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Photos on the cover starting at the top left and moving clockwise:

- Screen capture of home page of UWWBSI's Website for the Don Testing Labs - http://www.uky.edu/Ag/Wheat/wheat_breeding/USWBSI/DON_submission.html
- Jun Yingyang, Lab Assistant in NDSU's DON Testing Lab - Barley, preparing samples for derivatization; photo submitted by Paul Schwarz, North Dakota State University (NDSU)
- Diane Reaver (left) and Patty Gundrum (right), VA Tech DON Testing Lab, analyzing DON samples using a GC/MS; photo submitted by David Schmale, Virginia Polytechnic Institute and State University (VA Tech)
- University of Minnesota DON Testing Lab computer showing DON quantification using GCMS solution software (version 2.5 Su1); photo submitted by Yanhong Don. University of Minnesota
- Photo demonstrating initial grain sample processing and weighing: Center - Kelly Benson, Chemist in NDSU-Veterinary Diagnostic Lab (VDL), sorting through samples coming into the lab; Right - Todd Singer, NDSU-VDL technician, weighing out samples; and Left - Quincey Faul, NDSU-VDL work study student, placing analyzed samples into a hazardous waste container in the chemical fume hood. Photo submitted by Michelle Mostrom, NDSU-Veterinary Diagnostic Laboratory.
- Tyler Potts, Student Lab Technician in University of Minnesota's DON Testing lab, grinding grain samples using a Stein Mills (mode M-2) grinder under an exhaust hood; photo submitted by Yanghong Dong, DON Testing Laboratory, University of Minnesota

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PLENARY SESSION

Chairperson: Dave Van Sanford

OVERVIEW OF THE 2009 WHEAT CROP QUALITY WITH RESPECT TO VOMITOXIN IMPACT

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ABSTRACT

CII annually surveys three classes of wheat (HRS, HRW and SRW) in the United States for numerous quality factors. Also included in this survey is vomitoxin testing basis historical area composites.

Within this crop year, particularly in the Mid-Atlantic States, areas of concern have been identified. A brief overview of the seventeen states covered in this year's crop survey will identify these pockets of concern.

In the Soft Red Winter area, increased testing was requested by the clients of CII to help discover these patterns. High vomitoxins levels (>5.0 PPM) were identified in Ohio and Maryland. (Pennsylvania was not included in this survey, but the laboratory has some isolated samples from PA that ranged much higher than 5.0 PPM). A general review of the Soft Red Winter area illustrates a very widespread vomitoxin issue that was present in all states surveyed.

In the Hard Red Winter area, testing was continued on a composite basis with little change anticipated in the usual troublesome areas. As expected, increased vomitoxin was identified in eastern Kansas and South Dakota.

Hard Red Spring wheat testing identified only a few pockets, predominantly again in South Dakota.

In addition to providing the data in the form of graphics (maps) and some numerical data tables, the difficulties with collection, interpretation and sampling will be brought to light within this overview.

Quality issues that arise from the scab damage such as weak gluten and poor color bring about necessary creativity from both the buyer and miller to maintain the flour quality level that the end user has come to expect. The method to achieve that goal on the part of the buyer is appropriate purchases to be able to blend off and clean out the damaged kernels. The miller must monitor cleaning house procedures to eliminate the scab kernels. However, difficulty will persist on the scab kernels that are not as shriveled as the majority, thus carrying through the vomitoxin. The yield loss associated with the light and shriveled kernels illustrates the dramatic and persistent economic impact of the *Fusarium* problem.

SESSION 1:

FOOD SAFETY, TOXICOLOGY AND UTILIZATION OF MYCOTOXIN-CONTAMINATED GRAIN

Co-Chairpersons: Jim Pestka and David Schmale

RISK ASSESSMENT AND BIOMARKERS FOR DEOXYNIVALENOL

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ABSTRACT

Deoxynivalenol (DON) is the most commonly detected trichothecene fungal metabolite in cereal grains and processed food globally. Upon exposure, DON is rapidly distributed in animal tissues and induces proinflammatory cytokines (< 2 h). Longer term (> 2 wk) DON exposure reduces weight gain in many species through a poorly understood mechanism, thus creating uncertainties in human safety assessment and DON regulatory limits. Understanding of mechanism(s) and identification of biomarker(s) will increase the precision of DON regulatory limits. We hypothesized that DON-induced weight reduction is preceded by a dysregulation of proteins in the growth hormone pathway. Models of acute and chronic DON exposure were used to test this hypothesis. The results indicate that DON acutely induces hepatic suppressors of cytokine signaling (SOCS). The effect of SOCS on growth pathway was evaluated by measuring forms of insulin-like growth factor acid-labile subunit (IGFALS), a growth-related protein. Acute DON exposure (0.1-12.5 mg/kg) impaired growth hormone-induced IGFALS mRNA by 60-80%. Furthermore, dietary DON (20 ppm for 8 wk) suppressed IGFALS mRNA (65%), circulating IGFALS (66%), weight gain and elevated plasma DON (≤ 63 ng/ml). Circulating insulin-like growth factor 1 (a binding partner of IGFALS) was equally suppressed by dietary DON. Together, these data indicate that dietary DON consistently suppresses IGFALS in mice, while elevating plasma DON. Therefore, circulating IGFALS is a potential biomarker for DON's effect. Validation of this biomarker in human population will enhance epidemiological surveillance and might increase the precision of human risk assessment.

MULTI-YEAR SURVEYS ON FUSARIUM HEAD BLIGHT AND MYCOTOXINS IN COMMERCIAL WHEAT GRAINS FROM RIO GRANDE DO SUL STATE, BRAZIL

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ABSTRACT

Spring wheat is grown mainly in the states of Paraná and Rio Grande do Sul (RS), the major producers in Southern Brazil. In spite of the current significance of this disease in Brazil, systematic epidemiological surveys on FHB epidemics and mycotoxins on commercial grain are scarce. Since 2006 we collected extensive field and laboratory data to monitor and assess FHB disease-related parameters and mycotoxins in wheat growing regions of RS state. Assessment of kernel damage on 139 samples originating from 54 different municipalities surveyed in the 2006-2008 period revealed that over 90% of the samples presented some physical damage by FHB. Sixty-five samples taken from the same period were analyzed for the occurrence and levels of deoxynivalenol (DON), nivalenol (NIV) and zearalenone (ZEA) quantified using LC-MS/MS. DON was found in all but one sample. NIV and ZEA were present in 83% and 40% of the samples, respectively. DON levels averaged 0.55 mg/kg (ppm) with most samples showing DON<0.5 mg/kg (44/65) and 10 samples exceeding 1 mg/kg. NIV levels averaged 0.3 mg/kg with 10 samples in the range of 0.5 to 1 mg/kg. ZEA concentration was in trace levels. While mean DON varied among years and regions mean NIV was more consistent among the years. In our field surveys on FHB, 62 fields arbitrarily selected in the main growing regions were assessed for incidence in 2008 growing season. Mean incidence was lower than 10% with several fields showing trace levels. In the current 2009 season, both FHB incidence and severity were assessed and our preliminary results for 36 fields showed disease incidence averaging 41% (10-90%). However, severity levels averaged approximately 2%. The widespread occurrence of DON, but specially NIV, in commercial grain from the surveyed regions supports our previous molecular evidence of a toxin potential for a less predominant *F. graminearum* population spread across the state. Moreover, the monitoring of NIV should be regularly performed given its relatively high levels found and toxicological implications to animal and human health. Our further epidemiological analysis of survey data on several disease/toxin-related parameters will help to better determine local and regional factors associated to FHB and mycotoxin contamination in southern Brazil.

COMPARISON OF WEIGHT GAIN AND PLASMA INSULIN-LIKE GROWTH FACTOR ACID LABILE UNIT (IGFALS) SUPPRESSION FOR DETERMINING THE NO-OBSERVED EFFECT LEVEL (NOAEL) IN MICE FED DEOXYNIVALENOL

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ABSTRACT

Deoxynivalenol (DON) is a trichothecene mycotoxin that contaminates wheat and barley during Fusarium head blight in temperate regions throughout the world. The potential for adverse health effects in persons consuming DON is a public health concern, particularly with respect to growing children. The current no observed adverse effect level (NOAEL) for DON exposure in mice is 100 µg/ kg body weight / day which is based on impaired growth observed in a 2-year Canadian feeding study. This level has been used as a basis for current European Union (EU) regulations.

Recently, our laboratory discovered that acute DON exposure decreases the mRNA expression of insulin-like growth factor 1's (IGF1) binding partner, acid labile subunit (IGFALS). Furthermore, upon subchronic exposure to DON, mice exhibit decreased IGF-1 and IGFALS suggesting these to be sensitive biomarkers for DON adverse health effects. These hormones are critical for growth and likely will impact weight gain.

In this study, we compared the NOAELs for weight gain and IGFALS suppression in weanling (4-week old) B6C3F1 mice fed DON subchronically (9 wk) in a pelleted diet at concentrations of 0, 0.4, 0.7, 1.9, 3.6, and 5.8 ppm. To determine the NOAEL, average daily feed consumption was measured (2.7 g). Then the dose with no adverse effect was multiplied by the amount eaten per day, and divided by the average weight of mice (0.02 kg) to yield the NOAEL reported as "DON consumed per kg bw/day".

Mice fed DON did not exhibit weight gain inhibition at 3.6 ppm which is equivalent to a NOAEL of 490 µg/kg bw/ day. Using IGFALS as a biomarker of expression, the NOAEL was 260 µg/ kg bw/ day based on no significant decrease in plasma IGFALS after at 9 weeks at 1.9 ppm. Like IGFALS, there was also a trend toward suppression of IGF-1 by DON at 5.8 and 3.6 ppm but not at 1.9 ppm. The results suggest that IGFALS suppression is a predictive biomarker of weight gain inhibition. In both cases, the observed NOAELS in this subchronic study were higher than that of the aforementioned 2 year Canadian chronic study (ie. 100 µg/ kg bw/ day) currently employed to establish current EU regulations. Future studies will determine how food matrix (pellet vs. powder), exposure duration, gender, strain and age affect NOAEL determination using IGFALS as a biomarker of DON toxicity.

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FUSARIUM MYCOTOXIN CONCENTRATIONS IN THE STRAW, CHAFF, AND GRAIN OF SOFT RED WINTER WHEATS EXPRESSING A RANGE OF RESISTANCE TO FUSARIUM HEAD BLIGHT

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ABSTRACT

Fusarium graminearum Schwabe (teleomorph *Gibberella zeae* (Schwein.), which causes Fusarium head blight (FHB) or scab, is an increasingly important problem in the north-central region of the United States. During years of heavy FHB infection, veterinary diagnostic laboratories have, on occasion, unexpectedly found unusually high concentrations of both deoxynivalenol (DON) and zearalenone (ZEA) in wheat straw, as well as FHB-infected grain. Because swine are sensitive to concentrations of DON and ZEA as low as 1 $\mu g\ g^{-1}$, these mycotoxins are particularly problematic when wheat straw is used for bedding in less than optimal production settings. Similarly, where straw is used as a source of roughage for cattle in total mixed rations (TMRs), concentrations of mycotoxins, to which ruminants are fairly resistant, might be found at clinically relevant, high concentrations in straw. Although there is a large body of literature on mycotoxin content in FHB infected grain, little is known of both the range and concentrations of mycotoxins in wheat straw. As such, a preliminary study was undertaken to investigate mycotoxin concentrations in the straw, chaff, and grain of the 60 soft red winter genotypes comprising the 2008 Uniform Northern Fusarium Head Blight Nursery. The nursery was grown at the Bradford Research and Extension Center near Columbia MO and spray-inoculated at 75% heading with a macroconidial suspension of *F. graminearum* concentrated to 50,000 macroconidia/mL. It was maintained under overhead mist irrigation through heading and evaluated for incidence and severity 18 - 21 d after inoculation. The field FHB index for each genotype was determined as incidence x severity expressed as a percentage. At harvest, a 3-meter long sample of each genotype was cut at ground level, dried and separated in three components including grain, chaff, and straw. Samples were sent to North Dakota State University where they were analyzed by GC/MS in the SIM mode for DON, 15-ADON, zearalenol, and ZEA. FHBI for the 60 genotypes evaluated ranged from a low of 9.9% to a high of 61.9% and averaged 35.7%. Significant concentrations of DON and ZEA were detected in the grain, chaff and straw while 15-ADON and zearalenol concentrations were negligible (<0.5 $\mu g\ g^{-1}$) in the grain but higher levels were present in the chaff and straw. 3-ADON and nivalenol were not detected. In the grain, DON and ZEA concentrations were relatively low averaging 4.7 and 4.4 $\mu g\ g^{-1}$, respectively, across the 60 genotypes and were significantly correlated with resistance level (r=0.56 and r=0.51 for DON and ZEA, respectively). In chaff samples, both mycotoxins were present at higher concentrations averaging 16.9 $\mu g\ g^{-1}$ (DON) and 42.9 $\mu g\ g^{-1}$ (ZEA) and were poorly correlated with resistance (r=0.32 and r=0.37; DON and ZEA, respectively). In the straw, DON concentrations were again low, averaging 3.5 $\mu g\ g^{-1}$ over entries but, surprisingly, the ZEA concentrations were extraordinarily high (55.5 $\mu g\ g^{-1}$) and the correlation with resistance was much lower for both mycotoxins (r=0.21), indicating that *Fusarium* mycotoxin concentrations in the straw could not be predicted by the resistance level of the cultivar. These findings are, potentially, very clinically relevant to livestock producers. The need for a more rigorous, replicated study over different environments is warranted.

FORMATION OF THE BIOMARKER ZEARALENONE-4-O-GLUCURONIDE BY HUMAN UDP-GLUCURONOSYLTRANSFERASES AND ENGINEERED YEAST

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ABSTRACT

Various xenobiotic compounds (medicinal drugs, plant and fungal secondary metabolites, including *Fusarium* mycotoxins) are efficiently detoxified in animals and humans by formation and excretion of toxin-conjugates. The UDP-glucuronosyltransferases (UGTs) are the most relevant enzymes of phase II detoxification (conjugate formation). The main goal of the project was to test whether it is possible to engineer this detoxification pathway in *Saccharomyces cerevisiae* (baker's yeast), where it does not naturally occur. The toxicologically relevant *Fusarium* mycotoxin zearalenone served as model substances in this study.

A ZON-4-O-glucuronide standard was synthesized via the Koenigs Knorr procedure using a bromosugar activated by silver carbonate. LC-MS techniques to detect the conjugate were developed.

Since the catalytic domain of mammalian UDP-glucuronosyltransferases is located in the lumen of the endoplasmatic reticulum, and yeast does not contain the UGT co-substrat UDP-glucuronic acid (UDP-GlcUA), a gene encoding a UDP-glucose dehydrogenase (UGDH) for synthesis of UDP-GlcUA and in addition a gene encoding a membrane transporter allowing UDP-GlcUA to enter the ER were introduced. Such yeast strains additionally expressing different cDNAs of human UDP-glucuronosyltransferases formed predominantly zearalenone and zearalenol glucosides instead of the desired glucuronides, despite the strong overexpression of UGDH, which led to depletion of UDP-Glc in favour of UDP-GlcUA. Therefore, relocation of the enzyme into the cytosol as N-terminal GST-fusion protein was also attempted.

Only small amounts of glucuronides of the tested mycotoxins were found in the supernatant of toxin treated yeast cultures. It was therefore not possible to utilize the engineered yeasts as cell factory for production of mycotoxin conjugates, as previously demonstrated for ZON and ZOL-glucosides. A problem of the yeast heterologous expression system is that the product ethyl-glucuronide is formed by the expressed UDP-glucuronosyltransferases in the presence of the competitive inhibitor ethanol. The enzymes expressed in yeast or in insect cells are useful to test *in vitro* which of the multiple UGT isoforms are most relevant for inactivation of ZON.

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A TARGET FOR THE *FUSARIUM* MYCOTOXIN ZEARALENONE IN PLANTS: INHIBITION OF HSP90 ATPASE

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ABSTRACT

The *Fusarium* mycotoxin zearalenone (ZON) is well known for its strong estrogenic activity in animals. Plants do not have an estrogen receptor, and it is an open question whether the *Fusarium* metabolite ZON has a biological role in plant-pathogen interaction. We have identified a prominent target for zearalenone: Hsp90. Zearalenone and more strongly beta-zearalenol (bZOL) inhibit ATPase activity of purified yeast Hsp90 (ScHsp82p) *in vitro*. Hsp90 is necessary for the stability of many client proteins such as signal transduction components, and has been shown to be essential for plant defense [1]. Microarray experiments of ZON treated *Arabidopsis* plants showed marked changes in gene expression. ZON is able to suppress the root phenotype of a mutant with a defect in a cell wall biosynthetic gene, which leads to constitutive activation of ethylene responsive genes. Many genes encoding proteins with a role in cell wall remodeling and especially peroxidases were repressed by ZON. In contrast, small heat shock proteins and *AtHSP90-1* were upregulated by ZON treatment of *Arabidopsis*. Also many putative candidate detoxification genes (e.g. glucosyltransferases, sulfotransferase) were induced. ZON was found to be rapidly converted into ZON-4-O-glucoside and ZON-4-sulfate in plants. Both conjugates do not inhibit Hsp90 ATPase *in vitro*.

The finding that ZON and its biosynthetic precursor bZOL are Hsp90 inhibitors also raises the question about the mechanism of self resistance in the toxin producing fungus. We have engineered yeast strains with increased ZON sensitivity (deletion of several ABC transporters) and with deletions of the endogenous yeast Hsp90 genes (hsp82 hsc82), that express as sole source of Hsp90 either the yeast or *F. graminearum* gene. We further transformed *Fusarium graminearum* with a deletion construct removing both *PKS*4 and *PKS13*, which are required for ZON biosynthesis. These strains will be used to re-address the question whether zearalenone biosynthesis contributes to *Fusarium* virulence.

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ASSESSMENT OF THE ACCURACY OF SINGLE-KERNEL NEAR-INFRARED TECHNOLOGY TO SORT WINTER WHEAT KERNELS BASED ON SCAB AND DEOXYNIVALENOL LEVELS

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ABSTRACT

Fusarium head blight (FHB, scab), caused by *Fusarium graminearum*, causes significant losses in winter wheat by reducing yield and grain quality. Kernels damaged by FHB, commonly referred to as *Fusarium*-damaged kernels or FDK, appear shriveled and/or discolored. *F. graminearum* produces the toxin deoxynivalenol (DON) which accumulates in grain during the grain filling period. Grain contaminated by DON usually is discounted at the elevator or can be rejected altogether. For purposes of quality assurance, DON concentration usually is determined in grain or the products made from it. The most commonly used method for determining DON concentration is gas chromatography. This method is accurate and can determine DON concentrations as low as 0.5 ppm. However, the method is destructive because grain must be ground to flour before it can be tested for DON. For purposes such as breeding for resistance to FHB where grain (seed) destruction may be undesirable; thousands of samples may need to be screened for DON in a short period of time; and knowledge of whether the grain from a given line has a low or high DON concentration is all that is needed to make a decision on whether or not to advance the line in a breeding program, alternative methods of determining DON concentration in grain are needed.

Single-kernel near-infrared (SKNIR) technology provides a non-destructive and quick alternative to gas chromatography in the determination of DON concentration in wheat grain. The present study examined the accuracy of a SKNIR system to sort winter wheat grain based on FDK and DON calibrations. *Fusarium*-damaged kernels were visually sorted from grain (cultivar Jagalene) harvested in 2007 and 2008 from FHB-affected experimental plots at the University of Nebraska Agricultural Research and Development Center near Mead, Nebraska. The FDK from each year was mixed with healthy grain in increasing proportions by weight of FDK ranging from 0% FDK to 100% FDK in 5% increments. Six replicates of a total of 21 samples (treatments) each weighing 5 g were obtained for each year. The samples were first sorted using a scab calibration into four fractions (Bin 1 for sound kernels and Bins 2-4 for FDK with increasing severity of scab damage), and the number of kernels collected in each bin was recorded. Kernels were mixed together again and placed in the corresponding packets for later sorting on DON estimates.

Two SKNIR instruments were used for sorting. For each treatment/year combination, replicates 1-3 were sorted in SKNIR 1 (old machine) and replicates 4-6 were sorted using SKNIR 2 (new machine). Using a DON calibration, DON levels in single kernels were estimated and placed in respective bins as follows: BIN 1 - kernels with non-detectable DON levels; BIN 2 – kernels with DON values between 1 - 60 ppm; BIN 3 – kernels with DON values between 61-160 ppm; BIN 4 – kernels with DON val-

ues above 161 ppm. After sorting, the number and weight of kernels in each bin were recorded. The sorted kernels from each bin (4 bins x 21 samples x 3 replications x 2 SKNIR machines x 2 years) were bulked by replication for each bin, SKNIR machine, and year and sent to the North Dakota Veterinary Diagnostic Laboratory for DON determination using gas chromatography. The three replications from each sample were bulked because replication samples were too small for DON analysis. DON was also determined in three replications of each of 21 similarly prepared samples not sorted by the SKNIR system.

The average number of kernels from the 21 samples sorted into Bin 1 (sound kernels), Bin 2, Bin 3, and Bin 4 (increasing severity of scab damage) was 22 (range: 5-39), 111 (range: 98-130), 74 (range: 11-113), and 10 (range: 0-22), respectively, from 2007 samples and 27 (range:4-61), 76 (range: 55-97), 82 (range: 6-158), and 66 (range: 0-141), respectively, from 2008 samples. The majority of kernels sorted into Bin 1 were from samples with 0 to 50% FDK whereas the majority of kernels sorted into Bins 3 and 4 were from samples with 50 to 100% FDK. Kernels sorted into Bin 2 were evenly distributed among the 21 source samples (0 to 100% FDK).

DON results from the SKNIR 1 system are presented (bin number, range in ppm of actual DON as determined by gas chromatography, and average DON in ppm in the bin).

2007. Bin 1: range, 0-17.6 ppm; average, 4.2 ppm
Bin 2: range, 0.8-44.5 ppm; average, 16.1 ppm
Bin 3: range, 0.8-39.6 ppm, average, 25.3 ppm
Bin 4: range, 25.7-84.5 ppm, average, 63.0 ppm

Unsorted by the SKNIR system: 0.8 ppm in 0% FDK samples – 36.2 ppm in 100% FDK samples

2008. Bin 1: range, 0-23.1 ppm; average, 3.3 ppm
Bin 2: range, 0-10.5 ppm; average, 3.7 ppm
Bin 3: range, 3.9-35.0 ppm, average, 21.7 ppm
Bin 4: range, 80-173 ppm, average, 141.6 ppm

Unsorted by the SKNIR system: 0.0 ppm in 0% FDK samples – 50.5 ppm in 100% FDK samples

DON levels were higher in 2008 samples (up to 173 ppm) than in 2007 samples (up to 84.5 ppm). Based on the results obtained from this study, the SKNIR system's accuracy in estimating DON was highest for samples from both years sorted into Bin 2 (calibrated for 1-60 ppm) and for 2008 samples sorted into Bin 4 (calibrated for > 160 ppm). The SKNIR system was able to sort wheat kernels into four fractions with the Bin 1 fraction having the lowest DON concentration and the Bin 4 fraction having the highest DON concentration. Due to the high precision of the SKNIR system in sorting kernels based on scab and DON calibrations, kernels in Bin 4 had a much higher DON content compared to the visually sorted and bulked 100% FDK samples (84.5 ppm SKNIR versus 36.2 ppm visually sorted and bulked in 2007 samples and 173 ppm SKNIR versus 50.5 ppm visually sorted and bulked in 2008 samples. Compared to DON determination techniques that estimate DON in unsorted bulk samples, sorting wheat grain into several fractions based on DON levels would provide breeders with more detailed information and would be a way of enriching resistance in segregating populations.

SESSION 2:

FHB MANAGEMENT

Co-Chairpersons: Christina Cowger and Marcia McMullen

AGGRESSIVENESS AND DON PRODUCTION OF *FUSARIUM GRAMINEARUM* 3ADON AND 15ADON POPULATIONS AS AFFECTED BY WHEAT CULTIVAR RESISTANCE AND FUNGICIDE TREATMENT, UNDER ND FIELD CONDITIONS, 2009

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INTRODUCTION

Fusarium head blight (FHB) is primarily caused by *Fusarium graminearum* in North America. The disease affects yield, by reducing harvestable kernel numbers and weight, and affects quality, by contaminating grains with mycotoxins produced by the fungal pathogens. FHB management is mainly through use of wheat cultivars with moderate FHB resistance and through use of fungicides, when warranted. Population studies indicate that *F. graminearum* isolates can be identified having one of three chemotypes (15ADON, 3ADON and NIV) and isolates of 15ADON chemotype were predominant in the population of North America (Ward et al. 2002; Gale et al. 2007). However, several recent studies have shown that the frequency of 3ADON isolates have increased dramatically in recent years (Burlakoti et al. 2008; Gale et al. 2007; Ward et al. 2008). The newly emerging 3ADON population appears to be more aggressive than the 15-ADON population based on growth rate, virulence and DON production in culture (Ward et al. 2008). Characterization of the *F. graminearum* isolates collected from 1980 to 2000 (old collection) and those collected in 2008 (new collection) in North Dakota showed that 3ADON isolates accounted for only 3% of the old collection, while 45% of the isolates in the new collection was of 3ADON chemotype (Puri and Zhong et al., unpublished results). Greenhouse inoculation studies in North Dakota also indicated that most of the 3ADON isolates were more aggressive and produced higher DON than the 15ADON isolates on susceptible and resistant cultivars (Puri and Zhong et al., unpublished results). However, little information is available on aggressiveness and DON production of *F. graminearum* 3ADON and 15ADON populations under field conditions. The objective of this study was to compare the 3-ADON and 15-ADON populations for FHB development and DON production in spring wheat under North Dakota field conditions, as affected by cultivar susceptibility to FHB and as affected by fungicide treatment.

MATERIALS AND METHODS

Two wheat cultivars, Alsen (with the fhb1 gene from Sumai 3 and moderately resistant to FHB) and Briggs (susceptible to FHB), were planted at the NDSU Agricultural Experiment Station at Fargo on May 8, 2009. A split plot experimental design was used with three replications, where cultivars were main plot, inoculum type was sub plot, and fungicide application was sub subplot. The experiment was conducted in a field where soybean was planted in the previous year. The plot size was 10 x10 feet. Three plots of each cultivar, with or without fungicide treatment, were inoculated (100K spores/ml) at anthesis (Feekes 10.51) on July 3 with either spore suspensions from ten 3ADON isolates (A), spore suspensions from ten 15ADON isolates (B), or a balanced mixture of spore suspensions from A and B isolates. The *F. graminearum* isolates used in the inoculation were a random subset of isolates from the large collection of Dr. Robert Stack from 1980 to 2000 and isolates from a new 2008 collection from farmers' fields in different counties of North Dakota. The chemotypes of these isolates were determined by PCR using the primers and the conditions described by Ward et al (2002).

For plots treated with fungicide, "Prosaro" (6.5 fl oz/acre) was applied 12 hrs prior to inoculations. The mixture of ten isolates of each chemotype was used to mimic a population of each type and minimize genetic background differences between the two populations. Three plots of each cultivar were left un-inoculated and unsprayed as checks. The disease incidence and severity data was recorded on July 23 (three weeks after inoculation) when the cultivars were at early dough stage (Feekes 11.1). To test for DON accumulation, 50 spikes with a disease severity of >66 %, based on the rating scale of Stack and McMullen (1995), from each treatment were tagged, harvested at maturity and kept separately in zip lock bags. These grain samples were sent to the Veterinary Diagnostic Laboratory, NDSU, for DON analysis.

RESULTS AND DISCUSSION

FHB incidence and severity in spring wheat inoculated with 3ADON and 15ADON isolates

Due to the prolonged cool and dry weather throughout 2009 wheat growing season in North Dakota, conditions were not conducive for FHB development. This was reflected in the relative low disease incidence in all non-inoculated plots. The checks (without inoculation and without fungicide treatment) had traces of FHB, with very low incidence (2.0% and 2.3% in Briggs and Alsen, respectively) and low head severity (7.0% and 4.6% in Briggs and Alsen, respectively), suggesting that natural infection was very low and would not have a large impact on the results of the inoculated plots. As expected, disease incidence and severity were higher in the inoculated plots compared to the checks in both susceptible and resistant cultivars. In the plots without fungicide treatment, Briggs had higher disease incidence and severity than Alsen, but no significant differences in incidence or severity were observed between 3ADON and 15ADON isolate inoculations in either of the two cultivars (Table 1). Fungicide treatment significantly reduced FHB incidence and severity in both cultivars, generally by 50% or greater, compared to the untreated, but no significant differences were observed between the 3ADON vs 15ADON isolate inoculations, except for a higher severity in Briggs with the 3ADON isolate inoculations. Further field experiments are needed to test the interactions among fungicides, chemotypes and wheat genotypes.

DON production in grains harvested from spring wheat inoculated with 3ADON and 15ADON isolates

DON analysis from non-fungicide treated grain heads showing 66%=> FHB severity indicated that the 3ADON isolates produced approximately double (149.5 ppm) the amount of DON produced by the 15ADON isolates (77.7 ppm) in the FHB susceptible wheat cultivar Briggs. The DON level accumulated in the FHB moderately resistant cultivar Alsen was approximately one-third that found in susceptible Briggs, but the 3ADON isolates produced 32% higher DON (43.2 ppm) than the 15ADON isolates (32.7 ppm) (Table 1). It is notable that in non-fungicide treated Briggs, both 3ADON (2.4 ppm) and 15ADON (2.6 ppm) in addition to DON were detected in grains inoculated with the 15-ADON isolates, while only 3ADON was detected in grains inoculated with the 3ADON isolates. In the treatments with fungicide application, DON level was reduced compared to the untreated inoculated plots. DON accumulation was reduced with fungicide treatment by about 46% in Briggs for the inoculations with 3ADON isolates and by 41.7% for the 15ADON isolate inoculations. However, in fungicide treated plots of Briggs, the DON also was higher (80.8 ppm) in grains inoculated with the 3ADON isolates than those inoculated with the 15-ADON isolates (45.3 ppm). In Alsen, fungicide treatment reduced DON by 63.6% for the inoculations with 3ADON isolates and by 63.9% for those with 15ADON isolates (Table 1).

In summary, these preliminary studies have indicated that the 3ADON isolate inoculations accumulate higher DON than the 15ADON isolate inoculations in either of the cultivars tested although disease severity differences were not significant between the two inoculations. These preliminary studies have

also indicated the value in using variety resistance and fungicide treatment in reducing FHB disease and DON levels, regardless of the chemotype of the inoculum source.

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Table1. Effect of *Fusarium graminearum* 3ADON and 15ADON populations on FHB development and DON production under field conditions in North Dakota.

Treatment	FHB		Trichothecene (ppm)		
	Incidence (%)	Severity (%)	DON	3ADON	15ADON
Briggs inoculated with A	22.00	43.87	149.50	8.20	<0.5
Briggs inoculated with B	22.00	43.29	77.70	2.40	2.60
Briggs inoculated with A+B	26.00	49.59	101.00	5.10	2.00
Briggs sprayed with F and inoculated with A	5.00	27.87	80.80	4.30	0.5
Briggs sprayed with F and inoculated with B	5.30	18.82	45.30	0.60	3.10
Briggs sprayed with F and inoculated with A+B	11.00	26.24	73.00	5.10	1.20
Briggs without treatments	2.00	7.00	<0.50	<0.50	<0.50
Alsen inoculated with A	9.66	31.46	43.20	2.00	<0.50
Alsen inoculated with B	9.66	29.78	32.70	<0.50	1.30
Alsen inoculated with A+B	18.33	34.28	34.40	1.00	0.50
Alsen sprayed with F and inoculated with A	4.33	15.33	15.70	<0.50	<0.50
Alsen sprayed with F and inoculated with B	4.00	14.25	11.80	<0.50	0.50
Alsen sprayed with F and inoculated with A+B	4.33	10.47	3.80	<0.50	<0.50
Alsen without treatments	2.33	4.66	<0.50	<0.50	<0.50

Briggs = FHB susceptible variety; Alsen = FHB moderately resistant variety with Sumai3 heritage
A=a mixture of spore suspensions (100K spores/ml) from ten 3ADON isolates, B= a mixture of spore suspensions from ten 15ADON isolates, F=fungicide "Prosaro" (a mixture of prothioconazole and tebuconazole, applied at 6.5 fl oz/acre at anthesis, Feekes 10.51)

EFFECTS OF WITHIN-FIELD CORN DEBRIS IN MICROPLOTS ON FHB AND DON IN TEN U.S. WHEAT ENVIRONMENTS IN 2009

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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 FHB. Our experimental objective was to quantify the relative contribution of within-field corn debris as an inoculum source of *Gibberella zeae* for Fusarium head blight and DON contamination in ten variable wheat environments in 2009, all in regions where corn is the predominant crop in the agricultural landscape and corn debris is left on the land surface over large areas. Our research is based on the hypothesis that spores of *Gibberella zeae* that are deposited on wheat spikes and that result in Fusarium head blight come primarily from well-mixed, atmospheric populations in an area. The research was conducted in two commercial-scale wheat fields in Illinois, Missouri, Nebraska, New York, and Virginia, each following an FHB nonsusceptible crop. Over these environments we encountered six severe epidemics (in Illinois, Missouri, and Virginia), two moderate epidemics (in New York), and two mild epidemics (in Nebraska). Locally overwintered, natural corn stalks were collected in spring from two different sources in each state or locale by placing a 33 inch diameter plastic 'Hoola Hoop' onto four arbitrarily selected areas in a corn stubble field, and then removing all of the stubble within the hoop and placing it in a paper bag. Replicated (four) microplots containing corn debris and without debris were set out in each field and were separated by a minimum of 100 ft in each dimension. Debris was secured within the source circles by using cages fashioned of 2 ft high hardware cloth and shaped with the same 33 inch diameter plastic 'Hula Hoop', fastened with plastic zip-ties, and secured to the soil with metal ground staples. Wheat heads above each microplot were rated at soft dough stage for FHB incidence, severity, and index. At grain maturity, at least 100 heads from each microplot were harvested, dried and shipped to Cornell where grain was threshed from a subsample of heads and sent to Virginia Tech for DON analysis. Only in one field in Virginia did wheat heads from microplots containing locally overwintered corn debris show a slight but statistically significant increase in FHB incidence and index over those from microplots with no corn debris. The astounding result is that DON level did not differ significantly between corn debris and no debris microplots in any of the ten wheat environments. By inference of our results, it appears that elimination of corn debris from single wheat fields in a major corn producing region may have rather limited benefits in terms of reducing FHB and especially of reducing DON contamination of grain. The experiments will be repeated in ten additional environments in 2010.

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HOST RESISTANCE TO *FUSARIUM* METABOLITES: RELEVANCE OF MASKED MYCOTOXINS FOR RESISTANCE BREEDING AND TOXICOLOGY

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ABSTRACT

The working hypothesis of the special research program FUSARIUM at BOKU is that secondary metabolites of the fungal pathogen can suppress defense responses in host plants, thereby causing a lack of gene-for-gene interactions and the ability to cause disease on a broad range of host plants. According to our hypothesis, quantitative and polygenically inherited differences in host resistance are due to differences in the ability to antagonize such secondary metabolites. This can be achieved by multiple mechanisms. The products of multigene families are responsible for drug efflux across the plasma membrane (e.g. PDR genes), detoxification by conjugation to sugars (UDP-glycosyltransferases, UGTs) or glutathione (GSTs), and sequestration of conjugates in the vacuole (Multidrug Resistance-Related Proteins, MRPs). The *Fusarium graminearum* genome encodes multiple predicted secondary metabolite biosynthetic genes/clusters, such as terpenoide synthases, polyketide synthases and nonribosomal peptide synthases. For most of these the corresponding metabolites and their mode of action *in planta* are currently unknown, and the virulence function is expected to be masked by redundancy. The trichothecene deoxynivalenol (DON), a known virulence factor, is also acutely toxic for humans and animals and therefore received most of the attention. It was shown previously that the ability to detoxify DON into DON-3-O-glucoside (D3G) co-localized with wheat DON resistance and a major QTL for *Fusarium* spreading resistance. Since this QTL is heavily used by breeders, it is toxicologically relevant to which extent D3G accumulates and whether it is a "masked mycotoxin". D3G is heat stable and also unaffected by the acidic pH in the stomach, and it is not hydrolyzed by the product of the human cytosolic β-glucosidase, or the commonly used almond β-glucosidase. Yet, certain intestinal bacteria can hydrolyze D3G, and reform the parental toxin DON. UGTs are encoded in diploid grasses by a family of 140+ genes. Recently we succeeded to identify a D3G forming UGT of barley. Furthermore, evidence for formation of unstable glutathione conjugates of DON has been obtained. For breeding purposes it seems important to consider also other virulence mechanisms of *Fusarium*. For instance, we could show that the metabolite zearalenone (ZON), which is known for its estrogenic activity in animals, is an inhibitor of ATPase activity of heat shock protein 90 (Hsp90). Formation of zearalenone-4-O-glucoside (Z4G) leads to a loss of ATPase inhibitor activity. Hsp90 is an important player in plant resistance. ZON-inactivation could therefore also be relevant for resistance breeding, at least in maize.

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POPULATIONS OF *BACILLUS* STRAINS APPLIED TO WHEAT HEADS FOR BIOLOGICAL CONTROL OF FHB: RESULTS OF BROOKINGS, SD 2009 FIELD PLOTS

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ABSTRACT

Following spray application of biological control agents (BCAs) onto grain heads for control of FHB, evaluating numbers of BCAs on the inoculated grain heads is important to understand how the BCAs colonize and grow on plant surfaces, and how they might act against FHB. We have focused our research on *Bacillus* strains 1BA and 1D3 for use as BCAs to control FHB. In the 2009 biocontrol plot trials conducted at Brookings, SD using most probable number (MPN) methodology employing high temperature and high salt selection in the MPN growth media, control plots that did not receive spray application of BCAs had very low bacterial numbers, being at most in the hundreds of cells per gram fresh plant mass, indicating that a small number of native bacteria can tolerate the high salt and temperature conditions used in recovering and counting the BCA bacterial strains. The plots inoculated with BCAs also had very low bacterial numbers, not statistically different from the control plots. This is the first year since 2006 when we started tracking BCA populations on grain heads using the MPN method that there has not been a noticeable difference between control and treatment plots.

Although BCA numbers were apparently low, results of the BCA trails in Brookings indicated several significant differences between control and BCA treatments. A new growth medium formulation was used in summer of 2009 for growing the BCAs, which might help account for both the change in MPN counts of BCAs and the desirable effects the BCAs apparently had on reducing measures of FHB in the Brookings plots. Whether the modified growth medium enhanced production of antifungal lipopeptides by the BCAs, resulting in the treatment differences in the Brookings plot trial, is not yet clear. Laboratory studies with *Bacillus* strains 1BA and 1D3 grown in the new broth formulation showed apparent differences between the two strains in the amount of lipopeptide produced, as indicated by an oil droplet collapse assay. *Bacillus* strain 1D3 appears to have greater biosurfactant activity than strain 1BA.

Wheat heads inoculated with the BCA strains were processed for extraction of bacterial DNA, and using primers specific for the surfactin and iturin genes, PCR was carried out on the extract to see if there was evidence of surfactin genes on the grain heads. The yield of DNA from grain heads was very low, making the PCR difficult. Future work will use a greater number of grain heads for DNA extraction, to provide enough DNA for PCR to be successful.

