backcross [1]
$CI = 530 / (N*R^2)$ for F_2 intercross [2]
$CI = 163 / (N*R^2)$ for RILs [3]
$CI = 132 / (N*R^2)$ for DH [4]

Where N is the number of lines in the mapping population and R^2 is the percentage of phenotypic variation explained by the identified QTL. Formulas [1] and [2] were deduced by Darvasi and Soller (1997) through simulation. The CI formulas [3] and [4] for RIL and DH, respectively, were derived via further detailed characterization of genetic parameters (Weller and Soller, 2004; Weller, 2007, personal communication).

Classification of FHB resistance QTL - Criteria similar to those reported by Guo et al. (2006) were used in the current study to classify QTL into three classes: (i) suggestive QTL if LOD < 4.0 (or p value > 0.0001), (ii) significant QTL if LOD e" 4.0 (or p value d" 0.0001) and, (iii) confirmed QTL if the QTL was identified in two or more separate studies (Lander and Kruglyak, 1995) and was significant in at least one study.

Meta-analyses of marker-QTL associations - Where the estimated CI of QTL regions overlapped, those QTL were grouped into one cluster. QTL alleles

within the same cluster were assumed to be the same (Guo et al., 2006). QTL were classified into different clusters if none of the estimated CI regions overlapped and were more than 20 cM apart.

RESULTS AND DISCUSSION

Significant and confirmed type II resistance QTL in the same cluster - Significant QTL from different sources but in the same cluster were distributed on chromosomes 3AS, 5A, 7AL, 3BS, 4B, 6B, and 2DS (Fig. 1). Separate mapping studies of several derivatives of Sumai3 (W14, CM82036, DH181, and Ning7840) provide evidence of a confirmed type II FHB resistance QTL located on chromosome 3BS (Fig. 1). Another confirmed type II FHB resistance QTL also located on chromosome 3BS is derived from Wangshuibai. The third confirmed QTL is on 5A of CM82036 and W14, two Sumai 3 derivatives (Fig. 1). These are confirmed and significant type II FHB resistance QTL, present in different sources and located in the same position along the respective chromosomes Markers flanking the most common regions of these QTL's CI can be applied in MAS to increase the efficiency.

Significant type II resistance QTL in different clusters - The type II resistance QTL of Renan is on chromosome 5AL, which is different from another cluster located around the centromere of chromosome 5A in other sources, such as CM82036, Ernie, Frontana and W14 (Fig. 1). Other type II resistance QTL have been located on chromosome 1BS of Fundulea 201R and 1BL of Wangshuibai and Arina (Fig. 1). A second type II resistance QTL in Renan was mapped near the centromere of chromosome 2B while the QTL in Dream is located on distal region of the same chromosome (Fig. 1). A QTL close to centromere of chromosome 3B in Ernie is in a separate cluster compared to the primary QTL located in the distal region of chromosome 3BS of Sumai 3 and its derivatives. The type II resistance QTL on chromosome 5BS of Wangshuibai does not overlap with the QTL of Arina on chromosome 5BL. These QTL belong to different clusters and, therefore, should provide different FHB resistance alleles for breeding. The flanking markers identified in the original studies should be validated to confirm their effectiveness in MAS prior to using them for pyramiding these QTL. The application of respective tightly linked markers to pyramid these QTL should be effective to breed durable FHB resistances.

Different Types of FHB Resistance QTL in the Same Sources and Clusters - Types I and II resistance QTL were found on chromosomes 3AS in Frontana, 5A in W14, and 4B in Wangshuibai. Types II, III, and IV resistance QTL were identified on chromosomes 3BSc and 5A in Ernie while types I, II, III, and IV resistance QTL were discovered on 3BS in W14, a Sumai 3 derivative (Fig. 1). QTL conferring different types of FHB resistance were identified and located in the same clusters suggesting a pleiotropic effect or association among them. FHB resistance types other than type II have been evaluated only in a limited number of resistant sources and environments with most of them being greenhouse studies. Therefore, the common QTL reported for these different types of resistance (Chen et al., 2006; Abate et al., 2007; Liu et al., 2007) need to be proved with more evaluation in additional sources and genetic backgrounds, and in studies specifically designed to assess and distinguish resistance types I, III and IV. Such studies are needed to elucidate whether these QTL have pleiotropic effects or if their interrelatedness is simply a function of the highly correlated effects that FHB assessment methods, particularly single floret point inoculation, has on multiple types of FHB resistance.

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DISCLAIMER

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.

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Figure 1a.

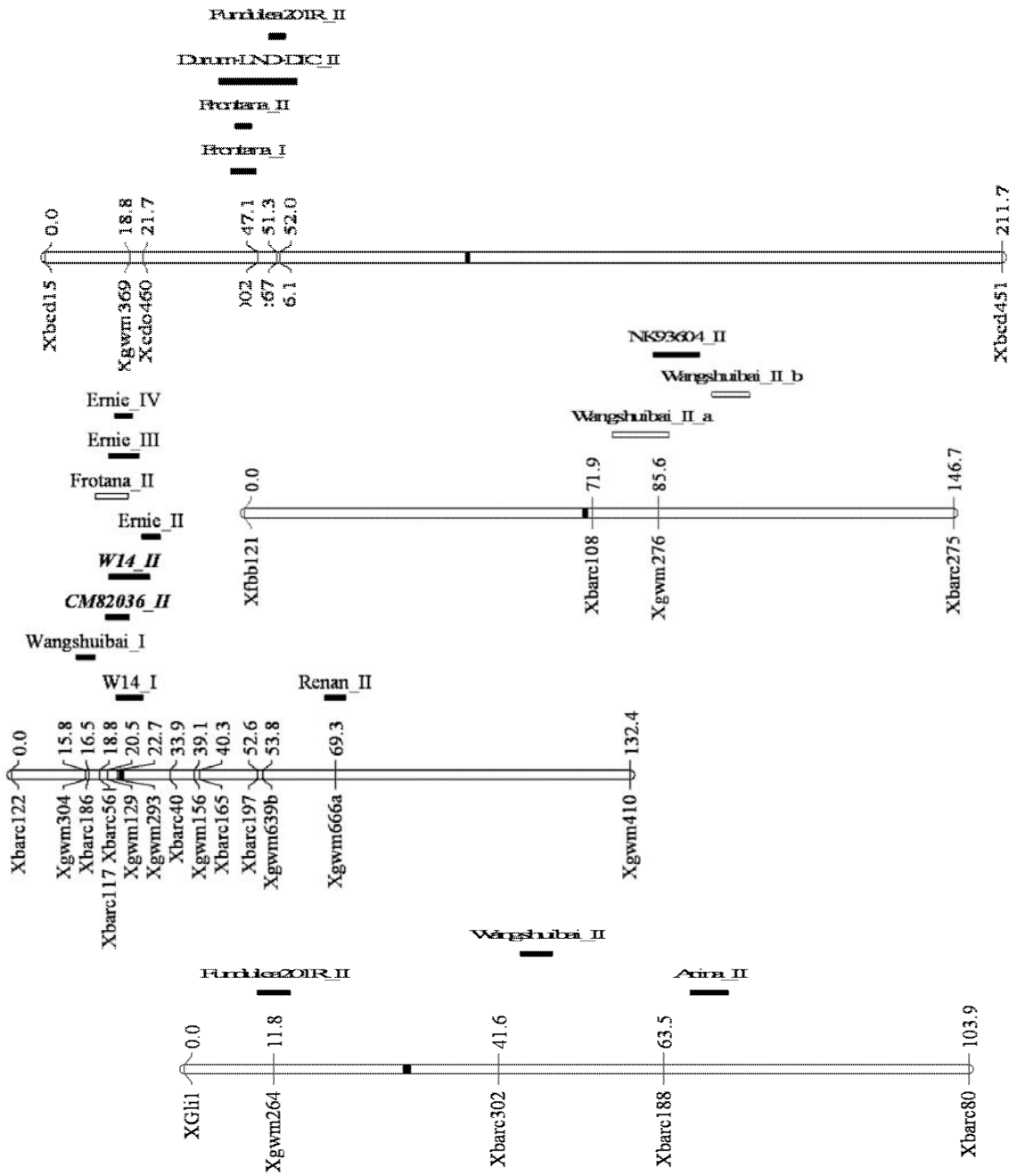
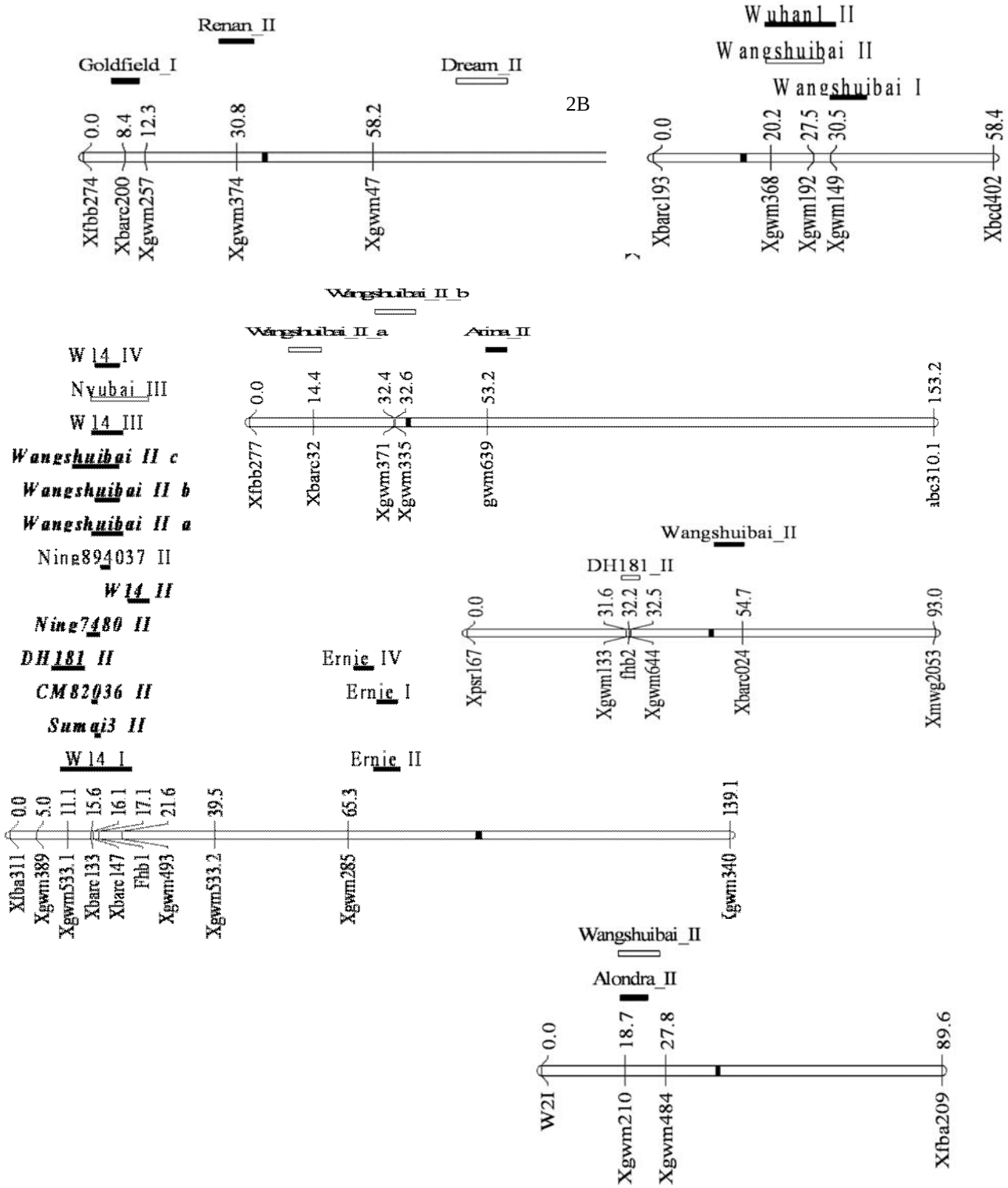


Fig. 1. The 95% confidence intervals of QTL associated with different types of Fusarium head blight (FHB) resistance. Significant QTL are represented by solid black bars (LOD > 4 or p <0.0001) above the respective chromosome region; suggestive QTL (LOD < 4 or p > 0.0001) are represented by open bars and; confirmed QTL are shown as solid black bars with bold and italic font-type. The QTL name is the source followed by an underscore and a Roman number which indicated the type of FHB resistance identified. The frame of ITMI genetic chromosome maps from Song et al. (2005) was used with the consensus map from Somers et al. (2004) as reference.

Figure 1b.


Renan_II
Goldfield_I
Dream_II
2B
Wuhan1_II
Wangshuibai II
Wangshuibai I
0.0
8.4
12.3
30.8
58.2
Xfbb274
Xbarc200
Xgwm257
Xgwm374
Xgwm47
0.0
20.2
27.5
30.5
58.4
Xbarc193
Xgwm368
Xgwm192
Xgwm149
Xbcd402
Wangshuibai_II_b
Wangshuibai_II_a
Arina_II
W14_IV
Nyubai_III
W14_III
Wangshuibai II c
Wangshuibai II b
Wangshuibai II a
Ning894037 II
W14 II
Ning7480 II
DH181 II
CM82036 II
Sumai3 II
0.0
14.4
32.4
32.6
53.2
153.2
Xfbb277
Xbarc32
Xgwm371
Xgwm335
gwm639
abc310.1
Wangshuibai_II
DH181_II
Ernie_IV
Ernie_I
0.0
31.6
32.2
32.5
54.7
93.0
Xpsr167
Xgwm133
fhb2
Xgwm644
Xbarc024
Xmwg2053
W14_I
Ernie_II
0.0
5.0
11.1
15.6
16.1
17.1
21.6
39.5
65.3
139.1
Xiba311
Xgwm389
Xgwm533.1
Xbarc133
Xbarc147
Fhb1
Xgwm493
Xgwm533.2
Xgwm285
Xgwm340
Wangshuibai_II
Alondra_II
0.0
18.7
27.8
89.6
W21
Xgwm210
Xgwm484
Xiba209

PYRAMIDING FHB RESISTANCE QTL USING MARKER-ASSISTED SELECTION IN WHEAT.

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ABSTRACT

This study was conducted to pyramid FHB resistance genes from the Chinese source Futai8944 and adapted sources Ernie and Tribute into adapted backgrounds using marker assisted selection. A three-way cross VA02W-713/Tribute//VA07W-120 was made. VA07W-120 is a backcross-derived line with the donor parent Futai8944 and the recurrent parent Ernie. This line contains QTL from both Futai8944 and Ernie and has exhibited a high level FHB resistance in both greenhouse and field tests. Marker assisted selection was applied in F_1 and F_2 generations in spring 2007 and will be applied in $F_{2:3}$ generation during winter 2007. Nineteen F_1 plants derived from the 3-way cross were selected for advancement on the basis of the presence of target alleles for 12 markers on chromosomes 2DS, 3A, 3BS, 5AS, and 6B. About 900 F_2 plants were characterized with 19 markers on 2BS, 2DS, 3A, 3BS, 5AS, and 6B. More than 200 F_2 plants having different combinations of target marker alleles for the five QTL regions were selected for advancement. All of these $F_{2:3}$ lines were planted as head rows this fall in a field scab nursery. Among the 210 $F_{2:3}$ lines, 31 having target marker alleles for two or more QTL fixed in a homozygous state also will be further evaluated and selected in greenhouse tests for plant phenotype, marker haplotypes, and Type II FHB resistance. Among 19 marker loci, these 31 progeny lines have only one to three loci in the heterozygous state and, thus, the primary goal is to identify desirable progeny having fixed target alleles in all five QTL regions. Based on the number of heterozygous marker loci in the progeny, 10 to 30 plants of each $F_{2:3}$ family will be further screened and selected using target markers. This study will assess the efficiency of marker-assisted selection for pyramiding different FHB resistance QTL.

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WINTER AND SPRING WHEAT PARENTAL DIALLEL ANALYSIS FOR SCAB RESISTANCE.

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ABSTRACT

Fusarium head blight (FHB), caused by *Fusarium graminearum*, is an important disease of wheat in South Dakota. The study was conducted to determine combining ability and gene effects in populations derived from mating among spring, winter and facultative wheat genotypes. Six genotypes consisting of susceptible winter wheat 'Nekota' and '2137', moderately susceptible winter wheat 'Harding', moderately resistant spring wheat 'ND2710' and 'BacUp' and resistant facultative wheat 'Ning7840' were crossed in a partial diallel mating design. $F_{4:5}$ lines were hand transplanted in May 2006 and 2007 and screened under mist-irrigated field conditions. Artificial inoculation consisted of corn spawn spread at jointing and inoculum suspension spray at flowering stages. Disease index percentage (incidence percentage * severity percentage/100) of the crosses was analyzed using Griffing's method 4 and model 1. General and specific combining abilities were highly significant ($P < 0.01$) for both years. The result showed that both additive and non-additive gene effects were involved in the inheritance of FHB resistance. The ratio of combining ability variation components $[2\sigma^2_{GCA}/(2\sigma^2_{GCA} + \sigma^2_{SCA})]$ was 0.85 and 0.81 in 2006 and 2007, respectively. The homogeneity of the data over two years was tested. The calculated F-value for the ratio of error variances (F = larger error MS/smaller error MS) for two years was 1.09 ($P = 0.10$, $Df_{num} = 846$, $Df_{den} = 867$). The test of homogeneity indicated that the two years data could be pooled. The pooled analysis showed that general combining ability was significant ($P < 0.01$) but not the specific combining ability ($P = 0.17$). Both the individual and pooled analysis showed that additive gene effects were more important than non-additive gene effects. Thus, progress in developing resistance in wheat can be made by selection.

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ROLE OF A PLASMA MEMBRANE CA^{2+}-ATPASE IN THE RESISTANCE OF POTATO CELLS TO *FUSARIUM SOLANI*.

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ABSTRACT

In natural conditions, plants are always under attack of numerous pathogenic micro organisms and remain, at that, resistant to most of them. To a large extent, the resistance is due to the main enzyme of plasma membrane – Ca^{2+}-ATPase, which plays an important role in the metabolism of the plant cells both in normal conditions and under pathogenesis. This research was aimed at studying the activity and some physical-chemical characteristics Ca^{2+}- ATPase of the potato varieties of different resistance level both in normal conditions and under infection of the fungus *Fusarium solani*. The research targets were the tubers and cells culture of the potato varieties *Tamasha* and *Santa*, which differ in their resistance to *Fusarium solani*. Membrane preparations were obtained by differential sucrose density-gradient centrifugation. The activity of Ca^{2+}-ATPase was evaluated by the number of Pi, obtained as a result of ATP hydrolysis. The fungus *Fusarium solani* was grown on the modified Chapek medium. The use of differential centrifugation method resulted in the isolation of pure preparation of plasma membrane, rich in ions Ca^{2+}. Maximal enzyme activity was identified at the ion concentration Ca^{2+}- 2,25mM, and pH – 7,0. The change of Ca^{2+}-ATPase activity under potation infection with the conidia of *Fusarium solani* was also studied. The increase of Ca^{2+}-ATPase transport activity is revealed in the first hours of infection with the fungus in the resistant potato varieties, where as no change of activity is observed with non-resistant potato varieties. In 24 hours, the increase of enzyme activity is observed in both resistant and non-resistant varieties. Activation of Ca^{2+}-ATPase during the fungus pathogens leads to the increased inflow of Ca^{2+} ions through plasmalemma, which results in the improvement of protective mechanisms. The studying of ATPase activity kinetics showed that for plasma membrane fractions, isolated from the resistant variety *Tamasha*, the maximum speed of hydrolysis from incubation time, was 1.5 higher than in the non-resistant variety *Santa*. The infected cells of potato, resistant to *Fusarium solani*, show the increase of K_m.