PROGRESS ON MODELING DEOXYNIVALENOL IN BARLEY

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INTRODUCTION

Fusarium head blight (FHB), caused by the fungus *Gibberella zeae* (Schwein) Petch (anamorph: *Fusarium graminearum* Schwabe), continues to be a serious problem for barley producers in the U.S. Northern Great Plains and elsewhere. Economic losses associated with FHB occur because of the blighting of florets (reduction in grain number), disruption of grain fill (shriveled kernels leading to lower test weight), and most importantly through the contamination of grain with trichothecene mycotoxins, primarily deoxynivalenol (DON). Tolerances for DON in malting barley are generally lower than those for food or feed-grade barley because of the association between DON, *G. zeae*-infested kernels, and gushing in beer (Garbe, 2009). DON concentration in grain is used to estimate this risk and crop rejection or severe discounts can be implemented if the level detected exceeds 0.5 parts per million (ppm).

Management of FHB in high-risk regions is currently accomplished with agronomic practices that limit in-field inoculum (e.g. rotation) and through the application of fungicides after spike emergence to reduce the risk of infection. Inoculum management has been documented to reduce both the number of *G. zeae* propagules reaching the spikes as well as the final DON concentration in the grain (Dill-Macky and Jones, 2000; Stein et al, 2009); however, this approach is not sufficiently effective in all situations since the spores of *G. zeae* can become airborne and travel moderate distances (Markell and Francl, 2003; Schmale et al., 2006). The application of fungicide is also not completely effective at preventing FHB and DON accumulation, but may provide some reduction in both (Yoshida et al., 2008). The timing of application is critical and therefore a need exists for a risk-advisory system that predicts the risk of an economic level of DON occurring in a malt barley crop.

OBJECTIVES

To identify the weather variables that were predictive of economic DON levels and to develop accurate risk model(s) that predict FHB and/or DON based on these variables.

MATERIALS AND METHODS

Experiments were conducted during the 2005-8 growing seasons using a set of regionally adapted, malting barley varieties at multiple locations in North Dakota and South Dakota (2005-8), and Minnesota (2005, 2007-8) or Montana (2006). At least three varieties, namely 'Conlon' (2-row), 'Robust' and 'Tradition' (both 6-row), were common at all locations. Plots were sown with a small-plot planter, a minimum of 1.5m x 4.6m in size, replicated four times in a randomized complete block design (RCBD), and maintained using standard agronomic practices for the region. The plots were not inoculated, nor were they misted or manipulated in any way to increase the probability of disease.

Crop growth was monitored regularly throughout the growing season and the date at which each variety was at 50% Feekes 10.5 (heads fully emerged) noted, hereafter this is referred to as the 'heading

day'. The incidence and severity of FHB was recorded on a minimum of 25 heads per plot at the soft-dough stage (18-21 days after heading). Plots were harvested at the end of the growing season using a small plot combine and DON concentration was determined from a random sample of 100 g grain from each plot. The mean DON concentration for all replicate plots of a singe variety at each location was used in the analyses. A binary response variable, eDON (economic DON), was created based on whether the mean concentration for each variety at every location*year met or exceeded 0.5 ppm. For example, if the mean DON concentration for a variety*location*year was 0.7 ppm it would be assigned a value of 1.

In addition to disease and mycotoxin data, nearby weather stations were used to record hourly environmental conditions for at least 10 days proceeding, and including, the heading day for each variety at all locations. At a minimum this included temperature, relative humidity (RH), and precipitation (incidence and rate). A total of 117 weather predictors were calculated from this data based on previous studies and trends observed in the data over 7- and 10-day intervals, which included the heading day itself (Andersen, 1948; De Wolf et al., 2003). For example, if a variety headed on June 30, predictors were obtained by summarizing the weather observations from June 24-30 (7-day) and June 21-30 (10-day).

Predictors were analyzed individually to evaluate their relation with eDON using univariate logistic regression and all predictors that were significant at a p-value of 0.25 were selected for further evaluation (Hosmer and Limeshow, 1989). The remaining predictors were divided into two subsets depending on the weather data interval used (i.e. 7- or 10-day intervals). Each subset was analyzed individually due to potential correlations between the predictors as the durations overlapped. A classification tree approach was used as a selection tool to identify the variables from the two subsets that were most associated with eDON (Harrell Jr., 2001). Box plots and stepwise regression methods were also used as a guide to obtain the most significant predictors from the two subsets. Interaction terms were calculated between the most significant predictors and evaluated using univariate logistic and stepwise regression procedures as noted above. The predictors that were most associated with eDON were reduced to five in total, three from 10-day subset and two from 7-day subset.

Logistic regression models were developed using the selected predictors from the two subsets by evaluating them in two and three variable combinations. Predictive power statistics such as generalized R^2 index of Nagelkerke (R^2_N), C-statistic, Somer's D_{xy} rank correlation, sensitivity, specificity, and prediction accuracy were calculated for each (Allison, 1999; Harrell Jr., 2001). In all cases, larger values correspond to stronger associations between the predicted and observed values. In order to achieve the greatest prediction accuracy, the probability of a positive eDON value (p*) was selected at which the sum of sensitivity and specificity was highest (De Wolf et al., 2003). Akaike's Information Criterion (AIC) was used to compare the regression models.

RESULTS AND DISCUSSION

A total of 43 location*years over four years were used in modeling process. The 6-row cvs. Robust and Tradition tended to have numerically higher DON concentrations than the 2-row cv. Conlon (Figure 1); however, variety was found to be not significant and was excluded from further analyses. Ninety-four predictors were selected in the univariate analyses with 46 and 48 being from the 7- and 10-day intervals, respectively. Overall, predictors that included some measurement of humidity were more strongly associated with eDON than those without.

The classification tree and stepwise procedures selected five predictors, of which three were from the 10-day subset and two were from the 7-day subset (Figure 1). For the 10-day subset, the remaining predictors were the number of hours the air temperature was 26-34^{o}C (T2634_10), maximum duration of hours where relative humidity was

continuously greater than 90% (DR_RH90_10) in the 10-day period, and the summation of a weighted-hour matrix (TR6_10). Specifically, TR6_10 was assigned a value of '0' for each hour if the temperature was <15°C or >28°C, a value of '2' was assigned if the temperature was 20-24°C, and a '1' assigned otherwise. For the 7-day interval subset, two predictors were selected: maximum duration of hours with relative humidity greater than 90% continuously (DR_RH90_7) in the 7-day period and number of hours the air temperature was 20-28°C with relative humidity greater than 90% (T2028RH90_7). All the two and three term interactions in both 10-day and 7-day subsets were deleted in the stepwise regression procedures since they were not significant.

Twenty logistic regression models from the five identified predictors in one, two, and three variable combinations were developed (intercorrelated variables were not used together in a regression model) and the prediction accuracies of all models ranged from 73-94% (data not shown). Two models were identified with $p^* \geq 0.3$, $R^2_N \geq 0.60$, Somer's Dxy $\geq$ 0.80 and C-statistic ≥ 0.90 (Table 1). Both models had smaller AIC values compared to the remaining models with same number of predictors. Model A had four false positives and six false negatives, whereas model B had three false positives and 11 false negatives. The fit statistics were higher for model A than model B, with the exception of sensitivity. However, lower p* for model B might increase the probability of misjudging a non-economic DON event as an eDON event, which is evident from the false negative rates of model A and model B (4% for model A and 11% for model B). Both models were under consideration and further analysis on validation of these two models is ongoing.

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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. Predicting power statistics for the selected logistic regression models.

#	Predictor1	Predictor2	AIC	C-Stat	R^2_N	Dxy	p*	Sensitivity (%)	Specificity (%)	Prediction Accuracy (%)
A	T2634_10	DR_RH90_10	61.1	0.92	0.67	0.85	0.4	83	96	94
B	T2028RH90_10	DR_RH90_10	69.1	0.90	0.61	0.80	0.3	88	89	89

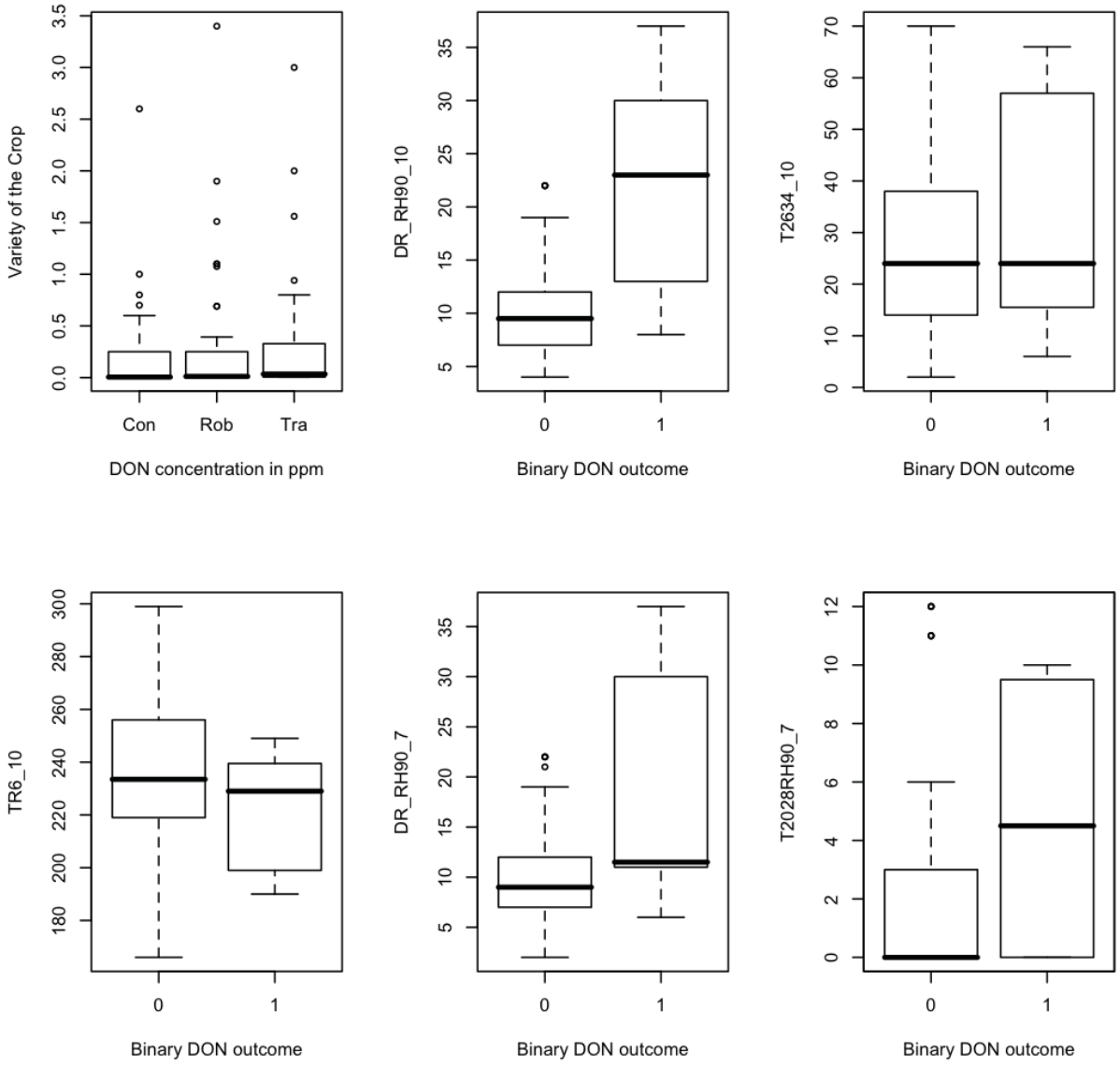

Figure 1. Box plots for variety of the crop based on the actual DON concentrations and the five most predictive variables divided based on non-economic (0) and economic (1) DON events.

USING FORECASTED WEATHER DATA AND NEURAL NETWORKS FOR DON PREDICTION IN BARLEY

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INTRODUCTION

Fusarium head blight (FHB), caused by the fungus *Gibberella zeae* (Schwein) Petch (anamorph: *Fusarium graminearum* Schwabe), continues to be a serious problem for barley producers in the U.S. Northern Great Plains and elsewhere. Economic losses associated with FHB occur because of the blighting of florets (reduction in grain number), disruption of grain fill (shriveled kernels leading to lower test weight), and most importantly through the contamination of grain with trichothecene mycotoxins, primarily deoxynivalenol (DON). Tolerances for DON in malting barley are generally lower than those for food or feed-grade barley and discounts can be implemented if the level detected exceeds 0.5 parts per million (ppm).

Predictive models are being developed for estimating the risk of economic DON levels in barley (i.e. 0.5 ppm) using the 10-day interval leading up to, and including, the date of full head emergence. Generating risk advisories from such weather data does not allow for the pro-active management of FHB. That is, growers learn of highly conducive conditions after infection has probably occurred. The timing of fungicide application to limit losses from *G. zeae* is critical (Yoshida et al., 2008) and growers would be best served by having accurate risk advisories based on forecasted weather data so that management decisions could be made before infection occurs.

OBJECTIVE

To develop Artificial Neural Network (NN) model(s) that predict the risk of economic DON accumulation in barley (>0.5 ppm) based on 5-day forecasted weather leading up to the date of heading.

MATERIALS AND METHODS

Quality Controlled Local Climatological Data (QCLCD) and extended range forecast model output statistics (MOS) weather data were collected from 36 locations in the U.S. Northern Great Plains for the months of June and July, over an eight-year period (2001-08). Selected locations represent three geographical regions; namely, Red River Valley: 11 stations from Minnesota (MN), three stations from North Dakota (ND), and one station from South Dakota (SD); western ND (nine stations); and eastern South Dakota (11 stations from SD and one station from MN). The daily risk of economic DON accumulation in barley was calculated with the QCLCD data over each 10-day interval using the best available barley-DON logistic regression model (see other report by Bondalapati in these proceedings). This represented the 'Gold Standard' and was binary, where '0' represented no risk and '1' represented risk. The proportion of risk to non-risk days in the complete data set was 1:7, respectively.

For each date, 120 h (5 days) of forecasted temperature and relatively humidity were calculated from

the MOS daily minimum and maximum values using an algorithm described previously (Baker and Kirk, 2007). Five additional data sets were created from this data using a combination of the QCLCD (true) and MOS (forecasted) hourly weather values by replacing 1-5 days of QCLCD data with an equal number of MOS data. That is, if June 10th was the day of full head emergence, weather data from June 1st-10th was used to predict the 'true' DON risk (response variable). In addition, QCLCD data from June 1st-9th plus MOS data from June 10th was used for a one-day forecast, QCLCD data from June 1st-8th plus MOS data from June 9th-10th was used for a two-day forecast, etc. In other words, increasingly larger proportions of the QCLCD (true) data set were replaced by MOS (forecasted) data until each 10-day interval consisted of 5 days each of QCLCD and MOS data.

To observe the impact of using forecasted data directly in the aforementioned barley-DON model, daily risk predictions were calculated for each combination of QCLCD and MOS data. That is, the risk was re-calculated for each 10-day interval after replacement one day of true (QCLCD) with forecasted (MOS) weather data, two days QCLCD with MOS, etc. These were then compared to those computed based on the QCLCD (true) weather and the accuracies for each combination were calculated.

Feed-forward back propagation NN with one hidden layer was then used to model the relationship between weather predictors and the outcome (Ripley, 1994). A random selection of 90% of observations was considered for training and the remaining were set aside for testing. Since NN models do not perform very well on unbalanced data sets (Ha et al., 2005), the training set was balanced by sub-sampling of the non-risk class so that the each set had an equal number of observations in both risk and non-risk classes. The package "*nnet*" from the statistical software R was used to perform the analysis (Venables and Ripley, 2002). Four variables were considered as input variables in NN models and were obtained by summarizing weather variables from the barley-DON logistic regression model over QCLCD and MOS periods individually. For example, if the mean temperature of 10-day period was used in the barley FHB model, two variables were constructed with one being the mean temperature of QCLCD weather interval and other being the mean temperature of MOS weather interval. The optimal number of hidden nodes in the hidden layer was considered where the misclassification error rate was minimum. A larger number of hidden nodes in a NN model is extremely flexible and can approximate any smooth function; however, too many hidden nodes can result in low prediction on validation set (Venables and Ripley, 2002) and lead to poor accuracy, no matter how powerful a model is (Ha et al., 2005).

As the main objective of this research is to develop the NN model to predict the risk using 5-days forecasted data, additional potential weather variables were calculated for the fifth data set in order to increase the prediction accuracy for the NN model developed in the previous step. To obtain the most predictive variables from the set of 102 variables, traditional regression methodologies, such as univariate logistic regression, stepwise variable selection, and regression trees were used. Variable selection prior to model development was recommended in order to reduce the noise from unnecessary predictors (Faraway, 2002). NN models were then developed on the selected predictors and the performance of the each was examined in the region as well as for sub-regions.

RESULTS AND DISCUSSION

The performance of logistic regression model decreased with the inclusion of forecasted days (Table 1). The total prediction accuracy was 98% in case of one-day forecast and only dropped to 93% when extended to five-day forecast. However, the sensitivity dropped rapidly from 91% to 52%. Here the high prediction accuracies with low sensitivities were due to high proportion of non-risk days. From Table 1, it is evident that the efficiency

of the logistic regression model was substantially reduced in predicting the true risk with the inclusion of forecasted weather data.

The optimal number of hidden nodes for NN models varied with the number of forecasted days (Table 2) due to complexity in the data. The number of hidden nodes was only 1 in case of the one-day forecast, whereas it was 5 in case of five day forecast. The performance of NN models were substantially better than the logistic regression models with the total prediction accuracies ranging from 94%-86% and sensitivities from 93% to 73%.

In the case of the five-day forecast, six variables were selected as the most predictive and were divided into two subsets. The first subset had four variables and second subset had five variables with three common to both subsets. Five and seven hidden nodes were selected as the optimal number based on the misclassification error rate and four NN models were developed with the optimal number of hidden nodes for each subset of predictors. The prediction accuracies of the four NN models varied from 81% to 84% (data not shown). Since four models had approximately equal prediction accuracies, the model with less number of parameters (four inputs and five hidden nodes) was selected for further evaluation and the prediction accuracies by each geographical region calculated (Table 2). The selected NN model had high accuracy in predicting risk days (sensitivity) in the Red River Valley region and high accuracy in predicting non-risk days (specificity) in western ND.

ACKNOWLEDGEMENTS

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Table 1. Prediction accuracies when comparing the logistic regression model response on historical data with logistic regression model response and NN model response after including the forecast data.

Number Forecast Days	Logistic Regression Model			Single hidden layer NN			
	Sensitivity (%)	Specificity (%)	Prediction Accuracy (%)	Architecture	Sensitivity (%)	Specificity (%)	Prediction Accuracy (%)
1	91	99	98	4-1-2*	93	92	92
2	82	99	97	4-3-2	86	95	94
3	73	99	96	4-5-2	81	93	92
4	63	99	94	4-5-2	80	89	88
5	52	99	93	4-5-2	73	88	86

* - 4 input variables, 1 hidden node and 2 output variables.

Table 2. Geographical prediction accuracy of NN model using five-day forecast data. The architecture of NN model is 4-5-2*.

	Sensitivity (%)	Specificity (%)	Prediction Accuracy (%)
Total Region	72	86	84
Red River valley region	74	84	82
Eastern South Dakota	71	84	82
Completely Dry region	68	91	89

* - 4 input variables, 5 hidden node and 2 output variables.

APPLICATION TIMINGS OF CARAMBA AND PROSARO FOLIAR FUNGICIDES FOR MANAGEMENT OF FHB AND DON

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ABSTRACT

As part of the USWBSI – funded uniform fungicide trial project for control of Fusarium head blight (FHB) and mycotoxins in wheat, a multi-state project was initiated to evaluate the efficacy of Caramba (metconazole; BASF Corporation) and Prosaro (tebuconazole + prothioconazole; Bayer CropScience) when applied at different Feekes growth stages (FGS). In field trials established at multiple locations in Arkansas, Illinois, Maryland, Michigan, Minnesota, North Dakota, and South Dakota, these two products were applied at FGS 10.5, 10.5.1, and five days following the 10.5.1 application. Caramba was evaluated at 13.5 fl oz/A, and Prosaro at 6.5 fl oz/A. A combination of Caramba + Proline (prothioconazole; Bayer CropScience) at 7 + 3 fl oz/A applied at FGS 10.5.1 also was evaluated. The trials were conducted on wheat cultivars in different market classes which included soft red winter, soft white winter, hard red winter, and hard red spring. The combination treatment of Caramba + Proline generally provided equal control of FHB and mycotoxins compared to Caramba or Prosaro treatments at most locations. In general, most applications of Caramba or Prosaro, regardless of application timing, significantly ($P \leq 0.05$) reduced FHB symptoms and mycotoxin levels in grain when compared to the untreated control; however, some application timings provided better fungicide performance than others, depending on location. These results indicate that the window of fungicide application for control of FHB and associated mycotoxins may be slightly wider than previously believed.

EFFECT OF PYRACLOSTROBIN APPLICATIONS TO WHEAT AT DIFFERENT GROWTH STAGES ON DON CONCENTRATIONS IN GRAIN

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ABSTRACT

As part of the USWBSI – funded uniform fungicide trial project for control of Fusarium head blight and mycotoxins in wheat, a multi-state project was initiated to evaluate the effect of pyraclostrobin (Headline fungicide applied at 6 fl oz/A; BASF Corporation) applications to wheat on deoxynivalenol (DON) concentrations in grain. Field research trials were established at multiple locations in Arkansas, Illinois, Maryland, Michigan, Minnesota, North Dakota, and South Dakota. These trials were conducted on wheat cultivars in different market classes which included soft red winter, soft white winter, hard red winter, and hard red spring. In addition to measuring DON concentration in grain, nivalenol (NIV) also was measured at the Arkansas location. At all locations, pyraclostrobin was applied at Feekes growth stage (FGS) 10.5. At some locations, pyraclostrobin also was applied at FGS 9 and 10.0. At the time this abstract was written, DON data were available for experiments at Fayetteville, Arkansas; Brownstown, Carbondale, Monmouth, and Urbana, IL; Clarksville, Michigan; Fargo, North Dakota; and Brookings, South Dakota. Concentrations of DON in the grain from the untreated controls ranged from 1.3 to 16.7 ppm. DON concentration was increased significantly ($P \leq 0.05$) in grain compared to the untreated control when pyraclostrobin was applied at FGS 10.5 at three out of eight locations, and when applied at FGS 10.0 at one out of six locations. Pyraclostrobin applications never significantly decreased DON concentrations compared to the untreated control. At the Arkansas location, pyraclostrobin applied at FGS 10.5 significantly increased NIV concentration compared to the untreated control (3.1 vs. 2.2 ppm). These results indicate that an application of a quinone outside inhibitor (QoI) or strobilurin fungicide such as pyraclostrobin to wheat at FGS 10.0 or 10.5 may increase mycotoxin concentrations in harvested grain.

INFECTION TIMING AND MOISTURE EFFECTS ON DON AND FDK IN WHEAT

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ABSTRACT

The USWBSI winter wheat forecasting tool provides forecasts of FHB severity that are timely for fungicide decisions based on weather in the seven days prior to anthesis (DeWolf *et al*, 2005). FHB severity and deoxynivalenol (DON) levels are also influenced by post-anthesis moisture (Cowger *et al*, 2009). Building on that foundation, our work has aimed to elucidate the influence of variable infection timing and post-anthesis moisture on disease development and DON concentrations. The questions addressed include:

1) When is wheat vulnerable; e.g., does it only need to be protected at anthesis? What factors change that window of vulnerability?

2) To what extent are visual FHB symptoms and *Fusarium*-damaged kernels (FDK) predictive of DON problems, and what factors influence that relationship? When should we be on the lookout for crops with higher DON levels than symptoms and FDK would predict?

3) What happens to DON levels during mid- and late grain-fill? How do environmental conditions at harvest affect DON? Can this information be helpful in high-DON situations, and should we modify our harvest-timing recommendations?

Field experiments with soft red winter wheat cultivars in North Carolina have been conducted to help clarify the relationship between DON, infection timing, and post-harvest moisture.

A) Infection timing

The effect of post-anthesis infection was examined by spray-inoculating individual heads of several wheat cultivars at anthesis, watery-ripe, or late-milk stages (0, 10, or 20 days after anthesis, or daa) with 10^5 *Fusarium graminearum* macroconidia/ml. Plots were subjected to 0, 10, 20, or 20 daa of misting. The threshed grain was assayed for FDK, DON, and (in one year) percent infected kernels. The experiment was conducted for three years.

The results indicated that the window of maximum FHB susceptibility of winter wheat in North Carolina is generally less than or close to 10 days after mid-anthesis. Wheat needs to be protected during this entire time period, not just during anthesis itself. In two of three years, FDK and DON were correlated for inoculations at anthesis and watery-ripe stage, but not at late-milk stage, and were more strongly associated for 0 or 10 daa of misting than for 30 daa of misting. In other words, late infection and protracted moisture reduced the association between FDK and DON. Samples with plump kernels (≤4% FDK) and unacceptable levels of DON (≥2 μg/g) were infrequent in two years (18-19%) but

were more frequent in a third year (41%). The "low-FDK, high DON" scenario was associated with late infections and was maximized by marginal disease conditions.

B) Changes in DON and kernel damage during grain development

A two-year experiment was conducted to assess the changes in FDK, infected kernels, and DON over the course of grain development. Plots of seven cultivars were spray-inoculated with 10^5 *Fusarium graminearum* macroconidia/ml at mid-anthesis and subjected to 0, 10, 20, or 30 daa of misting as described in (Cowger *et al*, 2009). On six dates about 10 days apart, from milk stage to about 20 days after harvest-ripeness, 30 spikes were blindly harvested from each plot. The spikes were threshed and the grain was weighed and assayed for FDK, DON, and percent *Fusarium*-infected kernels.

In both years, sample weights increased to a maximum in the 3rd sample, which was taken at hard dough stage approximately 7-10 days before harvest-ripeness. Mean DON per unit of sample weight, averaged across mist treatments, declined from the first to the second sample, and then remained roughly level. Mean DON per 30-spike sample, again averaged across mist treatments, fell by 42-47% between milk and harvest-ripeness. At the same time that DON levels were dropping, percent FDK and percent infected kernels increased until after harvest ripeness in 2006, and until harvest ripeness in 2007. Misting increased early DON levels, percent FDK, and percent infected seed, and decreased sample weights, to a greater extent in 2006 than in 2007.

These results are consistent with reports that DON biosynthesis occurs immediately following infection, with DON acting as a virulence factor that allows fungal hyphae to move into the wheat rachis and spread to other florets (Jansen *et al*, 2005). Early in grain fill, DON production apparently stops, and DON concentrations are reduced by host detoxification (Lemmens *et al*, 2005) micro-organism degradation, and/or by the leaching action of water. At the same time, according to our data, *Fusarium* continues to spread within spikes. Of interest for DON management are the changes in DON concentration in the period immediately before, during, and after harvest-ripeness. This is particularly relevant since growers confronted with severe FHB have traditionally been advised to harvest wheat early. This recommendation may make sense with respect to FDK. However, in 2006, DON continued to decline in the higher-DON treatments until the 5th sample, several days after normal harvest timing. In 2007, DON levels declined or stayed constant over the last three samples.

In summary, our work indicates that post-anthesis moisture increases disease symptoms, kernel damage, and initial DON levels by enhancing fungal spread within the head. Although this spread apparently continues to occur throughout grain-fill, DON concentrations decline over time. Late infection and extended post-anthesis moisture reduce the correlation between kernel damage and DON, and may account for occasional observations of high DON in apparently sound grain.

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ECOLOGY OF *BACILLUS SUBTILIS* ON WHEAT FLORETS IN RELATION TO BIOLOGICAL CONTROL OF FHB/DON

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ABSTRACT

The TrigoCor strain of *Bacillus subtilis* is one of a handful of biological control agents (BCAs) that show potential in the integrated management of FHB/DON. TrigoCor inhibits the growth of *Fusarium graminearum* in antibiosis assays, and has resulted in excellent and consistent reduction of FHB symptoms and DON accumulation in greenhouse experiments. Like other BCAs tested through the USWBSI, TrigoCor has shown inconsistent biocontrol in the field. The goal of our current USWBSI project is to identify strategies for enhancement of biocontrol by elucidating the ecology of interactions between *Bacillus* and *F. graminearum* on wheat florets under controlled conditions as well as under field conditions. Using TrigoCor as a model BCA, we are describing the dynamics of microbial populations and of *Bacillus*-generated antifungal metabolites relative to biological control. We examined populations of hand-sprayed *Bacillus* on wheat heads over critical infection periods in the greenhouse and in two upstate New York locations during the 2008 and 2009 field seasons. Using dilution plating, we quantified *Bacillus* populations on wheat heads at 0h, 24h, 72h, 7d, and 14d after *Bacillus* application. The population levels of *Bacillus* remained constant throughout the sampling period in the greenhouse (10^8 CFUs/head) and at both field locations in 2009 (10^6-10^7 CFUs per head), and increased over the first week from 10^4 CFUs/head to a constant level 10^6 CFUs/head in both field locations in 2008. In 2008 and 2009 we also recovered *Bacillus* from wheat heads in significant quantities (10^5 CFUs/head) at harvest, suggesting that this BCA may also be present in sufficient numbers to protect plants against late-season *Fusarium* infections. In addition to these hand-sprayed field trials, we also quantified population levels on field plots commercially sprayed with *Bacillus*. We consistently recovered *Bacillus* populations of 10^4-10^6 CFUs/head at 0h and 24h after *Bacillus* application from fields in New York, North Dakota, and Missouri in 2008, as well as throughout a 14d sampling period from a field trial in upstate New York in 2009. Treatment with TrigoCor did not provide significant reductions in FHB in any of the hand-sprayed or commercially sprayed trials in 2008 or 2009. The insufficient FHB control of *Bacillus* in the field compared with the greenhouse, despite its consistent persistence in both environments, suggests that some factor other than inadequate survival is responsible for the inconsistent performance of this BCA in the field. The absolute quantity of *Bacillus* on wheat heads in the field was lower than in the greenhouse, particularly in the 2008 field season and in the commercially sprayed plots, indicating that a reduction in the total amount of *Bacillus* on heads in the field may be responsible for its limited biocontrol efficacy in this environment. In addition to bacterial population dynamics, we are assessing the production and persistence of antifungal metabolites relative to biological control in field environments. Using LC and MS technologies we are currently evaluating the presence of *Bacillus* lipopeptides on wheat florets collected from the field at time points parallel to our population dynamics studies.

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EVALUATING THE USE AND POTENTIAL IMPACT OF FUSARIUM HEAD BLIGHT PREDICTION MODELS IN THE U.S., 2009

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ABSTRACT

A multi-state effort to predict the epidemics of Fusarium head blight continued during the 2009-growing season. These web-based prediction models provide daily estimates of disease risk for 24 states that can be used by growers to evaluate the need for disease management action. The prediction models combine estimates of temperature and relative humidity to predict the risk of an FHB epidemic with greater than 10% field severity. The prediction system incorporates weather observations generated by the Real Time Meso-scale Analysis (RTMA), and weather stations provided by the National Weather Service (NWS). This information is used to generate maps of disease risk throughout the region. Networks of weather stations independent from the NWS are also used by the system to display local estimates of disease risk. The independent nature of these weather networks allows for comparison of the map-based and local estimates of disease risk. In 2009, the prediction tools received more than 8,850 visits between April and August, the period when wheat is actively growing in the 24 states. A user survey conducted in the same year (n=593) indicated that 70% of these users were either farm advisors or farmers. Other users of the system included university extension personnel and members of the grain marketing and milling industries. The survey also indicated that 77% of the users applied the information provided by the prediction system for direct on-farm management decisions, or providing recommendations for disease management. In 2009, 92% of the users considered the information to be of high or moderate value for their farm operations or organization. The estimated net value of the disease prediction system to U.S. wheat growers exceeds $47 million.

ACKNOWLEDGEMENT AND DISCLAIMER

This material is based upon work supported by the U.S. Department of Agriculture, under Agreement No. 59-0790-7-072. 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.

CHARACTERIZATION OF THE SURFACE PROPERTIES OF WHEAT SPIKELET COMPONENTS

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OBJECTIVES

To characterize the physicochemical properties of the surfaces of glume and lemma tissues of wheat spikelets immediately before and after anthesis.

INTRODUCTION

The surface of the aerial parts of terrestrial plants are covered by a waxy cuticle which mediates the interactions between the plant and the environment. The chemical and physical properties of the plant surface determines the nature of these interactions. These properties play an important role in plant-microorganism interactions including influencing the likelihood of a microbial propagule adhering to the plant and subsequently colonizing the surface. In the case of Fusarium Head Blight (FHB), these propagules would include ascospores and macroconidia of *Gibberella zeae*, the primary causal agent of FHB. In addition, the propagules of some beneficial microorganisms also need to interact and adhere to the plant surface, such as biological control agents.

Knowledge of these physicochemical properties of the spikelet surface is also important for developing formulations of sprayable pest control products. Understanding how a spray droplet interacts with the target surface can help in guiding formulation decisions. In order for a spray droplet to adhere to a surface, the droplet must first be able to wet the surface. In general terms, for a liquid to wet a solid, the surface tension of the liquid must be lower than the surface energy of the solid. Most of the targets for pest control spray application are low surface energy targets (plant surfaces). These types of surfaces are commonly referred to as being hydrophobic, since they repel water or the interaction with water is not energetically favorable. Items with a low surface energy are difficult to wet with aqueous solutions, since the surface tension of water (surface energy in the case of a solid) must be reduced below that of the solid for wetting to occur. In order to reduce the surface tension of aqueous solutions low enough to wet these surfaces, surfactants are added, which greatly lower the surface tension of water.

These surface properties are also needed as parameters for accurate spray droplet adhesion models such as the model of Forster (Forster et al. 2005) which incorporates spray droplet size, velocity, surface angle, dynamic surface tension and leaf contact angle measurements. These models can help provide guide decisions when developing new formulations or application technology for a specific crop system.

MATERIALS AND METHODS

Wheat samples - Field trials were conducted in Peoria, IL in 2009. Soft red winter wheat cultivar Freedom (moderately resistant to FHB) and Pioneer Brand 2545 (susceptible to FHB) were grown using standard agronomic conditions (Schisler et al. 2006). Whole wheat heads were cut from plots at split boot (early Feekes 10.1), out of boot (Feekes 10.1), flowering (Feekes 10.5) and at 4, 8, and 12 days after flowering. Heads were stored in zip lock bags on ice until analysis.

Contact angle measurements – To determine the contact angle measurement of glume or lemma tissues, samples were removed from a wheat spikelet and affixed to a microscope slide using double-

sided tape. Contact angles were determined using a FTA4000 video drop shape analysis system (First Ten Angstroms, Portsmouth, VA). A droplet of 5 µl was deposited on to the sample and the image recorded every 100 milliseconds for 15 seconds. Under this time regime, the droplets reached equilibrium. The droplet images were analyzed using software provided by the instrument manufacturer. The images were fit to a non-spherical model. Ten replicate samples were tested for each sample and each liquid.

RESULTS AND DISCUSSION

Water contact angle measurements – The contact angle of a water droplet was determined for the wheat spikelet components (glume and lemma) for samples immediately before and after anthesis. The measurements were conducted on wheat cultivars Freedom and Pioneer Brand 2545. The results for glumes are reported in Figure 1A. The results show the glumes become slightly more hydrophobic leading up to and peaking at anthesis. Immediately after anthesis, a significant drop in hydrophobicity was observed. The most notable difference between the two wheat cultivars was during anthesis when moderately FHB resistant cultivar Freedom had a contact angle approximately 10 degrees higher than Pioneer Brand 2545.

The results for lemma tissue are reported in Figure 1B. The results show a similar pattern as found for glumes, with a drop in hydrophobicity after anthesis. The two cultivars were similar in their properties for the lemma at the different sample times.

Water/acetone contact angle measurements – The contact angle of a 1:1 (vol), water:acetone solution has been used to estimate the surface roughness of various leaves (Forester and Zabkiewicz 2001). It is also needed as a parameter in the spray droplet adhesion model(Forster et al. 2005). The results for the glume tissue for both cultivars are reported in Figure 2A. The results show a similar pattern as observed with the water only contact angle measurements. These results also suggests the glume of the two wheat cultivars may have a slightly different "roughness" or surface morphology. The acetone/water contact angle of the lemma tissue for both cultivars are reported in Figure 2B. The changes in the lemma tissue are less dramatic than those of the glumes although both show changes occurring at or immediately after anthesis.

Overall, these results suggest the surface chemistry and surface ultrastructure of the glume and lemma is changing during anthesis for these cultivars. These changes can also alter the ability of microorganisms to adhere to these surfaces (Lindow and Brandl 2003). It has previously be shown these properties can alter the binding of pathogen conidia to wheat surfaces (Stosch et al. 2007). These properties also play a role in pre-harvest sprouting, by regulating ear wetting via water repellence (King and Von Wettstein-Knowles 2000). Ultimately, it is our goal to determine the role of these properties in microbial colonization of these surfaces. This will include both colonization by the FHB pathogen and beneficial microbes (biocontrol organisms). This data will also be used to optimize pest control formulations for improved delivery and retention to the wheat head.

ACKNOWLEDGEMENT AND DISCLAIMER

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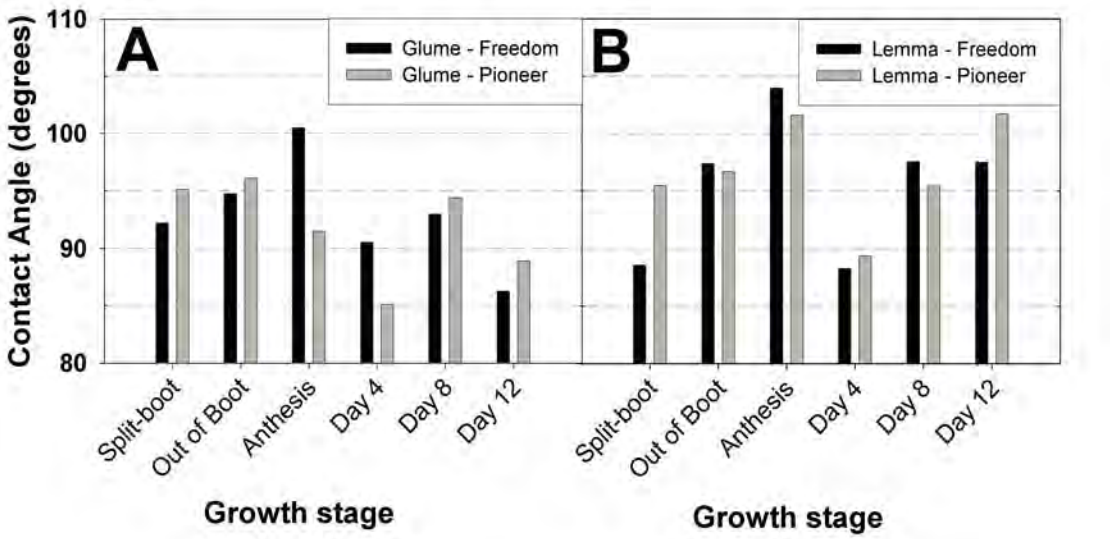

Figure 1. The contact angle of a water droplet on the: **A.)** glume and **B.)** the lemma of two wheat cultivars at different growth stages.

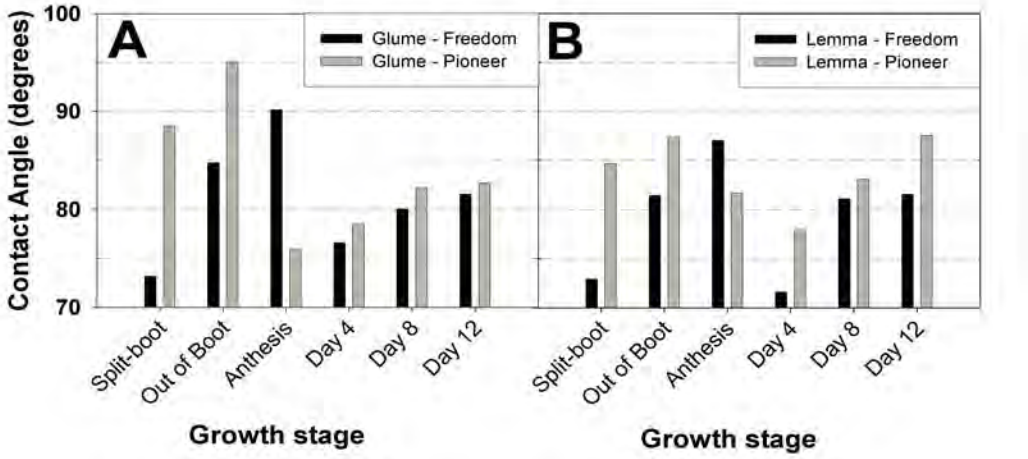

Figure 2. The contact angle of a 1:1 (vol), water:acetone droplet on the: **A.)** glume and **B.)** the lemma of Freedom and Pioneer Brand 2545 cultivars at different growth stages.

HEAD BLIGHTERS AND BLASTERS OF WHEAT: ARE WE READY?

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ABSTRACT

Wheat disease-causing agents can colonize different plant organs including the heads. These are of great importance because of the direct impact on the economic product of wheat – the kernels. In Brazil, there is a great concern about the increasing threat that two fungal diseases, known as wheat blast and head blight, now pose to wheat production. Wheat blast is incited by *Magnaporthe grisea* (*anamorph = Pyricularia grisea*). This disease was first described in wheat, in 1985, in northern Paraná, Brazil. Since then it has been reported in all wheat growing areas of the country. Up to this far, wheat blast occurrence is restricted to lower latitude wheat growing areas as those in Brazil, Paraguay and Bolivia. The disease may cause severe damage under conditions of high temperature (28°C) and high humidity (>93%). Fusarium head blight caused by *Gibberella zeae* is currently one of the most important diseases of wheat worldwide. Epidemics have been observed with a higher frequency in recent years in several regions, with damage on both yield and grain quality. In Brazil, the pathosystem has been studied for more than three decades, and recent reports indicate that a previous disease, previously sporadically outbreaking, achieved the status of major disease in wheat growing regions of southern Brazil causing significant economic impacts. Fusarium head blight is described as a disease of warm and humid climate, so that the rainfall and temperature are the main factors that influence the occurrence and severity of epidemics of this disease. Both fungi have other hosts besides wheat and can survive on crop residues. Depending on the region and time of the year, low temperatures or long dry periods may prevent growth and development of these fungi. Mechanistic simulations models have been developed for both diseases. The disease models take into account host development including details of the heading process (i.e., proportion of heads emerged, anther extrusion, grain filling and physiological maturity). Inoculum is considered not limiting. Hourly observed and 5-day forecast data for precipitation, relative humidity and temperature are used in mathematical equations to estimate infection risks. The models are implemented in a web platform which is intended to provide risk information to assist decision-making on crop management. An important wheat producing area in Brazil characterized by warm temperatures and moderately dry, is comprised by the North of Paraná, São Paulo and South of Mato Grosso do Sul states. This area, despite the likely occurrence of water stress during pre-flowering in some years, is considered a favorable environment for wheat production in terms of yield potential and quality. During the growing season of 2009, an abnormally high frequency of rainy days in July and August was observed. In the state of São Paulo, for example, July rainfall had record precipitation, four times the normal, since meteorological observations started in 1943. The resulting humid and warm climate observed in July coincided with the heading stage of wheat. Consequently, there were outbreaks of both Fusarium head blight and wheat blast. Crop yield declined 23% from pre-season estimates. The harvested product has been rejected by the milling industry due to low quality for bread and pasta making. Farmers suffered heavily from this climatic condition that resulted in crop failure due to head blighters of wheat. Continued global warming is likely to exacerbate Fusarium head blight problem. Moreover, it may contribute to the expansion of the geographical limits of new diseases like wheat blast. This hypothesis is supported by empirical evidence of the occurrence of wheat blast in

more southern regions of Brazil, which may relate to the warmer winters that have occurred in recent years. Therefore, efforts need to be made for better understanding of damaging head diseases of wheat in order to reduce impact on grain yield and quality, especially the mycotoxin issue related to Fusarium head blight. Genomic-based approaches promise to make a large and immediate impact through the identification of genes for disease resistance. However, the goal of lasting head blight disease control will depend on having an equally comprehensive understanding of the disease process from an epidemiological perspective.

RISK MAPPING FUSARIUM HEAD BLIGHT OF WHEAT IN BRAZIL

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ABSTRACT

Available epidemiological knowledge was previously used for developing a simulation model to predict Fusarium head blight infection risk in southern Brazilian conditions. The model has been successfully validated and incorporated into a web-based system to warn of FHB risk within-season using both site-specific observed and 5-day forecast weather. We have further used the model to assess disease risk under the influence of climate variability, especially under the effect of El Niño southern oscillation, and management practices (sowing dates) by using historical (50-year) records of weather data for a single location. We are now working on the development of tools to map disease risks over a broader geographic region. FHB risk maps are computer-generated images depicting the risk using special interpolation techniques within points indicated by the geographical location of automated weather stations. The final risk maps are made by color transparency layers which overlays a geographic map. Besides extending risk information for a large geographical region the use of intuitive images representing epidemic risks may facilitate dissemination and understanding of risks to guide decision-making on FHB management. In addition, maps may be useful for the fine tuning of wheat zoning and for the identification of post-harvest areas with lower probability risk of mycotoxin contamination.

AGRONOMIC FACTORS AFFECTING SPECIFIC MYCOTOXIN PRODUCTION IN FUSARIUM HEAD BLIGHT INFECTED WHEAT

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ABSTRACT

Several earlier studies have demonstrated a lack of correlation between the fungal biomass in grains from Fusarium head blight (FHB) infected ears of winter wheat and the level of mycotoxins such as deoxynivalenol (DON). However, the factors governing the specific mycotoxin synthesis are hardly known. In this study, results from experiments with four non-tillage crop rotations, three fungicide regimes and two cultivars run in two locations in Lower Saxony, Northern Germany, since 2007 are demonstrated. Pre-crops were wheat, sugar beets, maize and oil radish. A highly susceptible cultivar (cv. Ritmo) and a moderately resistant cultivar (cv. Centrum) were tested with three different fungicide regimes, one based on triazoles, a second on strobilurins and a third including chlorothalonil with no known physiological effects on the crop. Fungicides were applied twice on the foliage in growth stages (GS) 31 and GS 39, and no fungicides were applied after ear emergence or during anthesis. The FHB index was scored during milky ripening stage and compared with mycotoxin levels in grains after harvest. Furthermore, the fungal biomass of the predominant toxigenic species, *F. graminearum*, was measured with quantitative real-time PCR. The total DON content in wheat following maize was three times higher than following the other pre-crops. Similar levels of DON were recorded in wheat following either wheat or sugar beet pre-crops. Cultivar resistance reduced total DON content about 75%. Foliar application of strobilurins prior to ear emergence tended to increase the total mycotoxin level in grains. Specific mycotoxin production per µg *F. graminearum* DNA was not affected by fungicide treatments, while it was elevated in the susceptible cultivar and following maize and sugar beet pre-crops. However, specific toxin production was significantly affected by the location, indicating a predominant role for environmental factors in affecting the relative intensity of mycotoxin synthesis.

DEOXYNIVALENOL GENE EXPRESSION DURING WHEAT HEAD INFECTION BY *FUSARIUM GRAMINEARUM*

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ABSTRACT

The trichothecene mycotoxin deoxynivalenol (DON) is produced by several *Fusarium* species during infection of grain crops. *Tri5* encodes trichodiene synthase, necessary for DON production. Previous experiments, including Affymetrix GeneChip assays, indicated that *Tri5* is highly expressed during wheat infection. We dissected wheat heads from anthesis through kernel development with *Fusarium graminearum* and monitored *Tri5* gene expression using quantitative reverse transcript PCR (qRT-PCR). *Tri5* expression was compared with the expression of the housekeeping genes and relative abundances determined; the housekeeping genes also served as an indicator of fungal presence. The results present a detailed picture of fungal colonization and DON production during wheat head colonization.

EVALUATION OF BIOLOGICAL ALTERNATIVES FOR SINGLE TREATMENT FUNGICIDE ON HARD RED SPRING WHEAT FOR CONTROLLING FUSARIUM HEAD BLIGHT

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ABSTRACT

Several strategies using biological components were evaluated as alternatives to a single timing Prosaro fungicide treatment for reducing the effects of Fusarium head blight (FHB) in hard red spring wheat (HRSW) at Langdon, ND in 2008 and 2009. Biological alternatives tested were 1BA a *Bacillus* sp. provided by B. Bleakley from South Dakota State University, Brooking, SD, C3 *Lysobacter enzymongenes* from G. Yuen from University of Nebraska, Lincoln NE, TrigoCor 1448, *Bacillus subtilis* from G. Bergstrom, Cornell University, Ithaca, NY, a double yeast from D. Schisler of NCAUR, USDA-ARS Peoria, IL and Taegro, *Bacillus subtilis* var. amyloliquefacians from Novozyme Biologicals, Inc., Salem, VA, a product that was commercially available in 2008. Alternative strategies included a) biological treatment at Feekes growth stage (GS) 10.51, b) GS 10.54, c) a tank mix of Prosaro fungicide and a biological applied at GS 10.51 and d) Prosaro fungicide applied at GS 10.51 followed by a biological application at GS 10.54. A control treatment was included that received an application of water with the adjuvant Induce. All biological and fungicide treatments were applied with Induce at a v/v rate of 0.125%. The treatments were applied to Howard HRSW. Howard is a high yielding cultivar moderately susceptible to FHB from North Dakota State University. FHB incidence of the control ≥ GS 10.51 biologicals > Prosaro ≥ Prosaro/biological tank mixes ≥ Prosaro at GS 10.51 and GS 10.54 biologicals. FHB head severity of the GS 10.54 biological ≥ control ≥ GS 10.51 biologicals > Prosaro ≥ Prosaro/biological tank mixes ≥ Prosaro at GS 10.51and GS 10.54 biologicals. Deoxynivalenol (DON) concentrations from 2008 biologicals at GS 10.51> control > biologicals at GS 10.54 > Prosaro > Prosaro/biological tank mixes. Yield for the control ≤ biologicals at GS 10.51 and GS 10.54 < Prosaro/biological tank mixes and Prosaro fungicide.

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INTEGRATING RESISTANCE, BEST APPLICATION TIMING AND BEST FUNGICIDE DELIVERY TECHNIQUE FOR IMPROVED EFFICACY ON BARLEY, LANGDON, 2008

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ABSTRACT

Fusarium head blight (FHB) has reduced small grain yield and affected crop quality in the Northern Plains region of North America since 1993 and many other wheat and barley production regions worldwide. Genetic resistance to infection and spread of FHB will need to be combined with other management strategies to reduce losses in the short term. Control by fungicides has been inconsistent. This variability is due to environment, cultivar resistance, pathogen virulence, fungicide application method, coverage and fungicide timing. In the United States producers have been limited to one application of fungicide at the extended head growth stage in barley. Two cultivars were used in the trials including "Tradition" and a North Dakota Experimental labeled "ND20448". No differences were determined for FHB incidence, severity, index, test weight and plump. Foliar leaf disease was greater on the "ND20448" as compared to "Tradition", but foliar disease pressure was low in 2008. A significant interaction for yield was measured between cultivar and timing. The yield of "Tradition" barley was increased when fungicide was applied at Feekes GS 10.3 as compared to the GS 10.5. An interaction was measured for DON concentration between the cultivar and timing and also between the orifice orientation and timing. Deoxynivalenol levels decreased when the fungicide was applied to both cultivars at GS 10.5 as compared to 10.3. Deoxynivalenol levels also decreased further on cultivar ND20448 by the application timing at GS 10.5+5 days. A vertical oriented nozzle was less effective than a nozzle angled 30 degrees downward from horizontal and forward in depositing spray solution, 0.2 versus 0.34.

INTRODUCTION

Infection in small grains, by Fusarium head blight, also known as scab affects the food and feed quality of barley due to the toxin deoxynivalenol (DON). Damage from FHB affects the food and feed quality of barley because deoxynivalenol accumulates in the grain and cannot be cost-effectively removed from the end use product. The American Malting Barley Association recommends less than 0.5 ppm DON in barley used to brew beer. Grain with DON levels greater than 0.5 for barley are accepted for feed usage by some livestock industries but at reduced prices. Eastern North Dakota and northwest Minnesota growers have had barley yield and quality affected adversely by FHB since 1993 as environmental conditions have often been conducive for the development of the disease.

Applications of fungicide to headed barley are recommended as a management strategy to reduce damage in small grains caused by FHB. Fungicide application timing can be critical to obtaining favorable results. Other strategies such as planting resistant cultivars and using crop rotations are also recommended. Using resistant cultivars would be the preferred strategy by producers but

developing cultivars with levels of resistance that would not require use of fungicides is slow to be achieved. The environment, temperature, rainfall and humidity usually determine the severity of the FHB epidemic.

Results from fungicide applications are often highly variable and range from effective to poor. This variability can be caused by environment, differences in cultivar resistance, pathogen virulence, fungicide coverage, and fungicide timing. Temperature and water activity have been shown to produce an extreme impact on deoxynivalenol growth. Efforts are ongoing to evaluate application technologies that will increase the amount of fungicide deposited on the grain spike. In North Dakota producers have been limited to one fungicide application at heading to provide disease control making deposition and timing a very critical component to increased efficacy. FHB has a multiple disease cycle making control difficult with one fungicide application.

NDSU studies have examined the effect of fungicide application at varying development stages to determine the effects on FHB incidence and field severity, incidence, yield, test weight, plump, DON accumulation, and head coverage. The studies were designed to compare a commonly grown malt barley with an experimental that has been shown to have about 1/3 less deoxynivalenol accumulation in the grain.

MATERIALS AND METHODS

A study was conducted in 2008 at the North Dakota State University Langdon Research Extension Center, Langdon North Dakota. The principle objective of the study was to integrate and test three 'recommended strategies' for reducing the impact of Fusarium head blight (FHB) in barley. The three strategies included comparing a standard malt type barley 'Tradition' with an experimental 'ND20448'. 'ND20448' is from the North Dakota State University barley breeding program. In previous studies 'ND20448' has had about one-third less deoxynivalenol accumulation in the seed than 'Tradition' and is being tested by the brewing industry for consideration as a malt type. The second strategy compared applying Prosaro fungicide application at growth stage (GS) Feekes 10.3, 10.5 and five days after the 10.5 GS application. GS 10.5 is the recommended GS for applying fungicide to barley. The third strategy compares a delivery method with a vertically oriented nozzle orifice that is typically used for the application of herbicides to field crops. This configuration comes as a standard with sprayer systems when they are purchased. Our comparative parameter was the recommended configuration for fungicide application for controlling FHB. This configuration has the nozzle orifices oriented 30° downward from horizontal and forward in the direction of travel of the tractor. The study was designed as a randomized complete block with a split split plot arrangement and four replications. Mean treatment comparison measured coverage reported as absorbance for coverage, disease incidence, severity and index, deoxynivalenol concentration (DON) in the grain, yield, test weight and plump. Prosaro 421 SC (203kg a.i./ha) fungicide, a 50/50 blend of prothioconazole and tebuconazole, is marketed by Bayer CropScience, Research Triangle Park, North Carolina 27709, USA. Prosaro was applied at 474.5 ml ha^{-1}in a tank mix that included the non-ionic surfactant Induce (Helena Chemical Co., Collerville, Tennessee 38017) at 0.125% v/v. The previous crop was field pea. The soil type was a Barnes/Svea complex (fine-loamy, mixed superactive Frigid, Calcic Hapludolls/mixed superactive Frigid, Pachic Hapludolls). Blocks of 'Tradition', a malt type barley, and 'ND20448', were planted on 7 May with an Almaco double-disk drill with a row spacing of 15 cm. After emergence and weed control were completed, each block was divided into plots 3.6 x 9.1 m. After delineation of the plots, a *Fusarium* inoculum was hand-broadcast on each plot to encourage development of disease. A food grade dye, FD&C blue #1, was mixed with the fungicide solutions at a rate of 108.7 gm ha^{-1}. The dye was included as an indirect type measurement to determine differences in coverage on the grain head. After agitation, the treatments were applied with a tractor using a side-mounted spray boom.

The tractor traveled 2.68 m s^{-1} (6 mph) delivering a solution 93.5 l ha^{-1} at 276 kPa psi using Spraying Systems XR8002 nozzles. The sprayer was equipped with a CO_2 type delivery system instead of a standard pump. After applying the treatments, a sample of 15 heads were collected from each plot, deposited in 250 ml Erlenmeyer flasks, sealed with stoppers and placed on ice. 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 (Burrell Scientific Instruments and Laboratory Supplies, Model BT, Pittsburgh, Pennsylvania 15219). A sub sample of the solution was placed in a cuvette and placed in a Jenway photospectrometer (Jenway, Model 6300, Dunmow, Essex CM6 3LB England) 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 heads. After the fungicide was applied an impact type sprinkler irrigation system was installed (sprinkler heads were spaced on 9.1x 12.2 m centers) to modify the environment as needed and encourage the development of disease. North Dakota State University Extension recommended production practices for hard red spring wheat in Northeast North Dakota were followed. A visual disease evaluation was made from 20 samples per plot collected 20 days after the first fungicide application. The estimate of FHB incidence (number of spikes infected) head severity (average number of infected kernels per infected head), and FHB index (number of infected kernels per head divided by total kernels per individual spike), was determined for each plot. Five leaves were also sampled and scored to determine foliar disease severity. A rotary mower removed the front and back five feet from each plot prior to harvest to minimize any chance of interference by inaccurate application from the tractor sprayer when stopping or starting. Each plot was harvested with a Hege plot combine and the grain sample cleaned and processed for yield and test weight. A sub sample was ground and analyzed for deoxynivalenol (DON) by the North Dakota State University barley quality lab. 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 (Table 1).

RESULTS AND DISCUSSION

An untreated 'ND20448' and 'Tradition' were included in the trial for reference. However, data from the untreated plots were not included in the analysis because an untreated did not fit the timing and nozzle orientation factors. No differences were determined for FHB incidence, severity, index, test weight or plump. Foliar (leaf) disease severity was greater on the 'ND20448' compared to 'Tradition' (Table 2). The foliar disease pressure was quite low in 2008. Foliar disease was greater when the fungicide timing was Feekes 10.5 + 5 days. In other studies comparing the two cultivars, 'ND20448' has yielded less at times and had greater percentage plump kernels. Numerically, the 'ND20448' had both greater yield and plump. It has been theorized that the yield differences can possibly be attributed to differences in the cultivar's tolerance to root rot. This trial was conducted on previous crop field pea where the inoculum level in the soil may have been lower than trials conducted on previous crop small grains. A significant interaction for yield was measured between cultivar and timings. The yield of 'Tradition' barley was increased from 5649 kg ha^{-1} (105.1 bu a^{-1}) when the fungicide was applied at GS 10.3 to 6165 kg ha^{-1} (114.7 bu a^{-1}) at GS 10.5. Growth stage 10.5 would be the recommended fungicide application timing to maximize efficacy for control of FHB. Percentage plump of both cultivars were very good and numerically the 'ND20448' had greater plump. An interaction was measured for DON concentration between the cultivar and timing and also between the orifice orientation and timing. Deoxynivalenol levels were decreased when the fungicide applied to 'Tradition' was applied at GS 10.5 compared to 10.3. A similar decrease was measured on the 'ND20448'. An additional decrease was measured by the 5 day later fungicide application. While one study is not enough to change application timing recommendations, it certainly warrants further research. When the nozzle orifices were oriented forward the

application timing 10.3 had significantly greater DON than the other growth stages. This would concur with North Dakota State University Extension recommendations on application techniques and timing. When fungicide was applied with vertically oriented nozzles, a reduction in DON concentration was measured from GS 10.3 to GS 10.5 on 'Tradition' and GS 10.5 to 5 days after GS 10.5 on 'ND20448'. While this reduction could be partially related to the environment at the late spray date, perhaps more air turbulence increasing coverage, our data does not statistically support this conclusion. Numerically the vertical oriented nozzles had increased coverage as the fungicide timing was delayed. Statistically the coverage was greater with the two earlier timings compared to the late timing with the 30° forward facing nozzles. The maximum coverage obtained coincided with the lowest DON concentration which occurred at the fungicide application GS 10.5 with the 30° forward facing nozzles.

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Table 1. Source of variation, confidence intervals and coefficient of variation Langdon, 2008.

	Fusarium head blight			Leaf		Test			
	Incidence	Severity	Index	Severity	Yield	Weight	Plump	DON	Coverage
Cultivar (cult)	0.2362	0.0829	0.0894	0.0086	0.4497	0.8594	0.1421	0.9935	0.3946
Rep*cult	0.0949	0.0069	0.0042	0.8061	0.0934	0.0004	<0.0001	0.0015	0.0112
Orientation (orient)	0.3600	0.8041	0.9613	0.2610	0.5982	0.9536	0.6513	0.0497	<0.0001
Cult*orient	0.2346	0.5301	0.8864	0.6989	0.7494	0.9871	0.2149	0.5481	0.0914
Rep*orient	0.0258	0.3190	0.1700	0.2226	0.3785	0.4232	0.0858	0.7553	0.8739
Timing (tim)	0.2918	0.1757	0.1737	0.0141	0.8432	0.7518	0.8546	<0.0001	0.1665
Cult*tim	0.1250	0.3109	0.1995	0.3628	0.0224	0.9017	0.5501	0.0054	0.7822
Orient*tim	0.3924	0.4264	0.3841	0.2545	0.3520	0.2142	0.5159	0.0133	0.0004
Cult*orient*tim	0.7261	0.7962	0.8187	0.3731	0.3572	0.9799	0.2368	0.1493	0.2217
%C.V.	2.56	14.52	15.66	58.06	7.97	1.75	1.91	29.83	29.70

Table 2. Fusarium head blight incidence, severity and index, leaf severity, yield, test weight, plump, DON and head coverage by cultivar, nozzle orientation, and application timings (Feekes growth stage), Langdon 2008.

Cultivar, Nozzle Orientation or Appl. Growth Stage	Fusarium head blight Incidence %	Fusarium head blight Severity %	Fusarium head blight Index	Leaf Severity %	Yield kg ha^{-1} (bu a^{-1})	Test Weight kg m^{-3}	Plump %	DON ppm	Coverage
ND20448 untreated	100.0	11.9	11.9	8.2	5617 (104.5)	607	95.5	1.37	0.02
Tradition untreated	100.0	11.9	11.9	10.8	5531 (102.9)	618	88.0	1.43	0.03
ND20448	99.8	12.0	12.0	9.1	6122 (113.9)	616	95.9	0.62	0.29
Tradition	98.1	9.4	9.14	5.0	5934 (110.4)	618	89.5	0.62	0.24
LSD $_{(0.05)}$	NS	NS	NS	2.1	NS	NS	NS	NS	NS
Vertical	99.6	10.6	10.6	7.9	6068 (112.9)	617	92.9	0.67	0.20
30° F down from Horizontal	98.3	10.8	10.5	6.1	5988 (111.4)	617	92.5	0.57	0.34
LSD $_{(0.05)}$	NS	NS	NS	NS	NS	NS	NS	0.1	0.03
10.3	99.4	11.3	11.2	6.3	5999 (111.6)	618	92.6	1.01	0.28
10.5	98.1	10.5	10.3	5.1	6004 (111.7)	618	92.9	0.43	0.29
10.5 + 5 days	99.4	10.3	10.2	9.6	6085 (113.2)	615	92.6	0.41	0.24
	NS	NS	NS	3.0	NS	NS	NS	NS	NS
Cultivar by fungicide timing averaged across both nozzle orientations									
ND20448 10.3					6343 (118.0)			1.12	
10.5					5843 (108.7)			0.45	
10.5+5					6181 (115.0)			0.29	
Tradition 10.3					5649 (105.1)			0.90	
10.5					6165 (114.7)			0.42	
10.5+5					5993 (111.5)			0.54	
Nozzle orientation by fungicide timing averaged across both cultivars									
Vertical 10.3								0.98	0.17
10.5								0.48	0.18
10.5+5								0.25	0.24
30° F 10.3								1.04	0.39
10.5								0.39	0.39
10.5+5								0.57	0.23

LSD Yield: To compare two-subplot means at the same levels of the whole plot (a_0b_0 vs a_0b_1)=511 (9.5) and to compare two whole plot means at the same or different levels of the subplot (a_0b_0 vs a_1b_0)=769 (14.3) ; LSD DON: To compare two-subplot means at the same levels of the whole plot (a_0b_0 vs a_0b_1)=0.14 and to compare two whole plot means at the same or different levels of the subplot (a_0b_0 vs a_1b_0)=0.43 ;LSD DON: To compare two-subplot means at the same levels of the subplot (b_0c_0 vs b_0c_1)=0.19 and to compare two subplot means at the same or different levels of the sub-subplot (b_0c_0 vs b_1c_0)=0.018 LSD Coverage: To compare two-subplot means at the same levels of the subplot (b_0c_0 vs b_0c_1)=0.082 and to compare two subplot means at the same or different levels of the sub-subplot (b_0c_0 vs b_1c_0)=0.075.

REACTION OF WINTER WHEAT CULTIVARS TO FHB AND DON

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ABSTRACT

Fusarium head blight (FHB) of wheat, caused by *Fusarium graminearum*, produces significant losses resulting from yield reduction, kernel damage, and presence of the mycotoxin deoxynivalenol (DON). One strategy for management of FHB and DON is to plant resistant/tolerant cultivars. In 2009, an experiment was conducted to study the reaction of winter wheat cultivars to FHB and DON. Twenty cultivars (Overley, Overland, Jagalene, Mace, Hawken, Goodstreak, Bond CL, Wahoo, Wesley, Camelot, Postrock, Millennium, 2137, Harry, Settler CL, Art, Infinity CL, Hatcher, Bill Brown, and Alliance) were planted in the fall of 2008 in a commercial field near Paxton, Nebraska. Plots were irrigated and not inoculated, but there was heavy natural inoculum of *F. graminearum*. Experimental design was a randomized complete block with three replications. FHB severity and incidence were determined on the 2nd of July 2009, on 10 heads in each of 10 arbitrarily selected locations in each plot and used to calculate FHB index. Plots were harvested with a small plot combine. The percentage of *Fusarium*-damaged kernels (FDK) was measured with an automated single-kernel near-infrared system at the USDA ARS Grain Marketing and Production Research Center in Manhattan, KS. Grain samples from all plots were ground and sent to the North Dakota Veterinary Diagnostic Laboratory at North Dakota State University, Fargo, ND for DON determination. Linear correlation analysis was used to determine relationships between FHB index, FDK, DON, and yield. Differences among cultivars were highly significant for FHB index ($P < 0.0001$) and yield ($P < 0.0001$). Overley had the highest FHB index (20%) followed by Jagalene (13%), 2137 (12%) Bond (12%), and Wesley (12%). Goodstreak had the lowest FHB index (2%) followed by Mace (4%), Infinity CL (4%), Art (4%), and Overland (4%). Bond CL had the highest yield (93 bu/acre) followed by Camelot (90 bu/acre), Settler CL (89 bu/acre), Infinity CL (89 bu/acre), Jagalene (89 bu/acre), Wesley (88 bu/acre), Harry (87 bu/acre), Art (87 bu/acre), Wahoo (85 bu/acre), and Overley (85 bu/acre). Mace had the lowest yield (62 bu/acre) followed by 2137 (67 bu/acre). FHB index in the rest of the cultivars ranged from 5% to 10%. FDK and DON were generally low and not significantly different among cultivars at $P = 0.05$. DON concentration ranged from 0.2 ppm (Overland) to 4.3 ppm (Postrock) and FDK ranged from 6% (Overley) to 24% (Harry). There was a significant positive correlation between index and DON ($r = 0.62$, $P = 0.0037$), and between incidence and DON ($r = 0.60$, $P = 0.0048$). All other correlations were not significant at $P = 0.05$. Linear regression analysis with DON as the dependent variable showed that the relationship between index and DON was the strongest ($R^2 = 0.38$, $P = 0.0037$), followed by the relationship between incidence and DON ($R^2 = 0.36$, $P = 0.0049$). The relationship between severity and DON was not significant ($R^2 = 0.16$, $P = 0.0843$).

This study demonstrated differences among winter wheat cultivars in their reaction to FHB and DON. It was interesting to note that Overley, Jagalene and Wesley were among the most susceptible cultivars for the second year in a row. Based on the data from this study, the best predictor of DON concentration was FHB index.

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RELATIONSHIP BETWEEN FUSARIUM HEAD BLIGHT SEVERITY AND DEOXYNIVALENOL CONCENTRATION IN THREE WINTER WHEAT CULTIVARS

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ABSTRACT

Fusarium head blight (FHB) caused by *Fusarium graminearum* is a destructive disease of wheat. *F. graminearum* produces the mycotoxin deoxynivalenol (DON) which accumulates in grain and has serious food safety implications. Additionally, it can cause significant losses resulting from yield reduction and kernel damage. *Fusarium*-damaged kernels, commonly referred to as FDK, are shriveled and/or discolored. The higher the percentage of FDK in grain, the lower the yield, test weight, and grain quality. The relationship between FHB severity (FHBsev) and DON can be used to estimate the level of DON to expect in grain, enabling producers to make informed decisions early regarding the marketing or end use of grain from fields affected by FHB. The objectives of this study were to i) investigate the nature of the relationship between FHBsev and DON in three winter wheat cultivars, Jagalene, Harry, and 2137, and ii) determine if there were differences among the three cultivars in in the levels of DON they accumulated.

The cultivars were planted following corn in October 2008 at the University of Nebraska Agricultural Research and Development Center near Mead, NE. In addition to natural inoculum, plots were inoculated with 1×10^5 spores/ml of *F. graminearum* at early anthesis in May/June 2009 and were not irrigated. Cultivars were arranged in a randomized complete block design with three replications. FHBsev was determined 21 days after inoculation on 20 heads tagged in each of 11 disease severity categories in each plot: 0, 5, 10, 15, 20, 25, 30, 35, 40, 45, and 50%. After harvest, DON concentration in grain from each severity category was determined at the North Dakota Veterinary Diagnostic Laboratory.

There was a significant positive correlation between FHBsev in the 11 categories and DON for all three cultivars: Jagalene ($r = 0.91$, $P = .0001$), Harry ($r = 0.77$, $P = 0.0053$), and 2137 ($r = 0.73$, $P = 0.0101$). Linear regression analysis with DON as the dependent variable showed that the relationship between FHBsev and DON was strongest for Jagalene ($R^2 = 0.83$, $P < 0.0001$) followed by Harry ($R^2 = 0.60$, $P = 0.0053$), and 2137 ($R^2 = 0.54$, $P = 0.0101$).

This study demonstrated (i) a positive linear relationship between DON and FHBsev in three winter wheat cultivars and (ii) differences among the three cultivars in the levels of DON they accumulated. In 2007 and 2008, similar results were obtained.

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INFLUENCE OF CROP RESIDUES AND DISEASE RESISTANCE ON FHB IN VIRGINIA WHEAT

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ABSTRACT

Knowledge of the influence of crop residues and disease resistance on Fusarium head blight (FHB) in winter wheat is important for improving disease management strategies. Approximately 3.2 acres of the winter wheat cultivars Pioneer 26R12, Tribute, SS560, and Vigoro 9510 were planted in Blacksburg, Virginia in 2008. Pioneer 26R12 and SS560 are considered to be susceptible to FHB, and Tribute and Vigoro 9510 are considered to be moderately resistant to FHB. Varying amounts of corn stalk pieces (45g and 410g) infested with a clonal isolate of *G. zeae* were released in replicated 1 m diameter circular plots. Mature wheat spikes were collected at the released inoculum source, at a radius of 3.1 m (10 ft.) from the source, and from non-inoculated (control) locations separated 16.5 m (54 ft.) from the nearest released source. Spikes were observed for symptoms of FHB, disinfested, and plated onto a *Fusarium*-selective medium. Over 600 isolates of *G. zeae* were recovered from spikes approximately two weeks, four weeks, and six weeks after anthesis. For plots containing 45 g of inocula, disease incidence ranged from 55% to 79% and DON ranged from 18 to 45 ppm for all cultivars. For plots containing 410 g of inocula, disease incidence ranged from 88% to 100% and DON ranged from 19 to 109 ppm for all cultivars. Ongoing DNA-based methodologies are being used to determine the contribution of the released clone (relative to background sources) to FHB and DON in each of our experimental plots. Results from our first year of experimentation indicate that varying amounts of within-plot *G. zeae* inocula influence FHB and DON, despite varying levels of disease resistance that may be attributed to each of the cultivars used in this experiment.

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MULTI-STATE ASSESSMENT USING WINDOW PANE ANALYSIS CONFIRMING WEATHER VARIABLES RELATED TO FUSARIUM HEAD BLIGHT EPIDEMICS

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ABSTRACT

Fusarium head blight (FHB) of wheat, caused by *Fusarium graminearum*, is a sporadic disease that is dependent, at least in part, on weather and climatic conditions. The objectives of this research were to determine whether the annual variability in FHB in Ohio can be related to variability from year-to-year in environmental conditions over short or long time scales, and to compare findings with results found for three other states, Indiana, Kansas, and North Dakota. Historical records of FHB intensity in each state were used to address this issue. In Ohio, overall FHB intensity in the state was rated on an ordinal scale from 0 to 9 for 44 years (1964-2008). In Indiana, disease was assessed for 36 years (1973-2008) in variety nurseries by Purdue University researchers. In Kansas, disease index was assessed for 28 years (1978, 1980, 1982-2007) by Kansas State University, Kansas Department of Agriculture, and USDA-ARS personnel, based on surveys of wheat in the state. In North Dakota, FHB intensity was rated for 23 years (1986-2008) on an ordinal scale from 0 to 9 (same numerical scale as in Ohio), based on results from field surveys by North Dakota State University extension agents and specialists. Weather data were gathered from local weather stations within each state, and summary variables (such as average RH, precipitation, temperature) were calculated for a wide range of time windows and starting times of the windows during the growing season. The windows ranged from 10 to 280 days in duration, beginning around physiological crop maturity and proceeding backwards to the fall of the previous year (for winter wheat). This methodology is a form of data mining that has been termed 'Window-Pane' analysis.

The relationship between each summary environmental variable and disease intensity was quantified with a Spearman rank correlation coefficient for each of the window lengths and starting times. This rank-based nonparametric correlation was used due to the ordinal nature of the FHB intensity data in Ohio and North Dakota, and because of the non-normal data in the other locations. Based on Spearman rank correlations, the FHB rating in all states was significantly ($P < 0.05$) associated with the mean average daily relative humidity for short time windows during and shortly after anthesis (Feeks 10.5.1), covering the periods for infection, spike colonization, and early DON production. In Ohio, significant associations were also found around late April, early March, and late December, covering the period of spore production (April) and pathogen winter survival. In all locations, total daily precipitation was significantly associated with FHB intensity around the time of heading and flowering for various time-window lengths. There were no significant relationships found between the mean average daily temperature and FHB intensity for any time window in any location. In general, correlations between FHB intensity and weather variables were stronger with shorter window lengths, and weather toward the end of the growing season was the strongest indicator of FHB epidemics.

EFFECT OF VARIETY, LOCATION, AND ENVIRONMENT ON DEVELOPMENT OF FUSARIUM HEAD BLIGHT IN SOFT RED WINTER WHEAT IN WISCONSIN

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OBJECTIVE

To investigate how variety, location, and environment affect the risk of Fusarium head blight in soft red winter wheat in Wisconsin.

INTRODUCTION

During epidemic years, Fusarium head blight (FHB, scab, *Fusarium graminearum* group 2, teleomorph: *Gibberella zeae*) has the potential to cause significant reductions in both yield and quality of wheat grain and seed. Because infection occurs in the anthers, the risk of infection is greatest from flowering (Feekes 10.51) through the soft dough stage (Feekes 11.2) (Dill-Macky, 1997). During this period, disease development is favored by prolonged periods of moisture, high relative humidity, and temperatures from 64 to 80°F (Nyvall, 1999; Weise, 1989).

Symptoms of FHB are typically not observed until soft dough, and at this growth stage, it may be too late to implement management tactics. In Wisconsin, wheat acreage has increased and many growers lack the necessary information about how variety, location, and environment may interact to affect the risk of FHB development. Therefore, in order to improve recommendations for managing FHB in Wisconsin, we are investigating the role of each of these factors in FHB development.

MATERIALS AND METHODS

To examine the effect location, variety, and environment on the development of FHB, data were collected as part of the Wisconsin Winter Wheat Performance Tests (Results available at: http://coolbean.info). The performance tests were conducted at four locations around Wisconsin: Arlington, Chilton, Janesville, and Lancaster. At three of the locations (Arlington, Chilton, and Lancaster), 58 varieties of soft red winter wheat (*Triticum aestivum*) were planted in a randomized complete block design with four replications. At the Janesville location, the experimental design was a split-plot design with fungicide treatmentas the whole plot level and variety as the subplot level. Fungicide application (Quilt (R) Syngenta, azoxystrobin and propiconazole) was done at Feekes stage 9 for control of foliar diseases. Plots were 8' wide (7.5" row spacing) by 25' long, with seven center harvest rows and two non-harvest rows; all disease assessments were made in the non-harvested rows.

Fields were seeded at 1.5 million viable seeds per acre using a grain drill with cone units. The center seven rows were harvested using a self-propelled combine. Previous crop history, planting, flowering, and harvest date information are shown in Table 1.

Disease assessments were made for both foliar diseases and FHB. Assessments for foliar diseases were made at Feekes 4/5, Feekes 6/7, Feekes 8/9, and Feekes 10.51. During each assessment, 6 stems were destructively sampled from the non-harvested rows of each plot. Whole plant incidence and severity on the upper four leaves (flag, flag-1, flag-2, flag-3) were assessed for each stem. A weighted severity score was calculated for each plant as: weighted disease severity = (4 × severity on flag leaf) + (3 × severity on flag-1 leaf) + (2 × severity

on flag-2 leaf) + (severity on flag-3 leaf) (Lipps and Madden, 1989). Incidence and severity of FHB were assessed on 100 heads per plot at Feekes 11.2. The Fusarium head blight index (FHBI) value was also calculated for each plot as: FHBI = (% incidence × % severity) ÷ 100 (Conley *et al*, 2009; Paul *et al*, 2005). Following harvest, the percentage of *Fusarium* damaged kernels (FDK) was assessed for a 200 kernel sample from each plot.

Weather data during the spring and summer period were obtained using HOBO U30 weather stations (Onset Computer Corporation, Bourne, MA). Each station was equipped with sensors for temperature, relative humidity (hygrometer), rainfall, and leaf wetness (sensors at 76.2 cm and 121.9 cm above the ground, oriented according to manufacturer recommendations).