Infection leads to the change of physical-chemical enzyme parameters and, respectively, to the increased Ca^{2+} ion flow through membrane.

PROSPECTS FOR IDENTIFYING FUSARIUM HEAD BLIGHT RESISTANCE QTL BY ASSOCIATION MAPPING USING BREEDING GERMPLASM.

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ABSTRACT

In barley, there have been numerous quantitative trait loci (QTL) identified through bi-parental mapping, but very few have been utilized in marker assisted selection (MAS). A new tool, association mapping, may overcome some of the problems that accompany bi-parental mapping by: identifying QTL which are segregating in relevant germplasm, as well as give appropriate estimates of allelic effects. Use of association mapping may shorten the time to implementation of MAS. Two important considerations arise when using association mapping, the extent of linkage disequilibrium and the amount of phenotypic variation present in the mapping population. The objective of this study was to assess phenotypic variation, for FHB and DON, among representative breeding germplasm from four barley breeding programs in the upper Midwest. To accomplish this goal 768 lines were evaluated in seven environments over 2006 and 2007 with each line being assessed in at least four environments. Artificial inoculation, overhead spray and grain spawn, and mist irrigation were used to encourage disease development. Significant variation was found among lines for both traits, with heritabilities, calculated on an environment basis, of 0.45 to 0.52 (FHB) and 0.64 to 0.70 (DON). Histograms showed a range of phenotypic variation that is comparable to bi-parental mapping and therefore should be useful for mapping. Lines will be genotyped using 3000 SNP markers, which have been developed through the USDA Barley CAP. Mapping will be conducted using a mixed model approach to find significant markers.

BREEDING FOR FHB RESISTANCE IN WINTER WHEAT: WHAT'S AHEAD?

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ABSTRACT

Significant yield losses caused by *Fusarium graminearum* Schwabe (teleomorph *Gibberella zeae* (Schwein.), the pathogen known to cause Fusarium head blight (FHB), have occurred in Missouri for more than 70 years but have become more frequent in the last 15-20 years due to increased corn acreage, reduced tillage practices aimed at soil conservation, and the lack of effective cultural and/or fungicide control. Severe state-wide outbreaks in both 1990 and 1991 resulted in losses that were estimated at more than $250 million. In addition to the direct yield losses, test weights were reduced and associated deoxynivalenol (DON) accumulation in the grain prevented harvested grain from being marketed. In 1993, FHB resistance was included as a major breeding objective in the Missouri wheat breeding program and systemic screening of both Missouri breeding lines and germplasm that had been introduced through collaborations with CIMMYT became a routine part of the breeding effort. In 1998, germplasm screening efforts were augmented through U.S. Wheat and Barley Scab Initiative funding. Despite evaluating more than 10,000 genotypes from targeted regions globally between 1993 and 2005 we discovered that some of the best sources of resistance were in our own program in genetic backgrounds that were adapted in much of the soft red winter wheat region. It was clear that this 'native' resistance would lead to the most rapid release of resistant cultivars. Since 1994, three FHB resistant cultivars have been released from the Missouri breeding program including: 'Ernie' released in 1994; Truman, released in 2003; and Bess, an early maturing full-sib of Truman, released in 2005. All have been widely accepted in Missouri and Bess and Truman, which are more widely adapted than Ernie, are being grown on significant acreage outside of the state. The identification of native sources of resistance within the Missouri program has enabled us to have a productive pipeline of FHB resistant germplasm in adapted backgrounds and has led to our focus on this source of resistance. Truman and its early maturing full-sib Bess, have good to excellent levels of types I and II resistances coupled with low DON and good kernel quality retention. They are unique in that this high level of resistance is in an agronomic background that couples excellent yield and test weight with broad geographic adaptation. Haplotype data using known FHB resistance markers suggests that resistance alleles in Truman and Bess probably differ from those in other widely-used sources of resistance. Coupled with its potentially unique resistance alleles, Truman has excellent combining ability (both general and specific) for FHB resistance, producing progeny populations with a high percentage of agronomically desirable, FHB-resistant offspring. This paper will explore opportunities for further enhancing FHB resistance in winter wheat cultivars and accelerating their release by building on the broad-based native resistance available in the winter wheat region. It will focus on the use Truman and its early maturing, full sib, Bess.

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SCAB EPIDEMIC IN NEBRASKA.

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ABSTRACT

Fusarium head blight (FHB), caused by *Fusarium graminearum*, is an important disease of wheat. Natural epidemics of the disease may result in severe yield losses, reduction in quality, and contamination of the harvested grain by mycotoxins. Deoxynivalenol (DON) is the most important mycotoxin that affects all sectors of the wheat industry and it has serious food safety implications in marketing, exporting, processing, and feeding scabby grain. FHB is an episodic disease in the hard winter wheat region of the Great Plains that is known for its diverse and highly variable climate. In eastern Nebraska, the predominant rotation is corn-soybeans, but wheat acreage is increasing as wheat price increases, and wheat continues to be an important winter annual rotational crop. In the 2007 cropping season, a severe epidemic of FHB occurred in the eastern, southeastern, south central, and southwestern parts of Nebraska starting from Omaha to Ogallala (>435 km; >320, 000 ha of wheat). To gain an understanding of the impact of the disease, a sample of grain from each of sixty elite hard winter wheat experimental lines grown in four different locations (Lincoln, Mead, Clay Center, and North Platte) were tested for DON content. The overall mean DON level at each location ranged from <0.5 ppm to 2.3 ppm, the average across locations being 0.8 ppm. The level of DON was highest at Clay Center (3.9 ppm) followed by Lincoln (2.5 ppm), Mead (2.2 ppm), and North Platte (1.2 ppm). Of the sixty experimental lines NE05568, NE05418, and Overland had consistently low DON levels (a mean of <0.5 ppm) at all four locations and also in our mist-irrigated nursery at Mead. Two of the elite lines (NE04653 and Harry) which had the highest DON levels (a mean of >2 ppm) at all locations also showed elevated levels of DON in the mist-irrigated field nursery. This year's scab epidemic impacted many wheat growers in Nebraska. The ongoing FHB research will help in developing adapted FHB-resistant/tolerant cultivars.

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'FALLER': A NEW HARD RED SPRING WHEAT CULTIVAR WITH HIGH YIELD AND QUALITY ADDED TO COMBAT FUSARIUM HEAD BLIGHT DISEASE.

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OBJECTIVES

To develop a new improved hard red spring wheat (HRSW) cultivar which combines resistance to Fusarium Head Blight (FHB) disease and superior grain yield and bread-making quality.

INTRODUCTION

Scab or FHB has been a serious threat to wheat production throughout the world (Schroeder and Christenson, 1963; Bai and Shaner, 1994; McMullen et al., 1997; Stack, 2003). In North America, FHB is caused mainly by *Fusarium graminearum Schwabe* [telomorph *Gibberella zeae* (Schwein.)] (Bai and Shaner, 1994; McMullen et al., 1997). In the spring wheat region, FHB has been a major disease for HRSW produced in North Dakota and neighboring states since 1993. The most recent economic report (Nganje et al., 2004) estimate combined direct and secondary economic losses caused by FHB for all crops were at $7.7 billion. ND and MN account for about 68% ($5.2 billion) of the total dollar losses. Direct losses from 1993 trough 2001 for wheat only were estimated to $2.492 billion (Nganje et al., 2004). The use of genetically resistant cultivars is believed to be the most efficient and economical method of controlling this FHB in wheat. This has been demonstrated in 2002, 2003, and 2004 when 'Alsen', a moderate FHB resistance cultivar derived from the Chinese source 'Sumai 3', released in 2000 by NDSU (with the support of the scab initiative funds) was planted on more than 2.1, 2.4, and 1.9 million acres representing 30.8, 37.4, and 28.9% of ND wheat acreages, respectively (N.D. Agricultural Statistics Service, USDA. 2002; 2003; 2004). Similar scenario was repeated in 2007 when 'Glenn', the 2005 NDSU release jumped from about 2% to more than 20% of total HRSW grown in ND (N.D. Agricultural Statistics Service, USDA. 2006; 2007). The rapid increase in acreage planted to both Alsen and Glenn indicates the desire of ND wheat growers to produce such HRSW cultivars.

MATERIAL AND METHODS

Faller was developed using a modified bulk breeding procedure. It was selected from the "ND2857/ ND2814" cross made at NDSU in the fall of 1997. ND2857 (ND2709/ND688) is a hard red spring experimental lines that has good resistance to FHB originating from ND2709 line derived from the cross involving 'Sumai3' (PI 481542). Sumai3, a spring wheat from China, is arguably the most used source of resistance to FHB in the world. Both ND 2709 and ND688 are HRSW experimental lines developed by the NDSU breeding program. ND2814 ('KITT' (PI 518818)/'AMIDON' (PI 527682)//'GRANDIN' (PI 531005) /'STOA S' (PI 520297)) is a HRSW line developed by NDSU HRSW breeding program. Kitt is HRSW cultivar released in1975 by the Minnesota Agricultural Experiment Station and the USDA-ARS while Amidon, Grandin and Stoa are HRSW cultivars released by NDAES in 1988, 1989, 1984, respectively.

Faller was selected from a bulk of one purified F5 row-plot selected in 2001 at Christchurch, NZ. Faller was initially put in PYT in the summer of 2001. Subsequently, Faller was tested in the advanced yield trials (AYT) and elite yield trials (YET) at four locations in ND in 2002 and 2003, respectively. Faller was tested as ND 805 at 21 location-years in the North Dakota Variety Trials (NDVT) from 2004 to 2006 and in the HRSW Uniform Regional Nursery (URN) (18 locations) in 2005. The URN is conducted in the

states of North Dakota, Minnesota, South Dakota, Nebraska, Montana, Wyoming, Washington, and Manitoba, Canada. The first seed increase of Faller was grown in Prosper, ND in the summer of 2004.
Faller was tested for its reaction to different races of tan spot, leaf and stem rusts, SNB, STB, and FHB in the greenhouse and in the field during the period of 2001- 2006. The SNB, STB and tan spot are the major components of the leaf spotting disease complex of wheat in North America. A complex of these diseases occurs in nature. Hence managing leaf spots is difficult; however, resistant cultivars are the most effective and economical means of controlling leaf spot.

RESULTS

Faller was tested under experimental line ND 805 and was released because it combines very high yield (Table 1), resistance to FHB and leaf diseases (Table 2), and very good end-use quality (Table 3). The name of Faller was chosen as recognition to late James Faller, a former technician in the HRSW breeding program for almost three decades.

Based on 27 site-years of testing in the NDVT and AYT, grain yield of Faller (4467 kg ha-1) was significantly ($p<0.05$) higher than all previously NDSU released cultivars including Alsen (3763 kg ha-1), Glenn (3743 kg ha-1), 'Parshall' (3607 kg ha-1), 'Steele-ND' (4052 kg ha-1), 'Reeder' (3625 kg ha-1), and 'Howard' (3943 kg ha-1) (Table 1). In 19 site-years of testing in the URN trials conducted in 2006, Faller yielded 4055 kg ha-1 compared to 3631, 4095, and 2952 kg ha-1 for 'Keene', 'Verde', and 'Chris', respectively. Other agronomic traits including kernel weight, heading date, plant height and straw strength of Faller and other HRSW cultivars are reported in Table 1.

Quality parameter including Falling number, Flour extraction, dough and baking parameters for Faller and major grown NDSU HRSW cultivars are reported in Table 2. Mean grain volume weight of Faller (757 kg m-3) over 26 site-years in NDVT was similar to Reeder (753 kg m-3) and 'Dapps' (756 kg m-3), but significantly ($p<0.05$) lower than Glenn (797 kg m-3) and Howard (778 kg m-3) (Table 1). Similarly, grain protein of Faller (150 g kg-1) was comparable to Reeder (155 g kg-1) and Parshall (156 g kg-1), but lower ($p<0.05$) than Alsen (157 g kg-1) and Dapps (165 g kg-1) (Table 1).

The seedling and adult plant screening tests conducted under greenhouse conditions from 2003-2006 showed that Faller posses high level of resistance to pathotype THBL, the predominant race of leaf rust (caused by *Puccinia triticina* Eriks.) in the region (Table 3). Faller was also evaluated for resistance to stem rust (caused by *Puccinia graminis* Per.:Pers. f. sp. tritici Eriks. & E. Henn) and was found to be highly resistant to pathotypes Pgt-QCCJ, -QTHJ, -RTQQ, -TMLK, -TPMK, and –HPHJ (Table 3). Faller was screened in the greenhouse for *Septoria nodorum* [caused by *Stagonospora nodorum* (Berk.) Castellani & E.G. Germano] and tan spot [caused by *Pyrenophora tritici-repentis* (Died.) Drechs]. On a scale of 1 to 5 where 1 is resistant and 5 susceptible, Faller had average scores of 1.7, 2.1, 3.7, 2.7, and 3.1 in reaction to tan spot race, 1, 2, 5, *Septoria tritici*, and *Spetoria nodorum*, respectively (Table 3).

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Table 1. Summary of agronomic data for Faller hard red spring wheat (HRSW) and check cultivars tested in the ND HRSW Variety Trials (2003-2006).

Cultivar	Grain yield	Grain protein	Thousand kernel weight	Grain volume weight	Heading date	Height	Straw strength
	kg ha^{-1}	%	g	kg m^{-3}	days from 06/1	cm	score[†]
Faller	4467c[†]	15.0a	29.8cd	757a	29	85	0.6
Howard	3943b	15.3ab	29.0cd	778b	28 (<0.05)[‡]	85(<0.10)	0.5 (1.00)
Glenn	3743a	15.7b	30.2d	797c	28 (<0.05)	83 (<0.50)	0.5 (1.00)
Steele-ND	4052b	15.3ab	28.4bc	773b	28 (<0.05)	85 (<0.10)	2.0 (<0.01)
Dapps	3646a	16.5c	29.5c	756a	26 (<0.01)	93 (<0.01)	0.5 (1.00)
Alsen	3763ab	15.7b	26.9ab	777b	27 (<0.05)	80 (<0.05)	0.3 (0.50)
Parshall	3607a	15.6b	26.5a	770b	27 (<0.05)	86 (1.00)	0.2 (0.50)
Reeder	3625a	15.5ab	26.7a	753a	29 (1.00)	80(<0.05)	1.0 (0.50)
Observations	27	26	26	26	26	26	7

[†] Lodging score: 1=completely erect to 9=completely flat at harvest.

[‡] P values (in parentheses) represent the significance of the comparison between Faller and the respective check cultivar based on a Student's paired *t*-test procedure (SAS-JMP version 6.0.3, SAS Institute Inc., Cary, NC).

Table 2. Quality parameters for Faller hard red spring wheat (HRSW) and check cultivars tested in the ND HRSW Variety Trials (2003-2006).

Cultivar	Falling number	Flour Extraction	Mixing time	Mixing tolerance	Loaf volume	Water absorption
	sec	g kg^{-1}	min	min		%
Faller	423	71.1	8.1	15.8	1042	64.3
Howard	427	69.7	8.2	12.2	1007	64.5
Glenn	401	67.6	9.3	20.6	1102	64.9
Steele-ND	425	70.1	8.5	13.5	1011	
Alsen	412	68.6	9.0	16.2	1057	64.8
Parshall	415	69.2	8.3	14.9	1081	64.7
Reeder	431	67.8	7.0	12.0	1002	63.8
Observations	21	21	21	21	21	21

Table 3. Diseases reactions of Faller hard red spring wheat (HRSW) and check cultivars tested in the ND HRSW Variety Trials (2003-2006).

Cultivar	FHB[†]	Leaf rust		Stem rust		Tan spot			Septoria tritici	Septoria nodorum
		Greenhouse[‡]	Field	Greenhouse[§]	Field	Race 1	Race 2	Race 5		
	%					1-5[#]	1-5	1-5		
Faller	27	R[¶]	R	R	tR	1.7	2.1	3.7	2.7	3.1
Alsen	22	R	MR/MS	MR/R	5R	-	-	-	2.7	4.4
Traverse	-	R	MR/MS	R	R	-	-	-	2.9	2.6
Knudson	-	-	R	R	R	-	-	-	2.2	1.6
Reeder	55	R	S	MR/R	5R	-	-	-	2.9	2.2
Baart	-	-	S	S	50MS	-	-	-	-	-
Tatcher	-	S	-	-	-	-	-	-	-	-
Glenlea	-					4.3	2.0	1.9	2.4	3.7
Salamouni	-					1.4	1.4	1.3	1.7	1.7
6B662	-					1.7	1.7	4.0	1.9	1.4
6B365	-					1.7	4.1	1.9	2.1	1.7
Observations		9	5	4	9	6	6	6	4	4

[†] FHB (Fusarium Head blight) severity as described by (Stack and Frohberg, 2000).
[‡] Greenhouse reactions for leaf rust races MCDL and THBJ.
[§] Greenhouse reactions for stem rust races Pgt TPMK, TMLK, RTQQ, QFCQ, QTHJ, THTS, and TCMJ.
[¶] R=resistant, MR=Moderate resistant, MS=Moderate susceptible, S=Susceptible, tR= trace/Resistant.
[#] The 1-5 scale developed by Lamari and Bernier (1989) was used to score the genotype

PUTATIVE FHB RESISTANCE COMPONENTS RESISTANCE TO KERNEL INFECTION AND TOLERANCE IN THE SSRWW NURSERY, 2005-2007.

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ABSTRACT

The Southern Soft Red Winter Wheat Nursery has been tested in Hungary since 2003 with four isolates of *F. graminearum* and *F. culmorum* separately in three replicates, so four epidemic situations could be studied at the same time. Three bagged groups of heads served as controls. From 2005 we applied one inoculation date as the flowering differences were not larger than 4-5 days, and so an important source of mistake could be neutralized and the data become more suitable to estimate resistance components. After spraying the heads of 15-20 in a group with *Fusarium* suspension, polyethylene bags over 48 hrs secured the high humidity to initiate infection. As the members of the nursery changed from year to year, a repeatability of the Szeged results could not be tested over years, however, we provide a way to estimate the presence of the resistance to kernel infection and tolerance, which can be applied on the multilocated data set of the nursery. The statistical evaluation is as follows: A linear regression slope will be counted between FHB and FDK data. By using the function, the data points of FDK data for all FHB data points will be developed. From the original FDK data the counted FDK values will be extracted, so for each genotype we receive a difference. When this difference is larger than the LSD 5 % from the ANOVA of the FDK values, we will have two sorts of deviations. When the value will be negative, e. g. the predicted FDK is larger than the original data, we speak about the presence of the kernel infection resistance component, e. g. the kernel infection is lower than would be calculated from the function. On the opposite, for positive number the predicted value is smaller than the original data, we speak about an extra sensitivity. Both are important and this is one reason why FHB alone does not sufficiently describe resistance behavior of the given genotype. In 2007 for example, six genotypes of 45 provided resistance and seven revealed extra susceptibility. The estimation of tolerance has the same pattern. We think that such additional analyses of the data will help to evaluate additional resistance components and understand better behavior of the genotypes under different epidemic conditions.