Data were analyzed in SAS (v. 9.1.3, SAS Inc., Cary, NC) as a mixed model (PROC MIXED) for the following measures: FHB incidence and severity, FHBI, and percentage of FDK. Two analyses were conducted, the first specifically at Janesville to examine the effect of fungicide and variety, and the second a multi-location analysis (Littell et al., 2006). Fungicide-treated plots from Janesville were excluded from the multi-location analysis. Mean separations were based on a protected LSD to compare locations, varieties, and the interaction. The level of significance for all analyses was 0.05.

As a result of significant winterkill at the Arlington and Chilton locations, disease assessments were not made for a number of plots at these two locations (out of 256 total plots at each location: 172 plots were not assessed at Arlington and 162 plots were not assessed at Chilton). Due to the large number of missing values, the within-location effects on FHB incidence, FHB severity, and FHBI could not be analyzed for the Arlington and Chilton locations. However, grain was harvested from many of these unassessed plots, so the number of missing plots for the percentage of FDK analysis was smaller (96 of 256 plots were missing from Arlington, 51 of 256 plots were missing from Chilton, 3 plots were missing from Janesville, and 1 plot was missing from Lancaster) and statistical analysis of the within-location effects on percentage of FDK was carried out for all locations. All locations are also included in the among-locations analysis of the effects on FHB incidence, FHB severity, FHBI and percentage of FDK.

RESULTS AND DISCUSSION

Environmental conditions during the 7 days prior to flowering differed among locations (Table 2). The effect of fungicide and variety and the fungicide*variety interaction at Janesville on FHB incidence, FHB severity, FHBI, and percentage of FDK are shown in Table 3. There was a marginal effect of fungicide treatment on FHB incidence (P=0.065) (Table 3). This may indicate that the Feekes 9 fungicide application targeted toward control of foliar pathogens may provide some residual protection against FHB.

Table 4 shows the results of the among-locations analysis of the effect of location, variety, and the location*variety interaction on FHB incidence, FHB severity, FHBI, and percentage of FDK. The public variety Truman had consistently low incidence, severity, and FHBI values at both Janesville and Lancaster. The commercial variety Kaltenberg KW 70 had low severity at both Janesville and Lancaster. The varieties Growmark FS 637 and Kaltenberg KW 63 were both consistently high for incidence, severity and FHBI at Janesville and Lancaster. The variety Diener D 496W had a consistently high percentage of FDK at Janesville, Lancaster, and Chilton. The varieties PIP 717 and Pro Seed Genetics Pro 220 all had a consistently low percentage of FDK at Janesville, Lancaster, and Chilton.

The correlations between yield and FHB incidence, FHB severity, FHBI, and percentage of FDK for each location and across all locations are shown in Table 5.

These results demonstrate that variety selection is an important component for scab management in Wisconsin. Although there is variability among

locations, specific varieties performed consistently well across locations.

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Table 1. Previous crop history, planting, flowering, and harvest dates at each winter wheat performance trial location.

Location	Previous crop	Planting date	Flowering date	Harvest date
Arlington	Soybeans	26 September 2008	7 June 2009	30 July 2009
Chilton	Peas	30 September 2008	8 June 2009	5 August 2009
Janesville	Soybeans	13 October 2008	3 June 2009	29 July 2009
Lancaster	Alfalfa	26 September 2008	6 June 2009	4 August 2009

Table 2. Environmental conditions at each field location during the 7 days (168 hours) prior to flowering.

Location	Relative humidity (%)[1]	Rain (hours)[2]	Rain (inches)[3]	Temperature (hours)[4]
Arlington	63	1.3	0.19	44
Chilton	70	13.0	1.54	22
Janesville	67	3.5	0.28	44
Lancaster	65	1.0	0.04	52

[1]Average relative humidity.
[2]Hours (out of 168) during which rainfall was recorded.
[3]Total rainfall recorded.
[4]Hours (out of 168) during which the air temperature was 64 to 80°F.

Table 3. *P*-values for the effects of variety (Var), fungicide (Fung) and the fungicide*variety interaction (Fung*Var) on FHB incidence and severity, FHBI, and percentage of FDK at Janesville.

Location	Effect	FHB incidence	FHB severity	FHBI	% of FDK
Janesville	Fung	0.0605	0.5370	0.1392	0.0825
	Var	<0.0001	<0.0001	<0.0001	<0.0001
	Fung*Var	0.8794	0.3314	0.9972	0.9992

Table 4. *P*-values for the among-locations effects of location (Loc), variety (Var), and the location*variety interaction (Loc*Var) on FHB incidence and severity, FHBI, and percentage of FDK.

Effect	FHB incidence	FHB severity	FHBI	% of FDK
Loc	0.0001	0.0001	0.0001	0.0001
Var	0.0001	0.0001	0.0001	0.0001
Loc*Var	0.0001	0.0001	0.0001	0.0001

Table 5. Correlations between yield and FHB incidence and severity, FHBI, and percentage of FDK and *P*-values for those correlations.

	Correlations with yield			
Location	**Incidence**	**Severity**	**FHBI**	**FDK**
Arlington	-0.0214 (*P*=0.8522)	-0.1421 (*P*=0.2146)	-0.1097 (*P*=0.3392)	-0.2605 (*P*=0.0014)
Chilton	-0.0351 (*P*=0.751)	-0.0545 (*P*=0.6225)	-0.0773 (*P*=0.4845)	-0.0631 (*P*=0.4168)
Janesville	-0.0353 (*P*=0.5976)	-0.0277 (*P*=0.6785)	-0.0642 (*P*=0.3368)	-0.0707 (*P*=0.2902)
Lancaster	-0.2266 (*P*=0.0006)	-0.2050 (*P*=0.0019)	-0.2261 (*P*=0.0006)	-0.3257 (*P*<0.0001)
All locations	0.1206 (*P*=0.0027)	0.0073 (*P*=0.857)	-0.0040 (*P*=0.9217)	-0.0711 (*P*=0.0488)

EFFECT OF PRECEDING FORAGE CROPS ON DON CONTENT IN BARLEY

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ABSTRACT

In Northern Quebec, Canada (Saguenay-Lac-Saint-Jean area), barley (*Hordeum vulgare* L.) production is very important but Fusarium head blight (FHB) has become a major problem in this region. In 2002 and 2003, FHB affected 47% and 76 % respectively of total area seeded in barley. The economic losses associated with FHB in barley were estimated at 2 million Canadian dollars during those 2 years in the Saguenay-Lac-Saint-Jean region. In this region, dairy producers usually seed barley after 3-4 years of a forage crop. A study was conducted at the research farm of Agriculture and Agri-Food Canada in Normandin (Quebec, Canada), to evaluate if preceding forage crops could affect deoxynivalenol (DON) content in barley. Previous crops consisted in barley monoculture (seeded in 2006 and 2007), 10 different forage crops (seeded in 2006 and harvested in 2007 as recommended for each species) and a summer fallow. In fall 2007, glyphosate was applied at a rate of 2700 g a.e. ha $^{-1}$ on each treatment and in 2008, barley cv. Païdia was direct seeded. Some forage crops seemed to increase DON content. Higher DON content could have been induced by high lodging index. More data are needed to evaluate if some forage crops residues could be more conducive to FHB than others.

INTEGRATED MANAGEMENT OF SCAB IN WHEAT USING RESISTANT VARIETIES AND FUNGICIDE

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ABSTRACT

A study was conducted to assess the effectiveness of host resistance, Proline® or Prosaro® fungicide, and a combination of both control measures in reducing losses in grain yield and quality resulting from Fusarium head blight (FHB). Four soft red winter (SRW) wheat cultivars and eight winter durum wheat varieties were evaluated in a complete block design comprised of three replications and two treatments (varieties with and without fungicide). Experiments were conducted at two locations in Virginia in 2008 and 2009. Scabby corn seeds were applied to plots at the boot stage, and a spray inoculation using conidia of *Fusarium graminearum* was applied to each variety at 50% flowering in the mist-irrigated test at Blacksburg, VA. Plots in the mist-irrigated test at Mt. Holly, VA were inoculated using only scabby corn seed. Proline (2008) or Prosaro (2009) was applied at 5.5 oz/ac before flowering at both locations. Data were collected for test weight, grain yield, 100 grain weight, and FHB assessment parameters including incidence, severity, index, *Fusarium* damaged kernels (FDK), and DON concentration.

Variance analyses indicated that variety, fungicide treatment, locations and years had significant effects on most traits. Year and location interaction effects were common for all traits. All of the scab assessment parameters, except for severity, were significant and correlated ($r = 0.23$ to 0.79, $P < 0.001$) with each other. All of the scab parameters, except for FHB severity, had a significant ($r = -0.34$ to -0.89, $P < 0.001$) negative effect on test weight and grain yield. Results of this study indicate that a single fungicide application significantly reduced FHB incidence in 6 of 8 tested varieties. Significant reductions in FHB index, and FDK were also identified with a single fungicide application. As a result, the fungicide treatment significantly increased grain weight (3 varieties) and yield (5 varieties). Fungicide application resulted in a significantly higher yield in two SRW wheat varieties and five durum wheat varieties.

Fungicide application had the greatest effect on FHB infection in susceptible winter durum wheat varieties and less effect in moderately resistant SRW wheat varieties. Only the susceptible SRW cultivar Coker 9835 benefited from the fungicide treatment, which resulted in a significant reduction in scab infection and significant increase in test weight and grain yield. FHB resistance in the SRW wheat cultivars was more effective than in the winter durum varieties in reducing scab infection, and protecting grain yield and quality. Results of this study indicate that utilization of wheat varieties having moderate scab resistance provides a baseline of protection against FHB that is equal to or better than fungicide application to cultivars having little or no FHB resistance. Nevertheless, fungicide application is beneficial and critical under severe FHB epidemics especially when susceptible cultivars are grown.

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DEVELOPMENT OF THE *SCABSMART* WEB SITE - A QUICK GUIDE TO U.S. SCAB MANAGEMENT INFORMATION

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ABSTRACT

The U.S. Wheat and Barley Scab Initiative (USWBSI) supported the development of a website that provides scab management information for all small grain classes affected by this disease in the U.S. The website is called ScabSmart and can be found at www.scabsmart.org/. The purpose of the site is to allow ready access to collective information on management strategies that reduce head scab and the associated mycotoxins, including deoxynivalenol (DON). ScabSmart provides the latest information on variety resistance for eight grain classes, plus the ability to rapidly get basic information on other strategies, including fungicide management and disease forecasting, seed treatment, crop rotation, and residue management. This information resource is a result of a cooperative effort across regions and grain classes in the U.S. affected by this disease. The web site also serves as a portal for links to get further information from the USWBSI web site or from other, localized resources on management strategies for Fusarium head blight (scab), resources often available through many state's Extension and Experiment Station publications. The ScabSmart website tool will be updated periodically with new information as it becomes available. ScabSmart was launched on September 24, 2009, and its availability has been announced through press releases and contact with commodity organizations. Further use of such a tool may be aided by promotion of its availability through various grain industries.

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UNIFORM FUNGICIDE TRIAL RESULTS ON HRS WHEAT AND SPRING BARLEY, FARGO, ND 2009

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ABSTRACT

Nine fungicide treatments were compared to the untreated check for efficacy in reduction of Fusarium head blight (FHB) and deoxynivalenol (DON) in 'Tradition' spring barley and 'Steele ND" hard red spring wheat, at Fargo, ND. Both crops were planted on May 8 into previous wheat ground that had been chisel plowed twice prior to planting. Plots were 5' wide and 20' long, with 4 replicates per treatment, arranged in a randomized complete block design, and with winter wheat seeded between plots. At heading, an overhead misting system provided added water to the plots when the nighttime humidity dropped below 90%. Fungicides were applied between 6:00 am and 8:00 am with a backpack-type sprayer equipped with two XR8001 flat fan nozzles oriented toward the grain head at a 30° angle from the horizontal. The fungicides were applied at 18.5 gpa with 40 psi. Conidia (100,000 spores/ml) of *Fusarium graminearum* were applied to grain heads with a backpack sprayer in 30 gpa of water, on the same evening of the early full head emergence applications in barley (Feekes 10.5) and the anthesis (Feekes 10.51) applications in wheat. Disease notes were taken at soft dough stage of development and crops were harvested at kernel maturity. Sub-samples of the harvested grain were ground and analyzed for deoxynivalenol (DON) by the NDSU Veterinary Toxicology Laboratory using gas chromatography and electron capture techniques.

The fungicide treatments included: Proline (3 fl oz/A) + Caramba (7.0 fl oz/A) at Feekes 10.5 in barley, Feekes 10.51 in wheat; Prosaro (6.5 fl oz/A) or Caramba (13.5 fl oz/A) applied once at three separate growth stages - Feekes 10.3, 10.5 and 10.54 in barley and Feekes 10.5, 10.51 and 10.54 in wheat; and two treatments of Headline (6 fl oz/A), one at Feekes 10.0 and one at Feekes 10.5.

Very cold July temperatures (average temperature of 67°F and average lows of 55°F) and rainfall amounts 78% below normal resulted in very low FHB levels in Fargo in 2009, even with added mist. The untreated FHB field severity was 1.5 % in barley and 5.0 % in wheat. Despite low disease, all fungicide treatments reduced FHB field severity ($P = 0.05$) for both crops. DON levels were 1.4 ppm for untreated barley, and 2.0 ppm for untreated spring wheat. All fungicide treatments, except for the two Headline treatments, significantly ($P = 0.05$) reduced DON in both crops. The fungicide treatments applied before flowering in wheat and before full head emergence in barley resulted in greater DON levels than the other two fungicide timings. In wheat, only the two fungicide treatments applied at anthesis (Feekes 10.51) resulted in significantly improved yield.

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DEOXYNIVALENOL ACCUMULATION DURING MATURATION OF BARLEY GRAIN

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ABSTRACT

Fusarium head blight (FHB) associated with the presence of the fungus *Fusarium graminearum* is probably one of the most feared diseases in barley (*Hordeum vulgare*) production in Eastern Canada. In addition to reducing grain yields, the fungus produces a toxin (deoxynivalenol or DON) which can affect the health of livestock. A study was conducted at the research farm of Agriculture and Agri-Food Canada in Normandin (Quebec, Canada), to evaluate deoxynivalenol (DON) content in barley during the maturation of the grain. In 2007 and 2008, 6 barley cultivars were planted at a seeding rate of 375 grains m^{-2}. The fertilization was applied according to the provincial recommendations. Each plot consisted of 8 rows of 3.5 m long. The experimental design was a randomized complete block design with 6 repetitions. From Zadoks growth stage 73 (milky stage) to Zadoks 90 (maturity), a section of 1m long of each plot was harvested for DON determination. The experiment was conducted under natural conditions of infestation. In 2007, DON content was low for all cultivars. At the first harvest, DON content was 0.24 ppm and at maturity DON content reached 0.54 ppm. In 2008, FHB was more important. At Zadoks 73, DON content was 0.8 ppm. At maturity, DON content reached 6.0 ppm. It seems that DON is present very early during the grain formation and high and rapid accumulation could be observed during grain maturation of barley.

INTEGRATED MANAGEMENT OF FHB AND DON: A 2009 UPDATE

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ABSTRACT

Coordinated integrated management trials were conducted in 2007, 2008 and 2009 in vastly different regions of the country to evaluate the overall efficacy and consistency of an integrated approach relative to an approach based solely on fungicide application or cultivar resistance to manage FHB and DON in all major grain classes. The experimental design was a split-plot, with fungicide treatment and cultivar as the whole- and -sub-plots or vice versa. In a few trials, a split-split-plot treatment arrangement was used, with cropping sequence (crop rotation) or surface residue management as the whole-plot. One plot of each cultivar was treated with Prosaro (6.5 fl oz/A + 0.125% Induce) or Proline 3+3 (a tank mix of Folicur and Proline, each at 3 fl. oz/A + 0.125% Induce) at anthesis, while the other was left untreated. Application was made using a sprayer equipped with paired Twinjet or flat fan XR8001 nozzles, mounted at an angle (30° from the horizontal) forward and backward and calibrated to deliver at a rate of 10 to 20 gallons per acre. FHB, DON, FDK, yield, and test weight data were collected in all trials and analyzed to determine the effect of fungicide and cultivar resistance (and cropping sequence, were appropriate) on each of these variables.

In this summary, percent control relative to the untreated susceptible check (worst case scenario) will be used as the basis for evaluating efficacy against FHB and DON. Over the three years, disease intensity and DON contamination were low at most locations due to conditions unfavorable for FHB development; however, there were a few location-years with some level of disease and toxin, allowing for the comparison of treatments. Results thus far indicate that, at moderate to high levels of FHB and DON, the efficacy of both individual (fungicide or resistance) and integrated (fungicide + resistance or fungicide + resistance + residue management) approaches varies among trials, possibly reflecting, among other factors, differences in baseline levels of disease and DON, cultivar resistance (level and designation), overall weather conditions, and weather conditions at the time of anthesis of the individual cultivars. In spite of the relatively small dataset, a range of responses were observed over the three years. There were situations in which fungicide alone (applied to the susceptible check) was just as effective as resistance alone (resistant cultivar without fungicide) or resistance + fungicide. In other situations, resistance alone was more effective than fungicide alone (susceptible cultivar + fungicide) or just as effective as resistance + fungicide at reducing FHB index and DON. For trials with surface residue management or cropping sequence as a treatment factor, in most cases, there was a significant advantage in terms of disease and toxin reduction to planting wheat after a non-host crop as opposed to a cereal crop. The gain in FHB/DON reduction from using an additional control strategy was not always significant. However, in spite of these variations, a few general conclusions can be made. In general, moderately resistant cultivar + fungicide treatment combination resulted in higher percent control (relative to the untreated susceptible check) than that achieved by either approach used alone. Comparing trials with the same treatment combinations, but planted into different types of crop residue, non-host crop + moderately resistant cultivar + fungicide generally resulted in higher percent control than host crop + susceptible cultivar + without fungicide or other combinations of these variables. Under severe epidemic conditions, a three tier management approach of crop rotation with a non-host,

moderately resistant cultivars and fungicide application is required to achieve close to < 2 ppm DON and reduce index. Across all trials, based on FHB index, percent control ranged from 11.5 to 92.4% for fungicide or resistance alone and from 37 to 98% for fungicide + resistance. For trials with crop rotation or cropping sequence as a treatment factor, percent control (relative to the untreated susceptible planted into host residue) for non-host + resistance + fungicide combination ranged from 8.45 to 99% for index and from 33 to 96% for DON. (*note: trials with unusually high negative percent control [see Willyerd et al in this volume] were not considered in this summary, pending further information from the individual PIs*).

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FACTORS INFLUENCING THE ADOPTION FHB CONTROL PRACTICES IN ND AND MN: RESULTS OF A SURVEY

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ABSTRACT

In 2009 a survey was given to producers attending a wheat production workshop to determine the level of adoption of the various FHB control strategies and where they obtained the information they used to manage scab. Of the 161 respondents, 82% had adopted the use of tolerant varieties, 79% fungicides applied at heading, and 63% crop rotation to control FHB. The decision on whether to apply fungicide was largely based on the perceived likelihood of disease development by the growers themselves. Less than 20% used a disease forecasting model available through the internet, or guidance from crop consultants to help make that decision. Many growers reported the use of non-FHB tolerant varieties after having used them in the past. Farmers indicated that yield, and other desirable characteristics were the main reason from switching to non-FHB tolerant cultivars. Growers reported that the most important sources of information on scab control were extension meetings, crop consultants, articles in farm magazines and newspapers, and other extension publications. Less than 15% of the farmers considered the internet, other farmers, and personnel at the local elevator to be important sources of information on scab control.

2009 TRIAL FOR THE PERFORMANCE OF BIOLOGICAL CONTROL AGENTS FOR THE SUPPRESSION OF FUSARIUM HEAD BLIGHT IN SOUTH DAKOTA

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ABSTRACT

Fusarium Head Blight (FHB or scab) continues to be a potential problem for wheat and barley producers in South Dakota. The objective of this study was to continue evaluation of the efficacy of selected biological control agents (BCAs), alone or in combination with fungicide, that can suppress different measures of FHB under South Dakota conditions. Briggs hard red spring wheat was planted at Brookings, SD. Trial treatments included an untreated check; the fungicide premix Prosaro; *Bacillus* strain 1BA cultured in different broth formulations; *Bacillus* strain 1D3 cultured in different broth formulations; a combination of *Bacillus* strain 1BA and *Bacillus* strain 1D3; and combinations of Prosaro with one or both of the *Bacillus* BCAs. The treatments were applied at anthesis. Plots were treated with pathogen by spreading *Fusarium graminearum* (isolate Fg4) inoculated corn (*Zea mays*) grain throughout the field, and applying overhead mist irrigation each day for 10 days following anthesis. Following the treatments, 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).

Disease development was better than in some recent very dry years. The FHB incidence, FHB index, yield, and FDK were all significant for at least some of the BCA treatments. For FHB incidence, some BCA treatments resulted in significant differences in the absence of Prosaro. It appeared that there was desirable synergistic activity between Prosaro and one or more BCAs used in combination with the fungicide product. Data for DON will not be available until late 2009.

EFFECT OF VARYING COMBINE HARVESTER CONFIGURATIONS ON FUSARIUM DAMAGED KERNELS (FDK) AND DEOXYNIVALENOL ACCUMULATION IN WHEAT GRAIN HARVESTED FROM PLOTS WITH DIFFERENT LEVELS OF FUSARIUM HEAD BLIGHT

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OBJECTIVE

To evaluate the influence of varying combine harvester configurations on *Fusarium* damaged kernels (FDK) and DON in wheat grain harvested from plots with different levels of FHB.

INTRODUCTION

Fusarium Head Blight (FHB), caused predominantly by *Fusarium graminearum* Schwabe (teleomorph: *Gibberella zeae*) in North America, is a serious disease of wheat (*Triticum aestivum* L.) and other small grain in all wheat-growing regions. Infection of wheat spikes may cause significant grain yield and quality losses due to poor grain fill, high percentage of damaged (scabby) kernels, and low test weights. In addition, infected grain accumulates deoxynivalenol (DON), a mycotoxin produced by this pathogen (Bai and Shaner, 1994, and McMullen et al, 1997). DON, also known as vomitoxin, represents a health threat to humans and livestock, therefore mycotoxin-contaminated grain is either rejected or priced down in commerce. Research has shown that DON levels are positively correlated to *Fusarium* damaged kernel (FDK) and other visual estimates of FHB (Paul et al, 2005). As such, reducing FDK generally leads to reduction in DON. Integration of resistant cultivars, fungicide applications and agronomic practices are commonly recommended to reduce FDK and DON in harvested grain (McMullen, 2007). Management recommendations also include strategies to eliminate scabby, light-weight kernels during harvest by adjusting combine settings. However, specific information pertaining to how combine configurations should be adjusted to accomplish this is lacking. In response to this lack of specific information, research is currently being conducted at the Ohio Agricultural Research and Development Center (OARDC) to evaluate the influence of varying combine harvester configurations on FDK and DON.

MATERIALS AND METHODS

Wheat plots of moderately susceptible SRWW cultivar Hopewell were established in a conventionally tilled field, previously planted with soybeans. A total of 60 plots (5-ft x 20-ft) were planted for the experiment. The experimental design was a randomized complete block, with three replicate blocks and harvester configuration and inoculation treatments in a split-plot arrangement. Configuration was the whole plot, whereas inoculum density was the sub-plot. Subplots were spray inoculated with five different inoculum densities (0, 1.5, 3, 4.5 and 6 $x10^4$ spores per mL) prepared using a 1:1 mixture of ascospores and macroconidia of 10 isolates of *Fusarium graminearum*. All inoculations were done at anthesis (Feekes 10.5.1). At soft dough (Feekes 11.2), FHB incidence and index (Stack and McMullen, 1998) were visually estimated in five groups of 20 spikes randomly chosen within each subplot. Assessments were done by estimating percent spike area diseased on an individual plant basis. Plots were harvested using an ALMACO SPC20 plot combine harvester. Prior to harvesting the research plots, the combine was calibrated on non-inoculated, disease-free plots

of Hopewell. Threshing, separation and cleaning devices, along with fan speed (airflow speed and volume) were regulated to minimize excessive removal of healthy kernels. Considering that the volume of air flowing through the combine is controlled by the shutter adjustment, configuration settings were regulated as follows: the initial setting, **C1** = Fan speed of 1375 rpm with Shutter opening adjustment of 2 ¾ inches, was used as the default (manufacturer-recommended setting). The three other configurations tested were: **C2** = Fan speed of 1475 rpm and Shutter opening of 2 ¾ inches; **C3** = Fan speed of 1475 rpm with Shutter opening increased to 3 ½ inches; and finally, **C4** = Fan speed of 1375 rpm and Shutter opening of 3 ½ inches. Throughout the harvest of this trial, Winnowing Blower and Threshing Cylinder speeds were held constant. Yield (bu/ac), test weight (lb/bu), and moisture (%) content were determined for each subplot. Grain harvested from each plot was visually rated for FDK (%). During the harvest of each plot, a sample of discarded material was collected at the back end of the combine using a sweep net-type collector attached to a pole. These samples were cleaned and examined for healthy and FDK. Samples of harvested and discarded grain from each subplot were ground and sent for DON analysis at the USWBSI-funded DON testing laboratory at the University of Minnesota

RESULTS AND DISCUSSION

Based on Linear Mixed model analysis (Littell et al, 2006), our results revealed significant differences ($P < 0.05$) among *F. graminerum* inoculation treatments for both FHB incidence (INC) and severity (IND). Mean INC and IND increased with inoculum density, reaching the highest levels in plots inoculated with 6 $x10^4$ spores/mL (Table 1). Based on Pearson's correlation coefficient (r), FDK values from harvested and discarded grain samples were positively correlated ($r = 0.62$ and 0.67, respectively, both with $P < 0.001$) with DON levels. Averaged across all disease levels (inoculum densities), configuration C3 yielded the lowest mean percent FDK and DON and the highest mean test weight (TW) for both harvested and discarded grain samples (Table 2). For harvested grain, the difference between C1, the default setting, and C3 was statistically significant for FDK and TW but not for DON. Comparing configurations at specific mean levels of FHB index, C3 consistently resulted in lower FDK and DON and higher TW than the other configurations (Figures 1, 2, and 3), however, the differences were not statistically significant at all levels of FHB. For instance, C3 resulted in significantly lower DON content than C1 at 29.38% index but not at the other levels of disease (Figure 2). C3 and C4 resulted in the highest percent reduction in FDK relative to C1 at all levels of disease (Table 3). For DON, C3 resulted in the highest percent reduction relative to C1 at the three highest index levels (Table 3). These results suggest that modifying combine configuration to discard scabby, lightweight kernels could minimize wheat grain quality losses due to FHB by reducing the FDK and DON levels of harvested grain. However, for the configurations tested, the effects varied with disease levels. C3 proved to be the most consistent configuration across all tested levels of disease.

ACKNOWLEDGEMENT AND DISCLAIMER

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Table 1. Mean percent FHB incidence (INC) and index (IND) for five different inoculum concentrations of *F. graminearum* applied at anthesis (Feekes 10.5.1) to SRWW cultivar Hopewell

Inoculum Concentration	**FHB Intensity (%)***			
(x 10 ^{4} spores/ml)	**INC** [x]		**IND**[y]	
0	19.50	a	6.72	a
1.5	43.92	b	23.47	b
3.0	53.75	c	29.38	c
4.5	57.92	cd	31.81	cd
6.0	62.92	d	34.82	d
Mean	47.60		25.24	

* Within each column, means followed by the same letter are not significantly different from each other at $P < 0.05$.
[x] INC = Fusarium head blight incidence (proportion of diseased spikes)
[y] IND = Fusarium head blight index (mean proportion of diseased spikelets per spike)

Table 2. Mean percent FDK, DON content (ppm), and test weight (TW, lb/bu) of wheat grain harvested with four different combine configurations from plots of SRWW cultivar Hopewell inoculated at anthesis (Feekes 10.5.1) with five different inoculum concentrations of *F. graminearum*

Combine Configuration[v]**: Fan Speed (rpm) / Shutter opening (inches)**	**Harvested Grain***						**Discarded grain***[w]			
	FDK1[y]		**DON1**		**TW**		**FDK2**		**DON2**	
C1 = 1375/ open 2 ¾	14.73	a	10.53	a	48.48	a	88.33	a	42.73	a
C2 = 1475/ open 2 ¾	11.47	ab	10.78	a	48.75	a	84.00	ab	36.08	a
C3 = 1475/ open 3 ½	8.33	b	8.72	a	50.69	b	71.00	c	18.15	b
C4 = 1375/ open 3 ½	8.60	b	9.35	a	50.37	b	78.00	b	36.17	a
Mean			5		57		.33		3.28	

* Within each column, means followed by the same letter are not significantly different from each other at $P < 0.05$
[v] Combine configurations, with C1 as the default (manufacturer recommended).
[w] Discarded grain collected at the back of the combine
[y] FDK = Percentage of visibly scabby kernels

Table 3. Percent reduction of FDK and DON in harvested grain for different combine configurations relative to the default setting (C1) for plots with different mean levels of FHB

Inoculum Concentration (x 10 ^{4} spores/ml)	Mean FHB Index	Combine Configuration	Mean		% Reduction*	
			FDK (%)	DON (ppm)	FDK	DON
0	6.72 %	C1	3.33	1.41	…	…
		C2	3.33	1.34	0	4.55
		C3	2.00	1.40	39.94	0.50
		C4	1.33	1.43	60.06	(1.85)
1.5	23.47 %	C1	17.00	5.63	…	…
		C2	8.00	8.00	52.94	(42.02)
		C3	7.33	7.83	56.86	(39.00)
		C4	6.00	7.13	64.71	(26.63)
3.0	29.38 %	C1	11.67	15.20	…	…
		C2	15.00	12.50	(28.53)	17.76
		C3	8.67	8.23	25.73	45.86
		C4	8.67	8.57	25.73	43.62
4.5	31.81 %	C1	18.33	14.13	…	…
		C2	16.00	15.63	12.71	(10.62)
		C3	11.67	12.07	36.33	14.60
		C4	10.33	12.73	43.64	9.91
6.0	34.82 %	C1	23.33	16.30	…	…
		C2	15.00	16.40	35.71	(0.61)
		C3	12.00	14.07	48.56	13.68
		C4	16.67	16.90	28.55	(3.68)

* Values in parenthesis are negative (Increments in FDK or DON relative to C1)

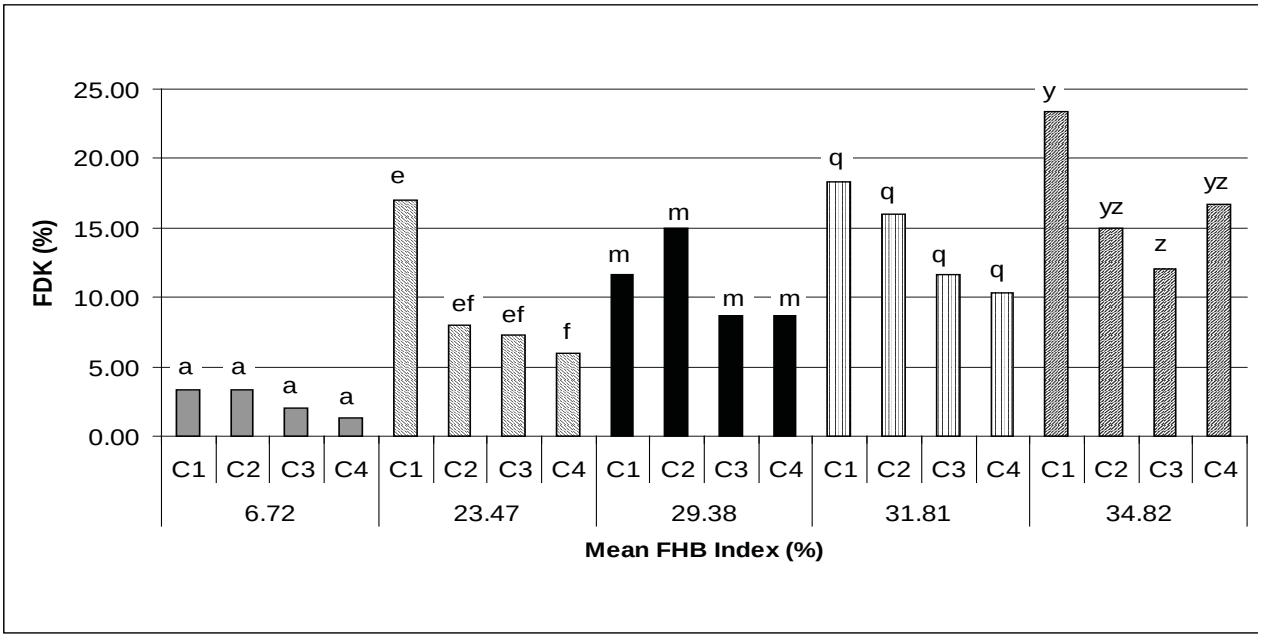

Fig. 1. Mean FDK in wheat grain harvested with four different combine configurations (C1, the default, and C2, C3, and C4 as described in the Materials and Methods) from wheat plots with different mean levels of FHB. Letters above the bars are for comparisons among configurations within, not across disease levels.

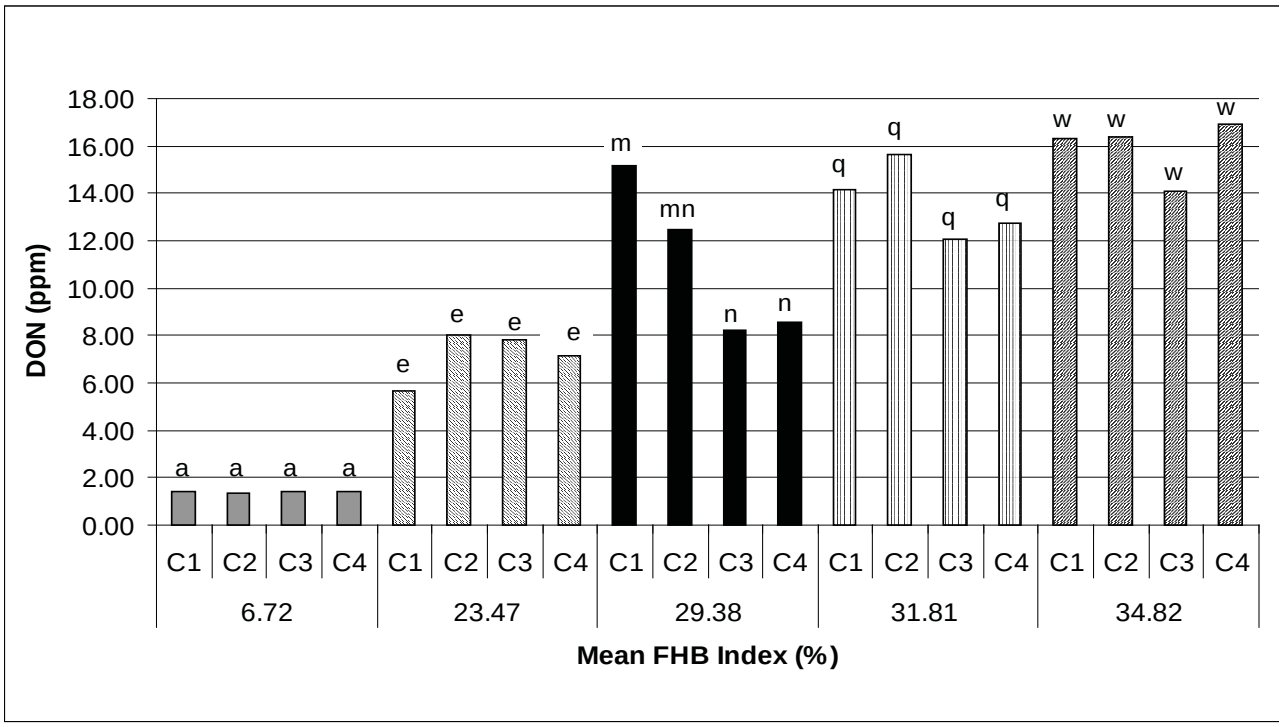

Fig. 2. Mean DON content of wheat grain harvested with four different combine configurations (C1, the default, and C2, C3, and C4 as described in the Materials and Methods) from plots with different mean levels of FHB. Letters above the bars are for comparisons among configurations within, not across disease levels.

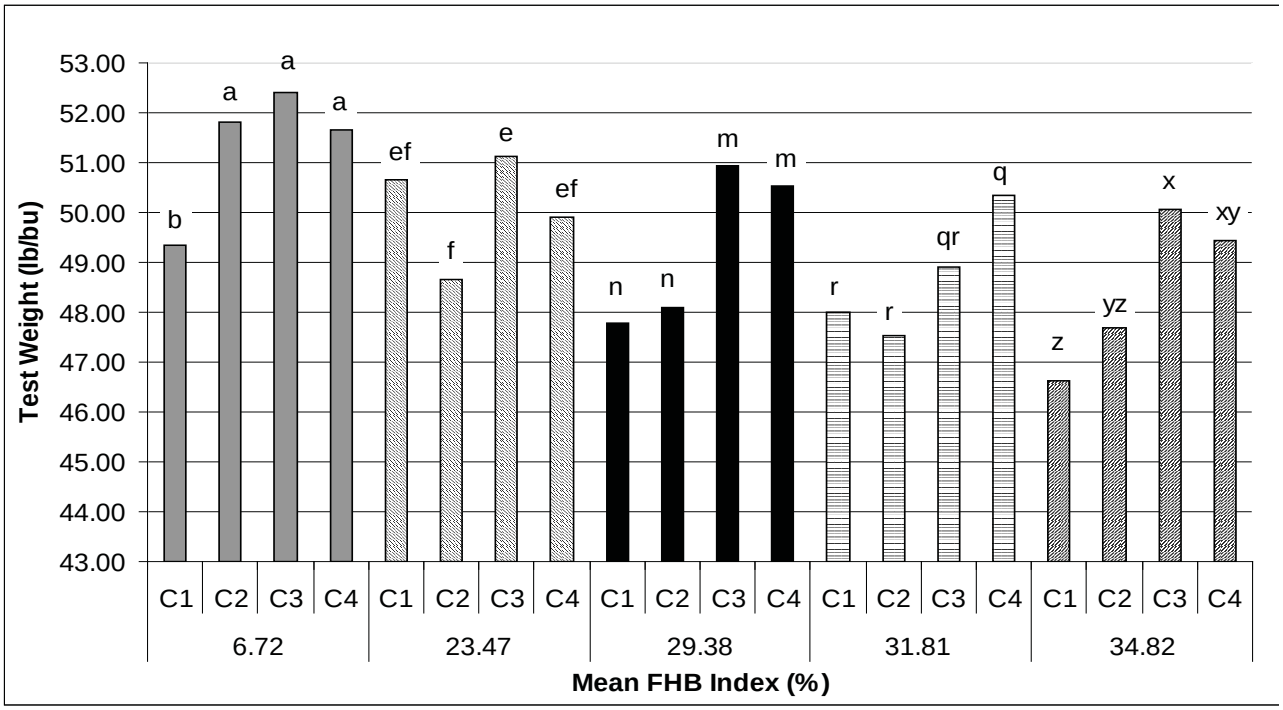

Fig. 3. Mean test weight (TW) of wheat grain harvested with four different combine configurations (C1, the default, and C2, C3, and C4 as described in the Materials and Methods) from plots with different mean levels of FHB. Letters above the bars are for comparisons among configurations within, not across disease levels.