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EVALUATION OF FHB PROFILES OF ADVANCED WHEAT BREEDING LINES TREATED WITH A FUNGICIDE.

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ABSTRACT

In evaluating FHB resistance of wheat breeding lines, breeders strive to estimate, as accurately as possible, the genetic component of resistance. The possible benefits of management tools, e.g., fungicides, are often ignored by the breeder. The purpose of this study was to evaluate the FHB profile of a set of advanced breeding lines in the presence and absence of the fungicide Prosaro. The study was conducted at Princeton and Lexington, KY. The Princeton location was non-irrigated and inoculated with a single application of scabby corn at Feeke's growth stage 9 and two conidial sprays (1×10^6 spores ml^{-1}) at flowering and one week later. The Lexington location was irrigated and inoculated with scabby corn at Feeke's growth stage 9. Rainfall across Kentucky was inadequate for FHB development, but measurable levels of disease were achieved in both nurseries. Diseases other than FHB were present in Lexington, but at very low levels.

A factorial design with 3 replications was used at each location. At Princeton the experimental unit was a conventional 6 row yield plot, 15 ft. long; at Lexington the experimental unit was a 4 row plot, 4 ft long, planted with a headrow planter. Plots at each location were treated at flowering with a tank mix of Prosaro fungicide (6.5 fl. oz. $acre^{-1}$) with Induce (0.125% w/v). Three replicates were left untreated for comparison. FHB symptoms were evaluated 21 days after flowering using a 5 point visual rating scale that encompasses both severity and incidence. After harvest, percentage *Fusarium* damaged kernels (FDK), deoxynivalenol concentration (DON), yield and test weight were measured.

In Princeton, where rain was a limiting factor, there was no significant difference between fungicide-treated and control plots for rating, FDK, DON, and test weight. There was a significant difference in yield, with the average yield of the control plots 11.5 % less than the average yield of the treated plots. In the irrigated Lexington nursery, FHB rating, FDK and DON were all significantly lower in treated than in controls. Yield and test weight were significantly higher for the treated plots than for the control plots. Particularly interesting was the DON level reduction in Lexington. Fungicide x genotype interaction was apparent. For instance, in the control plots, KY99C-1205-06-1 had the lowest DON with 15.4 ppm, but it was third lowest in the treated plots with 13.2 ppm, a 2.2 ppm reduction. In KY98C-1324-01-3 the reduction was 21.4 ppm. The study suggests that advanced breeding lines should routinely be screened with a fungicide as part of the candidate variety evaluation process.

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THE 2006-07 SOUTHERN UNIFORM WINTER WHEAT SCAB NURSERY.

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ABSTRACT

Most components of Fusarium Head Blight (FHB) resistance are greatly influenced by genotype by environment interaction which limits the heritability of resistance estimated by a single program in any given year. The Southern Uniform Winter Wheat Scab Nursery provides breeders in the public and private sectors with an opportunity for multi-environment, independent evaluations of FHB resistance in advanced generation breeding lines compared with the current most resistant check varieties Ernie and Bess. In addition, the nursery facilitates the sharing of the best resistant materials throughout the breeding community.

The 2006-07 nursery comprised 42 advanced generation breeding lines and three check cultivars, 'Ernie' and 'Bess' (partially resistant) and 'Coker 9835' (susceptible). Six U.S. public programs (Univ. of Arkansas, Univ. of Georgia, Louisiana State University, Univ. of Maryland, N.C. State Univ. and VA Tech.), and one private company, Agripro-Coker, submitted entries. The nursery was distributed to 11 U.S., one Hungarian, and one Romanian cooperator for field and / or greenhouse evaluations. In addition three USDA-ARS laboratories conducted evaluations for Hessian Fly resistance, milling and baking quality and haplotypes based on established SSR markers.

Several nights of uncharacteristic freezing temperatures during the April 6th to April 9th, 2007 period severely damaged the wheat crop throughout the southern US. As a result, no data were obtained by our Agripro-Coker and University of Georgia cooperators. Partial data were provided by the Univ. of Illinois and N.C. State Univ. Copies of the full report will be available at the 2007 National Fusarium Head Blight Forum and subsequently on line at the USWBSI web site: http://www.scabusa.org/.

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HAPLOTYPE STRUCTURE AND GENETIC DIVERSITY AT FUSARIUM HEAD BLIGHT RESISTANCE QTLS IN SOFT WINTER WHEAT GERMPLASM.

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INTRODUCTION AND OBJECTIVES

Several quantitative trait loci (QTLs) for resistance to Fusarium head blight (FHB) have been mapped in wheat. Among these, three were mapped in the Chinese cultivar Sumai 3 and its derivatives on chromosomes 3BS, 5AS, and 6BS (Anderson et al. 2001; Buerstmayr et al. 2002; McCartney et al. 2004; Yang et al. 2003; Zhou et al. 2002). The 3BS FHB resistance QTL (designated *Fhb1*) has been by far the most deployed by breeding programs worldwide (McCartney et al. 2004). Other FHB resistance QTLs have been mapped in Wuhan 1 on chromosomes 2DL and 4BL, (Somers et al 2003) and in the soft red winter wheat cultivar Ernie on chromosomes 4BL, 5A and 3BSc near the centromere (Liu et al. 2007). Haplotyping strategies make use of previous QTL mapping and molecular marker information. In our study we selected markers reported to be near FHB resistance QTL mapped in Sumai 3, Wuhan 1 and Ernie to haplotype a large set of eastern SW wheat lines submitted by breeders .

The objectives of this research were to (1) determine the genetic relationship among soft winter (SW) wheat lines with native and exotic sources of resistance using SSR markers data, (2) compare the SSR marker haplotypes of SW wheat lines with those of Sumai 3, Wuhan 1, and Ernie at known FHB resistance QTLs, and (3) identify lines with novel sources of FHB resistance.

MATERIALS AND METHODS

Two hundred forty-five SW wheat lines with moderately low to strong FHB resistance from native and/or exotic sources, including susceptible and resistant checks, were grown in screening nurseries at Wooster, OH; Urbana, IL; Lexington, KY; and Blacksburg, VA in 2005 to test for type I and type II resistance to FHB. Some exotic FHB resistant accessions (Sumai 3, Ning 7840, Futai 8944, W14, Wuhan 1, F201R, and VR95B717) were planted only for the purpose of obtaining DNA for the molecular marker analysis because they were in the pedigrees of some of the entries screened for FHB and they served as reference for alleles sizes for markers linked to FHB resistance. Forty-seven SSR markers and one STS marker that map near or at FHB-resistance QTLs on chromosome 2DL and 4BL based on Wuhan 1; 3BS, 5AS, and 6BS based on Sumai 3; and 3BS and 5A based on Ernie were selected.

Statistical analysis of phenotypic data was performed using the SAS software package (SAS Institute Inc., NC, USA), and wheat accessions were classified as resistant (R), moderately resistant (MR), moderate (M), moderately susceptible (MS), and susceptible (S). Scoring of polymorphic DNA fragments generated by SSR markers at each locus was conducted by using GeneMarker v1.5 (SoftGenetics LLC, State College, PA). The freeware package PowerMarker version 3.25 (Liu and Muse 2005) was used to perform the phylogenetic analysis and to obtain genetic

diversity estimates; polymorphism information content (PIC) values; and a total number of allele at each SSR marker locus. Haplotype numbers were calculated (1) on the basis of shared alleles at all loci to detect putative novel QTLs in germplasm lines, and (2) on the basis of the allelic distribution of SSR markers linked to 2DL and 4BL QTLs in Wuhan 1; 3BS, 5AS, and 6BS in Sumai 3; and 3BSc and 5A in Ernie.

RESULTS AND DISCUSSION

The table with haplotypes and the dendrogram of all the wheat lines/cultivars included in this study were too large to be included in these proceedings; this information can be found at http://www.cropsci.ncsu.edu/sggenotyping/index.htm.

Phenotypic Evaluation

Reaction of the SW entries evaluated was skewed toward resistance, with 59 lines classified as resistant, 116 moderately resistant, and 28 intermediate. Only 12 and 18 lines were considered moderately susceptible and susceptible, respectively. Of the resistant lines, 24 have exotic sources of resistance in the pedigree and the remaining resistant lines had only SW germplasm in their pedigrees. The SW wheat cultivars NC-Neuse and Truman were resistant to FHB. Of the seventy-three entries in the experiment having exotic sources of FHB resistance in their pedigrees, index scores ranged from resistant (VA 01W-476, IND = 4.1) to susceptible (VA41W-495, IND = 41.9). The exotic sources of resistance in pedigrees of lines in this study included Sumai 3, Ning 7840, Ning 8026, Ermai 9, Futai 8944, ZM10782, W14, Catbird, F201R, and VR95B717. The most common SW wheat cultivars in pedigrees with moderate resistance were Roane, Freedom, Patton, and Ernie.

Marker Diversity

The forty-eight SSR markers evaluated had PIC values that ranged from 0.175 (*Xgwm113*) to 0.922 (*Xcfd233*) with a mean value of 0.639. Only ten SSR markers had PIC values of less than 0.50. Two alleles were observed for STS marker *Xsts3B-256* that is closely linked to the *Fhb1* gene. The number of alleles observed for SSR markers ranged from two (*Xgwm508*) to 22 (*Xgwm601*) with a mean number of 10.42 alleles per locus. The 3BS QTL region had the lowest mean number of alleles detected by SSR markers (6.3), which resulted in the lowest mean PIC value of 0.493. In constrast, the 3BSc QTL interval had the highest mean number of alleles detected by SSR markers (12.1) and the highest mean PIC value of 0.7. A total of 251 haplotypes were detected by the 48 SSR markers, indicating that although there were several full-sib lines in included in the study, no entries had the exact same alleles at all loci.

Cluster Analysis

Entries were grouped into 16 clusters that were generally based on breeding program or geographic origin of lines. The Chinese wheat cultivars having the Sumai 3 haplotype at *Xbarc75*, *Xgwm533*, *Xgwm133*, *Xsts3B-256*, *Xbarc147*, and *Xgwm493*; and therefore the *Fhb1* resistance gene, were grouped separately from all other entries. One exception was inclusion of entry VA01W-476 (W14 x Roane) in this cluster. VA01W-476 had the highest level of resistance of all entries evaluated in the study.

Soft white winter wheat accessions were grouped together and with the resistant Chinese cultivar Wuhan 1, and the susceptible cultivars Madison and Pioneer 2545. In general, entries from breeding programs in the Corn Belt (IN, OH, MO and IL) were in different clusters than entries from the Southeast. The French line VR95B717 that was used as a resistance source by the Virginia Tech. breeding program was included in a cluster of resistant to moderately resistant Corn Belt entries.

Comparison of haplotypes of SW wheat with Sumai 3, Wuhan 1 and Ernie

Markers linked to the *Fhb1* locus had the lowest genetic diversity and linkage disequilibrium was observed between markers across the interval. Markers *Xgwm533* and *Xsts3B-256* proved to be the best available for identifying wheat lines with the *Fhb1* gene derived from Chinese sources. The *Fhb1* resistance gene was not present in all entries derived from crosses

with donor Chinese lines. However, all eight entries in this study that have the *Fhb1* resistance gene based on haplotype data were resistant in the field evaluation.

At the 6BS interval, no entries matched the Sumai 3 haplotype. The most common haplotype on this chromosome region was *Xgwm518* (224 bp), *Xgwm508* (Null), *Xwmc398* (168 bp), *Xgwm133* (131 bp), *Xwmc397* (178 bp), *Xwmc152* (Null), and *Xgwm219* (205 bp), which was shared by a group of moderately resistant to susceptible lines of diverse origin. The two moderately resistant SRW wheat cultivars Patton and Goldfield, while different from Sumai 3, had the most closely related haplotypes in this interval, with matching alleles at six of the seven SSR marker loci assayed.

Resistance QTLs located on chromosome 5A have been identified in a number of lines, including Sumai 3, Ning 7840 and Ernie. Chinese lines Futai 8944, Shaan 85-2, and Ning 7840 had the Sumai 3 haplotype across the ten loci at the 5AS interval analyzed. No SW wheat line had the complete Sumai 3 haplotype at all ten markers. Sumai 3 alleles at SSR marker loci *Xbarc117*, *Xgwm304*, *Xbarc186* and *Xgwm415* reported near the *Qfhs.ifa-5A* QTL peak were common in the SW entries evaluated, with frequencies of 0.42, 0.39, 0.31 and 0.26, respectively. However, when these four marker alleles were considered to form a Sumai 3 haplotype, only controls Ning 7840, W14, Shaan 85-2 and Futai 8944, and four resistant and moderately resistant SW lines derived from crosses with ZM10782 (OH904 and OH902), and with Ning 8026 (AR9700-2-1 and AR9700-2-2) had the donor parent haplotype.

No lines had the complete Ernie haplotype at all ten marker loci on 5A. Liu et al. (2007) reported the location of the *Qfhs.umc-5A* FHB resistance QTL between markers *Xbarc56* and *Xbarc40*. The alleles observed in Ernie at SSR marker loci *Xbarc56*, *Xbarc165*, *Xbarc40*, and *Xgwm156* were common in the SW germplasm in this study having allele frequencies of 0.53, 0.41, 0.34, and 0.40, respectively. When these alleles were considered as a haplotype, nine resistant SW wheat lines, 11 moderately resistant and 2 susceptible lines were identical to Ernie.

Wuhan 1 was not in the pedigree of any of the lines in this experiment. None of the SW wheat entries shared the complete haplotype of Wuhan 1 for markers in the 2DL or 4BL QTL intervals. The highest degree of similarity in the 2DL region was observed in soft white winter wheat lines from Michigan and New York. The most similar accession to Wuhan 1 was the susceptible soft white winter wheat cultivar Geneva. Wuhan 1 alleles for proximal SSR markers *Xwmc245*, *Xwmc144*, and *Xwmc601* were common among the soft white wheat lines. These haplotypes were not observed among the soft red winter wheat entries.

Resistance QTL were located on chromosome 4B in cultivars Ernie and Wuhan 1. The *Qfhs.umc-4BL* QTL peak in Ernie was located near marker *Xgwm495*. Only three backcross-derived lines had the Ernie haplotype for SSR markers spanning this interval (*Xwmc238, Xgwm165* and *Xgwm495*). Eighteen resistant to moderately resistant and 2 susceptible lines had the Ernie haplotype for markers *Xgwm165* and *Xgwm495*. Released cultivars Pat, Superior, Freedom, and Truman, as well as the French line VR95B717, shared this haplotype.

Wheat cultivars Freedom and Patton, and the SW wheat line VA04W-608 had the Ernie haplotype at all eight loci in the 3BSc QTL interval, and they showed moderately resistance reactions to FHB. Thirty-six moderately resistant to resistant lines had a partial Ernie haplotype for SSR markers *Xwmc625*, *Xgwm285*, *Xwmc307*, and *Xwmc418*, including the French line VR95B717, the soft red winter wheat cultivars Freedom, Roane, Patton, and their backcross-derived lines.

CONCLUSIONS

The *Xsts3B-256* and *Xgwm533* markers can be clearly used to identify lines with the *Fhb1* resistance gene. However, there is a need for fine mapping other regions in which FHB resistance QTLs have been located. This seems to be particularly important for resistance from Ernie. Allele sizes of Ernie at 5A and

4BL QTL intervals are common among Eastern soft wheat germplasm. The haplotypes at these loci, along with the 3BSc region, suggest that SW wheat cultivars such as Patton, Freedom, and Roane, that have been considered important sources of native FHB resistance, may share resistance QTL with Ernie. However, fine mapping and marker enrichment of these intervals could provide closer and better molecular markers to be used in germplasm characterization as well as marker-assisted selection programs.

There were 59 wheat entries with high levels of FHB resistance in this study. Many of the most resistant lines had the *Fhb1* QTL combined with native resistance. However, more than half of the resistant lines had no exotic parentage, including the cultivars NC-Neuse and Truman. Neither of these cultivars had the complete haplotype of the donor sources for any of the interval examined in this research. A number of other SW wheat breeding lines did not share any haplotype at known QTLs evaluated in this study. These lines likely carry novel sources of FHB resistance. In contrast, similarity of marker alleles in the 3BSc and 4BL regions suggests that resistance in the line VR95B717 may be due to the QTL identified in Ernie. These data are useful in prioritizing germplasm for QTL mapping and identifying diverse sources of resistance that can be combined to further increase the level of FHB resistance.

DISCLAIMER

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.

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SOLVING THE FHB PROBLEM: GROWERS, EXPORT MARKET AND WHEAT COMMODITY PERSPECTIVES.

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ABSTRACT

The Fusarium Head Blight (FHB) disease of cereal crops has become a very important disease in recent years. The favorable environmental conditions including wet weather during flowering and grain filling and major changes in cropping system (introduction of Maize and minimum tillage) has favored disease development. All classes of wheat produced in the United States, as well as barley, have been impacted by this disease, some regions and classes more than others with the most pronounced impacts in regions that tend to have higher precipitation during the growing season or a greater concentration of Maize in the crop rotation such the US Northern Plains.

In the Northern Plains region, FHB had a devastating impact on the wheat and barley production in 1993, and has continued to cause minor to major annual impacts on cereal crops since. The impact of FHB has not only caused significant economic loss to producers in the region but has also impacted domestic and export customers by threatening the quality reputation; particularly the accumulation of mycotoxins such as DON; and reliability of the Northern Plains in its ability to supply their demands. The Northern Plains has a strong history of being a quality source for hard red spring wheat (HRSW) and durum wheat, and malting and feed barley. Customers in the United States and in numerous export markets have come to rely on the regions wheat and barley production for making specialty breads, premium pasta and couscous and well known brands of beer.

Solving the FHB problem is a top priority for wheat, durum and barley research programs in the region, including developing varieties with higher levels of tolerance, research on optimal crop rotation and management practices that incorporate fungicide applications, and studying ways to help millers and processors handle the inherent quality problems that FHB has on the raw wheat and barley and their products. While notable gains have been made in wheat, durum and barley varieties during the past 15 years, further advancements in varietal tolerance and other tangent production research is needed. The significant financial contributions from the US Wheat and Barley Scab Initiative (USWBSI) have been complemented with State dollars and direct contributions from producers themselves through wheat and barley check-off programs, to help producers in the region and the customers they serve reduce the impacts from FHB.

The successful incorporation of resistance genes to FHB from the Chinese source 'Sumai 3' wheat and other sources such as wheat wild relative *Triticum dicoccoides* into hard red spring and other market classes wheat varieties like Alsen, Glenn, Steele-ND, Freyr, Truman, Neuse, Tribute, McCormick, and others has given producers some attractive options to ward off FHB pressures. An added bonus of many of these varieties is that they also maintain the end-use quality standards demanded by our domestic and international customers. For example, Glenn, released by NDSU and the mostly grown cultivar in the spring wheat region (1.3 millions in 2007) is currently the HRSW quality standard accepted by the US wh eat quality industry and for the regional breeding programs. Worldwide, Glenn has received numerous top quality ratings from key customers participating in the U.S. Wheat Associates Overseas Variety Analysis (OVA) program. In addition,

major improvements in forecast models, available fungicides and application methods have allowed producers to more effectively manage the disease in the field.