COLONIZATION OF WHEAT HEADS BY FUSARIUM HEAD BLIGHT ANTAGONIST *CRYPTOCOCCUS FLAVESCENS* OH 182.9 WHEN APPLIED ALONE OR IN COMBINATION WITH PROTHIOCONAZOLE AND THE TREATMENT EFFECT ON FHB DISEASE DEVELOPMENT IN FIELD GROWN WHEAT

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OBJECTIVES

Quantify the colonization of infection court tissues of field-grown wheat by FHB biocontrol strain OH 182.9 in the presence or absence of field rates of prothioconazole and 2) determine FHB disease development for the same treatments applied in the colonization work.

INTRODUCTION

Research results to date from laboratories worldwide indicate that it is unlikely that any single control measure will reduce Fusarium head blight (FHB) of wheat to economically acceptable levels when conditions favor disease development. The use of yeast biological control agent *Cyptococcus flavescens* OH 182.9 (NRRL Y-30216) as part of an integrated management strategy against FHB is understudied yet has considerable potential for significantly contributing to the reduction of FHB and deoxynivalenol (DON). We have isolated a prothioconazole-tolerant (PTCT) variant of OH 182.9 (OH 182.9 C3) that frequently exhibits enhanced biocontrol activity over its wild type progenitor strain (Schisler et al., 2009). This variant also is tolerant of tebuconazole. As part of an integrated control protocol, strain OH 182.9 could be applied to wheat and barley after flowering when fungicides are not approved for use. Alternatively, a tank mixed prothioconazole and OH 182.9 combination treatment applied at flowering would theoretically provide immediate protection from FHB and lasting protection due to OH 182.9 activity on wheat head infection courts after the fungicide component is no longer effective. The OH 182.9 component of this tank mix could be especially useful in limiting the total DON content in harvested grain by combating new DON producing infections by *F. graminearum* that can occur during early to late grain development (Del Ponte et al., 2007). By understanding the colonization dynamics of strain OH 182.9 under differing integrated application protocols, the direction of fermentation and formulation research could be focused on enhancing colonization of inadequately colonized infection courts and thereby improve biocontrol effectiveness.

MATERIALS AND METHODS

Selection of prothioconazole-tolterant (PTCT) variant C3

PTCT variant C3 of FHB antagonist *Cryptococcus flavescens* OH 182.9 (NRRL Y-30216) was generated by transferring log growth stage cells of wild type (WT) OH 182.9 into 10 ml of 1/5 strength Tryptic soy broth (TSB/5) containing 1 ppm of the fungicide prothioconazole (PTC) in 50 ml Erylenmeyer flasks, shaking flasks at 250 rpm for 5 days at 25 C, plating colonized broth onto 1/5 TSB agar (TSA/5) + 1 ppm PTC and repeating the process with increasing concentrations of PTC. Variant C3 was then evaluated for equivalence to the WT progenitor strain of OH 182.9 based on efficacy

in reducing FHB of wheat in greenhouse tests, log growth rate in TSB/5, and carbon utilization profile.

Integrated OH 182.9 and prothioconazole treatments: colonization and efficacy FHB

Field trials were conducted in Peoria, IL in 2009. Soft red winter wheat cultivar Freedom (moderately resistant to FHB) was grown using standard agronomic conditions (Schisler et al., 2006). Corn kernels colonized by native *G. zeae* were scattered through plots (~25-40 kernels/m^2) three weeks prior to wheat flowering. Biomass of WT OH 182.9 and PTCT variant C3 (~3 x 10^8 cfu/ml and 40 gal/acre) was produced in a B Braun Biostat B fermentors (B. Braun Biotech Inc., Allentown, PA) charged with 1 L of SDCL medium (Schisler et al., 2008). Treatments are shown in Table 1 and included OH 182.9 WT and PTCT variant C3 treatments, PTC at 6.5 oz/acre or 0.65 oz/acre, and combinations of PTC and OH 182.9 C3 applied at flowering (Feekes 10.5) or with OH 182.9 C3 applied 7 days after the PTC application at flowering. Sixteen, 88, 184 and 280 hours after treatment application to wheat heads at flowering, three replicate samples of glume and lemma tissues were taken from selected treatments (Figs 1 and 2, lemma data not shown) and plated on one-fifth strength tryptic soy broth agar (TSA/5) and TSA/5 with 50 ppm streptomycin and 5 ppm prothioconazole to enumerate "total" microbial populations and populations of yeast OH 182.9 C3, respectively. Thirty hours after treatment applications, mist irrigation was applied for 4 minutes per hour from 9 PM to 7 AM for two weeks. Additionally, four rainfall events occurred during the course of the colonization study (Fig 1). Heads were scored for disease severity and incidence and grain evaluated for 100 kernel weight and deoxynivalenol content. Analysis of variance and Fisher's Protected LSD test ($P \leq 0.05$) was used to compare all treatment means.

RESULTS AND DISCUSSION

Due to similar trends in colonization of lemma and glume tissues by strain OH 182.9 C3, only glume colonization data are presented. With no rainfall or mist irrigation after treatment application at 0 hours through the first monitoring of OH 182.9 C3 populations at 16 hours, log10 counts of OH 182.9 per gram of fresh glume tissue were low (<2) regardless of the presence or absence of PTC with cells of OH 182.9 C3 (Fig 1). Strain C3 made up approximately 10% or less of the total recovered microbial population (Fig 2). Initiation of misting irrigation prior to the 88 h sampling time corresponded with 1 log unit increases in OH 182.9 C3 that represented 40-70% of the total microbial population (Figs 1,2) though treatments did not differ. Substantial rainfall occurred immediately before application of the three treatments that included OH 182.9 C3 applied 7 days after flowering and in the 16 hours before the 184 hour assessment of OH 182.9 populations. The populations of OH 182.9 on glumes treated 16 hours before the 184 hour assessment were significantly higher in some cases, both on an absolute and percentage of the total population basis (Figs 1,2), than the glumes treated with OH 182.9 C3 seven days earlier. Yet all treatments except 1/10 PTC + OH 182.9 C3 applied at flowering (0 hours) supported OH 182.9 C3 populations that made up 60-95% of the total microbial population recovered (Fig 2) from glumes. Populations of OH 182.9 C3 dropped slightly by the 280 hour assessment for all treatments though even for treatments applied at 0 hours, OH 182.9 C3 made up 40-70% of the total recoverable microbial population (Fig 2). While additional studies are needed, these results support earlier work that demonstrated the competence of OH 182.9 in colonizing wheat head tissues and recovering from low populations once free moisture is available. There was no indication that field rates of PTC inhibited the colonization of OH 182.9 C3 compared to the treatment of OH 182.9 C3 alone. Preliminary results indicate that the hydrophobicity of the surfaces of glume and lemma tissues increase leading up to flowering and then are considerably lower after flowering (Dunlap and Schisler, this volume). Conducting colonization experiments under conditions of constant or controlled levels of moisture availability would help clarify if changes in the physiochemical nature of glume and lemma surfaces influence colonization of these surfaces.

Disease reduction associated with the various treatments supported the observation that the population of OH 182.9 C3 on infection court tissues was not inhibited by the presence of PTC and that treatments that contained both biocontrol and PTC provided the greatest arithmetic reduction in FHB symptoms and DON (Table 1). The discovery that PTCT variant C3 of OH 182.9 regularly exhibited enhanced efficacy in reducing FHB/DON and can be successfully combined with prothioconazole are key steps in the process of developing successful integrated FHB control strategies. We anticipate replicating these trials in two locations during the 2010 field season.

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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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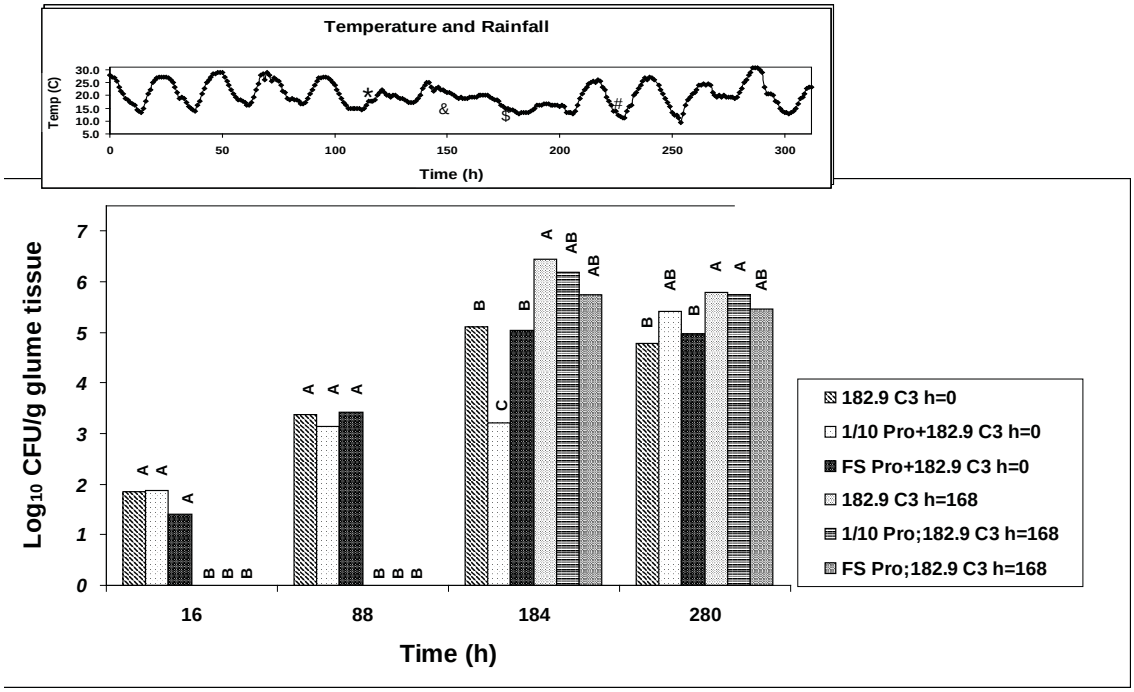

Fig 1 Log_{10} population of *Cryptococcus flavescens* OH 182.9 C3 on glume tissue when applied alone or in combination with prothioconazole at or seven days (168 hours) after wheat flowering. Rain events of 0.51 cm, 8.2 cm, 3.9 cm and 0.1 cm occurred between hours 120 and 144, 144 and 168, 168 and 216, and 216 and 240, respectively, and are designated on the temperature and rainfall graph with the symbols *, &, $, and #, respectively.

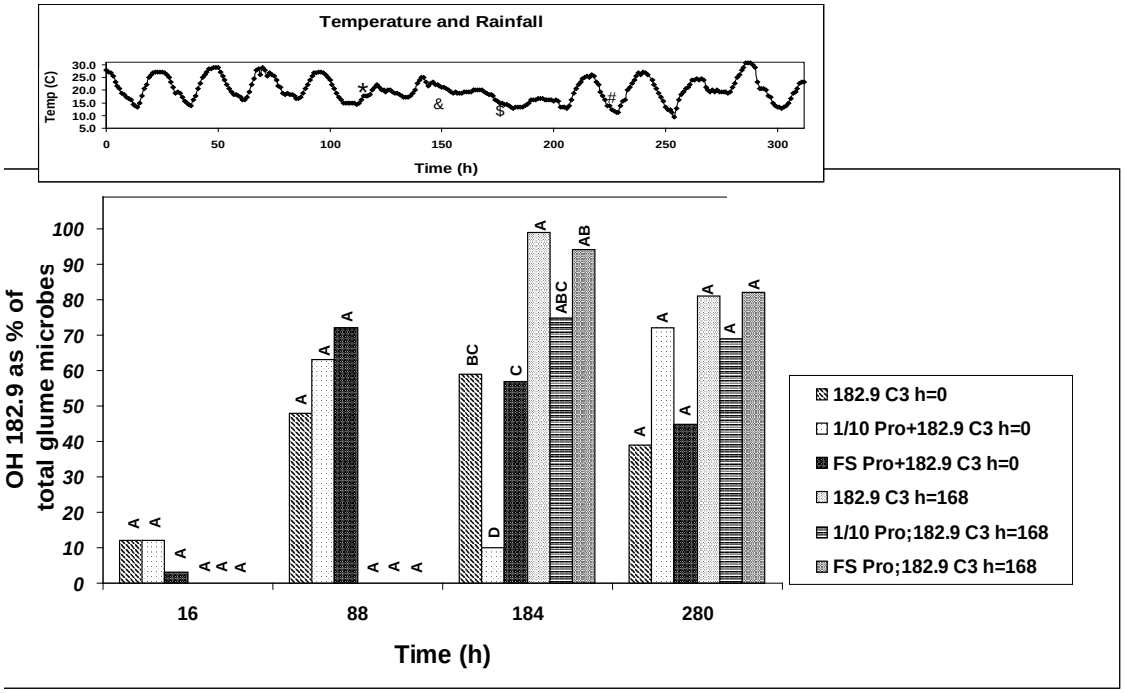

Fig 2 Population of *Cryptococcus flavescens* OH 182.9 C3 on glume tissue expressed as a percentage of the total recoverable microbial population when strain C3 applied alone or in combination with prothioconazole at or seven days (168 hours) after wheat flowering. Rain events are as described for Fig 1.

Table 1. 2009 field trial results at Peoria, IL: Influence of prothioconazole, yeast antagonist OH 182.9, prothioconazole tolerant variant C3 of OH 182.9 and combinations thereof on FHB disease parameters on winter wheat cultivar Freedom

	Wheat Cultivar Freedom			
Treatment[c]	DS (%)	DI (%)	100 KWT (g)	DON (ppm)
Untreated control	3.3^{A}	30.7^{A}	3.2^{FG}	8.6^{ABC}
182.9 C3 h=0	1.9 ^{CD}	20.0^{DE}	3.2^{EFG}	8.5^{ABC}
1/10 Pro+182.9 C3 h=0	1.3^{DE}	14.9^{EFG}	3.2^{EF}	7.1^{BCD}
FS Pro+182.9 C3 h=0	1.1^{EF}	11.2^{GH}	3.5^{B}	2.1^{F}
182.9 C3 h=168	1.9^{CD}	18.9^{DEF}	3.2^{GH}	9.3^{ABC}
1/10 Pro h=0;182.9 C3 h=168	2.7^{AB}	27.2^{AB}	3.2^{FG}	9.6^{ABC}
FS Pro h=0; 182.9 C3 h=168	0.9^{EF}	10.4^{GHI}	3.6^{A}	3.3^{EF}
1/10 Pro h=0	2.2^{CD}	18.7^{DEF}	3.3^{D}	7.6^{ABC}
FS Pro h=0	1.4^{DE}	13.9^{FG}	3.5^{B}	3.5^{DEF}
182.9 wild type h=0	2.5^{BC}	23.7^{BCD}	3.2^{EF}	10.6^{AB}
P value	0.05	0.05	0.05	0.05

[a]Within a column, means followed not followed by the same letter are significantly different (P<0.05, FPLSD mean separation)

[b]DS= Disease severity, DI= Disease incidence, 100 KWT= One hundred kernel weight, DON=Deoxynivalenol

[c]Pro (Prosaro)= Commercial fungicide formulation of prothioconazole applied at a rate equivalent to 6.5 oz/acre (FS=full strength) or at 1/10 this rate; 182.9 C3 (*Cryptococcus flavescens* variant C3)= prothioconazole tolerant variant of OH 182.9; 182.9 WT= Wild type strain of OH 182.9; h= time, in hours, of application of treatment (h= 0 represents application at flowering, h= 168 represents application seven days after flowering).

SPATIAL PATTERNS AND INCIDENCE-SEVERITY RELATIONSHIPS OF FUSARIUM HEAD BLIGHT EPIDEMICS ON WHEAT CROPS FOLLOWING SOYBEAN OR MAIZE IN RIO GRANDE DO SUL, BRAZIL

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ABSTRACT

A survey on Fusarium head blight (FHB) was conducted in 36 arbitrarily selected spring wheat fields in northern crop growing regions of Rio Grande do Sul State, southern Brazil. The fields varied in wheat cultivar and followed either corn or soybean. Surveys were made during October (approximately kernel soft dough stage). In each field, 20 sampling areas were randomly selected and 10 adjacent wheat heads were randomly sampled at the sampled area. In the laboratory, both incidence (I) and severity (S = FHB index) were visually estimated in each 10-head sample. Spatial pattern of FHB incidence was studied by calculating the mean incidence and the index of dispersion (D). The incidence-severity (I-S) relationship was studied by fitting empirical regression models to the data. FHB was present in all fields assessed with an overall mean incidence of 41.2% (0.6 – 90%). In most fields (30/36), the pattern of FHB was random. Three out of six fields where aggregation was detected were located in the same region that also had the highest mean incidence. Strong evidence of FHB aggregation among sampling areas ($P<0.01$) was verified in only three fields that followed soybean. However, fields following corn presented a slightly higher incidence levels. A model based on complementary log-log transformation of I and S performed well for the data set. Estimated slope from the fit of the model for the pooled data was 1.1. Our preliminary results confirm previous findings elsewhere and support our hypothesis that in areas where no-till system is intensive FHB spatial pattern is predominantly random given the abundance of regional inoculums levels. Once further incidence and severity data is collected, simple and robust models for predicting severity from incidence will be useful to perform faster and accurate FHB assessments.

INTEGRATED MANAGEMENT STRATEGIES FOR FUSARIUM HEAD BLIGHT OF SOFT RED WINTER WHEAT IN MISSOURI: SUMMARIZATION OF TRIAL DATA FOR THREE YEARS

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OBJECTIVE

To evaluate the importance of crop sequence, variety selection and fungicide application as components of an integrated management program for Fusarium head blight (FHB) of soft red winter wheat in Missouri.

INTRODUCTION

The severity of FHB or scab epidemics in the United States has caused enormous yield and quality losses in both wheat and barley over the last decade. The development of this disease is dependent on the genetics of the host, favorable environmental conditions, the prevalence of the causal fungus and the survival and spread of the causal fungus. Control of this disease has been difficult because of the complex nature of the host/pathogen interaction. Management of FHB and the associated mycotoxin DON have not been achieved by any single control measure. An integrated approach is critical to attaining the best possible management of FHB and DON in any given environment.

As a result of a workshop sponsored by the Chemical, Biological and Cultural Control Research Area of the U.S. Wheat & Barley Scab Initiative in 2006, a protocol for a multi-state project focusing on integrated management strategies for FHB was developed. The research portion of the project has been multi-state trials evaluating crop sequence, variety selection and fungicide application as an integrated management program for FHB.

The University of Missouri has participated in the multi-state integrated management project for the past three growing seasons. Results from the three years are summarized in this poster abstract.

MATERIALS AND METHODS

During the fall of 2006 two adjacent fields at the University of Missouri Bradford Research and Extension Center just east of Columbia, MO, were identified for this study. The fields had been in a corn/soybean rotation for at least five years prior to the initiation of the study and were separated by a small drainage ditch. The wheat trials were planted into standing corn residue or soybean residue on the same day. The remainder of each field was planted into the normal rotational crop of corn or soybeans. In subsequent years, the wheat trials were shifted to other areas of the same fields with the remainder of the fields planted to the normal rotational crop.

Five soft red winter wheat varieties with similar heading times and varying reactions to FHB were selected for the trial. The five varieties included the public varieties Bess and Roane which are widely grown in Missouri, the Agri-Pro variety Elkhart and the Pioneer varieties 25R47 and 25R54. The FHB resistance reactions for the five varieties are as follows: Bess is considered as tolerant, Elkhart as susceptible, Pioneer variety 25R37 as moderately susceptible, Pioneer variety 25R54 as moderately tolerant and Roane as moderately tolerant.

In the fall of 2006 the trials were planted no-tillage into either soybean residue or standing corn residue on the same day. Individual plots were 7 rows (~7.5" row spacings) by 30' in length. Each trial was set up as a split plot trial with fungicide application as the main plot and variety as the sub-plot. There were 6 replicates in each trial. Sub-plots

were separated by buffer plots. The foliar fungicide treatment Prosaro (6.5 fl oz/A) was applied at Feekes Growth Stage 10.51. A non-ionic surfactant was added to the fungicide at a rate of 0.125% v/v, and application was made using a CO2 pressurized backpack sprayer with TwinJet XR8002 nozzles mounted at an angle (30 and 60 degrees) forward and backward.

Plots were evaluated for incidence and severity of FHB, yield was taken, grain samples were submitted to North Dakota State University for DON analysis and grain samples were rated for percent of *Fusarium* damaged kernels (FDK). Data has been submitted annually to the regional coordinator for inclusion in the multi-state project report. Analysis of variance was used to determine the effects of variety, fungicide and their interactions on yield, DON levels, FHB index (average of 100 wheat heads per plot) and percent FDK for each residue type.

The trial was repeated following the same protocol during the 2007-2008 and 2008-2009 seasons.

RESULTS

Weather conditions during the 2006-2007 season were not conducive for the development of FHB at the Columbia, MO location. Conditions as the wheat crop was flowering were too dry for infection to occur and disease to develop. However, both the 2007-2008 and 2008-2009 seasons were quite conducive for the development of FHB. In both 2008 and 2009 weather conditions were unusually wet and cool as the wheat crop flowered and after flowering.

2007: Weather conditions were not conducive for the development of FHB. In both trials, the yield was statistically significantly different only by variety. For DON levels effects of residue type and residue type x variety were significant. All main and interaction effects were statistically significant for FHB index. Only residue type and variety were significant for % FDK. DON levels were slightly higher in all varieties in the corn residue trials than in the soybean residue trial.

2008: Weather conditions were quite favorable for the development of FHB and FHB developed in all five varieties in both residue types. In the corn residue trial all main and interaction effects were statistically significant for yield and DON levels. Overall, yields were higher and DON levels lower in the soybean residue trial than in the corn residue trial.

2009: Weather conditions again were very conducive for the development of FHB in all five varieties in both crop sequence trials. In the corn residue trial all main and interaction effects were statistically significant for both yield and DON. In the soybean trial main and interaction effects were statistically significant for DON. Although yields in the soybean residue trial tended to be lower than the yields of the same varieties in the corn residue trial, weed competition in the soybean trial may have been a factor. The DON levels for all varieties were lower in the soybean residue trial than in the corn residue trial.

Three Year Summary: Data from the three years for each crop residue type were analyzed using ANOVA. In corn residue, yields were statistically different for year, variety, year x variety and fungicide x variety but not for fungicide alone, year x fungicide or year x fungicide x variety. DON levels were statistically significantly different for all main and interaction effects. For both the FHB index and the percent FDK effects were statistically significant for all but year x fungicide x variety.

In soybean residue, yield was statistically significant for all main and interaction effects except year. DON levels and FHB index were statistically significant for all main and interaction effects. Percent FDK was statistically significant for year, fungicide, variety and year x variety but not for year x fungicide, fungicide x variety or year x fungicide x variety.

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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.

FUNGICIDES CONTROL OF FUSARIUM HEAD BLIGHT SYMPTOMS AND DEOXYNIVALENOL (DON) LEVEL CAUSED BY 15-ADON AND 3-ADON *FUSARIUM GRAMINEARUM* ISOLATES IN WHEAT IN ONTARIO

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ABSTRACT

Fusarium graminearum (Schwabe) causes Fusarium head blight (FHB), an important wheat disease. Deoxynivalenol (DON) is the most important mycotoxin produced by *F. graminearum*; 15-acetyl DON (15-ADON) and 3-acetyl DON (3-ADON) analogs are also produced. A shift in the presence of two *F. graminearum* chemotypes, 15-ADON and 3-ADON, has been reported in North America. The shift may influence current FHB management strategies including the use of fungicides. FOLICUR (tebuconazole) and PROLINE (prothioconazole) are two fungicides commonly used for FHB control in Ontario, while PROSARO has active ingredients from both fungicides. The objectives of this study investigated: 1) the effect of the fungicides on FHB symptoms and DON level after inoculation with 15-ADON and 3-ADON *F. graminearum* isolates in inoculated, misted wheat plots, and 2) the mycelium growth of different isolates of *F. graminearum* on PDA medium with and without fungicides. In 2008, both FHB index (%) and DON level were lower in cv. "Alsen" (moderately resistant) compared to cv. "Roblin" (highly susceptible) in all fungicide treatments and the untreated control, confirming that host resistance plays an important role in host-pathogen-fungicide interaction. Among all fungicide treatments, PROSARO and PROLINE produced the lowest FHB index DON concentration in the variety "Alsen", respectively. In addition, PROSARO resulted in the highest reduction of mycelium growth of both chemotypes compared to other fungicides.

EVALUATION OF INTEGRATED FHB MANAGEMENT METHODS UNDER MODERATE AND SEVERE EPIDEMICS IN NEW YORK

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OBJECTIVE

To evaluate the individual and interactive effects of moderately resistant cultivars, foliar fungicide (Prosaro), and a biological control agent (*Bacillus subtilis*) on wheat yield and the integrated management of Fusarium head blight (FHB) and deoxynivalenol (DON) under two natural environments in New York.

INTRODUCTION

In response to the USWBSI goal to validate integrated management strategies for FHB and DON, the Disease Management RAC of USWBSI initiated a multi-state, multi-year, coordinated field study. In New York during 2009, we conducted two separate experiments each with unique environmental conditions during flowering and early grain development.

MATERIALS AND METHODS

All experiments were performed at the Musgrave Research Farm in Aurora, NY following cultural practices recommended for winter wheat in the region. The two experimental wheat environments were characterized by the planting of winter wheat 1) no-till into soybean residue in late September 2008 and 2) no-till into corn residue in late October 2008. Each experimental design was a split plot with four wheat cultivars as whole plots and four spray treatments as subplots, and four replicate blocks. Main plots were planted with a10 ft wide commercial grain drill. Sprayed areas in each subplot were 8 ft wide by 20 ft long. Spray treatments applied at Feekes GS10.5.1 were 1) non-sprayed; 2) Prosaro 6.5 fl oz/A & Induce 0.125%; 3) *Bacillus subtilis* TrigoCor ca. 1.5×10^{14} cfu/A & Induce 0.125%; and 4) TrigoCor & Prosaro & Induce. Application was made with paired Twinjet nozzles mounted at an angle (30^{o} from horizontal) forward and backward and calibrated to deliver at 20 gallons per A. FHB and foliar diseases were assessed at soft dough stages. Grain was harvested from a 4 ft wide x 20 ft long area in each subplot using a Hege plot combine. Grain moistures, plot yields, and test weights were recorded and the latter two were adjusted for moisture. Means were calculated and subjected to Analysis of Variance. Fisher's protected LSD was calculated at P=0.05. Analysis of DON content in grain was conducted in the USWBSI-supported mycotoxin laboratory of Dr. Schmale.

RESULTS AND DISCUSSION

Due to moist weather through grain maturation, FHB occurred in both experimental environments. A moderate FHB epidemic was observed in the timely-planted plot following no-till soy, and a severe FHB epidemic was observed in the late-planted plot following no-till corn. Difference in epidemic severity for the two experiments is best explained by differences in flowering dates and moisture conditions through flowering. Wheat cultivars reached Feekes GS10.5.1 on June 5 and June 12 for the timely and late-planted experiments, respectively, while rain occurred frequently from June 9 through early July. The impact of crop residue type on FHB development was apparently less important than weather conditions as an adjacent experiment on plowed ground with no corn residue planted to Jensen wheat on the same date had a similar incidence of FHB (40%) as compared to nontreated Jensen (49%) in the late-planted management experiment into no-till corn.

Foliar diseases, including leaf rust and leaf spots, were observed in both experimental environments and were reduced significantly by application of Prosaro. In the timely-planted experiment, no foliar spray treatment (fungicide, biological control, or combination) had a significant effect on the yield of any cultivar. In the late-planted experiment, significantly greater yields due to fungicide treatment were observed in the two white cultivars, Jensen and Richland (Figure 1). Under the lower disease pressure of the timely-planted experiment, the fungicide application decreased DON levels to below 2.0 ppm for the two red cultivars, Pioneer 25R57 and Truman. Under the higher disease pressure of the late-planted experiment, Prosaro did not consistently reduce DON contamination and, when reductions were observed, remaining DON levels still greatly exceeded the 2.0 ppm threshold for sale at flour mills. Therefore, under severe epidemic conditions, the combination of the best available cultivar and fungicide did not reduce DON to satisfactory levels (Table 1). While TrigoCor alone was not able to inhibit FHB, it neither impaired disease control of the fungicide when applied as a mixture nor reduced yield. Moderate FHB resistance was observed with the cultivar Truman, averaging the lowest FHB incidence and DON levels of all of the cultivars in both experimental environments. While Truman is a lower yielding cultivar, the benefit of resistance under high disease pressure was shown by the yield results of the no-till corn plot. Designation of moderate resistance status of cultivars including Jensen is based primarily on observations of FHB symptoms at soft dough stage. The finding of very high DON levels in a cultivar designated as moderately resistant suggests that DON production should be given greater weight in future designation of cultivar reaction.

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Table 1. Main effect of treatment on grain yield, FHB incidence, and deoxynivalenol contamination at Aurora, NY.

	Adjusted grain yield (bu/A)		
Treatment:	Moderate FHB epidemic	Severe FHB epidemic	Average
No treatment	79.1	47.3	63.2
TrigoCor	80.0	48.6	64.3
Prosaro	87.7	58.5	73.1
Prosaro & TrigoCor	89.5	57.4	73.5
LSD (*P*=0.05)	NS	7.8	

	FHB incidence (%)		
Treatment:	Moderate FHB epidemic	Severe FHB epidemic	Average
No treatment	4.7	50.6	28
TrigoCor	4.8	47.3	26
Prosaro	1.1	22.8	12
Prosaro & TrigoCor	2.4	25.1	14
LSD (*P*=0.05)	1.7	10.3	

	Contamination of grain by DON (ppm)		
Treatment:	Moderate FHB epidemic	Severe FHB epidemic	Average
No treatment	2.0	26.4	14.2
TrigoCor	2.7	25.9	14.3
Prosaro	1.2	17.2	9.2
Prosaro & TrigoCor	1.7	21.4	11.5
LSD (*P*=0.05)	0.8	NS	

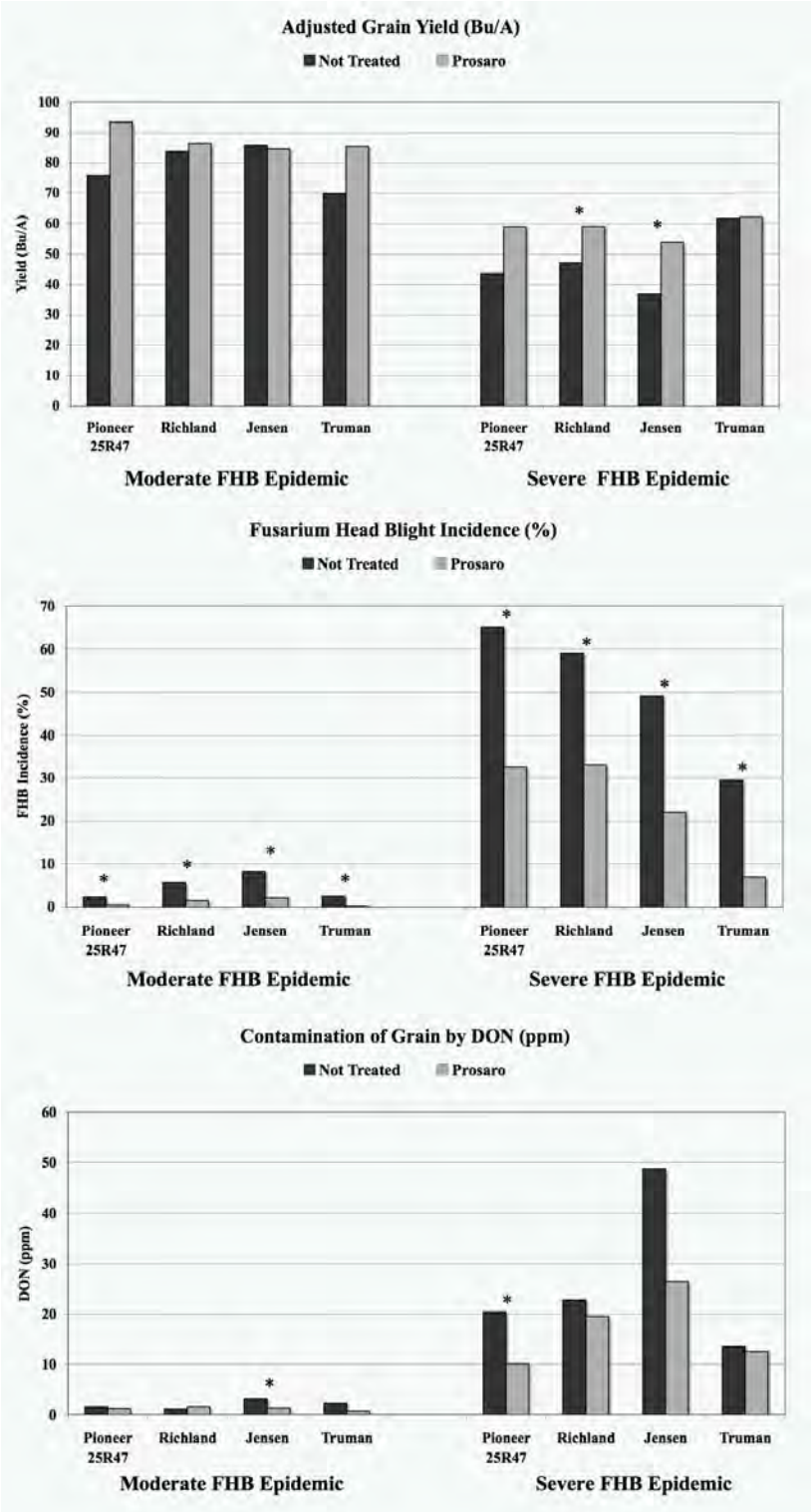

Figure 1. Effect of flowering stage application of Prosaro fungicide on yield, FHB incidence and DON contamination of four winter wheat cultivars in Aurora, NY. * denote treatment means that differ significantly at *P*=0.05.

INTEGRATED MANAGEMENT OF FUSARIUM HEAD BLIGHT AND DEOXYNIVALENOL IN WINTER WHEAT

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ABSTRACT

Fusarium head blight (FHB) is a destructive disease of wheat. In addition to lowering yield and grain quality, the causal fungus, *Fusarium graminearum*, also produces the mycotoxin deoxynivalenol (DON) which poses potential food and feed safety hazards. Integrating cultivar resistance and fungicide application is more effective in managing FHB than either strategy used alone. The objective of this study was to determine the effects of fungicide application and cultivar resistance on FHB and DON in winter wheat. Three cultivars differing in levels of resistance to FHB were planted following corn in the fall of 2008 at the University of Nebraska Agricultural Research and Development Center near Mead, NE. The cultivars were 2137 (susceptible), Jagalene (moderately susceptible), and Harry (moderately resistant). In the spring of 2009, corn kernels colonized by *F. graminearum* were applied to the soil surface in the wheat plots on May 20 at a rate of 50 g/m^2. Plots were not irrigated. The experimental design was a split plot in randomized complete blocks with six replications. Cultivars were the main plots and fungicide treatments (non-treated or treated with Prosaro at 6.5 fl. oz/acre + Induce non-ionic surfactant at 0.125% v/v) were the subplots. Plot size was 5 ft x 11ft. A CO_2-powered backpack sprayer and four Teejet 800-1 VS nozzles spaced 12 in. apart on a boom were used to apply fungicide to heads at early flowering. Fungicide was applied on May 28 (Jagalene and 2137) and on June 4 (Harry). Plots were inoculated with spores of *F. graminearum* (1 x 10^5 spores/ml) using a hand-pumped backpack sprayer. Inoculation dates were May 30 (Jagalene and 2137) and June 6 (Harry). Disease severity and incidence were assessed on 10 heads in each of five arbitrarily selected clusters in each plot and used to calculate FHB index. Disease assessment dates were June 20 (Jagalene and 2137) and June 27 (Harry). Plots were harvested with a small plot combine, which provided yield data. The percentage of *Fusarium*-damaged kernels (FDK) was measured by an automated single-kernel near-infrared system at the USDA ARS Grain Marketing and Production Research Center in Manhattan, KS. A grain sample from each plot was ground and sent to the North Dakota Veterinary Diagnostic Laboratory at North Dakota State University, Fargo, ND for DON determination. Disease levels were low due to dry weather in May. Differences in FHB index among cultivars were highly significant ($P < 0.0001$). FHB index in Harry (9.5%) was higher than that in either Jagalene (0.5%) or 2137 (1.0%). Fungicide application reduced FHB index, but not significantly ($P = 0.0748$). FHB index was 0.7, 1.3, and 10.3% for Jagalene, 2137, and Harry, respectively, in the non-sprayed treatment and 0.3, 0.7, and 8.7% for Jagalene, 2137, and Harry, respectively, in the Prosaro treatment. Fungicide treatment significantly ($P = 0.0051$) increased yield. Yield in the Prosaro treatment was higher (Jagalene, 34 bu/A; 2137, 29 bu/A; Harry, 36 bu/A) than that in the check treatment in all three cultivars. In the check treatment, yield of Jagalene (22 bu/A) was lower than that of 2137 (26 bu/A) or Harry (29 bu/A). Fungicide application significantly reduced FDK ($P < 0.0001$) and DON ($P = 0.0019$). FDK and DON in the Prosaro treatment were lower than in the check treatment for all three cultivars. In the check treatment, FDK (24%) and DON (0.52 ppm) in 2137 were lower than in Jagalene (41% FDK, 0.74 ppm DON)

and Harry (54% FDK, 5.36 ppm DON). In the Prosaro treatment, FDK (40%) and DON (2.86 ppm) in Harry were higher than in Jagalene (16% FDK, 0.44 ppm DON) and 2137 (21% FDK, 0.30 ppm DON). The winter wheat cultivars in this study differed in their reaction to FHB. Although fungicide application did not significantly ($P = 0.0748$) reduce FHB index, it reduced FDK and DON in all three cultivars. Late rains in early June coincided with flowering in Harry. Therefore, FHB index, FDK, and DON were all higher in Harry than in Jagalene or 2137.

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INTEGRATED MANAGEMENT OF FHB AND DON IN SMALL GRAINS: 2009 COORDINATED TRIALS

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OBJECTIVE

To evaluate the integrated effects of fungicide and genetic resistance on FHB and DON in all major grain classes in different cropping systems.

INTRODUCTION

Current FHB and DON control options include genetic resistance, cultural practices, chemical and biological control. However, when used individually, these control measures are not fully effective under environmental conditions favorable to disease development. Moderately-resistant wheat and barley cultivars may accumulate DON levels above critical thresholds for human and livestock consumption (Browne, 2009). Triazole fungicide efficacy varies among studies, with mean percent control between 40 and 60% for FHB index and 30 to 50% for DON accumulation (Paul *et al*, 2008). In general, more effective control is achieved when moderate resistance is combined with appropriate fungicide applications (Beyer *et a*l, 2006). However, this control is variable among grain classes and cropping systems. In 2007 and 2008, coordinated trials in multiple states evaluated the effects of grain class, crop rotation, cultivar resistance, and fungicide application on the management of FHB and DON (Paul *et al*, 2007 and Paul *et al*, 2008). This report summarizes results from trials conducted in 2009.

MATERIALS AND METHODS

Plots were established in fields previously planted with wheat, corn or soybean. At least three commercial small grain cultivars were planted in four to six replicate blocks in each trial. The standard experimental design was a randomized complete block, with a split-plot arrangement of fungicide treatment (whole-plot) and cultivar (subplot). A few trials used a split-split-plot arrangement with previous crop as the whole plot. Fungicide (Prosaro, 6.5 fl. oz/A) was applied at anthesis, using CO_2 powered sprayers equipped with Twinjet XR8002 or paired XR8001 nozzles, mounted at a 30 or 60^o angle, forward or backward. Protocol did not include artificial inoculations or supplemental misting to stimulate disease development. FHB index (plot severity) was assessed during the dough stages of grain development. Following harvest, yield, test weight and percentage of *Fusarium*-damaged kernels (FDK) were determined for each sub-plot. Milled grain samples were sent to a USWBSI-supported laboratory for toxin analysis. Analysis of variance (linear mixed model) was used to evaluate the effects of fungicide, cultivar, (previous crop, when appropriate) and their interactions

on FHB and DON. For severe epidemics, percent control was calculated to compare the effect of control measures to the untreated, susceptible check.

RESULTS AND DISCUSSION

Trials were conducted in 13 states (Arkansas, Illinois, Indiana, Kentucky, Maryland, Minnesota, Missouri, Nebraska, New York, North Dakota, Ohio, South Dakota and Wisconsin). FHB intensity and DON accumulation varied among locations. Trials with minimal disease and/or DON were not included in this summary (Arkansas, North Dakota, Ohio and Wisconsin). Data from Minnesota were forthcoming at publication time.

Illinois. Six soft red winter wheat (SRWW) cultivars were planted at four locations in a split-split plot design with previous crop (soybean or corn) as the main-plot, cultivar as the sub-plot and fungicide as the sub-sub-plot. FHB intensity varied greatly throughout the state (Table 1). In all locations, fungicide treatment had significant effects on index, while fungicide and cultivar had significant effects on DON. **Carbondale.** Prosaro-treated plots had significantly less disease than untreated controls, however FHB remained high (17.6 and 24.0%, respectively). Control measures provided minimal control of FHB index, but reduced DON levels, considerably (Table 1). In addition to cultivar and fungicide, previous crop and the cultivar x fungicide interaction had significant effects on DON accumulation. Generally, when moderately resistant cultivars were planted into soybean residue <2ppm DON accumulated in the grain, without the use of Prosaro (Table 1). **Dixon Springs**. Eight SRWW cultivars were used in this trial. The effects of cultivar and fungicide were significant for index at this location. Prosaro-treated plots had significantly lower mean index than the untreated checks, 3.9 and 12.0%, respectively. Cultivars Pro220, Exc5530 and Exc5170 had significantly lower index values than Cooper. Regardless of previous crop, Kaskaskia and P25R62 provided the highest levels of FHB control, compared to untreated Copper grown in corn residue (Table 1). DON data was unavailable for this site. **Monmouth**. Mean index values were <1% throughout this trial. Despite low disease intensity, DON levels were as high as 4.00ppm. Mean DON levels in Kaskaskia, P25R47and P25R54 were not significantly different from that of Cooper (mean 1.4, 2.3, 1.7 and 1.7ppm, respectively). **Urbana.** Three levels of previous crops (soybean, conventional corn and Bt corn) were used in this trial; however, this factor had no significant effect on index or DON. Kaskaskia had significantly higher levels of index (8.0%) and DON (3.4ppm) than Cooper (2.9%; 2.0ppm), despite providing some of the best disease control at the Dixon Springs location.

Indiana. Six SRWW cultivars were planted into corn residue. Index levels and grain DON content ranged from 0 to 6.5% and 0 to 1.7ppm, respectively. The effects of cultivar, fungicide treatment and their interaction were statistically significant for both index and DON, although mean DON values were <2ppm. P25R47 and P25R78 had significantly lower index than Hopewell, the susceptible check. Moderately resistant INW0412 and INW0801 had index levels that were not statistically different from Hopewell. Prosaro did not provide additional significant index reduction for Truman or P25R47.

Kentucky. SRWW cultivars were planted into corn residue near Princeton, KY. Index levels ranged from 0.3 to 6.5%, while DON ranged from 0.25 to 2.3ppm. The effects of fungicide, cultivar and their interaction were statistically significant for index and DON. AC9511 had significantly lower index and DON than Branson and P26R15 in untreated control plots. Combining AC9511 with Prosaro treatment did not provide any significant reduction in DON accumulation compared to the untreated control.

Maryland. SRWW cultivars were planted at two locations (Beltsville and Queensland) into both corn and soybean residues. Overall, FHB index was greater at Queensland (4.6%) than at Beltsville (1.8%) (DON data was unavailable at publication time). Index was slightly lower when the previous crop was soybean rather than corn. For both loca-

tions and previous crops, the effects of cultivar and fungicide were statistically significant for index. Bess, AC9511 and P26R15 had significantly less disease than susceptible SS8641 in all locations and cropping systems.

Missouri. Five SRWW cultivars were planted into corn and soybean residue. **Corn**. The effects of cultivar, fungicide and their interaction were statistically significant for FHB index and DON. Mean index and DON values were 25.7% and 5.6ppm, respectively. Moderately resistant cultivars, Roane and Bess had significantly lower disease and DON levels than other cultivars, however, mean index was still relatively high (Table 2). For each cultivar, the Prosaro treatment resulted in significantly lower DON than in the untreated check. Bess combined with Prosaro treatment was the only combination to achieve <2ppm DON. **Soybean**. The effects of cultivar, fungicide and their interaction were statistically significant for FHB index. Mean index and DON values were 29.1% and 2.8ppm, respectively. 'Roane' had significantly lower index than all other cultivars; however mean index for treated and untreated sub-plots was 17.10 and 19.97%, respectively (Table 2). Prosaro did not have a significant effect on index for Roane, compared to the untreated control. Only the effect of cultivar had a significant effect on DON. Roane, Bess and P25R54 accumulated significantly less DON than Elkhart and P25R47.

Nebraska. Three hard red winter wheat cultivars were planted into corn residue. For FHB index, only the effect of cultivar was statistically significant. The effects of cultivar, fungicide and their interaction were statistically significant for DON accumulation. Index and DON were significantly lower in Jagalene and 2137 than in the susceptible check, Harry. There was no significant reduction in DON between Prosaro-treated and untreated plots for Jagalene and 2137 (mean DON accumulation for these combinations were <1ppm).

New York. Two split-split plot trials were planted near Aurora, using two soft white and soft red winter wheat cultivars in each. Previous crop served as the whole plot factor, while cultivar and fungicide treatments served as sub- and sub-sub-plot factors, respectively. Overall, mean FHB index and DON levels were greater in SWWW than in SRWW (Table 3). **SWWW.** The effect of previous crop, fungicide, their interaction and the cultivar-fungicide interaction were statistically significant for index. All main and interaction effects, including the three-way interaction, were significant for DON accumulation. Jensen, the resistant cultivar, provided little control of FHB or DON; in fact, index and DON levels were often lower in Richland, the susceptible (Table 2). This seriously questions the resistance mechanisms Jensen may or may not possess. **SRWW.** The effects of previous crop, fungicide, cultivar and the crop x cultivar and crop x fungicide interactions were statistically significant for index. Only previous crop was statistically significant for DON in this trial, as levels were similar for both cultivars and treatments (Table 3).

South Dakota. A split-plot trial with 3 hard red winter wheat cultivars was planted into HRSW residue. Cultivar and fungicide treatment served as the whole plot and sub-plot factors, respectively, and were statistically significant for index and DON. The interaction between cultivar and fungicide was significant for DON. Overall, FHB index was significantly lower in Overland than Alice and Wesley. In all cultivars x fungicide interactions the difference between treated and untreated sub-plots was significant. Despite relatively low index (0.93%), Overland accumulated >2ppm DON without Prosaro.

CONCLUSIONS

In general, the greatest reductions in FHB intensity and DON accumulation were observed when moderately resistant cultivars were used. However, this coordinated effort demonstrated that cultivars, including Kaskaskia, P25R47, and P26R15, had variable FHB disease phenotypes at different locations. The effect of previous crop also had mixed results. In Illinois and Missouri, a non-host crop as the previous crop resulted in little control of FHB.

In Maryland and New York a non host crop resulted in reductions in index compared to a host crop as the previous crop. This warrants further study and suggests climate at these locations affects the efficacy of control measures. Under severe epidemic conditions, a three tier management approach of crop rotation with a non-host, moderately resistant cultivars and fungicide application was required to achieve < 2ppm DON and reduce index. Future work includes quantitative analysis of data from previous years' uniform trials, which will contribute to ongoing efforts to develop and disseminate "best management practices" for FHB and DON reduction.

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Table 1. Mean FHB Index, DON and estimated percent control for different previous crop–cultivar–fungicide combinations in two Illinois locations (DON data not available for Dixon Springs).

				FHB DISEASE		*DEOXYNIVALENOL*						*FHB DISEASE*	
Location (Grain Class)	**Previous Crop**	**Cultivar**	**Prosaro**	**Mean Index (%)**	**% Control**	**Mean DON (ppm)**	**% Control**	**Location (Grain Class)**	**Previous Crop**	**Cultivar**	**Prosaro**	**Mean Index (%)**	**% Control**
Carbondale, IL (SRWW)	corn	Cooper	NO	24.14	0.00	3.43	0.00	Dixon Springs, IL (SRWW)	corn	Cooper	NO	9.10	0.00
			YES	17.53	27.38	2.05	40.15				YES	4.17	54.21
		P25R47	NO	23.25	3.69	3.53	-2.92			P25R47	NO	20.40	-124.18
			YES	16.52	31.57	2.63	23.36				YES	4.03	55.68
		Kaskaskia	NO	20.75	14.04	2.18	36.50			Kaskaskia	NO	4.18	54.03
			YES	17.33	28.21	0.95	72.26				YES	0.93	89.74
		P25R54	NO	28.00	-15.99	3.00	12.41			P25R54	NO	5.63	38.10
			YES	18.61	22.91	1.13	67.15				YES	3.00	67.03
		E5530	NO	19.00	21.29	1.50	56.20			E5530	NO	13.45	-47.80
			YES	17.00	29.58	0.66	80.73				YES	7.87	13.55
		E5170	NO	28.25	-17.03	0.96	71.90			E5170	NO	17.03	-87.18
			YES	20.00	17.15	0.68	80.29				YES	11.02	-21.06
		…	…	…	…	…	…			Pro220	NO	9.13	-0.37
		…	…	…	…	…	…				YES	2.78	69.41
		…	…	…	…	…	…			P25R62	NO	5.32	41.58
		…	…	…	…	…	…				YES	1.93	78.75
	soybean	Cooper	NO	26.75	-10.81	2.03	40.88		soybean	Cooper	NO	23.00	-152.75
			YES	20.50	15.08	0.88	74.31				YES	4.53	50.18
		P25R47	NO	23.14	4.14	2.45	28.47			P25R47	NO	21.68	-138.28
			YES	14.31	40.72	1.38	59.78				YES	4.27	53.11
		Kaskaskia	NO	26.75	-10.81	1.09	68.18			Kaskaskia	NO	2.38	73.81
			YES	16.21	32.85	0.48	86.06				YES	0.07	99.27
		P25R54	NO	26.50	-9.78	1.50	56.20			P25R54	NO	5.23	42.49
			YES	18.25	24.40	0.75	78.10				YES	2.83	68.86
		E5530	NO	22.10	8.45	1.16	66.28			E5530	NO	18.45	-102.75
			YES	17.82	26.18	0.32	90.66				YES	7.52	17.40
		E5170	NO	19.76	18.14	0.52	84.74			E5170	NO	25.40	-179.12
			YES	17.00	29.58	0.17	95.11				YES	4.18	54.03
		…	…	…	…	…	…			Pro220	NO	8.30	8.79
		…	…	…	…	…	…				YES	2.77	69.60
		…	…	…	…	…	…			P25R62	NO	2.73	69.96
		…	…	…	…	…	…				YES	0.93	89.74

Table 2. Mean FHB Index, DON and percent control for different previous crop–cultivar–Prosaro combinations in Missouri.

				FHB DISEASE		*DEOXYNIVALENOL*	
Location (Grain Class)	**Previous Crop**	**Cultivar**	**Prosaro**	**Mean Index (%)**	**% Control**	**Mean DON (ppm)**	**% Control**
Missouri (SRWW)	corn	Elkhart	NO	47.07	0.00	15.98	0.00
			YES	30.11	36.03	6.03	62.25
		P25R47	NO	36.30	22.87	9.70	39.31
			YES	19.29	59.02	3.70	76.85
		P25R54	NO	32.73	30.46	6.22	61.11
			YES	15.86	66.31	2.15	86.55
		Roane	NO	22.30	52.63	4.52	71.74
			YES	17.12	63.62	2.18	86.34
		Bess	NO	21.76	53.76	3.78	76.33
			YES	14.13	69.99	1.42	91.14
	soybean	Elkhart	NO	48.57	-3.18	5.20	67.47
			YES	37.99	19.28	5.30	66.84
		P25R47	NO	28.74	38.94	4.78	70.07
			YES	29.99	36.29	3.60	77.48
		P25R54	NO	38.90	17.36	1.65	89.68
			YES	21.31	54.72	1.67	89.57
		Roane	NO	19.97	57.58	1.10	93.12
			YES	17.10	63.67	1.22	92.39
		Bess	NO	23.34	50.40	1.20	92.49
			YES	25.19	46.48	2.03	87.28

Table 3. Mean FHB index, DON and percent control for different previous crop-cultivar-Prosaro combinations in New York.

				FHB DISEASE		*DEOXYNIVALENOL*	
Location (Grain Class)	**Previous Crop**	**Cultivar**	**Prosaro**	**Mean Index (%)**	**% Control**	**Mean DON (ppm)**	**% Control**
New York (SRWW)	corn	Pioneer	NO	9.05	0.00	20.54	0.00
			YES	4.08	54.97	10.09	50.88
		Truman	NO	2.95	67.40	13.46	34.47
			YES	0.70	92.27	12.60	38.66
	soybean	Pioneer	NO	0.24	97.31	1.62	92.14
			YES	0.05	99.45	1.23	94.04
		Truman	NO	0.25	97.21	2.31	88.75
			YES	0.03	99.62	0.67	96.76
New York (SWWW)	corn	Richland	NO	6.38	0.00	22.79	0.00
			YES	3.43	46.27	19.53	14.31
		Jensen	NO	9.23	-44.71	48.77	-114.04
			YES	4.13	35.29	26.39	-15.82
	soybean	Richland	NO	0.78	87.71	1.13	95.06
			YES	0.22	96.57	1.65	92.78
		Jensen	NO	1.46	77.11	3.11	86.35
			YES	0.41	93.49	1.34	94.14

INHIBITION OF DEOXYNIVALENOL ACCUMULATION BY PREINOCULATION WITH NONTOXIGENIC *FUSARIUM GRAMINEARUM* - PRELIMINARY TESTS OF A NOVEL STRATEGY

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ABSTRACT

According to biological control theory, the best agents are those that share the same ecological niche and environmental requirements as the target. Many examples exist for hypovirulent pathogen isolates being used to control a virulent pathogen. This concept is based on the theory that hypovirulent isolates can compete with virulent isolates for limited niches and substrates and potentially can induce host resistance mechanisms. Extending this theory to Fusarium head blight (FHB), the ideal agent would be an isolate of the pathogen that does not produce deoxynivalenol (DON) and is hypovirulent. A naturally-occurring isolate of *Fusarium graminearum* (WG-9) was isolated from a wild grass from a remote, nonagricultural area in northern Minnesota by L.R. Gale. WG-9 has never produced detectable amounts of DON or other derivatives in inoculated spikelets in greenhouse experiments. Spread of WG-9 on point-inoculated wheat heads, however, varied between experiments from low to moderate compared to standard isolate PH-1. In this study, we are testing the concept that preapplication of a nontoxigenic (Tox-) hypovirulent strain, such as WG-9, to wheat heads can inhibit floret infection by a toxigenic (Tox+) virulent pathogen resulting in reduced DON accumulation in the grain. WG-9 was sprayed at 10^5 spores/ml onto flowering heads of a scab-susceptible spring wheat and then PH-1 was inoculated at the same spore concentration 1 day later. Scab severity was determined 7 to 9 days after pathogen inoculation. Upon seed maturation, the proportion of kernels infected by WG-9, PH-1, or both strains were determined using a multiplex PCR system with primers based on TRI3 and TRI12 gene sequences which reliably distinguished between the strains. In addition, kernels were assayed for DON content. When WG-9 and PH-1 were sequentially inoculated onto wheat at the high spore concentration, there was no reduction in total disease severity as WG-9 alone caused substantial scab. All of the seed was infected and shriveled. Consequently, DON content in kernels from PH-1 inoculated wheat heads pretreated with water were extremely high (average over 100 ppm). Pretreatment of wheat heads with WG-9 prior to PH-1 inoculation reduced the DON content by 10%. A lower proportion of seed was infected with PH-1 when the spikelets were pretreated with WG-9 as compared to pretreatment with water. These results support the hypothesis that a Tox- strain might compete with or exclude a Tox+ strain. The high disease severity caused by the Tox- strain alone is an obvious drawback. We will be exploring applications of WG-9 at much lower spore concentrations and the use of scab resistant cultivars as possible solutions. Field experimentation also is essential to confirm the benefits of pretreatment with Tox- strains.

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RESULTS OF 2009 UNIFORM BIOLOGICAL CONTROL TRIALS

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OBJECTIVE

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

INTRODUCTION

Various strains of biological control agents (BCA) have been demonstrated to be effective in separate studies (DaLuz et al., 2003; Jochum et al., 2006; Khan et al., 2004) in reducing FHB and deoxynivalenol (DON) in wheat and barley. But no one strain has shown to be consistently effective as a stand-alone treatment across diverse environments (Jochum et al., 2008; Yuen et al., 2007). Mixtures of BCA strains have improved the consistency of biocontrol in other pathosystems (Cruz et al. 2006). When this strategy was evaluated for FHB control using mixtures of bacterial strains, no benefits from applying the organisms in mixtures were found, presumably because the organisms were mutually antagonistic (Yuen and Jochum, 2004). The Uniform Biocontrol Trials in 2009 evaluated a novel organism mixture involving two yeast strains. This "double yeast" treatment consisted of *Cryptococcus flavescens* OH 182.9 (NRRL Y-30216) and *C. aureus* OH 71.4 (NRRL Y-30213). Schisler et al. (2007) showed that formulations containing both of these individually-effective, compatible strains had potential to reduce FHB symptoms on wheat in greenhouse trials. Despite the apparent advantages of applying strain mixes, the disadvantages for the manufacturer are capital costs, operation, maintenance, registration and management of a different fermentation for each strain used in a mix. One solution to this obstacle is to co-culture the strains together in one fermentor. Thus, for the current investigation, cultures containing both strains were produced in a fermentor in SDCL medium, and after the cells were concentrated, they were shipped frozen to cooperator field sites. At application, expected cell concentrations were 5 x 10^7 and 8 x 10^8 CFU/ml for strains OH 182.9 and OH 71.4, respectively.

As part of an integrated control protocol with fungicides, a tank mixed combination of BCA and fungicide applied at flowering could theoretically provide immediate protection from FHB and lasting protection due to BCA activity after the fungicide component is no longer present or effective. Alternatively, biocontrol agents could be applied separate from the fungicide after kernel development begins, a stage of development when fungicides are not approved for use. In either case, BCA could be especially effective in limiting the total DON content in harvested grain by combating new infections by the pathogen that can occur during early to late grain development (Del Ponte et al., 2007). In earlier investigations, tank-mixed combinations of bacterial strains with tebucanozole resulted in better performance than the organism or fungicide alone (DaLuz et al., 2003; Jochum et al., 2006; Khan et al., 2004). Subsequent studies with bacterial BCA tank-mixed with Prosaro 421 SC (a formulation of prothioconazole and tebucanozole; Bayer CropScience), however, revealed no improvement (Yuen et al., 2007; Jochum et al., 2008). The sequential application of a fungicide followed

by a BCA has not been tested. Therefore, another focus of the 2009 uniform biocontrol trials was the integration of BCA with a fungicide by combining the components as a tank mix or applying BCA as a follow-up treatment at late bloom.

MATERIALS AND METHODS

Six trials were conducted across four states on a range of wheat market classes (Table 1). The biological materials tested were the double yeast, supplied by D. Schisler, and Taegro (Novozymes Biologicals, Salem, VA), a commercial product containing *Bacillus amyloliquefaciens* FZB24. Treatments tested in these trials are listed in Table 2. All treatment liquids were amended with 0.125% Induce. One application was made per treatment at early flowering (Feekes 10.51) or 5 days later (late-bloom) in 20 gal/acre using a CO_2-pressurized sprayer. Pre-application samples of BCA at some locations were sent to G. Yuen for analysis of populations using dilution plating. The size and number of replicate plots varied among trials. Some of the trials were inoculated with *Fusarium graminearum*-infested corn kernels and utilized mist irrigation systems to stimulate infection. In all trials, FHB incidence, severity, and index were determined from at least 40 heads per plot around 3 weeks after anthesis. Plot yields, test weight, and the incidence of *Fusarium*-damaged kernels (FDK) were determined after harvest. Kernel samples from each plot were analyzed for DON content by the North Dakota State University Veterinary Diagnostic Laboratory in Fargo. Data from each trial were analyzed separately and pooled together using ProcMixed (SAS), with LSmeans separated by the LSD test at the 95% confidence level.

RESULTS AND DISCUSSION

Moderate to high FHB levels were recorded at Missouri and North Dakota trials. Dry weather conditions in Nebraska and Michigan hindered FHB development despite misting being provided. Nevertheless, significant treatment effects were found in Nebraska and Michigan for some in-field disease parameters. While none of the treatments reduced in-field disease measurements compared to the control in all trials, treatments involving Prosaro alone or combined with a BCA were efficacious in the majority of cases. The double yeast and Taegro applied alone were efficacious in Missouri on 'Elkhart' and in Michigan. When results from all trials were pooled, head severity and index in these biological treatments were significantly lower than the control. In only two instances did any treatment exhibit better efficacy than Prosaro alone; both involved a combination of Prosaro with Taegro. Interestingly, the treatment with Prosaro followed by the double yeast reduced DON by 34% and was the only treatment to significantly reduce DON when averaged across all locations. FDK was reduced only in North Dakota where all treatments had a significant effect. There were no differences in test weights and yields between any treatments at any location (data not shown).

The results with the biological treatments in these trials are promising. The treatments with the BCA alone or in combination with the fungicide were comparable in consistency to the standard fungicide and in a few instances provided higher levels of control than the fungicide. The reduction of DON by combining Prosaro and the double yeast may have resulted from the biocontrol agents inhibiting late infections by *F. graminearum* when reduced fungicide activity would be expected. Some of the instances in which the BCA were not effective could have been related to population levels of the organisms declining during shipment or storage. The viable cell concentration in the double yeast inoculum applied in North Dakota, in particular, was considerably lower than expected levels. Formulation to improve shelf life might provide more consistent performance in the future. In addition, populations of the two yeast strains in the double yeast co-culture differ by more than a log unit. Further tests may clarify whether efficacy improvements would be realized with a product containing equivalent populations of these two strains.

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Table 1. 2009 uniform biological control trial locations, wheat cultivars, and researchers.

State (location)	Crop market class and cultivar	PI and institution
MO (Columbia)	Soft red winter wheat 'Roane'	L. Sweets, University of Missouri
MO (Columbia)	Soft red winter wheat 'Elkhart'	L. Sweets, University of Missouri
NE (Mead)	Hard red winter wheat '2137'	G. Yuen, University of Nebraska
NE (Lincoln)	Hard red winter wheat '2137'	G. Yuen, University of Nebraska
ND (Langdon)	Hard red spring wheat 'Howard'	S. Halley, North Dakota State University
MI (Clarksville)	Soft white winter wheat 'Pearl'	W. Kirk, Michigan State University

Table 2. Treatments tested in 2009 uniform trials.

Treatment code	Treatment
Control	Nontreated
Pro	Prosaro 6.5 fl oz /acre at 10.51
Tae	Taegro 3.5 oz/acre at 10.51
Tae late	Taegro at late bloom
Pro + Tae	Tank mix of Prosaro and Taegro at 10.51
Pro early/Tae late	Prosaro at 10.51 followed by Taegro at late bloom
DYs	Double yeast at 10.51
Pro early/DYs late	Prosaro at 10.51 followed by double yeast at late bloom

Table 3. 2009 results from uniform biocontrol trials denoted by state and location (or cultivar).

Treatment	NE Mead	NE Lincoln	ND Langdon	MO 'Elkhart'	MO 'Roane'	MI Clarksville	LS means
INCIDENCE (%)							
Control	55	66	77	58	81	24	60
Pro	50	58	71*	38*	74*	20	52*
Tae	58	59	79	48*	79	21	57
Tae late	46	63	87	45*	78	20	56
Pro + Tae	38*#	59	63*	40*	74*	20	49*
Pro early/Tae late	49	54	54*#	40*	83	19	50*
DYs	47	52	83	43*	84	19	54*
Pro early/DYs late	No data	No data	63*	43*	71*	20	53*
P	0.0248	Ns	0.0001	0.0121	0.0008	Ns	0.0001
$LSD_{0.05}$	11	-	13	5	5	-	5
SEVERITY (%)							
Control	8	14	21	79	38	9	28
Pro	8	9	14*	55*	36	4*	21*
Tae	11	9	20	63*	39	4*	24*
Tae late	9	9	21	63*	41	3*	24*
Pro + Tae	7	10	15*	63*	38	4*	23*
Pro early/Tae late	9	14	11*	58*	33	4*	21*
DYs	9	10	20	63*	34	4*	23*
Pro early/DYs late	No data	No data	13*	61*	36	5*	23*
P	0.0831	0.0823	0.0013	0.0402	Ns	0.0010	<0.0001
$LSD_{0.05}$	-	-	5	12	-	2	3

Table 3 (continued)

Treatment	NE Mead	NE Lincoln	ND Langdon	MO 'Elkhart'	MO 'Roane'	MI Clarksville	LS means
INDEX (%)							
Control	4	9	13	45	31	2	17
Pro	4	5	8*	21*	27	1*	11*
Tae	6	5	13	30*	31	1*	14*
Tae late	3	6	16	28*	32	1*	14*
Pro + Tae	3	6	6*	25*	28	1*	11*
Pro early/Tae late	4	7	3*	24*	27	1*	11*
DYs	4	5	14	27*	28	1*	13*
Pro early/DYs late	No data	No data	5*	27*	26	1*	12*
P	0.0437	0.0806	<.0001	0.0011	Ns	0.0017	<0.0001
$LSD_{0.05}$	2		5	9		1	2
FDK (%)							
Control	1	1	5	23	12	12	9
Pro	1	1	1*	22	9	7	7
Tae	2	2	4*	18	9	9	7
Tae late	2	2	4*	27	7	9	8
Pro + Tae	2	<1	1*	19	9	6	7
Pro early/Tae late	1	2	1*	21	7	10	7
DYs	1	1	3*	24	9	5	7
Pro early/DYs late	No data	No data	2*	18	10	6	7
P	Ns	0.0995	<.0001	Ns	Ns	Ns	Ns
$LSD_{0.05}$			1				
DON (ppm)							
Control	<0.5	<0.5	Tbd	6.6	1.2	0.9	2.9
Pro	<0.5 - 0.5	<0.5	Tbd	5.2	0.7	1.8	2.6
Tae	<0.5	<0.5 - 0.7	Tbd	7.5	1.1	2.0	3.5
Tae late	<0.5 - 0.6	<0.5	Tbd	6.8	1.1	1.0	2.9
Pro + Tae	<0.5	<0.5	Tbd	4.8	1.4	0.5	2.2
Pro early/Tae late	<0.5 -0.5	<0.5	Tbd	4.8	0.7	1.5	2.3
DYs	<0.5	<0.5 - 0.5	Tbd	6.1	0.9	1.2	2.7
Pro early/DYs late	No data	No data	Tbd	3.9*	0.9	1.0	1.9 *
P	Ns	Ns		0.0155	Ns	Ns	0.0164
$LSD_{0.05}$				1.9			0.8

* = Value is significantly lower than the control at the 95% confidence level
# = Value is significantly lower than Prosaro at the 95% confidence level
Ns = not significant, i.e., *P*>0.1
Tbd = to be determined

SESSION 3:

VARIETY DEVELOPMENT AND HOST RESISTANCE

Co-Chairpersons: Anne McKendry and Kevin Smith

MAPPING QTL FOR FHB RESISTANCE AND DON ACCUMULATION IN BARLEY POPULATION COMP351 X M98-102

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ABSTRACT

Many previous studies have identified QTL for FHB resistance in barley, but most of these QTL co-locate to the same regions. To continue making progress in enhancing the FHB resistance of barley, additional unique sources of resistance must be identified. As an alternative means for identifying FHB resistance in barley, bulked seed of Composite Cross XXX-G was evaluated in China and over 350 early maturing, six-rowed lines with low (<5%) infection were selected for further evaluation in the Midwest. In subsequent screening tests, two selections (COMP 351 and COMP 355) consistently exhibited low levels of FHB and deoxynivalenol (DON). Crosses were made between COMP 351 and breeding line M98-102 to identify and map QTL loci conferring resistance to FHB and the accumulation of DON. A population of 137 $F_{4:5}$ lines derived from COMP351 x M98-102 was evaluated in seven field trials for FHB severity. Deoxynivalenol (DON) concentration, days to heading, spike angle, height, and spike density were evaluated in most, but not all of the environments. Lines were genotyped by Triticarte Pty. Ltd. using Diversity Arrays Technology (DArT) markers. Six hundred and fifty DArT markers were polymorphic between the parents and met the quality standards recommended by Triticarte for mapping. Joinmap 4.0 was used to construct the genetic maps, and QTL Cartographer (2.5) was used for single marker analysis and composite interval mapping. Six QTL for FHB severity and four QTL for DON accumulation were significant in two or more environments. The six QTL for FHB resistance were identified on chromosome 2H and 4H and four on chromosome 7H (0.21>R2>0.04). The four DON QTL were identified on chromosome 2H, 3H, 5H and 7H (0.22>R2 >0.05). QTL conferring heading date (0.37>R2>0.02) and height (0.24>R2>0.05) also were identified. Two QTL regions were coincident for FHB resistance and DON accumulation on chromosome 2H and chromosome 7H. The 2H QTL region was also associated with heading date and height in this study and has been described in other studies. Two of the four FHB QTL on chromosome 7H were associated with other traits. One was associated with DON and height and the other was associated with heading date. One major effect QTL for spike angle was identified on chromosome 5H (0.20>R2>0.12) and was not associated with FHB severity or DON accumulation. At least two of the FHB QTL on chromosome 7H appear to be novel and could be exploited for marker assisted selection.

ASSOCIATION ANALYSIS OF FHB RESISTANCE IN SOFT WINTER WHEAT

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ABSTRACT

Resistance to Fusarium head blight (FHB) has been identified in soft winter wheat (SWW) cultivars from the Eastern United States, although little information has been available about the genetic basis of resistance. Recently, QTL mapping has been done in bi-parental crosses involving SWW sources of resistance, but mapped QTL need to be validated in diverse genetic backgrounds. Conducting a genome-wide association analysis on SSW lines grown in uniform regional scab screening nurseries will enable breeders to identify and validate QTL associated with FHB resistance. Association analysis is amenable for analyzing multiple diverse populations for FHB resistance. The approach can offer greater power and precision to test QTL effects and associated markers for their diagnostic capacity. The Northern Uniform Winter Wheat Scab Nursery (NUWWSN) and Uniform Southern Fusarium Head Blight Nursery (USFHBN), grown at a total of 16 locations during 2008 and 2009, were rated for several phenotypic traits associated with the disease (INC, SEV, INDEX, FDK and DON). Nursery entries were genotyped with SSR markers targeted to regions previously associated with FHB resistance QTL and genome-wide DArT markers. Unlinked markers were used to assess the relatedness of lines using STRUCTURE 2.3.1, Principal Component Analysis (SASv9.1.3), and Kinship (TASSLE v2.1). Results of associations identified using mixed model analysis in TASSLE and SAS will be presented.

ACKNOWLEDGEMENT AND DISCLAIMER

This material is based upon work supported by the U.S. Department of Agriculture. 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.

DEVELOPMENT, MAPPING AND HAPLOTYPE ANALYSIS OF EST-BASED SNPS IN THE WHEAT *FHB1* REGION

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ABSTRACT

Fusarium head blight (FHB) is a destructive disease that reduces wheat grain yield and quality. The Chinese variety Sumai 3 and its derivatives such as Ning 7840 have a high level of resistance to FHB symptom spread within a spike (Type II resistance) and have been widely used as resistant parents in breeding programs worldwide. The quantitative trait locus (QTL) in chromosome 3BS (*Fhb1)* from Sumai 3 source has been identified to have the largest effect on FHB resistance to date. This QTL has been linked to restriction fragment length polymorphisms, simple sequence repeats (SSR), amplified fragment length polymorphisms and sequence tagged site (STS) markers. Single nucleotide polymorphism (SNP) is the most common form of genetic variation and will be the next generation marker system for mapping and marker-assisted selection (MAS). In this study, we developed SNP markers based on wheat expressed sequence tags (ESTs) associated with *Fhb1*. A total of 131 SNPs were identified between Ning 7840 (FHB-resistant) and Clark (susceptible) based on sequences of ten ESTs. SNPs were analyzed in a BC_7F_7 population derived from Ning 7840/Clark using the single base extension method. Six SNP markers mapped between *Xgwm533* and *Xgwm493*, SSR markers flanking the *Fhb1*. Four of these SNP markers clustered with five other SSR/STS markers and covered a 7.4 cM interval. This marker-dense region gave the highest R^2 (40-54%) and LOD values (9.16-11.80) and is the most likely location of *Fhb1*. Haplotype analysis of 63 wheat accessions from eight countries based on EST sequence (SNP), SSR and STS markers associated with *Fhb1* identified four major groups: (1) US-Clark, (2) Asian, (3) US-Ernie and (4) Chinese Spring cluster. The Asian cluster consisted of Chinese and Japanese lines that carry *Fhb1* and a marker *Xumn10* haplotype could differentiate these accessions from accessions in all other groups. All Sumai3-related accessions formed a sub-cluster within the Asian group and can be sorted out by the marker *Xsnp3BS-8* from all other accessions. The SNP markers identified in this study should be good for fine-mapping and MAS of *Fhb1*.

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SEQUENCE ANALYSIS FOR GENE DISCOVERY IN BARLEY CHR. 2H BIN 10 REGION

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ABSTRACT

Efficiently identifying candidate genes for FHB resistance is an important yet challenging goal. Without sequence data, gene discovery in barley must depend on syntenous organisms such as rice and the newly sequenced *Brachypodium distachyon*. The genetic map of barley chromosome (chr.) 2H bin 10 is well-saturated with only one gap over 2.2 cM and the syntenous region in rice has been mined for markers. *Brachypodium* synteny has already added seven markers to the region and provided further probes for creating a physical map. From probes throughout bin 10, we have created a minimum tiling path of 36 BACs covering nearly 3 Mb that are currently being sequenced at WSU in order to increase gene identification efficiency. Candidate genes will be identified by bioinformatic data analyses. The candidate genes will be used to further saturate the chr. 2H bin 10 genetic and physical maps. We now have two years of phenotyping data from our recombinant lines but though we have separated height and head type from FHB resistance, we still do not have markers that segregate with the disease. Mutagenesis of CIho4196 has provided us with FHB resistant lines that are 6-rowed, early, sterile and hence potentially promising as breeding parents. We are working to combine these traits in a single line.

SCAB RESISTANCE QTLS HAVE AN EFFECT ON AGRONOMIC AND QUALITY TRAITS OF SOFT RED WINTER WHEAT

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ABSTRACT

Fusarium head blight (FHB) is a disease that affects wheat world-wide. However, most of the quantitative trait loci (QTLs) in wheat which are responsible for resistance to FHB are derived from exotic spring wheat cultivars originating in Asia. The purpose of this research was to determine whether the introduction of exotic FHB resistance QTLs has an effect on the quality and agronomic traits of soft red winter wheat. Eighty-six F2 derived recombinant inbred lines were developed by crossing Ning 7840, a Chinese spring wheat with FHB resistance QTLs, with Pioneer 2643, an FHB susceptible soft red winter wheat. Using a complete block design, the recombinant inbred lines were evaluated for the presence of FHB resistance QTLs, agronomic performance and grain quality in 2009. Height was reduced by the 3BS QTL, lodging was increased by the 5A QTL, and seed weight was reduced by the 2DL QTL. The softness equivalent score was lowered by the presence of the 5A QTL. These results suggest that the introduction of FHB resistance QTLs into soft red winter wheat can have consequences on agronomic and quality traits.

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EXPLORATION, IDENTIFICATION, TRANSFERRING AND UTILIZATION OF NEW SCAB RESISTANCE IN WHEAT IMPROVEMENT

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ABSTRACT

It is widely considered that scab resistance is controlled by two or three major genes and several minor genes (QTL's). Recombination and convergence of different resistant components has been successfully used in wheat breeding for scab resistance worldwide. *Roegneria kamoji, Roegneria ciliaris* and *Leymus racemosus* have been identified with high scab resistance. They are new genetic resources and would be helpful for broadening the genetic basis of scab resistance. Three *Triticum aestivum-Leymus racemosus* disomic addition lines 5Lr.#1, 7Lr.#1 and Lr.7, one *T.aestivum-Roegneria kamoji* addition line 1Rk#1 and one *T.aestivum-R.ciliaris* addition line $2S^c$ with scab resistance have been developed in Nanjing Agricultural University. Similar as in common wheat, scab resistance of *Leymus racemosus* is controlled by at least three loci on different chromosomes. More than thirty wheat-*L.racemosus* translocation lines involving in chromosomes 5Lr.#1, 7Lr.#1 and Lr.7 with different chromosome segments have been developed by irradiation and gametocidal gene effect and characterized by chromosome C-banding, *in situ* hybridization, and molecular marker analysis. Their scab resistance was evaluated both in the greenhouse and field in multiple locations and multiple years. Three *T.aestivum-L. racemosus* translocation lines, NAU601 (T4BS·4BL-7Lr#1S), NAU617 (T6AL·7Lr#1S) and NAU635 (T1BL·7Lr.#1S), and several introgression lines with high scab resistance and good fertility were selected. A wheat-*R. kamoji* translocation line with scab resistance involving the short arm of 1Rk#1 was obtained. Intercrosses between different alien translocation lines with scab resistance and between alien chromosome lines and common wheat were made to pyramid different scab resistance genes. Varieties or elite lines were used as recurrent parents to improve agronomic characters of these resistant lines. A multiple translocation line with both scab and powdery mildew resistance and several advanced lines with scab resistance and good agronomic characters have been developed. These new genetic resources are being used as parents in wheat breeding program for scab resistance.

VALIDATION OF *FHB*1 IN SEVERAL SOFT RED WINTER WHEAT BREEDING POPULATIONS

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ABSTRACT

Marker assisted selection offers one potential strategy for developing wheat varieties resistant to Fusarium head blight (FHB). By incorporating resistance alleles at major quantitative trait loci, such as *Fhb*1, into genetic backgrounds with improved agronomic and quality characteristics, breeding lines with cultivar release potential should result. To quantify and validate the effect of *Fhb*1, we evaluated five different populations: 26R58/VA01W-476//KY97C-0574-01, 25R54/VA01W-476//KY97C-0574-01, 25R54/VA01W-476//KY97C-0554-02, 25R78/Cumberland//VA01W-476 and 25R23/KY93C-1238-17-1//VA01W-476. These three-way crosses were considered typical of those used in the University of Kentucky FHB resistance - breeding program. F_2 individuals were genotyped for the presence of resistance alleles at *Fhb*1. A total of 185 homozygous resistant and susceptible $F_{2:4}$ lines were rated for disease symptoms in the Lexington scab nursery in 2009. Grain was analyzed for percentage *Fusarium* damaged kernels (FDK) using air separation. In four populations, mean disease ratings (0-9) of lines containing *Fhb*1 were reduced from 3.3 to 1.7. Mean FDK was reduced from 24.7 to 15.5, despite the early generation of genotyping.