Development of tolerant varieties has been more of a challenge with barley and durum due to smaller germplasm pools. However, successful incorporation of tolerance has accelerated in recent years due to new breeding techniques. Advanced lines of both are showing more promise, and it now looks possible to have varieties available for commercial production within the next two to three years that could have the same level of tolerance as is found in some classes of red wheat.

Producers and end-users have already received significant dividends from the enhancement to breeding programs through the USWBSI and other complementary funding. Additional improvements are still needed however, and solving the FHB threat remains a top priority and will likely be for the foreseeable future for small grain producers and researchers in the Northern Plains and the entire US. Domestic and international customers need the additional research to ensure they are able to draw from quality cereal crop production to continue making premium priced, safe and wholesome food products. The producers in the United States and around the world need the additional research to help them maintain viable cereal crop options in their farming operations.

ASSOCIATION MAPPING OF COMPLEX TRAITS IN A DIVERSE DURUM WHEAT POPULATION.

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OBJECTIVE

To assess the potential of association mapping to complement existing mapping efforts in durum wheat.

INTRODUCTION

In plants, the traditional approach to quantitative trait loci (QTL) mapping is to develop a set of random lines derived from crossing two inbred lines that vary in phenotypic values for a particular trait of interest. Although this method has proven effective, there are a number of inherent limitations. First, QTL resolution is generally poor due to the low number of recombination events sampled (Nordborg et al. 2002). This limits potential for marker assisted selection and prevents identification of candidate genes coincident with the QTL. Second, allele variation is restricted to the alleles present in the two parents and QTL localization is restricted to loci segregating between the two parents. This prevents identification of novel alleles and QTL outside of the mapping population (Buckler and Thornsberry, 2002).

Association mapping (AM) mapping is employed in medical studies, and is expected to be a complementary strategy for describing associations between genotype and phenotype in crop plants. The most notable attributes of AM mapping are an improved level of molecular polymorphism as multiple alleles are detected at each locus. Second, accessions or breeding lines have been derived over many generations of meiotic events and this increases the number of crossover events in a defined chromosome interval which is expected to improve the resolution of trait/genotype associations. In plants, two strategies have been used for AM mapping: a) genome scans where the entire genome can be analyzed with molecular markers of sufficient density to localize the QTL (Kraakman et al. 2004; Rafalski 2002) or b) where a candidate gene has been identified, an association of polymorphic markers within the gene and a trait are examined (Thornsberry et al. 2001).

An attractive feature of AM is the ability to perform marker trait associations in well phenotyped breeding populations and locally adapted varieties. However, population structure, due to selection and high levels of co-ancestry, is expected in pedigree-based breeding programs, resulting in a high probability of identifying of spurious associations (Pritchard et al. 2000). However, computational methods have been developed to account for population structure and relatedness to reduce identification of spurious associations (Yu et al. 2005).

Studies on association mapping have been conducted in a number of crop species including barley (Kraakman et al. 2004) and wheat (Tommasini et al. 2007). However, most studies in wheat have focused on specific chromosomes where major QTL have previously been reported and further research to assess the potential of genome-wide association mapping for complex traits in wheat is needed. In this study, we performed genome-wide AM and report on a) the agreement of identified associations with previously published QTL b) and identification of novel QTL yet to be reported in the literature.

MATERIALS AND METHODS

AM Population and Molecular Analysis – Ninety-six (96) diverse durum wheat cultivars and breeding lines collected from breeding programs in Canada (25), Argentina (5), Australia (9), France (3), Italy (18), Germany (2), Mexico (3), Morrocco (3), United States (12), New Zealand (1), Russia (1), Iran (4), Spain (9), as well as one line of unknown origin formed the AM population. Genotyping was performed on an ABI 3100 capillary electrophoresis using M13-labeled microsatellites. A total of 241 microsatellite (SSR) markers were used to amplify 245 loci.

Trait Analysis and AM Mapping – Two traits were selected for genome wide AM study. Grain yellow pigment content (YP; mg kg^{-1}) was assessed on a plot basis using AACC approved method 14-50 on samples collected from replicated trials grown at Saskatoon and Swift Current, Saskatchewan, Canada in 2005 and 2006. Resistance to stem rust race TTKS (UG99) was also assessed in 2007 at an endemic nursery in Kenya. Prior to AM analysis, population structure was assessed using a selection of 147 SSRs were selected at 2 cM intervals within the program STRUCUTRE v.2 (Pritchard et al, 2000). Structure parameter settings were: linkage model, allele frequencies correlated, burn-in length 10,000, and 10,000 repetitions. The highest likelihood was observed for K (no. sub-populations)=5, but little difference was observed between K=3 and K=5. Therefore, the Q matrix was estimated as the average of five runs for K=3. Marker-trait associations were determined using a general linear model in TASSEL version 2.0.1 with the Q-matrix as covariates. Pair-wise linkage disequilibrium (LD) of each SSR with allele specific CAPS markers for phytoene synthase genes *Psy1-A1* and *Psy1-B1* was estimated as the squared allele frequency correlation (r^2) within TASSEL. In cases of multiple alleles, a weighted average of r^2 between loci was calculated.

RESULTS AND DISCUSSION

We first examined YP as a validation trait for AM as the genetics of this trait are well understood and QTL for have been well documented in the literature (Pozniak et al. 2007). Variation for YP was large, ranging from less than 5 mg kg^{-1} to greater than 12 mg ha^{-1}, regardless of testing environments. Heritability estimates were high, ranging from 0.95 to 0.99. Using the sub-population Q-matrix as covariates in a general linear model, marker associations for YP were identified on five chromosomes (Fig. 1) and were statistically significant ($P<0.01$) in all four environments. These associations were coincident with previously published QTL (Fig. 1). Additional markers on chromosomes 2B, 3A and 3B were identified, but these were not consistent among testing environments (data not shown).

Phytoene synthase (*Psy*) is the first critical enzyme in the biosynthesis of lutein, the major xanthophyll responsible for yellow pigment. Genes coding for *Psy1* have been mapped in durum, and *Psy1-B1* co-segregates with a QTL for YP on chromosome 7B (Pozniak et al. 2007). In genetic mapping studies, *Psy1-B1* mapped approx. 4 cM from *gwm146* and Pair-wise LD analysis revealed a CAPs marker for *Psy1-B1* was in strong LD with that SSR (Fig. 2). AM confirmed that this gene was also associated with variation in YP (Fig. 1).

Psy1-A1 was in disequilibrium with *cfa2257* on 7A and genetic studies have confirmed that this gene is linked to *cfa2257*. Using AM, *Psy1-A1* was associated with YP in the durum population (Fig. 1), and localizes to a region on 7A previously associated with YP (Elouafi et al., 2001). These results suggest that LD analysis can be used to correctly position genes in the durum wheat genome, and to determine their association with phenotypic variation. Interestingly, both genes were in LD with a region on 1A where a putative QTL for YP has been identified.

Given the apparent success of genome wide AM mapping for YP, similar analyses were performed for resistance to stem rust race TTKS. Nearly half of the durum wheat accessions evaluated in the 2007 Kenya nursery were scored as moderately to high resistant. The remaining lines possessed intermediate resistance (n=25), or were scored as being moderately susceptible (n=21) or susceptible (n=10). Marker associations for TTKS using numerical severity ratings were

identified on chromosomes 1B, 4B, and the group 5 and 7 chromosomes (Fig. 3).

Of these, only two regions identified are known to house mapped *Sr* resistance genes. Two regions were identified on chromosome 7A, one distal to the centromere, and a second at *gwm276* and *cfa2257* (Fig. 3). *Sr22* is linked to *gwm276*, and that gene is effective against TTKS (Jin et al., 2007). Linked markers on 4B, including the lipoxygenase gene *Lpx-B1.1*, were significantly associated with variation in disease resistance. Lipoxygenase is known to play a role in disease resistance and enzyme activity has been reported to increase in wheat treated with a rust fungal elicitor (Bohland et al. 1997). Sr gene *Tmp* from winter wheat cultivar 'Triumph 64' is effective against TTKS (Jin et al., 2007) and that gene is believed to reside on 4B. Marker associations were identified for *gwm291* and *wmc727* near the distal end of 5A and linked markers *wmc537* and *Cdu1* on 5B, were also significant. Although the association on 5B is in a region were *Sr* genes have yet to be identified, this region has recently been associated with stem rust resistance in hexaploid wheat using AM (Crossa et al., 2007). Interestingly *Psy1-B1* was associated with TTKS resistance (Fig. 3). Although linkage with leaf rust resistance with YP has been reported on 7A, linkage with stem rust has not been reported on 7B

CONCLUSIONS

Our results experimentally validate the potential of genome-wide AM to detect marker associations for complex traits in durum wheat. In the case of YP, marker associations were in agreement with previously identified QTL, suggesting that AM for that trait was effective in this population. For TTKS resistance, novel regions not associated with previously mapped *Sr* resistance genes were identified, and further validation of these marker-trait associations is a high priority. In particular, the association with *Lpx-B1.1* will need to be confirmed, as high enzyme activity results in undesirable colour loss in pasta products. Given the apparent success of AM in this population, we are currently in the process of performing additional genotyping and more detailed phenotyping for mapping of other important agronomic, end-use quality and disease resistance traits, including Fusarium head blight (FHB) resistance. Only a few FHB resistance QTL have been reported in durum wheat, but given the results of this study, AM could be used for identification of novel chromosome regions and alleles that confer some degree of resistance. Although FHB resistance has yet to be evaluated in balanced field trials in this population, sufficient variation for resistance is known to exist in and efforts to perform AM will be pursued. Inclusion of additional FHB resistant breeding lines known to carry similar resistance (i.e. progeny of known lines with improved tolerance) is being considered to improve statistical power for detecting relevant marker associations.

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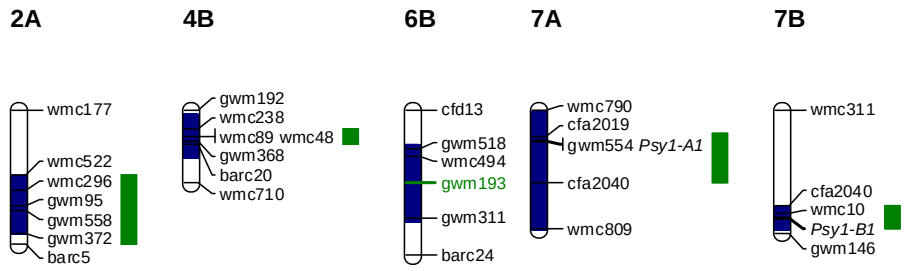

Figure 1. Marker associations for YP in the durum wheat association mapping population. Bars to the right of the linkage group regions where significant associations ($p<0.001$) were identified at all four testing environments. Highlighted regions in the centre of the linkage groups are QTL regions previously identified using bi-parental mapping populations. Only relevant regions of the linkage groups are shown.

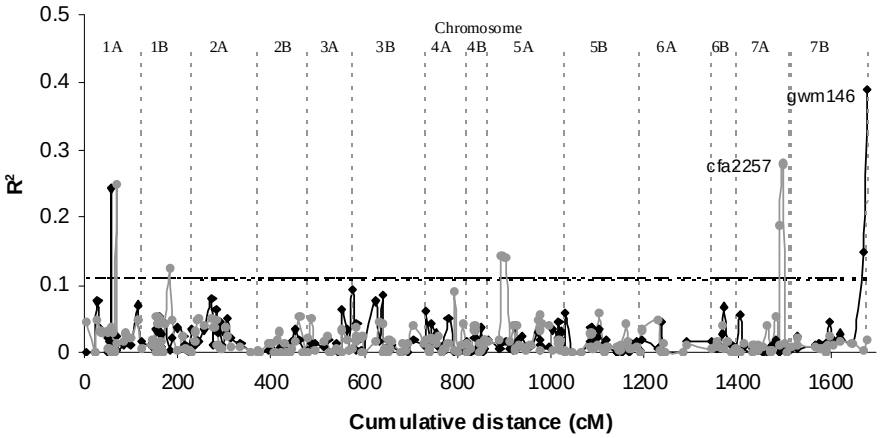

Figure 2. Pairwise LD (r^2) with *Psy1-B1* (—) and *Psy1-A1* (—). The dashed line represents where the cumulative frequency of genome wide r^2 reached 95%.

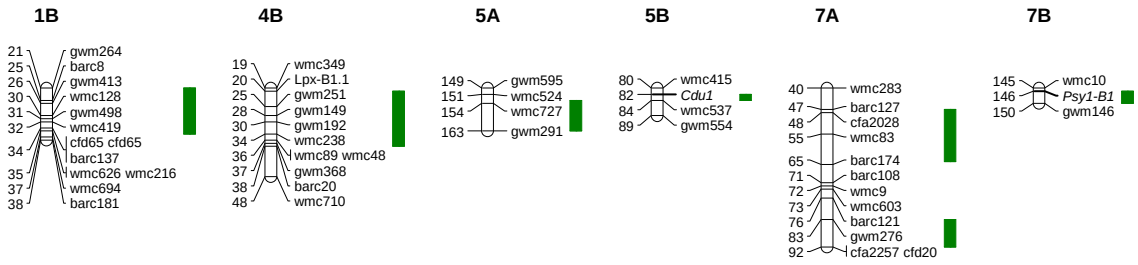

Figure 3. Marker associations for TTKS in the durum wheat association mapping population. Bars to the right of the chromosomes represent regions where significant associations ($p<0.001$) were identified. Only relevant regions are presented.

MOLECULAR CHARACTERIZATION OF A WHEAT-*LEYMUS* COMPENSATING TRANSLOCATION LINE CONFERRING RESISTANCE TO FUSARIUM HEAD BLIGHT.

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ABSTRACT

Fusarium head blight (FHB) resistance was identified in the alien species *Leymus racemosus* (syn *Elymus giganteus*). Several wheat-*Leymus* translocation lines with FHB resistance were developed using radiation treatment or gametocidal gene action. However, all of them are noncompensating, preventing their use in cultivar improvement. We have further screened 58 wheat-*Leymus* introgression lines from 14 siblings for their resistance to spread of infection within spikes. Of 24 lines with high levels of resistance to FHB, we determined that three lines (T01, T09, and T14) were lacking Sumai 3-type alleles at marker loci linked to *Fhb1*, indicating that the FHB resistance in these lines was most likely derived from *L. racemosus*. Previous cytogenetic data revealed that line T01 has a translocation, T4BS·4BL-7Lr#1S, and line T14 has a translocation, T6BL·6BS-5Lr#1L. Line T09 has an unknown wheat-*Leymus* translocation chromosome. A total of 33 RFLP markers selected from seven homoeologous groups of wheat were used to screen T09. Only short arm markers of group-7 detected *Leymus* specific fragments in line T09. The 7AS-specific RFLP fragments were missing in T09, indicating that this line has a compensating Robertsonian translocation involving the long arm of wheat chromosome 7A and the short arm of *Leymus* chromosome 7Lr#1. C-banding and genomic in situ hybridization (GISH) analyses using *Leymus* genomic DNA as probe confirmed the RFLP results. RFLP analysis was further conducted in lines T01 and T14, as well as the wheat-*Leymus* disomic addition lines of DA5Lr#1 and DA7Lr#1, with 11 group-5 markers (five on the short arm and six on the long arm) and eight short arm markers of group-7 chromosomes. The results revealed that both lines T01 and T14 were complex translocations involving the short arms of *Leymus* chromosomes 5Lr#1 and 7Lr#1. These two lines have similar segments from 5Lr#1S. However, the length of 7Lr#1S segment in T01 and T14 is different. Line T01 contains an almost complete short arm of 7Lr#1, whereas about 50% of the distal portion of 7Lr#1S is transferred to the translocation chromosome in the line T14. Three translocation lines and the disomic addition 7Lr#1 were consistently resistant to FHB, whereas the disomic addition 5Lr#1 was susceptible. Because three translocation lines share a common distal segment of 7Lr#1S, a novel scab resistance gene from *Leymus* most likely resides in the distal region of the short arm of chromosome 7Lr#1. Three PCR-based markers, BE586744-STS, BE404728-STS, and BE586111-STS, were developed to accommodate marker-assisted selection in breeding programs. Development of wheat-*Leymus* compensating recombinant lines with smaller alien segments that retain FHB resistance is underway by *ph1b*-induced homoeologous recombination.

FHB RESISTANCE OF WHEAT LINES NEAR-ISOGENIC FOR FIVE DIFFERENT FHB RESISTANCE QTLS.

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ABSTRACT

To continue improving FHB resistance in hard red spring wheat (HRSW), it is imperative that new FHB resistance genes from wheat and its relatives be introduced into the HRSW germplasm base. In 2001 we initiated a program to use marker-assisted backcrossing to individually introgress five confirmed or postulated FHB resistance QTLs from diverse germplasm sources into three FHB-susceptible HRSW backgrounds (Norm, Wheaton, Apogee). The QTLs include two from Sumai 3 (*Fhb1* and *Qfhs.ifa-5AS*) to serve as reference QTLs, one from the soft red winter wheat Freedom reportedly on chromosome arm 2AS, one from the Brazilian wheat Frontana on chromosome arm 3AL, and one from chromosome 3A of wild emmer (*Qfhs.ndsu-3A*). This individual QTL introgression permits evaluation of the effect of each QTL while simultaneously performing prebreeding introgression into HRSW germplasm. The development of the BC_4-derived QTL near-isogenic lines (QTL-NILs) is now complete. For each genetic background/QTL combination, 4 to 5 independent resistant and susceptible NIL pairs were developed, except for the QTL *Qfhs.ndsu-3A*, for which genetic barriers prevented introgression into Norm and Apogee. These lines have been subjected to comparative FHB resistance evaluations both in the field and greenhouse. Results of these evaluations reveal the effect of these QTLs on FHB resistance improvement in the different HRSW genetic backgrounds. Evidence from greenhouse and/or field evaluations has accrued to indicate enhancement of FHB resistance by each of the QTLs introgressed, with both genetic background and the efficacy of the molecular markers contributing to the outcomes. The QTL-NIL series we have developed represent new germplasm for HRSW FHB resistance breeding efforts, and they are a useful resource for additional research on FHB resistance. For instance, we currently are using the lines to examine epistatic interactions between these QTLs to determine which combinations most effectively reduce FHB symptoms. Additional scientific uses include exploring the molecular basis of the wheat-*F. graminearum* interaction as well as the biological basis both of type I and type II resistance.

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FAMILY BASED MAPPING OF FHB RESISTANCE QTLS IN HEXAPLOID WHEAT.

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ABSTRACT

Traditionally quantitative trait loci (QTL) mapping and marker aided selection programs have been two different ventures. For application in breeding programs, novel QTLs must be mapped, though mapping populations are generally not highly useful to breeders because the bi-parental cross is often composed of an unadapted parent. In contrast, breeding programs obtain the most success by creating a large number of breeding populations, or families, using adapted parental germplasm. We combined data from many breeding populations to map QTLs initially, and subsequently used markers of interest for selection. This single step approach is quick, simple, and employs pedigree information and variance component based linkage analysis to place QTLs with substantial effects. Experiments were conducted to validate the approach by mapping *Fhb1*. As part of an ongoing spring wheat breeding program, forty-five susceptible spring wheat genotypes were crossed in different combinations with at least one founder (i.e., a parent containing *Fhb1*). Eighty-three unique families were generated and 793 individuals were screened for resistance in the greenhouse using a point inoculation method. Genotyping was done using mapped simple sequence repeat markers. The QTL was placed in the same position as previous studies with a high probability value. These results demonstrate the usefulness of this approach to quickly map QTLs with relatively large effects, and should allow for marker aided selection as generations are advanced.