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EVALUATION OF *HORDEUM* ACCESSIONS FOR RESISTANCE TO FUSARIUM HEAD BLIGHT

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ABSTRACT

Fusarium head blight (FHB), caused by *Fusarium graminearum*, has devastated the malting barley industry in the Upper Midwest. The deployment of cultivars with resistance to *F. graminearum* and its associated mycotoxins is the best means for combating the disease. Over the past decade, 21,487 cultivated barley and 1,768 wild barley (*Hordeum vulgare* subsp. *spontaneum*) accessions were screened for FHB resistance in Hangzhou, China and/or St. Paul and Crookston, Minnesota. The diverse *Hordeum* germplasm was provided by the USDA National Small Grains Collection, Aberdeen, ID USA; N. I. Vavilov All-Russian Scientific Research Institute of Plant Industry [VIR] in St. Petersburg, Russia; Station federale de recherches en production vegetale de Changins [SFRSPP] in Nyon, Switzerland; Nordic Gene Bank [NGB] in Alnarp, Sweden; Institute for Cereal Crops Improvement [ICCI] in Tel Aviv, Israel; International Center for Agricultural Research in the Dry Areas [ICARDA] in Aleppo, Syria; and Plant Gene Resources of Canada (Agriculture and Agri-Food Canada) in Saskatoon, Canada. Using the six-rowed cultivar Chevron as the standard for resistance, only 279 cultivated (1.3%) and 26 wild (1.5%) barley accessions were selected as possessing a useful level of partial resistance to FHB. Seventy-seven of the 279 selected cultivated barleys have been evaluated for resistance in three or more years. Of these 77 accessions, 15 (19.5%) were six-rowed, 58 (75.3%) were two-rowed, and 4 (5.2%) were of unknown type. Within the group of 77 selected barleys, the highest frequency of resistance found was in accessions from Ethiopia (11.7%), Switzerland (10.4%), Japan (7.8%), Finland (6.5%), and Czech Republic and Ukraine (each with 5.2%). For wild barley, the highest frequency of resistance found was in accessions from Israel (65.4%), Iran (19.2%), and Azerbaijan, Iraq, Jordan, and Syria (each with 3.8%). These selected *Hordeum* accessions should provide diverse alleles for enhancing the level of FHB resistance in barley breeding programs.

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CHROMOSOME ENGINEERING OF T7A·7LR#1S FOR THE ISOLATION OF NEW RECOMBINANTS AND FIELD EVALUATION OF T7A·7LR#1S CHROMOSOME INTROGRESSION HARD WINTER WHEAT LINES FOR RESISTANCE TO FHB AND DON

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ABSTRACT

T7AL·7Lr#1S is a genetically compensating wheat-*Leymus* translocation line (T09) involving wheat chromosome 7AL arm and *Leymus racemosus* (Lr) chromosome 7Lr#1S arm in CS (Chinese Spring) and was consistently resistant to FHB in greenhouse point-inoculation experiments. The novel FHB resistance gene was designated *Fhb3* and resides in the distal region of the short arm of chromosome 7Lr#1. T7AL·7Lr#1S was backcrossed twice to Overley and Jagger and ten lines homozygous for T7AL.7Lr#1S, three in Overley and seven in Jagger background, were evaluated for FHB resistance in a field nursery in Manhattan. All of the translocation lines except 08-183 had significantly lower mean disease ratings compared to their susceptible parent Overley. Unfortunately, the other backcross parent Jagger was not included in the test; however, three of the translocation lines (08-193, 08-189, and 08-184) had significantly lower ratings than Jagalene, which is known to be identical to Jagger in its reaction to FHB. It appears that *Fhb3* increased resistance in these entries. Similarly, the same three translocation entries had significantly lower DON levels than those of Overley and Jagalene and were statistically similar to moderately-resistant Truman.

Simultaneously, chromosome engineering was initiated to reduce the genetic linkage drag associated with T7AL·7Lr#1S. Three PCR-based markers, BE586744-STS, BE404728-STS, and BE586111-STS, specific for 7Lr#1S, were developed to expedite marker-assisted selection of recombinants. Upon analysis of 1,118 progeny, three wheat-*Leymus* recombinants, one proximal (#124) and two distal (#679 and #989), have been isolated in homozygous condition. These lines along with resistant and susceptible controls, as well as 08-193, 08-189, and 08-184, will be evaluated for FHB resistance by single point inoculation method in the greenhouses.

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DEVELOPMENT AND EVALUATION OF HARD RED SPRING WHEAT QTL-NILS FROM DIVERSE FHB RESISTANCE SOURCES

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ABSTRACT

Fusarium head blight (FHB) resistance has been identified in many diverse wheat genotypes from around the world, and a multitude of FHB resistance QTLs has been reported. One challenge that wheat breeders face is deciding which QTLs reported in unadapted or exotic backgrounds will be worth the time and resource investment associated with their introgression into regionally adapted germplasm. Over the course of several years, we used backcrossing coupled to marker assisted selection to develop near isoline sets of the FHB-susceptible hard red spring wheat cultivars Norm, Wheaton, and Apogee that possess one of five reported FHB resistance QTLs from a range of unadapted and exotic wheat sources. The FHB resistance of these near-isoline series has been evaluated in multiple greenhouse and field studies. Results of these studies and future research directions based on the findings will be presented.

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MIXED MODEL ASSOCIATION ANALYSIS FOR FHB RESISTANCE IN TUNISIAN DURUM WHEAT POPULATIONS

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ABSTRACT

There are limited sources of resistance to Fusarium head blight (FHB) predominantly derived from Chinese hexaploid genotypes like Sumai3 and Wangshuibai. Therefore, there is a need to use more diverse sources of resistance to expand the number of genes that may be used in gene pyramiding. In this study we used 184 BC_1F_6 and 189 BC_1F_7 lines derived from crossing Tun7, Tun18, Tun34, and Tun36 with durum cultivars 'Ben', 'Maier', 'Lebsock' and 'Mountrail' for association studies. We evaluated all the parents and RILs in the greenhouse in two seasons for type II resistance to FHB using the single floret injection inoculation method. The data showed the Tunisian lines have good level of resistance varying from 22% to 10% infection rate through the spikes.

To have a full coverage of the genome for association analysis lines were genotyped utilizing the 2,300 Diversity Array Technology (DArT) markers showing 25% polymorphism between the parents. The cluster analysis of the polymorphic markers revealed three distinct groups. The major groups were the North Dakota derived durum cultivars and majority of Tunisian lines except for Tun7 that was in a separate group far from the others. As both Tun7 and Tun18 are resistance to FHB and have different genetic backgrounds, both could be considered as potential candidates for new sources of resistance.

Different association mapping strategies were performed and the best models were used to find the associated markers to FHB resistance. In total 537 polymorphic markers had allelic frequencies more than 5% and used in the analysis. Ten different models (Naïve, K, K_T, Pedigree, Q, PCA, QK, QK_T, PK and PK_T) were compared and best models were selected by considering the lowest mean of squared difference (MSD) between observed and expected p-values of all marker loci and percentage of observations below 0.05 in P (expected)-P (observed) plot. MSD values for the $K_{T(0.65)}$ and $QK_{T(0.7)}$ was the lowest among all other models. These two models had an MSD 50 times lower than the naïve model. The P-P plot also showed the mixed model performs better than the Naïve model.

A union output of the two different models showed 20 markers from 2A, 3B, 4A, 5B and 6B, and 15 markers with unknown locations are associated ($p<0.05$) with FHB resistance when analyzing the whole population derived from nine different crosses. Of these 35 markers, association of five markers was significant after correcting for multiple testing using positive false discovery rate (pFDR) criterion. All of these markers were from the same QTL located on 5BL. The other QTL found in this study were not confirmed by pFDR<0.1 although the QTL from 3BS seems promising as the pFDR criterion is very close to being significant. Tun 18 and Tun 7 which have both QTLs are the parents which show a good level of resistance in our study. We could also hypothesize the potential of having a suppressive gene(s) coming from susceptible cultivars that masks the effect of the resistance genes in the population. All

the susceptible cultivars are sensitive to FHB infection although they carry the 5BL QTL.

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AN ALTERNATIVE PATH TO FUSARIUM HEAD BLIGHT (FHB) RESISTANT WHEAT CULTIVARS: EXPRESSION RATHER THAN INTROGRESSION

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ABSTRACT

Decades of sustained effort attest to the difficulty of generating FHB-resistant spring wheat cultivars. The common assumption underlying these efforts is that discrete genes conditioning superior FHB resistance must be introgressed from sources such as Sumai 3 into elite germplasm. An alternative path could start from a very different assumption. FHB-susceptible near-isogenic lines of Sumai 3, like Sumai 3 itself, carry pathogenesis-related genes that are induced by *Fusarium graminearum* Schwabe. This suggests that the key to FHB resistance is the control of expression of critical genes that are already present. A scheme that might generate variation in expression was suggested when we observed that progeny derived by selfing of plants under pressure from systemic virus infection could vary visibly from type. We devised an iterative protocol which, even within small populations, selects such variants and identifies by their expression in subsequent generations those whose altered traits are heritable. Promising individuals are then advanced as founders of lines for testing. Within three years we have thus derived lines from the doubled haploid cultivar 'McKenzie' that express traits not seen in their progenitor: short stature, near-immunity to wheat streak mosaic virus, and improved resistance to leaf spot diseases and FHB. These new characteristics have been stably expressed over multiple generations.

LEVEL OF *FUSARIUM* MYCOTOXINS IN WHEAT GRAIN HIGHLY ASSOCIATED WITH PERCENTAGE OF SCABBY KERNELS

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ABSTRACT

Resistance to head blight of winter wheat is believed to be essential for managing the disease and achieving low levels of associated mycotoxins in grain. Resistance to mycotoxin accumulation has been hypothesized as one of the five types of head blight resistance. Relative to our understanding of resistance to initial infection and spread within a spike, little is known about resistance to mycotoxin accumulation, and there has been no definitive data on how best to select for low mycotoxin accumulation in grain. The objectives of this research were to develop an efficient method for selecting lines with low levels of mycotoxin accumulation in grain and to determine if resistance to mycotoxin accumulation is a separate, independent resistance component or simply a pleiotropic effect of other resistance mechanisms. A susceptible cultivar (Coker 9835) and 15 winter wheat lines with diverse sources of head blight resistance were evaluated in a series of field and greenhouse experiments. Lines were inoculated with deoxynivalenol (DON) and nivalenol (NIV) chemotype isolates of *Fusarium graminearum* and characterized for head blight severity, percentage of scabby grain, and level of mycotoxins in the grain. Correlation analyses were performed to determine the relationships of severity and percentage of scabby grain with the levels of mycotoxins in grain. Compared to the susceptible check, all resistant lines had significantly lower levels of DON and NIV in grain. In the greenhouse tests, DON and NIV levels in grain were positively correlated with the percentage of florets blighted 21 days after inoculation ($r = 0.87$ and 0.96, respectively) and with the percentage by weight of the scabby grain ($r = 0.96$ and 0.88, respectively). Furthermore, most of the mycotoxins were associated with the scabby grain, and healthy grain had low levels of mycotoxins. In the field experiments, DON levels in the grain were positively correlated with head blight severity at soft dough stage ($r = 0.83$ to 0.93) and with percentage of scabby grain ($r = 0.96$ to 0.97). Compared to DON levels for the susceptible check, DON levels for the most resistant lines were reduced up to 94%. The results of this study indicate that selecting wheat lines for lower disease severity, or more importantly for lower levels of scabby grain, also will select lines with lower levels of *Fusarium* mycotoxins in harvested grain. Furthermore, reduced mycotoxin accumulation in grain appears to be a pleiotropic effect of other resistance mechanisms rather than an independent mechanism. These findings should simplify the process of developing cultivars with lower levels of mycotoxin accumulation in grain, and the resistances in these lines would have a significant impact on *Fusarium* mycotoxin levels in grain if the resistances were incorporated into cultivars that replaced existing susceptible cultivars.

MAPPING QTL FOR FUSARIUM HEAD BLIGHT RESISTANCE IN WHEAT CHROMOSOME 7A

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OBJECTIVES

1) Characterize QTLs for type II FHB resistance in Wheat chromosome 7A using Chinese Spring – Sumai3 7A chromosome recombinant inbred lines. 2) Identify SSR markers associated with the QTL to be used in marker-assisted selection (MAS).

INTRODUCTION

Fusarium head blight (FHB), caused by *Fusarium graminearum*, has been an important cereal disease in tropical and sub tropical regions of the world for a century. Its epidemics not only reduce yield and quality, but also produce mycotoxins that result in a potential health hazard to humans and animals (Bai and Shaner, 2004).

FHB resistance is a quantitative trait (Grausgruber et al., 1999) therefore its expression is heavily influenced by the existing environmental conditions. Currently no single measure can completely control its epidemics. Integration of cultural practices, chemical applications and use of resistant cultivars have proven to be the most promising approach to reduce the losses caused by FHB. Among different types of resistance against FHB, resistance to disease spread within a spike (type II) is the most stable type of resistance (Bai and Shaner, 2004). QTL mapping discovered many QTLs associated with type II resistance in wheat (Chen et al., 2006; Ma et al., 2006b; Mardi et al., 2006; Yu et al., 2008). Pyramiding these QTLs into cultivars by marker-assisted selection will enhance cultivar resistance under FHB epidemics (Bai and Shaner, 2004).

Chinese landrace Sumai3 and its derivatives are excellent sources for type II FHB resistance. Sumai3 has been used as the resistant parent in many breeding programs worldwide (Bai and Shaner, 2004). QTLs with Sumai3 origin are reported in chromosome 3BS, 5AS, 6A, 6BS, 2D and 4D (Anderson et al., 2001; Ma et al., 2006b; Waldron et al., 1999), but never on chromosome 7A. However, QTLs for type II FHB resistance was reported in 7A chromosome of Wangshuibai and Frontana (Mardi et al., 2006; Yu et al., 2008). A previous study revealed a high level of type II resistance in Chinese Spring-Sumai3 chromosome 7A substitution lines (Zhou et al. 2002a), but mapping work did not find any QTL on the chromosome (Ma et al., 2006b). In this study, we developed 7A chromosome recombinant inbred lines to investigate the effect of QTL on 7A chromosome of Sumai3 origin.

MATERIALS AND METHODS

Planting Materials

An F_5 population of 191 Chinese Spring-Sumai3-7A chromosome recombinant inbred lines (CRIL) was derived from the cross between Chinese Spring and Chinese Spring-Sumai3-7A disomic substitution lines by single seed descent.

Planting, Inoculation, Disease Evaluation and Phenotypic Data Analysis

Disease evaluation was carried out in spring 2009 in a greenhouse at Kansas State University, Manhattan, KS. Experimental lines consisted of 191 Chinese Spring-Sumai3-7A CRILs and their parents. Fifteen seeds from each CRIL along with

the parents were planted in trays containing soil (Sungrow Metro-mix 360® growing medium) and vernalized in a growth chamber at 4°C for one month. The seedlings of each experimental line were divided into three replicates and transplanted in plastic pots containing soil. The pots were arranged in the greenhouse in a randomized complete block design. Greenhouse temperature was maintained at 20°C. Plants were watered and fertilized with Miracle-grow® as necessary throughout the season.

Fusarium graminearum inoculum was prepared by growing the Kansas strain GZ3639 in mung bean liquid medium (Bai and Shaner, 1996). The spore density was evaluated by counting them using a hemocytometer under a microscope. Inoculum concentration was adjusted to 100,000 conidial spores per ml. At anthesis, a single spikelet residing in the center of the spike was inoculated by dispersing 10 µl/spikelet using a syringe. Five to six heads were inoculated in each pot. The plants were placed in a humid chamber, and sealed by polythene sheets to facilitate disease development. After 48 hours the plants were moved back to a greenhouse bench. Watering was done as necessary. Disease was evaluated at 21 days after inoculation by counting the number of infected spikelets and the total number of spikelets/inoculated spike. Any spikelet with a dark brown water-soaked spot to a completely bleached spikelet was recorded as a diseased spikelet (Figure 1). Proportion of symptomatic spikelets (PSS) for each CRIL was calculated for QTL analysis.

DNA Extraction and Genotyping

Three-weeks-old seedlings of population F_6 were used for DNA extraction. Leaf tissues were collected into 1.1 ml strip tubes. The tissue samples were dried in a freeze dryer for three days. A 3.2 mm stainless steel bead was loaded into each strip tube and the Mixer Mill was used to ground the dry tissue to a fine powder by shaking the tubes for 6 minutes at a speed of 1200 rpm. DNA was extracted using a modified Cetyltrimethyl ammonium bromide method (Saghai-Maroof et al., 1984).

Parents were screened using 60 SSR markers (Somers et al., 2004) mapped on 7A chromosome and 28 polymorphic markers were used to screen the CRIL population. Polymerase chain reaction (PCR) was done with 14 µl of PCR mix containing a final concentration of 10X ASB buffer, 2.5 mM of $MgCl_2$, 200 µM of dNTP, 100 nM each of forward M13-tailed primer and M13-fluorescent-dye labeled primer, 200 nM of reverse primer, 1 U of *Taq* DNA polymerase and 50 ng template DNA. PCR was carried out in a GeneAmp® 9700 PCR system using a touchdown program with initial denaturing at 95°C for 5 min, 5 cycles of 96°C for 1 min, 68°C for 3 min with a reduction of 2°C in each following cycle and 72°C for 1 min, followed by 4 cycles again with a modified annealing temperature of 58°C for 2 min. The final step consisted of 40 cycles of 96°C for 20 sec., 50°C for 20 sec., 72°C for 30 sec. and ended with a final extension step of 72°C for 5 min.

PCR products with four M13-florescent dyes (FAM, VIC, NED and PET) were pooled using the Bechman Coulter 96-channels Biomek NXp Liquid Handling System and the pooled PCR products were analyzed in an ABI PRISM 3730 DNA Analyzer. Data were analyzed using GeneMarker v1.75 and CRILs were scored for the polymorphic alleles between the two parents.

QTL Mapping

Linkage maps were developed using JoinMap v3.0 using a LOD score of 4.00 and Kosambi mapping function. QTL maps were analyzed by composite interval mapping feature of QTL Cartographer v2.5 at a walking speed of 2.0 cM and a window size of 2.0 cM. Threshold value to claim a significant QTL was set using 1000 permutation at a significance level of 0.05.

RESULTS AND DISCUSSION

A population of CRIL created by crossing a susceptible cultivar to the same cultivars with one chromosome substituted by a chromosome from a resistant cultivar is an ideal mapping population

to study the QTL effects of individual chromosomes (Garvin et al., 2009; Kumar et al., 2007). Previous studies showed that Chinese Spring is a wheat line that is moderately resistant to FHB (Grausgruber et al., 1999) and Chinese Spring-Sumai3-chromosome-7A disomic substitution line is highly resistant to FHB (Ma et al., 2006a; Ma et al., 2006b; Zhou et al., 2002a). The same results were obtained for the parents in this study. Segregation for FHB was observed among F_5 CRIL with average proportions of symptomatic spikelets (PSS) ranging from 5% to 97%. The frequency distribution of PSS was bimodal (Figure 2). This suggests an existence of few QTLs with a major effect on type II FHB resistance in the population.

QTL mapping using polymorphic markers on chromosome 7A detected a major putative QTL for type II FHB resistance on the short arm of chromosome 7A with a LOD score of 5, flanked by markers *Xbarc174* and *Xwmc17* (Figure 3). The QTL explained 12% of the phenotypic variation in the population.

Ma et al. (2006b) used a recombinant inbred line population derived by crossing Chinese Spring-Sumai3-chromosome-7A disomic substitution line to a Chinese cultivar Annong 8455 and did not find any QTL associated with type II FHB resistance on chromosome 7A. Lack of marker polymorphism between the parents in the QTL region could have been one of the factors (Ma et al., 2006b; Zhou et al., 2002b).

To validate the findings from this study, the mapping population will be repeatedly evaluated for two more seasons under greenhouse conditions. Deoxynivalenol (DON) content in infected kernels will be measured to evaluate the effect of the QTL on reducing DON content. Comparative mapping will be conducted between the QTL region and the corresponding rice chromosome region to map functional ESTs and develop SNP markers in the region for further improvement of the linkage map resolution.

ACKNOWLEDGEMENT AND DISCLAIMER

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Figure 1: a) Susceptible spike of chromosome recombinant inbred line 57 b) Resistant spike of chromosome recombinant inbred line 119 after needle inoculation in the center spikelet of a spike

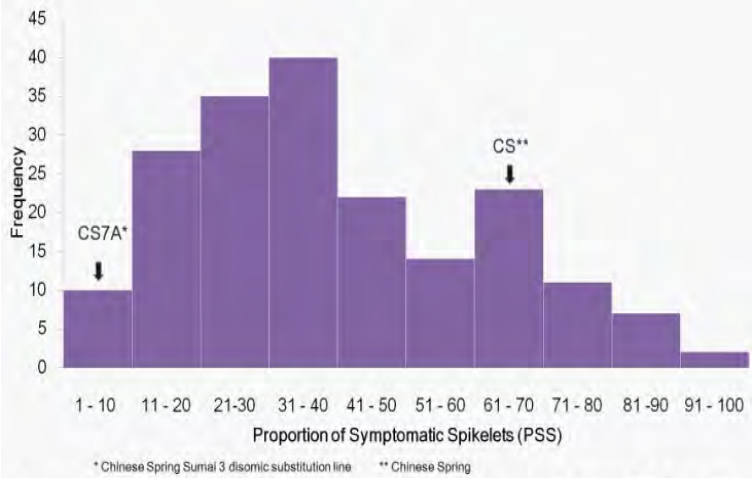

Figure 2: Frequency distribution of proportion of symptomatic spikelets in CS-Sumai3 7A chromosome recombinant inbred population.

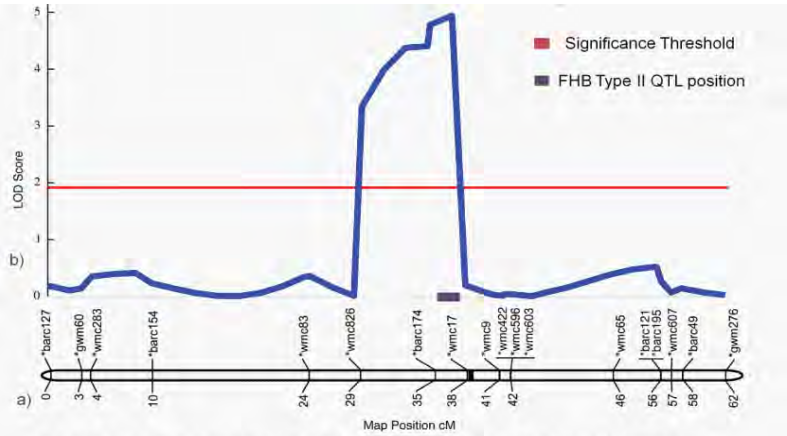

Figure 3: A genetic linkage map of Chromosome 7A (a) and a LOD score map to show possible QTL position in the map (b)

MARKER ASSISTED TRANSFERRING OF FUSARIUM HEAD BLIGHT RESISTANCE QTLS INTO LOCAL ADAPTIVESOFT RED WINTER WHEAT

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ABSTRACT

Fusarium head blight (FHB), also known as scab, is a potentially devastating winter wheat disease in southeast region where persistent rainfall could happen during the spring season. FHB can cause significant reduction in seed yields and quality. Infested seeds are often contaminated with trichothecene and estrogenic mycotoxins, which is a serious threat to animal health and food safety. Effect of chemical control of FHB was limited by the narrow window of application and cost increase. Development of resistant cultivars is the most efficient option to control FHB. Top crosses were made to introduce FHB resistance QTLs from VA04W-433, VA01-476, Sumai 3 derivatives, from Virginia and IN97397 from Indiana into our local adapted soft red winter wheat elite lines. Massive selections with molecular markers were carried out from the early generations to prevent the loss of FHD resistant QTLs which could happen if selection were carried out for economical and agricultural important traits in the early generations without marker assisted selection for FHB QTLs.

EVALUATION OF EXOTIC SCAB RESISTANCE QUANTITATIVE TRAIT LOCI (QTL) EFFECTS ON SOFT RED WINTER WHEAT

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ABSTRACT

Fusarium Head blight (FHB) of wheat, caused by *Fusarium graminearum*, is a disease that periodically strikes the mid-Atlantic region of the USA. Breeding for resistant wheat varieties is an effective method of disease control. The objective of this study was to evaluate the effects of exotic FHB resistance QTL, singly and in combination, on FHB resistance in soft red winter wheat. McCormick, a soft red winter wheat (SRWW) genotype adapted to the mid-Atlantic region, was used in a backcross program with the Chinese variety Ning7840. Eight Near-Isogenic Lines (NILs) were developed by marker-assisted backcrossing. Three FHB resistance QTLs on chromosomes 3BS, 2DL, and 5A were introgressed from non-adapted Ning7840 into the elite SRWW McCormick. The 3BS+2DL NIL showed higher resistance and lower deoxynivalenol (DON) content than other NILs in one greenhouse study conducted in College Park (MD) and also in two field studies conducted in Salisbury (MD) in 2008 and 2009 and in one field study conducted in 2009 in Lexington (KY). These results indicate that the 3BS+2DL NIL could be used in the mid-Atlantic region to breed for improved FHB resistance in soft red winter wheat.

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EVALUATION OF HOST PLANT RESISTANCE AND FUNGICIDE TREATMENT FOR SUPPRESSION OF FUSARIUM HEAD BLIGHT

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ABSTRACT

Fusarium head blight (FHB), caused by *Fusarium graminearum* has become a formidable opponent to wheat production in the United States over the past 25 years. A favorable climate, as well as high levels of inoculum has caused FHB to become a major disease of wheat that can cause significant economic losses. Until recently, there were few fungicides labeled for suppression of FHB. Numerous studies have shown that fungicides containing the active ingredient tebuconazole are very effective in reducing losses caused by FHB. While fungicides can be a useful tool for FHB suppression, they do not provide complete control, and their efficacy is greatly affected by timing. Planting varieties that are resistant to FHB infection provides farmers with continual protection against the disease. Our objective in this study was to examine the effectiveness of two foliar applied fungicide products on Fusarium head blight, and assess fungicide performance on varieties with varying levels of resistance. The experiment was set up as a split plot design, and conducted in both 2008 and 2009. The plots were grown in a grain spawn inoculated/ mist irrigated nursery in Urbana, IL with four replications. The main plots consisted of the fungicide treatment where the following were applied: an untreated check, Folicur® (tebuconazole), or Prosaro® (tebuconazole + prothioconazole). The sub plots consisted of twelve wheat cultivars with a range of FHB resistance, from very susceptible to resistant. Data were collected on incidence, severity, FHB index, *Fusarium* damaged kernels (FDK), ISK index (incidence/ severity/kernel quality index), deoxynivalenol (DON) concentration, yield, and test weight. Data from each year were combined when the year variance was homogeneous. Transformations were performed to correct for non-homogeneous year variance, as well as non-normally distributed residuals when possible. Data were analyzed and contrasts were made using the Proc Mixed procedure in SAS9.2. Both fungicide treatment and cultivar had a significant effect on all measures ($P<.05$). We found significant interactions between fungicide treatment and variety for *Fusarium* damaged kernels and yield. A high level of disease pressure was observed in both 2008 and 2009 with a wide range in incidence and severity. Also, we observed a broad range in yield for both years from 73 to 121 bu/A in 2008 and 64 to 114 bu/A in 2009. When split into two groups (6 resistant and 6 susceptible) and averaged over all treatments, the resistant cultivars significantly ($P<.05$) outperformed susceptible cultivars for all measured values; this was also the case when the resistant cultivars were compared to the susceptible cultivars with no fungicide applied. The resistant cultivars reduced mean incidence by 26 percent and mean severity by 17 percent while increasing yield by 12.7 bu/A. Both Folicur® and Prosaro® provided a significant benefit for all measures when compared to untreated checks. Mean incidence was reduced by an average of 32 percent when Prosaro® was applied and by 20 percent when Folicur® was applied. Prosaro® increased yield by an average of 12.4 bu/A while Folicur® increased yield by an average of 9.0 bu/A when compared to the untreated plots. Prosaro® and Folicur® significantly improved test weight. Prosaro® consistently provided more improvement in the measured variables than Folicur®; however, the Prosaro® treatment was not significantly ($P<.05$) different than Folicur® application for test weight, mean incidence, or yield. When the six susceptible cultivars treated with Prosaro® and Folicur® were compared to the six resistant cultivars with no fungicide treatment, the fungicide treated

cultivars provided significantly better results for most disease measures; however, the untreated resistant cultivars did not exhibit a significant response in yield due to fungicide application. While there were slightly lower yields when the resistant varieties were untreated, the resistant varieties were still able to provide acceptable yields and DON concentrations under heavy disease pressure. Based on the data from these two years, suppression of FHB can be achieved by planting resistant varieties and applying a fungicide such as Folicur® or Prosaro®. The results confirm the importance of planting a resistant variety. In some cases, fungicide application may not be possible or the timing may not be optimal; therefore, it is imperative for farmers to plant resistant cultivars.

SUCCESSES IN DEVELOPMENT OF FUSARIUM HEAD BLIGHT RESISTANT SOFT RED WINTER WHEAT VARIETIES USING PHENOTYPIC EVALUATION

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ABSTRACT

Development of high-yielding, well-adapted Fusarium head blight (FHB) resistant wheat varieties is essential for reducing the damage and substantial economic losses due to FHB when conditions favor infection. FHB resistance has been an important breeding objective of my program since about 1995. We have been evaluating FHB resistance in a mist-irrigated, inoculated FHB nursery since 1997. Each year we make at least 200 two-way and 200 three-way or four-way crosses involving one or more sources of FHB resistance. In 2009 we made 433 crosses, and 93.7 % had at least one FHB resistant parent. Many of the resistance sources we are now using are breeding lines from our program or other soft red winter wheat programs, and many crosses now involve more than one source of FHB resistance. Although we are using marker assisted selection to enrich F_2 populations in some crosses, for most populations we use phenotypic selection to select FHB resistant lines derived from F_3 or F_4 bulk populations. In addition to evaluation of lines in six cooperative nurseries and the Illinois Wheat Variety Trial, we evaluate all University of Illinois breeding lines in the misted, inoculated FHB field nursery each year. Approximately 2500 rows are evaluated each year in the replicated FHB nursery. Grain spawn (corn kernels cultured with 6-10 FHB isolates) is used to inoculate the nursery. Experiments have either two or three replications. Data are collected on incidence, severity and percent *Fusarium* damaged kernels (FDK). Data on incidence are based on visual assessment of the percentage of heads in a row that show symptoms. Severity is assessed by counting, or estimating, the number of infected spikelets per head on 7 to 10 heads per row. Percentage of FDK is determined by visual assessment compared to standards with known FDK percentages. FHB and ISK indexes are calculated. Grain samples are harvested sent to the University of Minnesota for DON evaluation. Data are analyzed with Agrobase and SAS software. In addition, about 1850 breeding lines (first year after headrows) are evaluated in a single row in the misted, inoculated field nursery. A single observation for FHB resistance is performed on each of these rows to identify the susceptible lines for discard at an early stage of evaluation. Producers will not adopt FHB resistant breeding lines unless they are yield competitive; therefore, breeding lines are evaluated for an array of traits at multiple locations. Advanced breeding lines are evaluated in three replication performance tests at four locations. Preliminary breeding lines are also evaluated at four locations but with fewer replicates. The first year after a line is selected in a headrow it is evaluated in single plot nurseries at two locations. In 2010 we will have 1944 entries in the single plot nursery excluding checks (Total plot number at both locations with checks = 4320). We will have 3960 plots in 2010 in replicated performance trials, and will evaluate 428 Illinois breeding lines in replicated tests. Breeding lines from the University of Illinois program have regularly been among the most resistant lines in the NUWWSN and the PNUWWSN. There are currently at least eight University of Illinois breeding lines with FHB resistance in commercial production or in various stages of advanced or regional evaluation and seed increase.

RECENT PROGRESS IN BREEDING FOR FHB RESISTANCE IN CANADIAN BARLEY

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ABSTRACT

Fusarium head blight (FHB) caused by *Fusarium graminearum* continues to be the most destructive disease of barley in Canada. Good progress has been made over the past decade by Canadian barley breeding programs in developing FHB resistant cultivars and germplasm with low deoxynivalenol (DON) accumulation, particularly in two-row classes which occupy the largest acreage in Canada. Progress has generally been lagging in six-row germplasm. The two-row feed barley cultivar CDC Mindon, developed by the Crop Development Centre (CDC), University of Saskatchewan, Saskatoon, SK, was registered in 2007, and has set the standard with about 40 to 50% lower DON content than AC Metcalfe over many years of testing. Two new cultivars, Norman (TR05915) and HB705, resulting from *in vitro* selection (IVS) using *Fusarium* mycotoxins during doubled haploid (DH) production at the Agriculture and Agri-Food Canada (AAFC) Brandon Research Centre, were registered in 2009. Norman, developed jointly by AAFC-Brandon and the CDC, is a two-row malting cultivar selected from CDC Kendall with 25-30% lower DON content than its parent, while maintaining CDC Kendall's desirable quality profile. HB705, a two-row hulless cultivar with malting quality potential selected from the CDC Freedom/Rivers cross at AAFC-Brandon, combines reduced DON content relative to other hulless cultivars with high malt extract, which may be attractive to the malting and brewing industry. Most programs are in the second or third breeding cycle, and have better parents available for crossing purposes to enhance FHB resistance. Use of exotic parents, such as the two-row Chinese accession Harbin, has been attempted with limited success. TR08203, a promising two-row malting line developed at AAFC Brandon that traces back to Harbin, has DON levels intermediate between AC Metcalfe and CDC Mindon and was advanced to a second year in the 2009 Western Cooperative Two-row Barley Registration Test. Numerous breeding lines with promising FHB resistance at various stages of development are being evaluated. In 2009, the FHB project in western Canada will replace selection based on visual symptoms for most advanced breeding lines in the FHB nursery with preliminary selection for DON content using near infrared reflectance (NIR) spectrometry to identify lines for further DON testing with standard methods. Although funding constraints may affect future progress, more new cultivars with low DON accumulation should be released over the next few years.

ASSOCIATION ANALYSES OF SNP MARKERS WITH SCAB RESISTANCE IN WINTER FEED BARLEY

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ABSTRACT

Two Barley OPAs, consisting of 3,072 SNPs, were used to genotype 284 barley breeding lines from Virginia Tech evaluated in the Barley CAP project. Among them 102 lines were screened for scab resistance in a mist-irrigated nursery inoculated with scabby corn seed and a conidial suspension spray from 2006 to 2009. Scab incidence, severity, and DON toxin concentration were determined in each year. FHB index was calculated using incidence and severity data. Association analysis was conducted to identify SNP markers linked to scab resistance. Each set of barley CAP lines from 2006, 2007 and 2008 were analyzed separately based on two sets of SNP data. Scab data collected from the field were averaged over two or three years for each set of barley CAP lines including the maximum number of common lines. The following preliminary results are from the analyses of 46 lines from barley CAP 2006 based on scab data averaged over three years from 2006 to 2008. Nine chromosome regions were associated with at least one type of scab resistance using OPA1. Among these nine regions, five regions were also identified from analyses using OPA2 SNP data. Important SNPs were identified on chromosomes 2H, 3H, 5H, and 7H and explained a range of variation in scab resistance. Of particular interest is a region on chromosome 5H at 151 cM which explained 9% of DON toxin levels, 11% of FHB incidence, and 15% of FHB severity. Another region on chromosome 7H explained 15% of DON toxin levels and 18% of FHB index. Barley CAP lines in 2007 and 2008 will be analyzed in a similar way and overlapping or common regions will provide barley breeders with useful information regarding putative FHB resistance QTL. SNP markers will be validated in breeding populations and can be applied in marker-assisted selection.

ACKNOWLEDGEMENT AND DISCLAIMER

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SATURATION MAPPING OF SCAB RESISTANCE QTL IN ERNIE AND IDENTIFICATION OF DIAGNOSTIC MARKERS FOR BREEDING SCAB RESISTANCE

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ABSTRACT

Fusarium head blight (FHB) has decreased wheat yields and quality significantly under epidemic conditions in the eastern and southern U.S.. Many QTL for scab resistance have been mapped in exotic and native sources. However, only a few QTL have been widely deployed in breeding programs using marker-assisted selection (MAS) due to the lack of diagnostic and tightly linked markers for most QTL. Four major QTL for type II resistance were mapped on chromosomes 5A, 4B, 3BSc and 2B of Ernie. A set of 243 RILs were evaluated in inoculated, mist-irrigated scab nurseries at Columbia, MO and Blacksburg, VA in 2008 and at Blacksburg and Warsaw, VA in 2009. Phenotypic data were obtained for FHB infection and severity, DON toxin accumulation, and *Fusarium* damaged kernels. Forty-seven new microsatellite markers were mapped to saturate these four QTL target regions and other regions based on field scab resistance. Overlapping and distinct QTL were identified for different types of resistance in Ernie. Markers linked to QTL on chromosome 4B are associated with greenhouse and field severity, and grain weight with R^2 values at 12%, 5%, and 12% over years. The awn suppressor gene on chromosome 5AL, B_1, explained variation in field incidence, severity, and grain weight at 6%, 8% and 5% over years. Tightly linked markers were tested in a wide range of Chinese, European and America sources. The most diagnostic markers were used in marker-assisted selection to pyramid various QTL.

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SATURATION MAPPING QTL FOR SCAB RESISTANCE IN A VIRGINIA WHEAT CULTIVAR MASSEY

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ABSTRACT

Fusarium Head Blight (FHB) or scab is a serious disease which reduces yield and quality of wheat in warm and humid areas worldwide. Planting resistant varieties is an economically beneficial and environmentally sound way to manage this disease. Identifying new sources of resistance and characterizing native sources of resistance are both major components in developing scab resistant wheat varieties. Massey, a cultivar released by Virginia Tech in 1985, has adult plant resistance to powdery mildew as well as being moderately resistant to scab. A set of 589 Diversity Array Technology (DArT) markers were mapped onto all 21 chromosomes in a Becker/Massey mapping population comprised of 152 recombinant inbred lines. Phenotypic data for FHB severity were obtained from a greenhouse test conducted in Virginia. Data for FHB incidence and severity, *Fusarium* damaged kernels (FDK) and DON toxin concentration were collected in field tests conducted in Virginia (2007, 2008, 2009), Missouri (2008), and Kentucky (2008). Within each test, FHB incidence was significantly correlated to FHB severity ($P < 0.001$). A set of fifty-eight simple sequence repeat markers were mapped to target regions. Three major QTLs conferring resistance to FHB in Massey were located on chromosomes 3B, 4B, 4D on the basis of field data. The QTL on chromosome 3BSc was associated with greenhouse severity, field severity, and FDK with R^2 ranging from 7.1% to 16.6%. The QTL on chromosomes 4B, close to *Rht1* gene, explained 9.5% of field index based on three year data, 22% of grain weight, and 26% of FDK based on data from 2008. The QTL on chromosome 4D, close to *Rht2* gene, explained 9% to 35% of field incidence, severity, FDK and grain weight. However, the R^2 might be overestimated due to the low marker density at this target region. More markers derived from wheat EST or rice synteny regions will be mapped to the target regions. Diagnostic markers will be validated and applied in marker-assisted selection.