FACING THE FHB CHALLENGES TO MALTING BARLEY AND BREWING THROUGH BARLEY BREEDING.

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INTRODUCTION

Fusarium head blight has posed the biggest challenge to the malting and brewing industries, possibly since Prohibition. While disease has lead to reduced yield and quality problems for farmers, DON has caused scarcity of high quality malting barley and relocation of production areas farther from the bricks and mortar (malt houses and breweries) of the industry. In response, BARI Seed Research integrated breeding for resistance into its domestic 6 rowed spring malting barley program with an emphasis on resistance to DON accumulation.

THE CHALLENGES

All malting barley cultivars grown in the 1990s were susceptible to FHB. Breeding programs had little or no resistance in their up-and-coming lines. The Challenge - *Where to get resistance?*

Even under high disease pressure, it was risky to rely on natural infection in the field. Field trials are expensive and require many location years. Greenhouse inoculations, while giving high levels of disease and DON, overwhelmed the low levels of resistance available. The Challenge - *How to insure good screening?*

FHB is difficult to evaluate and DON is costly to determine. The Challenge - *How to measure DON in thousands of breeding lines?*

Meeting and overcoming these challenges has required a collaborative effort involving the entire global barley scientific community. What is reported here includes results from that global effort.

SOURCES OF RESISTANCE

Sources of resistance have been identified from the National Small Grains Collection (NSGC), Composite lines, ICARDA/CIMMYT breeding program, Swiss landraces, the Vavilov Collection in Russia and others. Screening of the NSGC was done by Skoglund and Menert at BARI (Skoglund and Menert, 2002) from 1998-2001 and Steffenson and Scholz at NDSU (Steffenson and Scholz, 2001) from 1999-2001. Over 8100 6 rowed spring barley accessions were screened by one or both groups. Screening was carried out in the field and in the greenhouse. The BARI group identified 15 accessions for possible breeding and the NDSU group identified 10. Only one accession, CIho 6613 (Seed Stocks 1148-1) was identified by both groups.

BARI began collaboration with the ICARDA/CIMMYT barley breeding program in 1999. Since then there has been 1) exchange of germplasm, 2) crossing of elite malting germplasm to resistant sources and 3) screening of various germplasm in nurseries run by BARI and collaborators in North Dakota and Minnesota. Some of the best parents from ICARDA/CIMMYT are listed in Table 1.

Dr. Brian Steffenson (University of Minnesota) has been instrumental in identifying and distributing sources of resistance from other, less accessible sources. These include Composite Cross XXX, Swiss landraces, the Vavilov Collection in Russia and Nordic Gene Bank (Steffenson, 2003; Steffenson and Dahl, 2003: Steffenson, Dahl and Luskutov, 2005). Table 2 has a list of the accessions used by BARI.

FHB NURSERIES AND COLLABORATIVE SCREENING

A number of misted, inoculated nurseries have been established around the world (Table 3). Data collected usually include FHB severity and, in some cases, DON concentration. BARI has collaborated with these institutions to have breeding lines tested. These nurseries have proven invaluable as insurance against low DON years in our yield trials.

There are a number of collaborative screening trials in which BARI participates, some intended for FHB only and others that are primarily for yield and agronomic characteristics. The North American Barley Scab Evaluation Nursery (NABSEN) is an international screening nursery that includes six breeding programs and is planted in 8-10 locations, both dryland and irrigated. Data collected includes FHB incidence and severity, DON and any other (heading date, etc.). The Mississippi Valley Barley Nursery and the Midwest Coop are examples of trials that are grown by multiple collaborators in multiple locations. Where feasible, these are evaluated for resistance to FHB and DON.

DON TESTING

For the past 8-10 years, we have placed high priority on screening for DON accumulation in the BARI breeding program. This has been facilitated by the establishment and/or expansion of a number of facilities (Table 4). We primarily rely on the NDSU DON Testing Lab. Also, BARI Seed Research has collaborated with Dr. Nick Hill, Agrinostics Inc, in testing an ELISA-based technique for quantifying *Fusarium graminearum* mycelium in grain.

WHERE ARE WE NOW?

Progress has been painfully slow due to lack of major genes with large effects in a background close to that needed for brewing. Legacy (accepted in 2001) has reduced DON accumulation to about 30% of that typically found in Robust. Lines in the program now reduce DON by 30-50% of Robust. This progress is the result of incremental integration of genes available within the BARI germplasm as well as UM and NDSU germplasm. Overall, this approach has been a matter of integrating multiple genes with minor effects into an elite line that has acceptable malt quality – not an easy task.

Many additional sources of resistance to FHB/DON are in use at BARI. Projects are currently underway at various universities to determine their genetic diversity. Meanwhile, the most advanced of these were planted as F6 headrows in summer 2007. Selections from these were planted in the nursery in China in Fall 2007. Some of these should advance to first year yield trial status in 2008. These include crosses to the following resistant parents:

- COMP 351 & 355
- HV 746, 779 and 531
- B2027/5/Ataco/Bermejo//Higo/3/CLN/ Gloria/Copal/4/Chevron
- Legacy/Chamico
- Merit//Canela/Zhedar#2
- Merit/MSEL
- Merit/4/Gob/Humai10//Canela/3/Aleli
- Arupo/K8755//Mora/3/Gob/ Humai10/4/ Shyri

Seed Stocks 1148-1 (CIho 6613) was crossed with Legacy in 2000. Though several selections did well in field trials, they failed to advance through our selection process. HV 527, VIR 16537, 28797 and 28807 and NGB9443 are in the current crossing block.

THE FINAL CHALLENGE

Most elite lines never make it through AMBA testing and Anheuser-Busch acceptance. The Final Challenge - *How to get resistant cultivars into the beer?*

BARI continues to submit our best resistant lines to AMBA for testing, as do NDSU and UM. At this time, two lines have made it through AMBA and will be tested in brewing trials with the 2008 crop. These are the UM line M122 and the NDSU line ND20448.

CONCLUSION

Malting barley breeding programs are making progress and meeting the challenges faced by industry. There have been incremental improvements in DON accumulation in advanced lines using a variety of genetic resources. These lines are slowly progressing through the testing and acceptance procedures on their way to farmers' fields and the brew house.

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Table 1. Two rowed and six rowed spring barley lines developed by ICARDA/CIMMYT with resistance to Fusarium head blight and other diseases.

LINE	TYPE
SVANHALS-BAR/MSEL//AZAF/GOB24DH	2 rowed
GOB/HUMAI10/3/ATAH92/ALELI	2 rowed
TOCTE//GOB/HUMAI10/3/ATAH92/ALELI	2 rowed
CANELA/ZHEDAR#2	2 rowed
ATACO/BERMEJO//HIGO/3/CLN/GLORIA/COPAL/4/CHEVRON	2 rowed
CHAMICO	6 rowed
MADRE SELVA	2 rowed
PENCO/CHEVRON	6 rowed

Table 2. New sources of resistance to Fusarium head blight utilized in the Busch Agricultural Resources, Inc. barley breeding program.

ACCESSIONS	TYPE	YEAR	SOURCE	STATUS
COMP 351	6 rowed	2003	Composite Cross	Active
COMP 355	6 rowed	2003	Composite Cross	Active
HV 746	6 rowed	2003	Swiss Landrace	Active
HV 779	6 rowed	2003	s Swiss Landrace	Active
HV 527	2 rowed	2003	Swiss Landrace	Active
HV 531	2 rowed	2003	Swiss Landrace	Dropped
VIR 20738	6 rowed	2004	Vavilov Collection	Dropped
VIR 20733	2 rowed	2004	Vavilov Collection	Dropped
VIR 16537	2 rowed	2007	Vavilov Collection	Active
VIR 28797	6 rowed	2007	Vavilov Collection	Active
VIR 28807	2 rowed	2007	Vavilov Collection	Active
NGB 9443	6 rowed	2007	Nordic Gene Bank	Active

Table 3. Misted and inoculated Fusarium head blight nurseries available to barley breeding programs.

INSTITUTION	LOCATIONS	# BARLEY ENTRIES
NDSU	Osnabrock, ND	21000 hills, 1200 rows, 90 plots
UM	St. Paul, Morris and Crookston, MN	12000 rows
BARI	Fort Collins, CO	350 rows
AAFC	Brandon, MB	18000 rows
ZHEJIANG UNIVERSITY	Hangzhou, China	10000 half rows
CIMMYT	El Batan, Mexico	3000-3500 rows

Table 4. DON testing facilities available to barley breeders.

INSTITUTION	# BARLEY SAMPLES	USWBSI FUNDING	COMMENT
NDSU	18000 in 2006 12000 in 2007 (est)	Yes	Up from 4000 samples in 2000
UM	5000 in 2006/2007	Yes	
AAFC	7000-9000 samples/yr	No	Private lab - 3000 samples
CIMMYT	unknown	No	Est. by Dr. Lucy Gilchrest

DEVELOPMENT OF BARLEY VARIETY CANDIDATE M122WITH ENHANCED RESISTANCE TO FHB.

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ABSTRACT

M122 is a barley variety candidate with enhanced FHB resistance, good malting quality and yield performance similar to currently grown varieties in the Midwest. This line was developed from the sources of resistance Chevron and Zhedar 1 over three breeding cycles. Based on 27 field experiments conducted between 2002 and 2007, M122 has FHB severity and DON levels that are 47% and 52% of the common variety check Robust, respectively. M122 was developed using field-based screening for FHB resistance and low DON in harvested grain. Simultaneous selection was imposed for agronomic performance and malting quality. M122 does not appear to contain resistance source alleles at known and validated QTL for FHB or DON.

Development

M122 is an experimental breeding line, with enhanced FHB resistance, from the University of Minnesota that has potential as a new six-rowed malting variety for the Upper Midwest barley production area. M122 is an F5 derived selection from the cross FEG18-20 / M110 (Figure 1). FEG18-20 was a line selected for its FHB (Fusarium Head Blight) tolerance over several years of disease screening from the cross MNBrite / SI4-29. SI4-29 was also a line selected for its FHB tolerance from the cross Zhedar 1 / Stander // Foster, which was given to us by Rich Horsley as F2 seed in 1994. M122 was advanced by single seed descent from the F1 thru F4 generations. It was selected from an FHB tolerant population (FEG65) in 2002 as an F4.5 line, then seed from a single F5 plant was increased in New Zealand for preliminary yield testing and malt quality evaluations in 2003. M122 was included in the Mississippi Valley Nursery in 2005 (limited locations), 2006 and 2007. M122 was entered into AMBA Pilot Malt evaluations with the 2005 and 2006 crops. M122 is scheduled to be evaluated in plant-scale brewing evaluations with the 2008 crop. From 2002 – 2007, M122 was evaluated for FHB resistance and DON level in numerous FHB nurseries.

FHB Screening and Performance

Screening for resistance that lead to the development of M122 was conducted entirely in inoculated and mist-irrigated field nurseries in Minnesota or in an off-season nursery in China. Disease evaluation in the barley improvement project is a large collaborative effort that involves personnel from the barley breeding project, Ruth Dill-Macky and Char Hollingsworth's pathology programs, and staff at the UM Research and Outreach Centers at Morris and Crookston. Off-season screening in Hangzhou China was done in collaboration with Dr. Bingxin Zhang at Zhejiang University in Hangzhou. Disease screening of M122 began 2002 in replicated F4:5 plots. M122 has been evaluated for resistance to FHB and DON in 25 field experiments conducted between 2002 and 2007. M122 has FHB severity and DON levels that are 47% and 52% of the common variety check Robust, respectively.

Agronomic Performance

In Minnesota State Variety Trials (2004-2007) based on the 12 location mean, M122 was the top yielding variety (Table 1). In on-farm trials conducted in Minnesota, M122 was among the higher yielding lines although had slightly more lodging (Table 2). In North Dakota trials in 2007, M122 yielded better than Ro-

bust but not as high as Lacey, Tradition and Legacy (Table 3).

Graphical Genotype

We assessed M122 and all of the parents in the pedigree (Figure 1) with DArT markers (Wenzl et al., 2006). A total of 884 markers provided a good signal in the assay and 165 of those were polymorphic between the parents of M122. We examined chromosome 2H and 6H since these chromosomes have been implicated in FHB resistance in mapping studies with Chevron, Zheddar 2 and other sources of resistance(de la Peña et al., 1999; Dahleen et al., 2003; Canci et al., 2004). In the two QTL regions that have been mapped and validated in Chevron, M122 appears to carry the susceptible parent allele. We were also able to confirm that M122 does not carry the Zhedar 1 allele in these regions. This indicates that we should be able to improve resistance in M122 via MAS for the Chevron or Zhedar 1 alleles at these QTL.

ACKNOWLEDGEMENT

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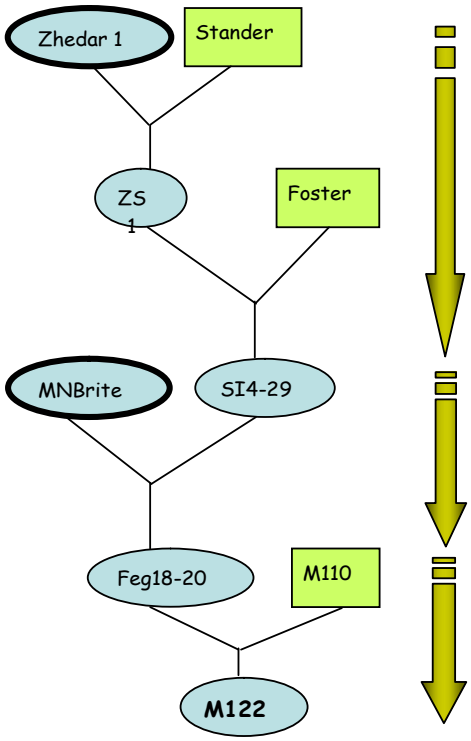

Figure 1. M122 was developed over three breeding cycles and has two resistant sources in its pedigree (Zhedar 1 and MNBrite) MNBrite derives its resistance from Chevron. Lines or varieties in boxes are susceptible to FHB and those in ovals have enhanced resistance to FHB.

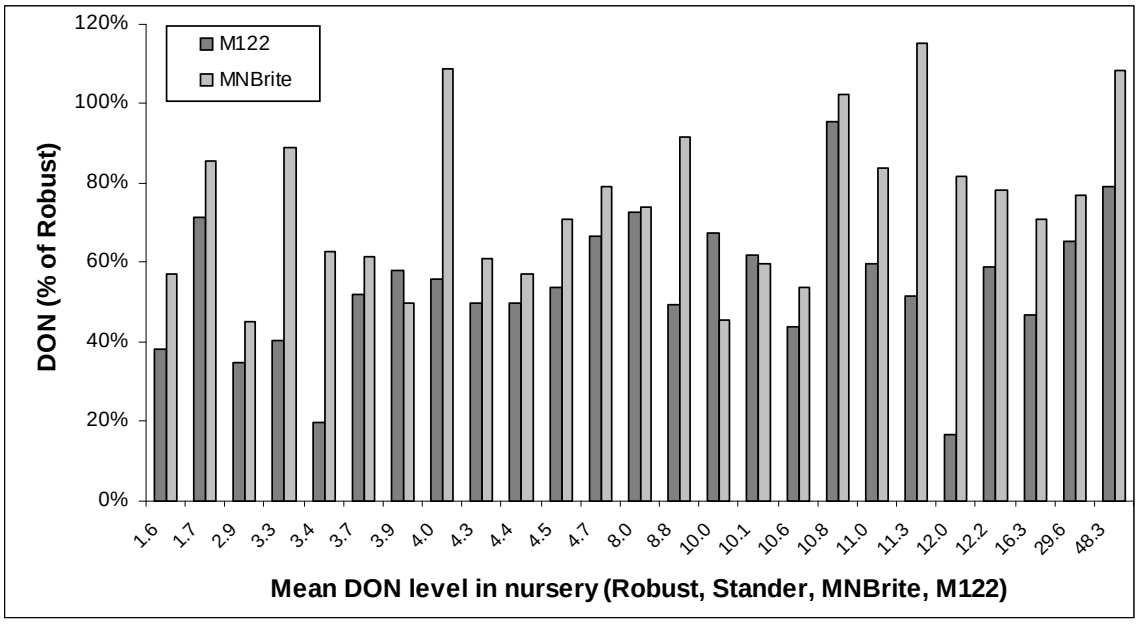

Figure 2. DON levels as a percent of Robust for M122 and MNBrite in 25 trials representing a wide range of disease pressure.

Table 1. Yield comparisons of M122 compared to check varieties 2005-2007 in Minnesota State Variety Trials (mean of 12 trials).

	Crookston		Morris		Stephen		St.Paul		Roseau		
	3 yr.		3 yr.		2 yr.		2 yr.		2 yr.		**12 Sta.Yr.**
Variety	Ave.		Ave.		Ave.		Ave.		Ave.		Ave.
Robust	91		71		84		100		86		85
Stander	94		77		86		98		92		88
MNBrite	92		70		86		93		74		83
Lacey	94		79		99		96		76		88
Drummond	93		72		92		106		84		88
Stellar ND	96		66		94		90		84		85
Legacy	95		79		103		106		85		92
Tradition	96		80		101		95		88		91
Conlon	97		69		96		81		73		83
M122	**103**		**77**		**96**		**113**		**81**		**93**

Table 2. Yield comparisons of M122 compared to check varieties 2006-2007 in Minnesota On-Farm trials (mean of 5 locations).

Variety/Line	Yield (Bu/A)	Test Weight	Protein	Plump	Plant Height	Lodging*
Drummond	107.5	43.9	13.2	72.5	32.6	1.0
Lacey	105.5	45.6	13.6	73.9	31.4	1.0
Legacy	99.1	41.5	13.0	61.8	31.2	1.3
Robust	100.1	44.6	13.8	69.4	32.4	1.8
Stellar	107.2	44.2	12.7	77.1	31.9	1.7
Tradition	101.7	44.5	13.2	74.7	31.7	1.0
M122	**105.5**	**43.4**	**13.3**	**68.7**	**32.6**	**3.2**

Data provided courtesy Jochum Wiersma
*data from 2007 only

Table 3. Yield comparisons of M122 compared to check varieties 2007 in North Dakota State Variety Trials (mean of 5 trials).

Variety / Line	Days to Heading (June)	Plant Height (inches)	Lodging (%)	Stem break (1-5)	Yield (Bu/A)
Robust	25.0	33	33	2.5	75.7
Lacey	24.7	32	30	2.5	84.0
Drummond	24.4	32	25	1.9	78.1
Stellar-ND	24.3	32	26	2.6	76.7
Legacy	26.5	32	34	2.2	82.8
Tradition	25.2	32	31	1.5	80.5
M122	**24.9**	**31**	**31**	**2.3**	**79.0**

Data provided courtesy Richard Horsley

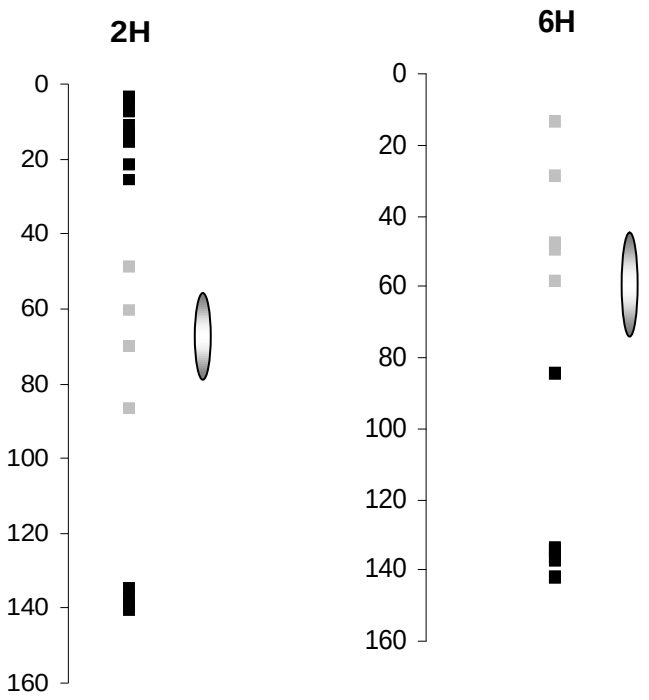

Figure 3. Graphical genotype of DArT markers for M122 on chromosomes 2H and 6H. Numbers are centimorgans. Dark squares indicate that M122 carries the resistant parent (Feg18-20) allele at the marker locus and grey squares indicate M122 carries the susceptible parent allele (M110). Ovals indicate position of QTL for FHB.

REPORT ON THE 2006-07 NORTHERN UNIFORM WINTER WHEAT SCAB NURSERIES (NUWWSN AND PNUWWSN).

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OBJECTIVES

This is a summary of the report on the 2006-2007 Northern Uniform Winter Wheat Scab Nursery (NUWWSN) and the Preliminary Northern Uniform Winter Wheat Scab Nursery (PNUWWSN). A full report will be available on the USWBSI web site prior to the 2007 forum. The objective of these tests is to screen winter wheat genotype adapted to the northern portion of the eastern US for scab resistance.

MATERIAL AND METHODS

The traits assessed and locations that reported data are listed in Table 1. Entries for the NUWWSN came from 13 programs while the PNUWWSN entries came from nine programs (Table 2).

RESULTS

There are eight FHB traits for each trail. Entries with means that were not significantly different than the lowest mean for six or more FHB traits are shown in Tables 3 and 4 (eg entries with at least 6 "l"s). Only three entries had DON < 2 ppm (entries 5, 22, and 26 in the PNUWWSN, see Tables 4 and 5).

Table 1. Traits assessed in the 2006-07 PNUWWSN and NUWWSN tests.

Code	Trait	Description	PNUWWSN Locations*	NUWWSN Locations*
SEV	Disease severity from field tests	% of infected spikelets in an infected head.	IL,KY,MI,MO,ON,VA	IL,KY,MD,MI,MO,NE,NY,OH,ON, VA
INC	Disease incidence	% of heads with at least one infected spikelets	IL,KY,MI,MO,ON,VA	IL,KY,MD,MI,MO,NE,NY,OH,ON, VA
IND	Disease index	IND = (SEVxINC)/100	IL,IN,,KY,MI,MO,OH,ON, VA	IL,IN,KS,KY,MD,MI,MO,NE,NY, OH,ON,RO,VA
KR	Kernel rating	A visual assessment of the percent infected kernels	IL	IL,KS
PSS	Percent scabby seed	Percent of scabby seed by weight	KY,MO	KY,MD,MO,NE,RO
ISK	Composite of head and kernel traits	ISK Index = .3 (Severity) + .3 (Incidence)+.4 (% FDK or PSS)	IL,KY,MO	IL,KY,MD,MO,NE
DON	DON (vomitoxin)	PPM of vomitoxin in grain	IL,KY,VA	IL,KS,KY,MD,NE,NY,VA
GH	Greenhouse severity	Same as SEV except from greenhouse	IL	IL,MO

* ON and RO indicate Ontario Canada, and Romania, respectively

Table 2. Entries in the 2006-07 PNUWWSN and NUWWSN.

NAME	PNUWWSN PEDIGREE
ERNIE	Moderate Resistant Check
TRUMAN	Mod Resistant/Resistant Check
FREEDOM	Moderate Resistant Check
PIONEER 2545	Susceptible Check
P.981129A1--17	92829A1/Patton
P.99751RA1--94	92212/961331/5/92212/4/F201R/3/9547//Patterson/Ernie
P.0128A1-44-1-7	981129A1/981312A1
P.03528A1-10	INW0315/9895C1/3/INW0301/INW0304//981542A1
P.03630A1-18	99751RA1/INW0315//981358C1/97462
SE981089-34	P25R57/SE1694-12
SE91 1492-4	TAISHANG1/GR863//CARDINAL
SE94-1012-25	T814/L880119
M04-4843	KM2186-92/M94*1649//Patton
M04-4788	Pio26R61/Patton
M04*5109	VA94-54-479/Pio2628
M04-4258	Madison/Roane
M04-4393	M94*1586-1/Roane
RCUOGGold.Val	N/A
RCUOGL15	ACRONxSVP/R/FR.#1
RCUOGL4	2737W x EX9806/TF13
RCUOGL17	SVPx ACRON/TF18
RCUOG10/18	ACRON x R/FR. #1
IL03-18438	IL97-3574 / IL95-4162
IL03-15452	IL96-2526 / IL97-3574
IL03-453	Ernie/ IL95-4162
IL01-34159	IL84-2191 / IL87-2834 // IL90-6364 / IL96-24851
IL79-002T-B-B	IL94-6727 / IL96-6472
KY99C-1298-08-1	KY 89C-804-11/KY 89C-225-5//2540
KY99C-1051-03-1	2552/2684//2540
KY99C-1176-02-1	NC96 BGT 6/2552//25R26
MO 050600	Kingraze/Bess 'S'
MO 050699	950016/3/950016//90X54-1-1/MO 91-1009
MO 050917	Truman 'S'/MO 960815
MO 050921	Ernie/Truman 'S'
VA06W-598	P89118RC1-X-9-3-3-1/TRIBUTE//M94-1069
VA06W-557	IL 94-1549/AGS 2000,F8
VA06W-595	P88288C1-6-1-2-8/VAN98W-346//RC STRAT.
VA06W-608	FREEDOM/NC96-13374//RC STRATEG, F7
VA06W-627	IL 94-1549/VA97W-375//COKER 9025, F7
OH03-183-32	15497 /897A
OH03-235-2	OH552 /HOPEWELL
OH03-41-45	IL91-14167 /OH599
OH03-97-6	P88288C1-6-1-2 /OH536
OH03-75-58	HOPEWELL /OH655

NAME	NUWWSN PEDIGREE
ERNIE	Moderate Resistant Check
TRUMAN	Mod Resistant/Resistant Check
FREEDOM	Moderate Resistant Check
PIONEER 2545	Susceptible Check
NY88046-8138	Susquehanna/Harus
NY93285SP-7343	SuMei Comp: 92002
NY93285-7110	SuMei Comp: 92002
NY91028SP-9082	Harus/4/CS/A.Curvif//Glenn/3/Ald/Pvn(M-30)
NY93306-7091	18cc-59/Pio2548

NAME	NUWWSN PEDIGREE (CONTINUED)
KS04HW47-3	X921012-A-7-1/TGO
KS04HW101-3	98HW423/98HW170
P.011035A1-71	981128A1/981477A1//92145E8
P.011036A1-14	981128A1/97462A1//92145E8
P.02444A1-23-6	981129A1/99793RE2//INW0301/92145E8
P.03647A1-1	981477A1/INW0315//981517A1/97462A1
P.04287A1-10	INW0315*2/5/INW0304/4/9346/CS5A//91202/3/INW0301/INW0315
NE01643	NE94482 (=ARA/ABILENE//NE86488)/ND8974
HARRY	NE90614 /NE87612
NI04421	NE96644//PAVON/*3SCOUT66/3/NE94653
NE04653	N97S084//W96-500W/N95L158
NE03490	WI90-540W/NE93554
MD01W233-06-11	MCCORMICK/CHOPTANK
M03-3002	Winter/Winter FHB Bulk
M03-3104	Hopewell / M94-1107
M03-3616	Hopewell / Patton
M03*3877	T8141 / D93-6093
M03*3861	Pio2552 / M94-1407
RCUOG19/21	AC Ron/WEK0609H3xACRon
RCUOGF110202D/4	SD97060 x Ringo Star
RCUOGF111202A/3	Freedom x Harding
RCUOGDHACF1109O2D	SD97060 x Freedom
RCUOGNS984-1	Not available
IL00-8530	IL89-1687 // IL90-6364 / IL93-2489
IL01-11445	IL87-2834-1 / IL95-678
IL01-11934	IL90-6364 / IL94-1909
IL02-19463	Patton / Cardinal // IL96-2550
IL02-23168	IL94-1909 / Pioneer25R26 // IL95-4162
KY97C-0540-01-03	COKER 9803/L910097//2552
KY97C-0554-03-06	VA94-54-549/Roane//Kristy
KY97C-0554-04-05	VA94-54-549/Roane//Kristy
KY97C-0508-01-01A-1	FFR 555W/VA94-52-25//2568
KY97C-0554-03-02	VA94-54-549/Roane//Kristy
MO 040165	Bess RS, earlier
MO 050101	Bess RS, same
MO 050143	Bess RS, shorter
MO 050197	MO 12278/Pioneer 2552
MSU Line E3023	CALEDONIA/NY85020-395
MSU Line E5015	CALEDONIA/PIONEER_25W33
MSU Line E6001	PIONEER_25W60/CJ9306
MSU Line E6002	VA96W-403WS /CJ9403
MSU Line E6003	VA96W-403WS /W14
VA06W-600	P89118RC1-X-9-3-3-1/TRIBUTE//M94-1069,F7
VA06W-602	P89118RC1-X-9-3-3-1/TRIBUTE//M94-1069, F7
VA06W-587	ROANE//OH 552/AGS 2000, F7
VA06W-594	P88288C1-6-1-2-8/VAN98W-346//RC STRATEGY
VA06W-585	Roane / Ernie//McCORMICK,F8
OH02-15978	PATTERSON/HOPEWELL
OH02-12678	FOSTER/HOPEWELL//OH581/OH569
OH02-12686	FOSTER/HOPEWELL//OH581/OH569
OH02-13567	OH581/IN83127E1-24-5-2//5088B-D-32-1/OH601

Table 3. Best entries (top) and worst (bottom) from the 2006-07 NUWWSN. Summary statistics are for all 60 entries.

	NAME	SEV		INC		IND		KR		PSS		ISK		DON		GHSEV		#l	#h
2	TRUMAN	11.2	l	26.7	l	6.1	l	17.5	l	12.6	l	16.6	l	3.9	l	3.4	l	8	0
51	MSU Line E6003	14.0	l	18.3	l	6.1	l	24.4	l	17.4	l	12.6	l	4.8	l	8.4	l	8	0
43	MO 040165	20.2	l	34.7		12.2	l	16.2	l	15.4	l	25.6	l	3.1	l	4.6	l	7	0
45	MO 050143	19.5	l	38.1		12.2	l	20.6	l	7.1	l	26.7	l	3.9	l	6.2	l	7	0
44	MO 050101	21.4	l	40.4		12.7	l	26.2	l	7.3	l	26.3	l	3.9	l	4.5	l	7	0
50	MSU Line E6002	17.5	l	27.1	l	12.9	l	35.6		13.9	l	26.7	l	4.6	l	25.5	l	7	0
59	OH02-12686	20.2	l	35.5		14.6	l	25.0	l	17.1	l	26.0	l	2.1	l	24.0	l	7	0
46	MO 050197	17.7	l	33.7		11.6	l	17.9	l	8.6	l	21.3	l	3.7	l	39.1		6	0
31	RCUOGDHACF 1109O2D	18.3	l	21.4	l	12.1	l	47.1		22.7	h	26.7	l	3.9	l	25.4	l	6	1
49	MSU Line E6001	19.1	l	41.5		14.2	l	33.1		14.4	l	25.0	l	4.6	l	4.9	l	6	0
29	RCUOGF110202D/4	22.9		25.7	l	14.7	l	32.1		11.1	l	23.5	l	1.7	l	10.7	l	6	0
58	OH02-12678	19.4	l	39.5		17.0		15.3	l	11.2	l	25.2	l	4.2	l	13.3	l	6	0
5	NY88046-8138	38.6	h	54.1	h	24.8	h	44.6		28.0	h	48.5	h	7.8		46.6	h	0	6
32	RCUOGNS984-1	38.0	h	61.1	h	28.3	h	45.6		26.6	h	42.6	h	8.2		46.9	h	0	6
24	M03-3104	31.4	h	64.3	h	25.5	h	50.9	h	22.6	h	49.5	h	8.4		59.1	h	0	7
4	PIONEER 2545	39.0	h	62.3	h	30.0	h	49.8	h	18.5	lh	50.3	h	11.6		50.5	h	1	7
10	KS04HW47-3	41.5	h	62.7	h	34.1	h	65.0	h	21.2	h	46.1	h	16.6	h	70.6	h	0	8
	AVERAGE	24.1		40.4		17.0		33.3		16.2		30.5		5.7		26.1		4.8	2.1
	LSD	11.2		14.3		10.0		17.6		14.8		14.1		3.9		28.8			
	R2	0.53		0.73		0.52		0.79		0.48		0.52		0.8		0.71			
	CV	46.7		34.7		66.2		27.6		64.9		32.6		60.9		57.8			
	n	10		10		13		2		5		5				2			

l,h indicate a mean that is not significantly different than the lowest (l) or highest (h) mean in that column

Table 4. Best entries (top) and worst (bottom) from the 2006-07 PNUWWSN. Summary statistics are for all 44 entries.

	NAME	SEV		INC		IND		KR		PSS		ISK		DON		GHSEV		#l	#h
2	TRUMAN	16.9	l	38.4	l	7.3	l	13.0	l	9.4	l	17.8	l	2.2	l	12.3	l	8	0
26	IL01-34159	18.9	l	40.1	l	8.0	l	8.0	l	5.5	l	14.9	l	0.3	l	3.2	l	8	0
31	MO 050600	21.7	l	39.1	l	10.0	l	20.0	l	9.8	l	18.9	l	1.8	l	18.0	l	8	0
34	MO 050921	19.9	l	42.7	l	10.4	l	15.0	l	6.4	l	17.2	l	2.7	l	4.0	l	8	0
27	IL79-002T-B-B	19.4	l	40.3	l	11.1	l	11.0	l	4.0	l	11.8	l	3.4	l	21.1	l	8	0
40	OH03-183-32	19.5	l	46.1	l	12.2	l	15.0	l	7.7	l	19.7	l	2.0	l	32.9	l	8	0
23	IL03-18438	23.1	l	54.7	l	13.3	l	18.0	l	5.1	l	20.2	l	4.1	l	12.5	l	8	0
5	P.981129A1--17	23.7	l	47.0	l	13.4	l	23.0	l	19.2	l	22.4	l	0.4	l	3.8	l	8	0
6	P.99751RA1--94	23.3	l	47.9	l	15.2	l	20.0	l	15.6	l	23.8	l	3.9	l	4.1	l	8	0
37	VA06W-595	25.0	l	49.9	l	16.7	l	25.0	l	13.7	l	22.3	l	3.4	l	37.5	l	8	0
8	P.03528A1-10	16.9	l	43.2	l	9.6	l	30.0		5.6	l	22.2	l	3.6	l	28.8	l	7	0
43	OH03-97-6	18.8	l	47.7	l	10.0	l	33.0		18.6	l	24.1	l	4.0	l	37.8	l	7	0
24	IL03-15452	22.0	l	40.0	l	11.0	l	12.0	l	7.6	l	15.1	l	3.7	l	58.2		7	0
9	P.03630A1-18	21.0	l	50.8	l	11.2	l	27.0		10.5	l	24.0	l	2.6	l	3.4	l	7	0
32	MO 050699	22.6	l	50.8	l	12.6	l	25.0	l	10.6	l	22.5	l	4.9		6.0	l	7	0
22	RCUOG10/18	31.0		48.0	l	16.8	l	11.0	l	6.8	l	16.9	l	1.3	l	17.4	l	7	0
1	ERNIE	22.8	l	44.5	l	12.9	l	33.0		12.7	l	23.6	l	4.9		28.6	l	6	0
44	OH03-75-58	23.6	l	50.0	l	13.3	l	60.0		12.5	l	32.5		3.5	l	40.8	l	6	0
25	IL03-453	24.3	l	57.5		15.8	l	25.0	l	3.0	l	23.5	l	3.7	l	59.3		6	0
33	MO 050917	24.3	l	55.7	l	16.0	l	37.0		6.9	l	31.0		4.4	l	27.5	l	6	0
16	M04-4258	29.4	l	56.8		17.9	l	22.0	l	9.1	l	24.9		4.1	l	4.8	l	6	0
13	M04-4843	32.3		42.5	l	20.4		15.0	l	10.8	l	20.0	l	2.6	l	36.1	l	6	0
4	PIONEER 2545	38.1		69.0	h	30.9	h	47.0		28.6	h	39.8	h	6.5	h			0	5
12	SE94-1012-25	49.6	h	60.0		33.5	h	63.0	h	32.0	h	43.5	h	6.7	h	62.1	h	0	7
10	SE981089-34	47.8	h	64.4	h	36.4	h	80.0	h	42.8	h	48.0	h	9.9	h	38.8	l	1	7
18	RCUOGGoldenValue	53.3	h	80.4	h	38.9	h			40.1	h	46.7	h	8.3	h	85.4	h	0	7
	Average	26.5		50.3		16.3		31.7		13.6		24.9		3.8		36.7		4.8	1.2
	LSD	13.9		17.5		11.6		17.8		19.6		12.8		4.3		38.6			
	R2	0.6		0.6		0.6		0.8		0.7		0.8		0.9		0.4			
	CV	42.5		42.5		65.5		33.4		65.3		29.4		55.3		90.1			
	# Locations	6		6		8		1		2		3		3		1			

l,h indicate a mean that is not significantly different than the lowest (l) or highest (h) mean in that column

Table 5. Summary of results of the 2006-076 PNUWWSN.

	NAME	SEV	INC	IND	KR	PSS	ISK	DON	GHSEV	#l	#h
1	ERNIE	22.8 l	44.5 l	12.9 l	33.0	12.7 l	23.6 l	4.9	28.6 l	6	0
2	TRUMAN	16.9 l	38.4 l	7.3 l	13.0 l	9.4 l	17.8 l	2.2 l	12.3 l	8	0
3	FREEDOM	28.3 l	47.5 l	15.6 l	37.0	12.5 l	26.2	6.0 h	5.0 l	5	1
4	PIONEER 2545	38.1	69.0 h	30.9 h	47.0	28.6 h	39.8 h	6.5 h		0	5
5	P.981129A1--17	23.7 l	47.0 l	13.4 l	23.0 l	19.2 l	22.4 l	0.4 l	3.8 l	8	0
6	P.99751RA1--94	23.3 l	47.9 l	15.2 l	20.0 l	15.6 l	23.8 l	3.9 l	4.1 l	8	0
7	P.0128A1-44-1-7	27.6 l	56.9	17.1 l	40.0	17.1 l	30.3	3.0 l	4.9 l	5	0
8	P.03528A1-10	16.9 l	43.2 l	9.6 l	30.0	5.6 l	22.2 l	3.6 l	28.8 l	7	0
9	P.03630A1-18	21.0 l	50.8 l	11.2 l	27.0	10.5 l	24.0 l	2.6 l	3.4 l	7	0
10	SE981089-34	47.8 h	64.4 h	36.4 h	80.0 h	42.8 h	48.0 h	9.9 h	38.8 l	1	7
11	SE91 1492-4	31.7	55.0 l	21.5	32.0	13.0 l	28.7	4.9	44.5	2	0
12	SE94-1012-25	49.6 h	60.0	33.5 h	63.0 h	32.0 h	43.5 h	6.7 h	62.1 h	0	7
13	M04-4843	32.3	42.5 l	20.4	15.0 l	10.8 l	20.0 l	2.6 l	36.1 l	6	0
14	M04-4788	38.4	42.1 l	16.4 l	37.0	20.1 l	29.2	2.8 l	100.0 h	4	1
15	M04*5109	25.0 l	53.7 l	16.0 l	50.0	4.6 l	25.6	5.8 h	21.5 l	5	1
16	M04-4258	29.4 l	56.8	17.9 l	22.0 l	9.1 l	24.9	4.1 l	4.8 l	6	0
17	M04-4393	24.7 l	59.7	14.7 l	35.0	14.0 l	29.1	5.6 h	65.4 h	3	2
18	RCUOGGoldenValue	53.3 h	80.4 h	38.9 h		40.1 h	46.7 h	8.3 h	85.4 h	0	7
19	RCUOGL15	29.8 l	52.1 l	17.9 l	43.0	23.1	35.6 h	4.6 l	76.2 h	4	2
20	RCUOGL4	37.8	53.8 l	22.6	47.0	13.9 l	31.1	6.9 h	40.3 l	3	1
21	RCUOGL17	31.6	51.8 l	17.7 l	40.0	11.2 l	25.6	4.5 l	66.7 h	4	1
22	RCUOG10/18	31.0	48.0 l	16.8 l	11.0 l	6.8 l	16.9 l	1.3 l	17.4 l	7	0
23	IL03-18438	23.1 l	54.7 l	13.3 l	18.0 l	5.1 l	20.2 l	4.1 l	12.5 l	8	0
24	IL03-15452	22.0 l	40.0 l	11.0 l	12.0 l	7.6 l	15.1 l	3.7 l	58.2	7	0
25	IL03-453	24.3 l	57.5	15.8 l	25.0 l	3.0 l	23.5 l	3.7 l	59.3	6	0
26	IL01-34159	18.9 l	40.1 l	8.0 l	8.0 l	5.5 l	14.9 l	0.3 l	3.2 l	8	0
27	IL79-002T-B-B	19.4 l	40.3 l	11.1 l	11.0 l	4.0 l	11.8 l	3.4 l	21.1 l	8	0
28	KY99C-1298-08-1	30.0 l	50.4 l	19.9	35.0	13.7 l	24.3 l	7.8 h	40.9 l	5	1
29	KY99C-1051-03-1	33.3	67.8 h	25.0	43.0	14.8 l	33.6	9.1 h	52.1	1	2
30	KY99C-1176-02-1	31.7	63.3 h	19.7	37.0	28.8 h	34.4	7.5 h	62.2 h	0	4
31	MO 050600	21.7 l	39.1 l	10.0 l	20.0 l	9.8 l	18.9 l	1.8 l	18.0 l	8	0
32	MO 050699	22.6 l	50.8 l	12.6 l	25.0 l	10.6 l	22.5 l	4.9	6.0 l	7	0
33	MO 050917	24.3 l	55.7 l	16.0 l	37.0	6.9 l	31.0	4.4 l	27.5 l	6	0
34	MO 050921	19.9 l	42.7 l	10.4 l	15.0 l	6.4 l	17.2 l	2.7 l	4.0 l	8	0
35	VA06W-598	28.9 l	51.4 l	22.0	30.0	11.3 l	25.7	4.0 l	60.7	4	0
36	VA06W-557	35.5	62.4	27.1	50.0	26.3 h	30.5	8.4 h	42.4	0	2
37	VA06W-595	25.0 l	49.9 l	16.7 l	25.0 l	13.7 l	22.3 l	3.4 l	37.5 l	8	0
38	VA06W-608	30.9	62.6	17.8 l	35.0	16.0 l	30.8	5.6 h	32.5 l	3	1
39	VA06W-627	35.1	62.7	27.8 h	38.0	19.6 l	34.4	7.2 h	66.0 h	1	3
40	OH03-183-32	19.5 l	46.1 l	12.2 l	15.0 l	7.7 l	19.7 l	2.0 l	32.9 l	8	0
41	OH03-235-2	28.7 l	61.4	19.6	38.0	27.3 h	30.8	6.3 h	61.5 h	1	3
42	OH03-41-45	34.3	56.6	21.6	30.0	14.5 l	29.4	4.7	52.7	1	0
43	OH03-97-6	18.8 l	47.7 l	10.0 l	33.0	18.6 l	24.1 l	4.0 l	37.8 l	7	0
44	OH03-75-58	23.6 l	50.0 l	13.3 l	60.0	12.5 l	32.5	3.5 l	40.8 l	6	0
	Average	28.5	52.7	17.8	31.7	14.9	26.9	4.6	36.7	4.8	1.2
	LSD	13.9	17.5	11.6	17.8	19.6	12.8	4.3	38.6		
	R2	0.6	0.6	0.6	0.8	0.7	0.8	0.9	0.4		
	CV	42.5	42.5	65.5	33.4	65.3	29.4	55.3	90.1		
	# Locations	6	6	8	1	2	3	3	1		

l,h indicate a mean that is not significantly different than the lowest (l) or highest (h) mean in that column

Table 6. Summary of results of the 2006-07 NUWWSN (l,h indicate a mean that is not significantly different than the lowest (l) or highest (h) mean in the col.).

	NAME	SEV	INC	IND	KR	PSS	ISK	DON	GHS	#l	#h
1	ERNIE	27.1	45.7	16.8	40.6	4.3 l	30.7	6.2	18.0 l	2	0
2	TRUMAN	11.2 l	26.7 l	6.1 l	17.5 l	12.6 l	16.6 l	3.9 l	3.4 l	8	0
3	FREEDOM	24.0	47.2	15.2 l	38.4	7.1 l	34.9	5.8	20.7 l	3	0
4	PIONEER 2545	39.0 h	62.3 h	30.0 h	49.8 h	18.5 lh	50.3 h	11.6	50.5 h	1	7
5	NY88046-8138	38.6 h	54.1 h	24.8 h	44.6	28.0 h	48.5 h	7.8	46.6 h	0	6
6	NY93285SP-7343	25.6	33.3	18.4	24.4 l	33.2 h	32.6	4.4 l	22.3 l	3	1
7	NY93285-7110	34.1 h	32.7	15.8 l	26.3 l	24.4 h	29.5	4.2 l	32.8	3	2
8	NY91028SP-9082	29.9	58.7 h	20.4	36.9	25.1 h	42.2 h	11.6	42.6 h	0	4
9	NY93306-7091	24.2	52.2 h	17.1	45.6	31.4 h	37.9 h	10.1	42.6 h	0	4
10	KS04HW47-3	41.5 h	62.7 h	34.1 h	65.0 h	21.2 h	46.1 h	16.6 h	70.6 h	0	8
11	KS04HW101-3	33.8 h	49.2	24.5 h	41.3	31.8 h	35.4	8.8	40.5	0	3
12	P.011035A1-71	29.7	53.9 h	23.4	31.5	21.4 h	43.4 h	4.2 l	22.1 l	2	3
13	P.011036A1-14	32.6 h	46.9	23.0	32.7	18.6 lh	39.0 h	4.9 l	10.6 l	3	3
14	P.02444A1-23-6	24.7	46.2	17.1	17.1 l	16.1 l	39.8 h	3.9 l	19.8 l	4	1
15	P.03647A1-1	23.6	38.7	13.8 l	22.9 l	17.8 l	27.6	3.4 l	10.2 l	5	0
16	P.04287A1-10	30.4 h	51.2 h	23.8	39.4	13.2 l	34.7	6.6	21.8 l	2	2
17	NE01643	22.5	44.1	19.2	41.6	10.9 l	30.3	6.7	21.4 l	2	0
18	HARRY	23.2	51.1 h	17.6	57.1 h	16.4 l	35.9	11.2	14.1 l	2	2
19	NI04421	28.5	56.8 h	20.3	46.6	25.7 h	39.2 h	10.3	19.5 l	1	3
20	NE04653	27.2	51.5 h	20.6	45.6	20.6 h	31.1	8.8	19.7 l	1	2
21	NE03490	29.1	49.8	21.8	41.0	16.8 l	37.5 h	7.2	25.9 l	2	1
22	MD01W233-06-11	27.2	48.3	19.1	25.2 l	24.4 h	31.1	3.7 l	25.2 l	3	1
23	M03-3002	33.1 h	50.8 h	26.0 h	36.5	12.7 l	43.7 h	6.1	35.6	1	4
24	M03-3104	31.4 h	64.3 h	25.5 h	50.9 h	22.6 h	49.5 h	8.4	59.1 h	0	7
25	M03-3616	28.2	45.2	20.5	26.5 l	27.0 h	36.5 h	4.9 l	21.7 l	3	2
26	M03*3877	27.5	61.8 h	23.3	37.9	20.2 h	37.8 h	8.5	26.7 l	1	3
27	M03*3861	27.6	61.6 h	21.9	44.1	11.7 l	34.1	8.7	20.3 l	2	1
28	RCUOG19/21	28.6	44.9	15.8 l	34.0	14.2 l	29.5	7.8	31.2 l	3	0
29	RCUOGF110202D/4	22.9	25.7 l	14.7 l	32.1	11.1 l	23.5 l	1.7 l	10.7 l	6	0
30	RCUOGF111202A/3	35.4 h	58.5 h	21.9	32.5	22.4 h	42.5 h	5.9	25.5 l	1	4
31	RCUOGDHACF1109O2D	18.3 l	21.4 l	12.1 l	47.1	22.7 h	26.7 l	3.9 l	25.4 l	6	1
32	RCUOGNS984-1	38.0 h	61.1 h	28.3 h	45.6	26.6 h	42.6 h	8.2	46.9 h	0	6
33	IL00-8530	30.3 h	48.4	20.0	18.4 l	19.9 h	30.9	3 l	14.7 l	3	2
34	IL01-11445	32.5 h	39.2	22.3	15.9 l	7.0 l	34.0	3.5 l	11.1 l	4	1
35	IL01-11934	24.0	40.2	18.0	15.0 l	11.9 l	26.3 l	3.1 l	11.6 l	5	0
36	IL02-19463	34.7 h	37.4	22.5	21.0 l	9.7 l	34.8	4 l	25.3 l	4	1
37	IL02-23168	28.8	45.3	20.5	24.1 l	19.1 lh	32.4	4 l	31.8 l	4	1
38	KY97C-0540-01-03	35.4 h	57.9 h	25.6 h	30.0	14.5 l	40.3 h	7.1	39.6	1	4
39	KY97C-0554-03-06	23.7	55.7 h	16.0 l	38.8	22.5 h	42.9 h	4.4 l	12.1 l	3	3
40	KY97C-0554-04-05	24.0	48.6	16.8	23.5 l	30.4 h	42.3 h	4.1 l	9.8 l	3	2
41	KY97C-0508-01-01A-1	23.8	42.8	17.9	45.9	27.7 h	31.9	6.4	28.5 l	1	1
42	KY97C-0554-03-02	20.9 l	50.7 h	16.4	22.9 l	15.5 l	29.2	3.5 l	8.8 l	5	1
43	MO 040165	20.2 l	34.7	12.2 l	16.2 l	15.4 l	25.6 l	3.1 l	4.6 l	7	0
44	MO 050101	21.4 l	40.4	12.7 l	26.2 l	7.3 l	26.3 l	3.9 l	4.5 l	7	0
45	MO 050143	19.5 l	38.1	12.2 l	20.6 l	7.1 l	26.7 l	3.9 l	6.2 l	7	0
46	MO 050197	17.7 l	33.7	11.6 l	17.9 l	8.6 l	21.3 l	3.7 l	39.1	6	0
47	MSU Line E3023	35.4 h	50.0 h	22.0	28.1	12.7 l	38.7 h	7.3	50.7 h	1	4
48	MSU Line E5015	27.2	55.3 h	20.8	29.1	23.6 h	33.9	10.7	17.1 l	1	2
49	MSU Line E6001	19.1 l	41.5	14.2 l	33.1	14.4 l	25.0 l	4.6 l	4.9 l	6	0
50	MSU Line E6002	17.5 l	27.1 l	12.9 l	35.6	13.9 l	26.7 l	4.6 l	25.5 l	7	0
51	MSU Line E6003	14.0 l	18.3 l	6.1 l	24.4 l	17.4 l	12.6 l	4.8 l	8.4 l	8	0
52	VA06W-600	30.1	45.1	27.3 h	29.4	22.5 h	42.2 h	4.1 l	39.5	1	3
53	VA06W-602	32.9 h	49.6	27.7 h	19.0 l	18.5 lh	38.1 h	4.1 l	26.8 l	4	4
54	VA06W-587	33.7 h	55.4 h	23.6	20.9 l	23.4 h	43.1 h	4 l	34.4	2	4
55	VA06W-594	25.9	52.3 h	18.3	24.4 l	13.8 l	35.8	4.8 l	18.8 l	4	1
56	VA06W-585	21.2 l	47.4	18.6	9.4 l	15.1 l	34.2	3.5 l	19.1 l	5	0
57	OH02-15978	25.7	41.5	17.5	37.9	14.1 l	31.3	5.7	50.2 h	1	1
58	OH02-12678	19.4 l	39.5	17.0	15.3 l	11.2 l	25.2 l	4.2 l	13.3 l	6	0
59	OH02-12686	20.2 l	35.5	14.6 l	25.0 l	17.1 l	26.0 l	2.1 l	24.0 l	7	0
60	OH02-13567	18.8 l	43.8	12.8 l	28.5	15.8 l	29.6	5.2 l	12.3 l	5	0
	AVERAGE	26.9	46.2	19.2	31.9	18.0	34.1	5.9	24.9	3.1	1.9
	LSD	11.2	14.3	10.0	17.6	14.8	14.1	3.9	28.8		
	R2	0.53	0.73	0.52	0.79	0.48	0.52	0.8	0.71		

DEOXYNIVALENOL ACCUMULATION AND FUSARIUM HEAD BLIGHT SEVERITY IN WINTER WHEAT AFTER SPRAY-INOCULATION WITH MIXTURE OR SINGLE ISOLATES OF *FUSARIUM GRAMINEARUM*.

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OBJECTIVES

1) to compare aggressiveness of four *F. graminearum* isolates and their mixture based on FHB severity and DON accumulation in grain, after spray-inoculation of winter wheat cultivars with known FHB resistance and 2) to test the influence of isolates, wheat cultivar, year and their interactions on level of FHB symptoms and DON accumulation to ensure that there was no isolate-specific resistance from different wheat cultivars with respect to DON accumulation.

INTRODUCTION

Fusarium head blight (FHB), caused by *Fusarium graminearum* (Schwabe), is an important wheat disease. In Canada FHB is caused primarily by *Fusarium graminearum* (Schwabe) [teleomorph: *Gibberella zeae* Schw. (Petch)]. Apart from yield and quality losses, the most serious concern associated with FHB infection is the contamination of the harvested crop with mycotoxins. Deoxynivalenol (DON) is the mycotoxin most commonly recovered from wheat grain in Canada. In Ontario, losses in winter wheat production from yield and quality reductions, were more than $ 100 million CAD in 1996 (Schaafsma , 2002).

Host specific strains have not been demonstrated and resistance to FHB in wheat is generally considered horizontal (race-nonspecific) (Van Euwijk et al., 1995).

When breeding for FHB and DON resistance, a breeder often has to rely on FHB symptoms rather than to perform costly DON analysis. However, FHB severity and DON content are not always well correlated; we have experienced correlations, from as low as r=0.18, to as high as r=0.70, depending on the year and level of FHB resistance in cultivars/lines tested in *F. graminearum* inoculated nurseries (unpublished data). Resistance to FHB infection and DON content may be controlled by independent loci or genes (Somers et al., 2003; Tamburic-Ilincic et al., 2002).

MATERIALS AND METHODS

Winter wheat cultivars were planted in mid October in 2003, 2004 and 2005 at Ridgetown, Ontario, Canada in single rows, 4 m long, spaced 17.8 cm apart, containing 270 seeds each. The experiments were arranged in a 5 x 4 factorial randomized complete block design with four replications. There were four cultivars (FHB susceptible (S) 'AC Ron' and three FHB moderately resistant (MR): 'Wisdom', 'Vienna' and 'AC Morley') and four isolates of *F. graminearum* (DAOM178148, DAOM234041, DAOM234042 and DAOM234043) and a mixture of the four.

Spray-inoculations of each row with a suspension of 50-ml of macroconidia (50,000 spores/ml) of each of four *F. graminearum* isolates (1-4) and their mixture (5) were done, when primary wheat heads were at 50% anthesis for each cultivar (Zadoks Growth Stage, ZGS 65) (Zadoks et al., 1974) using a back-sprayer. The suspension was produced in liquid shake culture using Bilay's medium. *F. graminearum* isolate #1 (DAOM178148) was obtained from Agriculture Canada (ECORC, Ottawa, Ontario, Canada) and was isolated from wheat. Isolates #2 (DAOM234041), 3 (DAOM234042), and 4 (DAOM234043), were isolated from spring barley varieties 'Chapais', 'AC Stephen', and 'C231-0141', respectively in 2000 at Elora Research Station, Guelph, Ontario, Canada by

Tamburic-Ilincic. After isolation the isolates were kept in liquid nitrogen and they have been submitted to the Canadian collection of fungal cultures in Ottawa and are available as DAOM234041, DAOM234042 and DAOM234043. All the plots were misted daily beginning after the first inoculations with about 7.5 mm of water each day. The mist system was engaged until three days after the last cultivar was inoculated. In each year, cultivars were assessed for visual symptoms when the early dough stage (ZGS 83) was reached. Disease levels were estimated as Fusarium head blight severity on a scale of 1-9 where 1 was disease free and 9 was total. The entire grain sample for each cultivar was harvested in mid July in 2004, 2005 and 2006 and finely ground through a ROMER mill (Model 2A, Romer Labs, Inc. Union, MO). Deoxynivalenol (DON) content was estimated from the three replications with highest mean FHB severity using a quantitative fluorometric test-FluoroQuan (Romer® Labs, Inc, Union MO).

PROC UNIVARIATE (SAS Institute Inc., 2003) was used to test ANOVA assumptions to determine if transformations were needed. DON content was transformed by log (x+0.5). The data was analyzed using PROC GLM (SAS Institute Inc., 2003) where year, cultivar, isolate and their interactions were sources of variation.

RESULTS AND DISCUSSION

FHB severity and DON accumulation in this experiment depended on year, isolate and cultivar and the interaction between year and isolate and year and cultivar, but there was no significant interaction between isolates and cultivars (Table 1), suggesting that variation in resistance in these winter wheat cultivars was independent of the variation in aggressiveness of the *F. graminearum* isolates tested. The results from the present study are also in agreement with Mesterhazy (1997) who also reported that DON level in wheat depends on both cultivar and isolate. FHB severity and DON content in winter wheat cultivars after inoculation with four isolates and their mixture during three years are shown in Fig. 1 and Fig. 2.

Aggressiveness of F. graminearum isolates

High isolate x year interaction was reported in the present study (Table 1). Average FHB severity after spray-inoculation with isolates #3 (DAOM234042), #4 (DAOM234043) and the mixture of all (5) four across the cultivars was higher in 2004 than in 2005 or 2006 (Fig. 1a). Average DON accumulation across the cultivars was also higher in 2004 than in 2005 or 2006 (Fig. 1b). In 2004, after spray-inoculation with isolate #4 (DAOM234043) the DON level across the cultivars was significantly higher than after inoculation with the three other isolates or the mixture of all four (Fig. 1b). The summer of 2005 and 2006 was hot and dry and was not favorable to DON accumulation in winter wheat in Ontario. DON content measured in a survey of winter wheat in Ontario in 2005 and 2006 ranged from 0.1 ppm to 1.0 ppm and from 0.1 ppm to 0.3 ppm, respectively, which was much lower than found in a similar survey in 2004, where the highest level of DON was 4.9 ppm (Tamburic-Ilincic et al., 2006).

FHB susceptibility and DON accumulation in winter wheat cultivars

In 2004 and 2005, the average FHB severity was the highest for the FHB-susceptible cv. 'AC Ron' and significantly higher than three FHB MR cultivars (Fig. 2a). In 2006, cv. 'AC Ron' had significantly higher FHB severity than 'AC Morley' and 'Wisdom' (Fig. 2a).

'AC Ron' had the highest average DON level after inoculation in all three years; while the relative ranking of the three MR cultivars was slightly different in each year (Fig. 2b).

In 2004, 'Wisdom' had the lowest DON level (Fig. 2b); 'Wisdom' and 'Vienna' accumulated the least DON in 2005 (Fig. 2b). There was no significant difference in DON accumulation among MR cultivars in 2006 (Fig. 2b).

In conclusion, it is cautiously suggest that perhaps the selection of isolates may be less important when

screening for FHB symptoms than when screening for the propensity to accumulate DON. Relatively similar FHB ratings between years resulted in very different DON levels between years. Although inconclusive the data suggest that a mixture of isolates might contribute some stability in results over years. This possibility would need further study with more cultivars under more environments and years. It seems from the results that high DON-producing isolates would allow for differentiation among cultivars which differ in their propensity to produce DON, and are recommended for screening wheat cultivars for simultaneous FHB resistance and DON accumulation. A highly pathogenic isolates of *F. graminearum*, that simultaneously produce a high level of FHB symptoms and DON, or a mixture of isolates is recommended for breeding programs targeting FHB resistance and reduced DON production in winter wheat.

ACKNOWLEDGEMENTS

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Table 1. Analysis of variance for the effect of year, *Fusarium graminearum* isolate, winter wheat cultivar, and interactions on: a) FHB severity (1-9) and b) transformed (log (x+0.5)) deoxynivalenol (DON) content (ppm) in winter wheat. Ridgetown, ON, 2003-2004, 2004-2005 and 2005-2006.

a) FHB severity (1-9)

Source	df	Mean square	*F*	*P>F*
Year	2	6.254	19.16	<.0001
Isolate	4	4.235	12.98	<.0001
Cultivar	3	1.249	3.83	0.0098
Year*Isolate	8	4.192	12.84	<.0001
Year*Cultivar	6	3.249	9.95	<.0001
Isolate*Cultivar	12	0.474	1.45	0.1459
Year*Isolate*Cultivar	24	0.547	1.68	0.0310

b) DON accumulation

Source	df	Mean square	*F*	*P>F*
Year	2	90.034	427.24	<.0001
Isolate	4	1.584	7.52	<.0001
Cultivar	3	5.027	23.86	<.0001
Year*Isolate	8	1.710	8.11	<.0001
Year*Cultivar	6	2.125	10.09	<.0001
Isolate*Cultivar	12	0.202	0.96	0.4912
Year*Isolate*Cultivar	24	0.195	0.93	0.5671

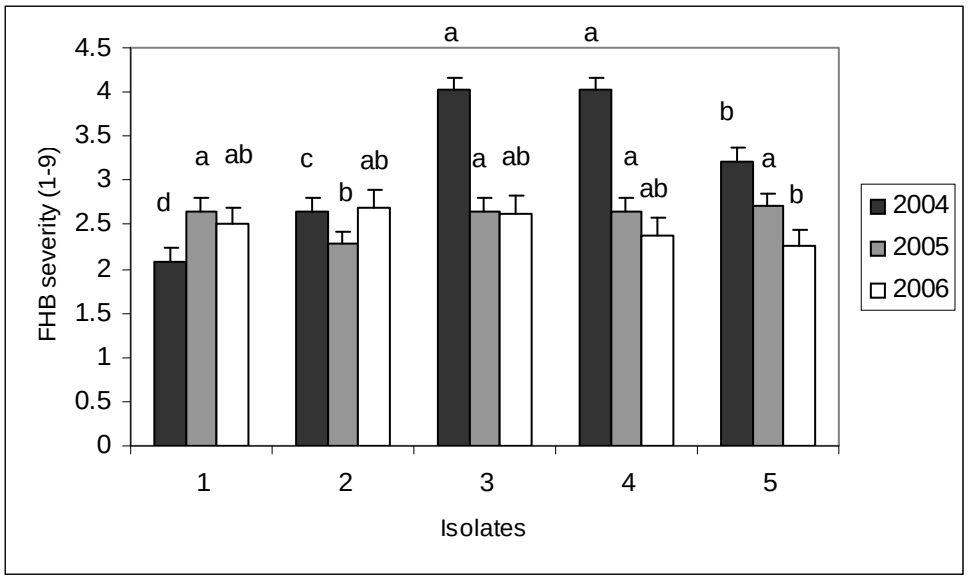

Figure 1 a

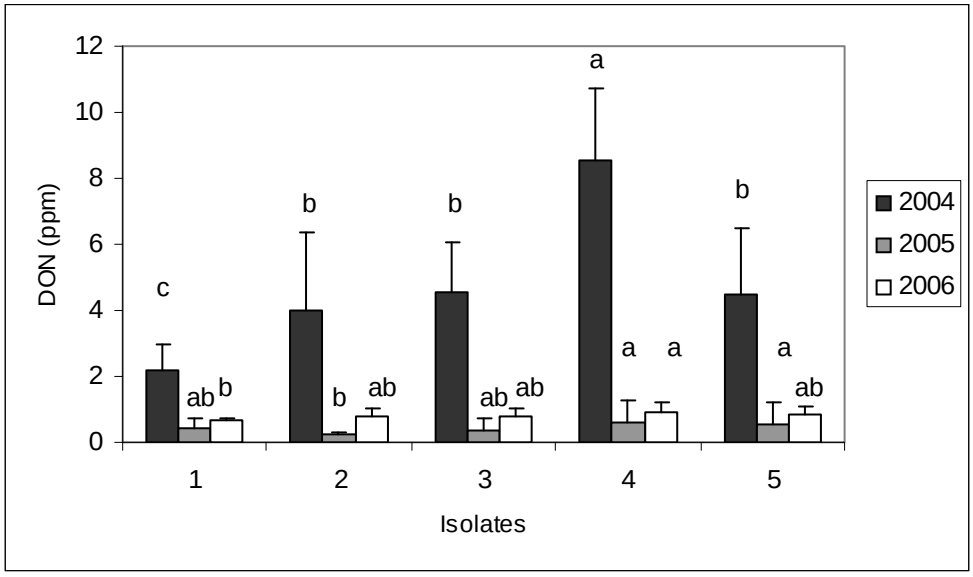

Figure 1 b

Figure. 1. The effect of *Fusarium graminearum* isolates (1-4) and their mixture (5) (± SE) on: a) FHB severity (1-9) and b) deoxynivalenol (DON) content (ppm) across winter wheat cultivars. Ridgetown, ON. Means within years followed by the same letter are not different according to Fisher's protected least significant difference test (P= 0.05).

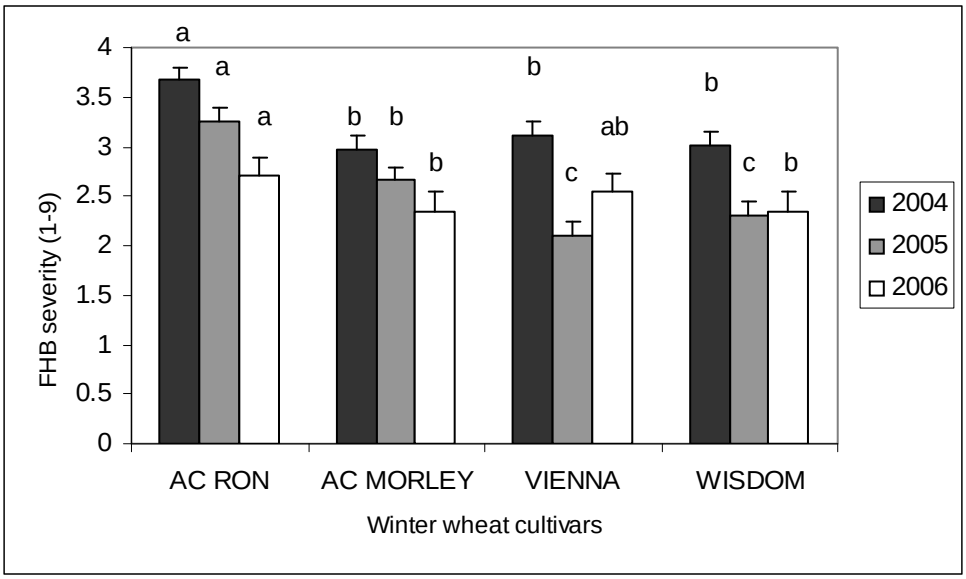

Figure 2 a

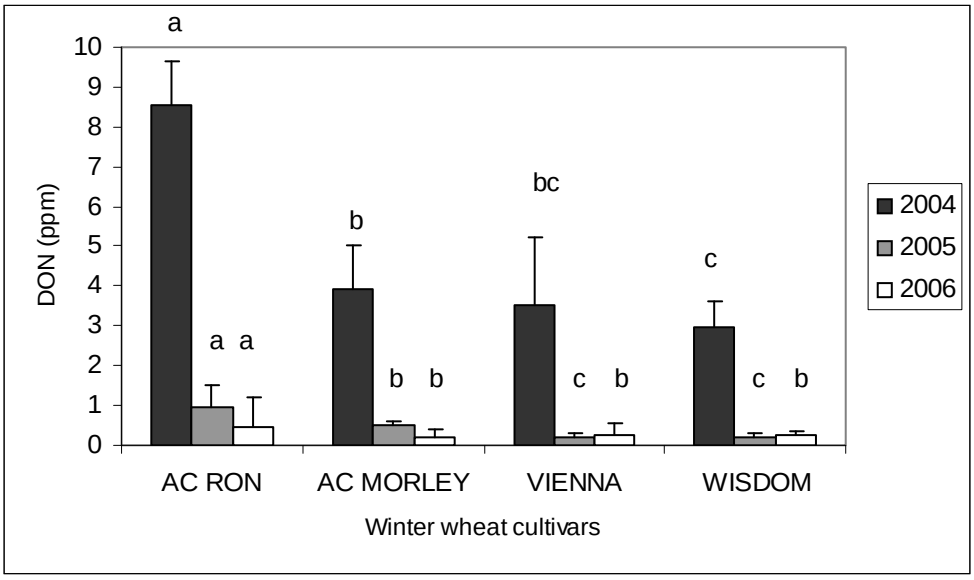

Figure 2 b

Figure. 2. The effect of winter wheat cultivars ('AC RON', 'AC Morley', 'Vienna' and 'Wisdom') on: a) FHB severity (1-9) and b) deoxynivalenol (DON) content (ppm) (± SE) after spray-inoculation across *Fusarium graminearum* isolates. Ridgetown, ON. Means within years followed by the same letter are not different according to Fisher's protected least significant difference test (P= 0.05).

THE EFFECT OF *FUSARIUM SOLANI* METABOLITES ON PEROXIDASE ACTIVITY IN POTATO.

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ABSTRACT

The fungus *Fusarium solani* cause the dry rot of potato tubers. The disease advances in tubers during storage. It leads to great losses of harvest. The reveal of qualities of host- pathogen interaction and natural biochemical mechanisms of tolerance is an actual scientific problem. It is known that so-called "oxidative burst" is one from early defense reaction of plant cells to attack of pathogens. The formation reactive oxygen species such as hydrogen peroxide, superoxide radicals etc. to result from "oxidative burst". The many enzymes involved into cascade of oxidative reaction. The peroxidase (POD, EC.1.11.1.7) is one antioxidant enzymes which play important role in defense reaction. POD to oxidize substances with use hydrogen peroxide or molecular oxygen and participate in biosynthesis of toxic compounds for pathogen and processes of lignification or suberinization for forming barriers against pathogens. The various POD activate on different stages of protective mechanism. The role of soluble and cell-wall bound forms of POD in protective reactions of host-pathogen system "Solanum tuberosum – *Fusarium solani*" was studied. The changes of enzyme activity in tubers and *in vitro* cultivated cells of potato by additional different fractions of fungal metabolites were investigated. Changes showed response reactions of cells depended from plant's sensitivity, localization of enzyme and chemical composition of fungal isolates. The effect of proteins and non-proteins isolates of cultural filtrate and mycelium on the disks of tubers was studied. The proteins of cultural filtrate stimulated rapid induction of soluble (in 2-2,5 time) and especially cell-wall bound (in 5,5-8 time) forms of POD as in tolerance as in sensitivity sorts. The non-proteins isolates also insignificantly induced soluble PODs (in 1,8-2 time). Induction of cell-wall bound PODs depend from resistible of plant. In tolerance sort was more significant increase (in 4-6 time) of enzyme activity than in sensitivity (in 1,2-1,5 time). The influence of metabolites of cultural filtrate and mycelium on POD activity in suspension cells was studied. The metabolites of cultural filtrate induced activity only of soluble forms POD. The magnitude of induction depended from filtrate concentration. The low concentrations (dilution 1:50) increased POD in sensitivity, but high (dilution 1:5) – in resistible cells. Metabolites of mycelium along with cytotoxic effect to suspension cells caused exchanges as soluble as cell-wall bound PODs. The metabolites of ethanol fraction of mycelium induced rapid increase of soluble forms in 2 time, but metabolites of acetone and water-soluble fractions increased activities only cell-wall bound PODs in 2-3 and 3-5 time accordingly and independently from initial sensitivity of cells to fungus.

SEARCHING FOR NEW SOURCES OF FHB RESISTANCE IN THE RELATIVES OF WHEAT.

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ABSTRACT

Epidemics of Fusarium head blight (FHB), caused mainly by *Fusarium graminearum* Schwabe, have threatened the production of bread wheat (*Triticum aestivum* L.) and durum wheat (*T. turgidum* L., subsp. *durum*) in North America in recent years. Deployment of FHB-resistant cultivars has been considered the most efficient and cost-effective strategy to combat this disease. However, only limited sources of FHB resistance are currently available, especially in durum, which makes the development of FHB-resistant varieties difficult. In an effort to identify novel sources of FHB resistance, we have screened about 900 accessions of wheat relative species and their derived lines for Type II resistance in greenhouse and in field nurseries (Fargo and Langdon, ND) during the past four years. A number of accessions and derived lines of the relative species have exhibited resistance or moderate resistance to FHB in these screening experiments. Resistant lines include 16 *T. carthlicum* and 20 *T. dicoccum* accessions, two synthetic hexaploid wheat lines, one *T. timopheevi*-derived hexaploid line, one 'Fukuhokomuji'-*Elymus rectisetus* disomic addition line, and two 'Chinese Spring'- *Thinopyrum junceum* disomic addition lines. These materials likely represent new sources of FHB resistance for durum and bread wheat. The resistant tetraploid wheat accessions are currently utilized for developing durum wheat germplasm resistant to FHB. Introgression of FHB resistance from the derived lines of wild species is currently in progress.

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COMPARISON OF BARLEY SEED PROTEOMIC PROFILES ASSOCIATED WITH FUSARIUM HEAD BLIGHT REACTION.

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ABSTRACT

Plants have evolved a complex array of chemical and enzymatic defenses expressed both constitutive and inducible, that influence pathogenesis and disease resistance. To better understand the constitutive molecular mechanisms associated with the differential reactions to FHB, mature seed of barley genotypes and sister lines differing in FHB reactions were subject to proteome analysis using two dimensional gel electrophoresis (2DE). A total of 38 protein spots were correlated with FHB resistance and susceptibility. Several of these proteins were previously identified as important for disease resistance or as pathogen related proteins (PR-protein). Aldehyde dehydrogenase (BIS1) upregulated in resistant lines has been previously identified as a PR-protein upregulated in barley during stem infection. Protein spot #155 also upregulated in resistant sister lines, was identified as aconitate hydratase. Aconitate hydratase is important for Pto-mediated plant defense response. Putative NADP Malic enzyme found elevated in resistant barley lines, has been previously identified in EST libraries prepared from the barley lemma, palea and FHB infected spikes. Alpha amylase inhibitor (BDAI-1) also upregulated in resistant lines is also known to have antifungal activities. Several proteins were identified to ESTs or proteins, for which their functions were unknown. Selecting for constitutively expressed proteins or enzyme activities that correlate with enhanced FHB resistance may allow the development of new *in vitro* tissue test that could be used to select for improved FHB resistance in new barley lines.

NOVEL FUSARIUM HEAD BLIGHT RESISTANCE IN *TRITICUM AESTIVUM* REVEALED BY HAPLOTYPING WITH DNA MARKERS ASSOCIATED WITH A KNOWN RESISTANCE QTL.

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ABSTRACT

Fusarium head blight (FHB) is a devastating disease of wheat (*Tritcium aestivum* L.) that causes reduced grain yield, and the fungus, *Fusarium graminearum*, produces a mycotoxin, deoxynivalenol (DON), that renders infected wheat grain to be unfit for food or feed. Several sources of resistance in wheat and certain related grass species have been identified. However, research to date indicates that several resistance genes need to be combined to result in highly effective resistance. Thus, there is need to identify additional novel sources of resistance. Xing 117, a wheat line obtained from China that has FHB resistance, the seven other wheat lines Ning 7840, Frontana, Wangshuibai, Arina, Renan, F201R, and Chokwang, all previously identified as having partial FHB resistance, as well as P9762 and P9774 that are susceptible to FHB, were haplotyped at marker loci that are associated with FHB resistance of the seven partially resistant wheat lines. Xing 117 was polymorphic at all previously identified marker loci of the seven wheat lines with partial FHB resistance, as well as these marker loci of P9762 and P9774, indicating that the FHB resistance of Xing 117 is likely novel compared to the resistances of the seven wheat lines in this study.

U.S. Wheat & Barley

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