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This material is based upon work supported by the U.S. Department of Agriculture, under Agreement No. 59-0790-4-102. 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.

INTEGRATION OF 3BS (*FHB1*) FHB QTL USING MARKER-ASSISTED BREEDING INTO HARD RED WINTER WHEAT (*TRITICUM AESTIVUM* L.) OF NEBRASKA

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ABSTRACT

Fusarium head blight (FHB), also known as scab, is a prevalent disease of wheat (*Triticum aestivum* L.) in warm, humid regions where flowering coincides with rainy periods. Natural epidemics of the disease may result in severe yield losses, reduction in quality, and contamination of the harvested grain by mycotoxin. The use of host resistance has long been considered the most practical and effective means to control FHB. The objectives of this study were to evaluate FHB severity under visual based (phenotypic) and marker based (genotypic) selection procedures. In order to supplement the existing native tolerance to FHB, spring wheat cultivar 'Alsen' was crossed with two elite adapted hard winter Nebraska lines through a three way cross [(spring x winter) x winter]. In this study a population of 116 $F_{3:4}$ lines were genotyped for the 3BS QTL using 5 diagnostic molecular markers and also field evaluated under mist irrigation at two sites in Nebraska (Lincoln and Mead) during the 2008 and 2009 cropping seasons. Out of the 116 $F_{3:4}$ lines 42 of them showed at least four 3BS markers from Alsen. The population showed significant differences for the measured phenotypic traits that included incidence, severity, and index in all the individual testing environments and for the combined analysis. Lines with and without the 3BS allele were compared for their field resistance. Generally, the lines identified with the 3BS QTL have good field FHB resistance and can be used as adapted resistance sources in the future hard winter wheat breeding program.

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EVALUATION OF DIFFERENT GREENHOUSE INOCULATION MODELS FOR PREDICTION OF FHB INFECTION RATES IN FIELD

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ABSTRACT

Fusarium Head Blight is a fungal disease in wheat, caused by *Fusarium graminearum*. It causes severe losses in yield and grain quality and is responsible for considerable economic losses. Susceptible wheat heads are infected at the time of anthesis, and late infections can also occur. Varieties are known to exhibit varying levels of resistance to the initiation of infection (Type 1 resistance) and the spread of infection (Type 2 resistance). There have been questions on the ability of greenhouse evaluation of FHB to effectively predict field performance (which is due to an interaction of both type 1 and type 2 resistance). We have examined four greenhouse inoculation protocols- 1) Point inoculation at anthesis, 2) Point inoculation at 7 days post anthesis, 3) Spray inoculation at anthesis and 4) Spray inoculation 7 days post anthesis for their correlation with field symptoms. The study was conducted on 26 varieties adapted to Michigan; which included both soft red and soft white winter wheat lines with varying levels of resistance to FHB. The infection observed was measured as the number of infected spikelets per total number of spikelets in an individual head, and observations were recorded at two time points in both the greenhouse and the field. This poster presents visually observed infection levels. Further work will involve studying the correlation of toxin accumulation in the field vs. greenhouse, and validation of the most effect greenhouse method identified here.

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THE 2008-09 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, Bess and Jamestown. Valuable data are provided on milling and baking quality of entries, and genotypic analyses identify alleles present at numerous important loci. In addition, the nursery facilitates the sharing of the best resistant materials throughout the breeding community.

The 2008-09 nursery comprised 54 advanced generation breeding lines and four check cultivars, 'Ernie', 'Bess', 'Jamestown' (partially resistant) and 'Coker 9835' (susceptible). Seven U.S. public programs (Univ. of Arkansas, Univ. of Georgia, Louisiana State University, Univ. of Maryland, N.C. State Univ., VA Tech., and USDA-ARS, 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 marker genoypes based on established diagnostic markers.

Mean performance results are shown in Table 1. Copies of the full report will be available at the 2009 National Fusarium Head Blight Forum and subsequently on line at the USWBSI web site: http://www.scabusa.org

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Table 1. Mean performance of the 57 entries in the 2008-09 Southern Uniform Winter Wheat Scab Nursery evaluated at up to 11 locations for components of FHB resistance.

	Cultivar/ Designation	FHB ncidence	RANK	FHB Severity	RANK	FHB Index	RANK	FDK	RANK	ISK	RANK	DON	RANK	G'hse # Florets	RANK	Heading Date	RANK	Plant Height	RANK	Fhb1 3BS
1	ERNIE	45	16	25	4	17	6	17	6	39	17	16	46	2	10	129	1	33	8	no
2	COKER 9835	65	57	56	56	50	57	47	57	65	56	15	42	11	43	133	43	32	1	no
3	BESS	40	4	18	1	12	1	12	1	29	2	8	4	2	10	131	17	36	39	no
4	JAMESTOWN	44	9	27	10	20	14	22	21	30	3	8	4	12	45	129	1	32	1	no
5	AR 97002-2-1	41	5	26	6	16	5	20	15	36	8	6	1	1	2	130	6	34	20	no
6	AR99039-5-2	49	36	36	32	30	45	35	45	49	40	17	49	25	56	136	56	35	29	no
7	AR 99263-7-1	46	23	25	4	19	10	19	12	40	20	12	25	9	34	133	43	39	55	no
8	VA05W-510	48	30	28	11	22	22	19	12	42	26	11	19	7	28	130	6	34	20	no
9	B030543	46	23	28	11	21	18	16	4	41	22	7	2	9	34	131	17	35	29	no
10	LA01164D-94-2	48	30	29	18	24	28	18	10	45	33	11	19	4	19	132	34	34	20	yes
11	LA01162D-131-8	44	9	29	18	21	18	21	19	33	4	11	19	5	22	131	17	35	29	yes
12	LA01162D-136-8	50	38	37	34	29	42	29	36	41	22	12	25	18	52	133	43	33	8	yes
13	GA 031454-DH7	53	44	44	48	32	48	31	38	50	43	10	17	1	2	131	17	38	50	no
14	GA 031307-DH14	59	52	46	50	30	45	31	38	54	51	14	35	9	34	130	6	32	1	no
15	NC05-21090	50	38	37	34	27	35	23	22	47	36	15	42	22	55	131	17	33	8	no
16	AR 99254-7-1	46	23	28	11	22	22	19	12	39	17	19	51	2	10	134	52	38	50	no
17	AR 99054-4-1	41	5	35	28	19	10	16	4	33	4	14	35	7	28	133	43	38	50	no
18	AR 99071-7-2	49	36	33	25	27	35	31	38	48	38	16	46	20	53	132	34	36	39	no
19	MD02W81-08-6	44	9	31	21	23	25	35	45	43	29	11	19	15	49	133	43	36	39	no
20	MD01W255-08-1	45	16	34	26	23	25	24	27	41	22	11	19	11	43	132	34	35	29	no
21	M05-1531	45	16	28	11	20	14	20	15	36	8	9	9	9	34	130	6	36	39	no
22	B0390207	54	45	44	48	29	42	27	33	48	38	13	31	3	14	129	1	34	20	no
23	03M1539#031	45	16	35	28	23	25	18	10	33	4	13	31	6	25	130	6	35	29	no
24	03M1599#0007	54	45	50	52	35	50	42	53	51	46	12	25	10	41	130	6	34	20	no
25	MH06-2370	44	9	31	21	20	14	21	19	39	17	9	9	9	34	131	17	35	29	no
26	ML07*7571	46	23	26	6	18	9	17	6	38	14	13	31	3	14	131	17	34	20	no
27	ML07-7758	43	8	24	3	17	6	20	15	36	8	7	2	1	2	131	17	39	55	het
28	VA04W-90	48	30	35	28	28	38	24	27	43	29	9	9	6	25	131	17	35	29	no
29	VA05W-534	37	1	28	11	14	2	14	2	28	1	9	9	8	33	130	6	35	29	no
30	VA06W-575	48	30	38	37	26	31	23	22	41	22	12	25	3	14	130	6	32	1	no
31	VA06W-587	44	9	31	21	19	10	17	6	38	14	8	4	1	2	129	1	34	20	no
32	VA07W-568	44	9	30	20	17	6	15	3	37	12	8	4	1	2	131	17	35	29	no
33	VA07W-607	45	16	28	11	20	14	17	6	44	31	8	4	12	45	131	17	33	8	no
34	VA05W-640	46	23	38	37	24	28	33	42	49	40	14	35	4	19	130	6	34	20	no
35	LA01141D-98-6-2	64	56	53	54	43	55	43	54	66	57	14	35	1	2	133	43	32	1	yes
36	LA03187C-2	48	30	48	51	34	49	39	51	56	52	14	35	4	19	132	34	36	39	no
37	LA01164D-43-7-B	38	2	34	26	19	10	25	30	36	8	12	25	3	14	131	17	37	48	no
38	ARGE97-1048-6	60	54	52	53	35	50	35	45	49	40	20	54	3	14	131	17	36	39	no
39	GA 991209-6E33	61	55	54	55	35	50	38	50	53	48	15	42	1	2	130	6	36	39	no
40	GA 031454-DH38-7	45	16	31	21	21	18	20	15	42	26	9	9	6	25	132	34	33	8	no
41	GA 031454-DH38-8 (11?)-	46	23	28	11	21	18	28	34	45	33	10	17	5	22	131	17	34	20	no
42	GA 991109-1-G1	54	45	40	41	26	31	23	22	42	26	14	35	1	2	129	1	33	8	no
43	GA 991109-1-G2	58	50	43	45	28	38	28	34	50	43	11	19	0	1	130	6	35	29	no
44	ARS03-5358	51	40	43	45	28	38	39	51	53	48	23	56	9	34	134	52	41	57	no
45	ARS03-3806	39	3	22	2	15	3	23	22	37	12	9	9	2	10	132	34	36	39	no
46	ARS03-4736	58	50	37	34	28	38	32	41	52	47	15	42	9	34	132	34	37	48	no
47	ARS04-1249	46	23	35	28	29	42	36	48	50	43	23	56	16	50	135	54	36	39	no
48	ARS05-0443	45	16	40	41	24	28	43	54	40	20	18	50	12	45	133	43	33	8	no
49	ARS05-0242	55	48	58	57	43	55	44	56	61	55	19	51	10	41	133	43	33	8	no
50	ARS05-1044	44	9	26	6	15	3	24	27	38	14	20	54	5	22	131	17	38	50	no
51	ARS05-1234	48	30	42	44	35	50	34	44	57	54	19	51	14	48	135	54	38	50	no
52	NC05-23015	51	40	39	40	26	31	25	30	45	33	14	35	20	53	131	17	32	1	no
53	NC05-20671	52	43	40	41	27	35	29	36	47	36	9	9	7	28	131	17	32	1	no
54	NC05-21937	59	52	43	45	40	54	36	48	56	52	16	46	29	57	133	43	33	8	yes
55	NC06-20288	55	48	38	37	30	45	33	42	53	48	9	9	7	28	132	34	33	8	no
56	NC07-23170	51	40	36	32	26	31	23	22	44	31	13	31	17	51	132	34	33	8	no
57	NC07-22927	41	5	26	6	22	22	26	32	35	7	12	25	7	28	136	57	33	8	het
	Mean	48.5		36		25		27		44		13		34		131		35		
	LSD (0.05)	21.0		26		20		24		17		10		35		3		3		
	CV%	22.0		37.1		40.8		44.9		19.3		40.5		50.7		1.3		4.0		

ASSOCIATION MAPPING QTL FOR FHB RESISTANCE IN SIX-ROW BARLEY BREEDING LINES

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ABSTRACT

Association mapping is a strategy to identify significant associations between markers and quantitative trait loci (QTL) that does not rely on bi-parental mapping populations. We used association mapping to identify QTL for FHB resistance using six-row spring barley breeding lines from North Dakota State University and the University of Minnesota six-rowed breeding programs. Lines were evaluated in mist-irrigated and inoculated field nurseries. FHB severity, rated as a percentage of diseased kernels, and deoxynivalenol concentration in harvested grain was collected over two years in several environments. The software TASSEL v. 2.1 was used to identify associations between two sets of 1536 barley Oligo Pool Assay (BOPA I and II) single nucleotide polymorphism (SNP) markers and disease data. Markers were evaluated in two sets in each breeding program; BOPA I alone and BOPA I/BOPA II combined. Three models, naïve association, structure matrix (using principal component analysis), and structure plus kinship matrix were used to detect significant associations. We will report analyses that examine the effect of marker number on detection of QTL and compare QTL identified in the two breeding programs.

PROGRESS ON DEVELOPMENT AND APPLICATION OF SINGLE KERNEL NIR SORTING TECHNOLOGY FOR ASSESSMENT OF FHB RESISTANCE IN WHEAT GERMPLASM

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ABSTRACT

We are developing Near Infrared (NIR) spectroscopic methods to sort *Fusarium* damaged wheat kernels (FDK) from sound kernels and to nondestructively determine deoxynivalenol (DON) levels of FDK. Our objective is to facilitate the rapid and objective evaluation of varieties for *Fusarium* resistance. We herein report progress and highlight research results in the development and use of our single kernel NIR (SKNIR) scab sorting and DON estimation techniques and other scab related studies.

Since December 2008 we have sorted 216 kernel samples for *Fusarium* damage from North Dakota State University (NDSU) and 216 samples from University of Nebraska, Lincoln (UNL) wheat breeders. DON analysis of sorted FDK fractions of NDSU samples for two seasons confirmed that SKNIR sorted FDK fractions had significantly higher DON levels. Therefore, this technique may be employed to obtain a more detailed characterization of host plant resistance mechanisms compared to characterizations that are based on DON analyses of composite samples.

We investigated the NIR absorbance characteristics of DON and that of sound and *Fusarium* damaged wheat kernels and showed that DON has NIR absorption bands with peaks at 1408, 1904 and 1919 nm. Therefore NIR may be absorbed by DON in *Fusarium* damaged wheat kernels indicating the suitability of NIR spectroscopic technique for objective evaluation of *Fusarium* damage on the basis of kernel DON levels. In collaboration with UNL, we have also completed a study to assess the accuracy of SKNIR to sort kernels based on scab and DON levels using our scab and DON calibrations.

We studied the distribution of DON levels among single kernels in artificially inoculated wheat spikes. The concentration of DON among single kernels above and below the point of inoculation varied between two varieties studied. Results indicated the existence of asymptomatic kernels with high DON levels as well as scabby kernels without DON in infected spikes. This may in part explain the failure to observe a consistent relationship between the intensity of scab infestation and DON levels.

We have developed a NIR moisture calibration for the SKNIR to estimate single kernel moisture content in samples having sound and *Fusarium* damaged kernels. This will be helpful to non-destructively estimate DON and other constituent levels of single kernels at a constant moisture basis.

We have initiated work on using Raman Spectroscopy to detect *Fusarium* damage in wheat kernels. Raman spectroscopy has the advantage of being insensitive to water, whereas NIR detection of DON is very sensitive to interference from strong NIR water absorption bands found adjacent to NIR absorption bands of DON. Our preliminary work to study the Raman spectra of pure DON using a 785 nm Raman system showed that DON is a Raman active compound. However, due to heavy fluorescence interference, 785 nm Raman system was not suitable for scanning intact single kernels. Therefore, we expect to use a 1064 nm Raman system for scanning single kernels in the future.

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QTL MAPPING OF FHB RESISTANCE TRAITS IN THE JAPANESE WHEAT LANDRACE, PI 81791

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ABSTRACT

The Japanese wheat landrace, PI 81791 (Sapporo Haru Komungi Jugo), has consistent resistance to FHB in field and greenhouse studies. A population of 150 recombinant inbred lines was developed from a cross between this genotype and the susceptible spring wheat variety, Wheaton. Phenotypic data for resistance to initial infection (type 1), and resistance to the spread of infection (type 2) was collected in four field environments. Post-harvest resistance traits, including grain weight, visually scabby kernels (VSK), and DON accumulation were analyzed from grain harvested from these same field experiments. Type 2 data also was collected from two greenhouse-based point inoculation experiments. Approximately 500 SSR markers were used to construct linkage maps for all chromosomes. Composite interval mapping was used to identify QTL for resistance and single marker analysis was used to identify markers associated with these QTL. Markers with P <0.01 for grand averages for each trait and P<0.05 for at least 3 out of 4 environments were considered significant and explored for marker assisted selection. These analyses identified QTL on chromosomes 2B, 2D, 3A, 3B, 3D, 4A, 4D, 5A, 5B, and 6B. Two QTL on both 3BL and 5AL and a single QTL on 4D were associated with several different resistance traits, including type 1 and 2 field resistance, post harvest grain traits, and greenhouse type 2 resistance. The QTL on the distal end of 5AL is near marker Xwmc727 (R^2=2-6.6%), and may represent, or lie adjacent to, a QTL identified in the winter wheat varieties Apache, Pirat and Arina (Holzapfel *et al.*, *Theor Appl Genet*, 2008). The QTL on the proximal end of 5AL is near marker Xwmc415 (R^2=3.1-6.8%), and maps at or near a QTL for DON accumulation identified in Nyubai. The QTL on 3BL are both located in the central region. One is near Xgpw94037 (R^2=3.0-8.6%) and a QTL for type 2 resistance identified in Ernie, and the other appears to be a novel QTL near Xgwm108 (R^2=3.2-10.9%). The QTL on 4D may be problematic to utilize as the resistance QTL is flanked by QTL for plant height and heading date (morphological traits that can influence resistance). However, selection with marker Xgwm192 (R^2=3.1-9.0%) may help avoid confluence of resistance and morphological traits, as this marker is significantly associated with resistance traits, but not with morphological characters. Major QTL for type 2 greenhouse resistance were identified on chromosomes 2B (Xgwm120, R^2=1.1-5.7%) and 3A (Xbarc1057, R^2=12.4-15.8%; Xgwm30, R^2=12.2-16.0%). The 2B QTL also was significant for field and grain resistance. These QTL map in regions previously identified for type 2 resistance in Ning7840 and Strongfield. The QTL on 3A, however, did not provide field-related resistance in our population even though it is in a region associated with FHB resistance QTL in other wheat varieties. Overall, markers Xgwm120 (2B), Xbarc1057 and Xgwm30 (3A), Xgpw94037, Xbarc229, and Xgwm108 (3B), Xbarc98 and/or Xgwm192 (4D), Xwmc415 and Xwmc727 (5A) represent QTL for a variety of resistance traits and can be used for validation purposes as well as marker assisted selection. The markers Xwmc111 (2D, adjacent to heading date QTL), Xwmc656 (3D), Xbarc233 (4A), Xbarc156 (5B), and Xgwm219 (6B) can be used in addition to the markers listed above to select on minor QTL for field, greenhouse, and grain resistance traits.

MAPPING AND INTROGRESSION OF FHB RESISTANT QUANTITATIVE TRAIT LOCI FROM TWO SPRING WHEAT GENOTYPES USING A FAMILY-BASED APPROACH

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ABSTRACT

Finding new quantitative trait loci (QTL), and introgressing them into adopted genetic backgrounds is a very important objective in the development of wheat cultivars resistant to the Fusarium head blight (FHB). We previously reported the use of a family-based method for QTL mapping in plant breeding families that allows for simultaneous mapping, marker validation, and marker-aided selection. We are applying this method to map QTLs in two resistant genotypes "SD3934" and "Mult 757". Three- and four-way crosses were made to develop families and each family had one of the resistant genotypes as a parent. The SD3934 based population consisted of 90 families with an average size of 13. Similarly, the Mult 757 population consisted on 86 families with an average size of 10. Genotyping was performed using simple sequence repeat (SSR) and sequence tagged sites (STS) markers elucidated on an ABI 3031*xl* genetic analyzer. Phenotyping F_1 plants for FHB resistance was performed in the greenhouse through artificial inoculation of a mixture of isolates collected from South Dakota. Mapping was performed using both family-based linkage (variance component linkage and pedigree-wide regression) and association (quantitative transmission disequilibrium test) approaches. Results from SD3934 suggest the presence of a QTL on chromosome 3BS. Selected individuals carrying the resistance allele were further advanced to the F_2 generation. Similar procedures will be followed with Multi 757 families. Results of this experiment further document the usefulness of a family-based method for simultaneous mapping, marker validation, and marker-assisted selection, within adapted genetic backgrounds.

RESULTS FROM THE SECOND *FUSARIUM* INTERNATIONAL SPRING WHEAT NURSERY (FIEPSN)

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ABSTRACT

The *Fusarium* International Elite and Preliminary Spring Wheat Nursery (FIEPSN) was assembled after merging the *Fusarium* International Elite Spring Wheat Nursery (FIESWN) and *Fusarium* International Preliminary Spring Wheat Nursery (FIPSWN) and distributed in 2009. Entries were tested for Type-I resistance and DON content at CIMMYT in Mexico, both in 2008 and 2009. Plants were inoculated artificially using precision CO_2 backpack sprayers equipped with flat fan nozzles at a defined pressure of 40 psi. Inoculum concentration was adjusted to 50,000 conidia ml^{-1} and 39 ml of inoculum per meter were applied. The inoculum consisted of a mixture of 5 different *F. graminearum* strains collected during the preceding year in naturally infected fields. Haplotyping was conducted at USDA-ARS (Fargo, ND) to determine the presence or abscence of different QTLs: 3B, 5A and 6B (Sumai #3), 3A and 5A (Frontana), 2D and 4B (Wuhan 1), 2D (CJ9306) and 3A and 7A (*T. dicoccoides*). Sumai #3 (resistant) and Flycatcher (Ocoroni F 86) and Gamenya (both susceptible checks) were used as controls.

The FHB index ranged from 0.87% (Sumai #3) to 68.95% (Gamenya) in 2008 and from 0.06% (Sumai #3) to 93.7% (Gamenya) in 2009, respectively. Drought stress in July 2009 resulted in lower FHB indices for most genotypes in comparison to 2008 in spite of a higher FHB severity observed in Gamenya. The drought conditions seemed not to influence the DON content. No correlation could be observed between FHB index and DON content in both years: r = 0.27 in 2008 and r = 0.05 in 2009, respectively, This observation may result from two explanations. (i) Mostly tolerant and nearly resistant material have been tested and showed low FHB indices in most cases whereas Type-I resistance does not necessarily offer protection against high DON contamination. (ii) The materials were tested under high artificial inoculation pressure which can lead to much higher DON contamination than natural infection. The haplotyping showed the diversity of sources of resistance within the FIEPSN. Only 4 lines of the nursery had the QTLs from Sumai #3 which suggest that new sources of resistance to FHB have become available.

SCREENING FOR NEW SOURCES OF FUSARIUM HEAD BLIGHT RESISTANCE IN CHINESE WHEATS FROM CIMMYT GERMPLASM BANK

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ABSTRACT

The evaluation of 583 Chinese wheat genotypes from CIMMYT germplasm bank was initiated in 2009 to identify new sources of Fusarium Head Blight resistance. This preliminary trial was not replicated due to the small amount of seed available.

Plants were tested in hill plots for Type-I resistance at CIMMYT in El Batan (Mexico) under artificial inoculation using precision CO_2 backpack sprayers equipped with flat fan nozzles at a defined pressure of 40 psi. The inoculum concentration was adjusted to 50,000 conidia ml^{-1} and 39 ml of inoculum per meter was applied. The inoculum consisted of a mixture of 5 different *F. graminearum* strains collected during the preceding year in naturally infected fields. Five tillers that flowered at the same time were scored 30 days after inoculation. A total of 491 entries out of 583 genotypes could be evaluated. Of these 491 lines, 13 genotypes (2.9%) showed a FHB index of 0%, a level of severity similar or lower than resistant check Sumai #3 which had a FHB index of 0.05%. The susceptible check Gamenya showed the highest FHB index (96.6%). Of the 491 lines 313 genotypes (63.7%) showed a FHB index below 7% whereas 134 lines (27.3%) scored below 1%.

Genotypes with a FHB index lower than 7% will be planted in hillplots at CIMMYT's field station in Ciudad Obregón for seed increase to allow the confirmation of preliminary results under FHB artificial epidemic using larger plots in 2010 at El Batan. In Ciudad Obregón, genotypes will also be selected for leaf rust resistance and agronomic type.

FAMILY-BASED ASSOCIATION ANALYSIS FOR PLANT POPULATIONS

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ABSTRACT

Association analyses (AA) test the association between marker loci and phenotypic variation. Plant geneticists have generally used a population-based AA (PBAA) where a single statistic is computed across all levels of population structure and then tested for significance. PBAA models must model the structure (subgroups, lineages, etc) of a population and adjust for its effect on type I error, a process that can be inadequate in small highly structure populations. In family-based AA (FBAA), statistics are computed within lineages of related individuals, then compiled over the lineages and tested for significance. As such, population structure does not lead to type I error and significance in a FBAA requires linkage between marker loci and QTL.

FBAA and PBAA were first developed in mammalian genetics where FBAA has been used extensively. Only recently have human geneticists started to use PBAA, motivated by reasons that are not applicable to plants genetics. First, family data is expensive to generate in humans whereas it is cheap in plants due to the extensive phenotyping conducted by large breeding programs. Second, human populations used in human genetics have little structure due to experimental design and careful *apriori* sampling of case/controls from all levels of a population's structure. This minimizes the impact of the structure on type I error from PBAA. In contrast, plant populations used in PBAA so far are very structured and perhaps poorly suited for PBAA despite their success. Uses of simple FBAA approaches in plant breeding populations for QTL discovery and QTL validation will be discussed.

REPORT ON THE 2008-09 NORTHERN UNIFORM WINTER WHEAT SCAB NURSERIES (NUWWSN AND PNUWWSN)

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OBJECTIVES

This is a summary of the report on the 2008-2009 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 after the 2009 forum. The objective of these tests is to screen winter wheat genotypes 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. The 60 entries in the NUWWSN came from 13 programs while the 46 entries in the PNUWWSN entries came from nine programs (Table 2).

RESULTS

Many entries in the NUWWSN showed very good resistance to FHB (Table 3). Over 33.3% (21/60) were not significantly different from the most resistant entry for all seven FHB traits: seven of these were also more resistant than the most susceptible entry for all seven traits. Only three entries had DON levels < 5 ppm from these inoculated and listed nurseries. FHB resistance was lower in the PNUWWSN (Tables 4,5) than in the NUWWSN (Table 6) as only 6.5% (3/46) of the entries were not significantly different that the most resistant entry for all six traits and none had DON levels < 5 ppm.

Table 1. Traits assessed in the 2008-09 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,IN,IN,KY,MI,MO,ON,VA	IL,IN,IN,KY,MD,MI,MO,NE,NY,OH ON,VA
INC	Disease incidence	% of heads with at least one infected spikelets	IL,IN,IN,KY,MI,MO,ON,VA	IL,IN,IN,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,KY,MD,MI,MO,NE,NY,OH ON,VA
FDK	Fusarium damaged kernels	Percentage of grain ishowing sypmotoms of Fusarium infection	IL,IN,KY,MO,RO	IL,IN,IN,KY,MD, MO,NE,RO
ISK	Composite of head and kernel traits	ISK Index = .3 (Severity) + .3 (Incidence)+.4 (FDK)	IL,IN,KY,MO	IL,IN,KY,MD,MO,NE
DON	DON (vomitoxin)	PPM of vomitoxin in grain	KY,VA	KY,MD,NE,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 2008-09 PNUWWSN and NUWWSN.

NUWWSN NAME	NUWWSN PEDIGREE
ERNIE	CHECK
TRUMAN	CHECK
FREEDOM	CHECK
PIONEER 2545	CHECK
P.03615A1-4-4	Ernie/INW0316//981358/97462
P.04704A1-2-1-1	INW0316*2//Ernie/9346
P.053A1-6-7	2754/INW0412/Truman/INW0303
P.0537A1-7-12	INW0411/2754//INW0412/98134
P.0128A1-22-22	L4/Foster/4/Gfd/X117/3/VA54-429//92145
MOCHA	OH489/OH490
SHAVER	NASW84-345/Coker9835//0H419/OH389
RUBIN	MO800071-56/PION2545//KY88C
ARENA	NASW84-345/Coker9835//0H419/OH389
CANON	MV 17/RUBY
NE06469	Unknown
NI04420	NE96644//PAVON/*3SCOUT66/3/WAHOO SIB
NI04427	KS98HW22//W95-615W/N94L189
NE05459	IN92823A1-1-4-5/NE92458
NE06471	W95-610W/WAHOO//NE98574
NY03179FHB-10	NY7387/Caledonia//Caledonia-2///Caledonia
NY03180FHB-10	NY7387/Caledonia//Caledonia-2///Caledonia
NY03179FHB-12	NY7387/Caledonia//Caledonia-2///Caledonia
NYW103-21-9183	Cayuga/ Caledonia
NYW103-102-9103	Cayuga/ Caledonia
IL02-18228	Pio25R26/9634-24437//95-4162
IL04-7874	G65201/ IL98-12212
IL04-7942	G65201/ IL98-12212
IL04-10721	IL95-4162/ IL97-7010
IL04-10741	IL95-4162/ IL97-7010
MD02W81-08-2	Freedom/Ning7840//VA97W533
MD02W81-08-4	Freedom/Ning7840//VA97W533
ACF213003B	
ACF126103	
ACF12004	
RCUOGTr34	
RCUOGTr35	
M05-1531	LA87167-D8-/P92118B4-2
B0390207	BL931167/Pioneer 2643
03M1539#031	GIBSON/92226E2-5-3
03M1599#0007	M99*3038/Pioneer 25R49
MO 050101	Bess reselection
MO 050921	Ernie/980521
MO 041020	MO 960429/960112
MO 050219	MO 010708 RS
MO 050144	MO 010708 RS
KY00C-2059-19	KY91C-170-3/2552
KY00C-2515-02	SS 550/KY93C-0721-34
KY00C-2059-24	KY91C-170-3/2552
KY00C-2567-01	SS 520/25W33
KY00C-2143-08	KY90C-048-59/KY90C-160-14
MSU Line E6003	VA96W-403-WS / W14
MSU Line E7035R	MSU Line D6234 / W14
OH04-264-58	OH645/HOPEWELL
OH04-268-39	HOPEWELL/VA96-54-372
OH05-248-38	OH685/OH686

NUWWSN	(continued)
VA07W-580	Goldfield /TRIBUTE//IL4162
VA07W-600	OH 552/SS550//RC-STRATEGY,F8
VA07W-672	REN3260*2//W14/ REN3260 /3/ REN3260
VA06W-558	96W-348/P92823A1-1-4-4-5 //McCORMICK
VA06W-615	ROANE/OH 552//RC STRATEGY

PNUWWS NAME	PNUWWSN PEDIGREE
ERNIE	CHECK
TRUMAN	CHECK
FREEDOM	CHECK
PIONEER 2545	CHECK
P.0513A1-2-3	Truman/INW0731//Fdm/F201R
P.0527A1-9-15	99751/2754//97462/INW0412
P.0558A1-5-5	INW0412/L3//F201R/97462
P.0570A1-7-6	9017/92823//F201R/04302
P.05218A1-6-31	INW0304/9346//97395/INW0411
OH02-12686	FOSTER/HOPEWELL//OH581/OH569
SILAS	OH546/SE1694-12
LINUS	5-TIEGANMAI/PION 25R26
OKIE	F285N3-111/65343(spelt)
PENZO	5-TIEGANMAI/PION 25R26
AJAX	T63/PION2737W
IL04-11003	IL96-3073/ Roane
IL04-17762	IL97-3578/ IL97-7010
IL05-15079	NEL-1538/ KY93C-38-17-1
IL05-27333	IL96-24851-1/ IL97-3574// IL97-3950
IL05-27522	IL96-24851-1/ IL97-3574// IL99-2536
MH06-2370	COOPER/SS550
MH06-2410	M98-1660//PATTON/Pioneer 2552
ML07*7571	VA98W-586/HONEY
ML07-7758	COKER 9025/Pioneer 25R57
MO 050771	MO 960120/MO 960304
MO 041687	MO 960304/MO 960815
MO 071411	MO 980429/P86958RC4-2-1-1-10
MO 071722	MO 980429/Ernie
MO 071522	MO 003013/MO 980525
KY02C-3007-41	25R18/Allegiance
KY02C-3005-25	25R18/McCormick
KY03C-2170-24	VA01W-476/Roane
KY03C-2170-06	VA01W-476/Roane
KY02C-3007-45	25R18/Allegiance
MSU Line E5024	MSU Line D6234 / Pio25W33
VA07W-643	COKER 9474/ McCormick"S"
VA06W-580	Roane / Pion 2684//OH 552
VA07W-591	FREEDM/NC96-13374 // RC-STRATEGY
VA06W-578	Roane / Pion 2684//OH 552
VA04W-90	SS 520/PION2552//ROANE
OH05-101-1	HOPEWELL/PIONEER 25R26
OH05-72-6	PIONEER 25R18/VA97W-375
OH05-249-32	OH685/OH686
OH05-152-68	OH685/PATTON
OH05-164-76	PIONEER 25R18/OH686
OH05-200-74	OH629/HOPEWELL

Table 3. Best (top) and worst (bottom) entries from the 2008-09 NUWWSN. Summary statistics are for all entries.

NAME	INC		SEV		IND		FDK		ISK		DON		GHSEV		#l	#h
MSU Line E6003	40.0	l	10.3	l	6.2	l	7.9	l	23.9	l	5.2	l	19.6	l	7	0
MD02W81-08-4	43.6	l	14.2	l	9.1	l	18.9	l	27.6	l	3.5	l	14.1	l	7	0
MD02W81-08-2	47.3	l	17.9	l	12.1	l	17.0	l	29.6	l	7.0	l	11.3	l	7	0
IL02-18228	35.4	l	19.0	l	12.3	l	9.9	l	25.1	l	2.3	l	35.0	l	7	0
RCUOGTr34	42.3	l	19.7	l	12.8	l	19.6	l	33.3	l	5.8	l	28.4	l	7	0
MO050101	48.0	l	18.2	l	12.9	l	9.1	l	31.3	l	6.6	l	6.8	l	7	0
NYW103-102-9103	40.6	l	22.7	l	13.7	l	16.2	l	29.4	l	6.1	l	10.0	l	7	0
TRUMAN	41.3	l	15.4	l	9.4	l	12.9	l	28.1	l	9.0	hl	4.4	l	7	1
MO050921	42.9	l	19.3	l	11.3	l	14.8	l	27.7	l	12.8	hl	10.5	l	7	1
P.0128A1-22-22	52.4	hl	22.3	l	12.0	l	11.1	l	33.8	l	4.3	l	15.9	l	7	1
IL04-10741	43.4	l	24.5	hl	15.3	l	18.0	l	32.1	l	7.0	l	23.5	l	7	1
RCUOGTr35	47.3	l	26.1	hl	15.4	l	23.9	l	36.9	l	4.3	l	32.0	l	7	1
MO041020	52.2	hl	18.9	l	13.5	l	15.8	l	34.9	l	13.2	hl	10.9	l	7	2
MO050144	56.2	hl	17.7	l	13.5	l	17.3	l	32.4	l	7.7	hl	12.1	l	7	2
VA07W-600	53.0	hl	20.2	l	14.4	l	15.5	l	34.9	l	12.4	hl	14.3	l	7	2
ERNIE	50.8	hl	22.5	l	14.9	l	20.5	l	33.3	l	11.6	hl	19.8	l	7	2
P.0537A1-7-12	58.0	hl	22.8	l	16.1	l	17.0	l	38.5	l	9.2	hl	27.9	l	7	2
VA06W-558	52.9	hl	24.5	hl	16.9	l	21.0	l	33.4	l	5.4	l	17.3	l	7	2
MO050219	55.1	hl	22.2	l	17.1	l	15.5	l	37.6	l	12.6	hl	27.0	l	7	2
IL04-7942	46.2	l	24.8	hl	17.3	l	19.4	l	32.3	l	8.7	hl	16.5	l	7	2
M05-1531	53.9	hl	25.5	hl	17.9	l	23.0	l	38.2	l	4.6	l	12.3	l	7	2
RUBIN	59.3	hl	37.9	h	27.4	h	35.4	h	49.0	h	9.0	hl	60.8		2	6
CANON	69.4	h	35.9	h	28.5	h	31.1	h	50.4	h	7.4	hl	24.6	l	2	6
MOCHA	68.2	h	38.8	h	31.4	h	39.8	h	52.5	h	10.7	hl	21.9	l	2	6
SHAVER	72.0	h	42.3	h	32.7	h	36.4	h	53.0	h	8.9	hl	32.1	l	2	6
P.03615A1-4-4	67.5	h	35.7	h	26.0	h	29.5	h	51.4	h	14.2	h	11.5	l	1	6
NE05459	67.9	h	33.9	h	26.9	h	35.9	h	50.4	h	9.4	hl	40.0		1	6
KY00C-2059-24	68.4	h	34.0	h	26.9	h	26.9	hl	48.7	h	17.9	h	66.5		1	6
P.053A1-6-7	63.3	h	36.3	h	29.8	h	27.4	h	51.0	h	13.8	h	21.5	l	1	6
NI04420	69.9	h	35.8	h	29.9	h	38.9	h	52.7	h	15.7	h	28.2	l	1	6
03M1599#0007	70.4	h	44.2	h	34.6	h	33.4	h	52.3	h	7.5	hl	67.3		1	6
KY00C-2059-19	70.4	h	32.3	h	25.4	h	27.9	h	45.5	h	18.4	h	42.8		0	6
KY00C-2515-02	64.0	h	37.2	h	27.4	h	35.6	h	50.8	h	13.6	h	67.4		0	6
OH05-248-38	69.0	h	40.0	h	30.4	h	36.0	h	51.3	h	14.3	h	84.6		0	6
ARENA	73.9	h	37.3	h	30.9	h	36.6	h	51.4	h	14.9	h	47.1		0	6
B0390207	62.3	h	40.7	h	30.9	h	30.2	h	50.7	h	14.7	h	80.4		0	6
PIONEER 2545	72.4	h	43.7	h	37.4	h	44.2	h	58.6	h	14.0	h	53.1		0	6

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

Table 4. Best (top) and worst (bottom) entries from the 2008-09 PNUWWSN. Summary statistics are for all entries.

NAME	INC		SEV		IND		FDK		ISK		DON		GHSEV	#l	#h
KY02C-3005-25	47.8	l	15.9	l	8.2	l	8.6	l	28.7	l	8.4	l	6.2	6	0
IL05-27522	49.6	l	18.0	l	11.0	l	23.5	l	24.6	l	13.2	hl	13.7	6	1
TRUMAN	35.3	l	20.1	l	11.3	l	18.1	l	29.5	l	8.9	l	3.5	6	0
KY02C-3007-45	52.3		16.2	l	10.3	l	19.1	l	35.6	l	12.3	hl	6.2	5	1
KY02C-3007-41	53.6		17.1	l	10.6	l	20.5	l	36.3	l	13.4	hl	3.4	5	1
MO 050771	50.1		20.7	l	10.9	l	22.0	l	33.4	l	20.9	hl	17.0	5	1
MO 071411	54.4		19.1	l	11.0	l	24.5	l	31.4	l	15.4	hl	37.8	5	1
IL04-17762	54.3		22.6	l	12.3	l	24.5	l	37.2	l	11.9	hl	43.2	5	1
ML07-7758	56.3		22.9	l	12.9	l	30.3	hl	33.8	l	9.7	l	2.7	5	1
OH05-101-1	61.9		22.4	l	13.3	l	28.4	hl	36.2	l	8.9	l	23.3	5	1
VA06W-580	54.2		26.9	l	14.3	l	22.0	l	38.7	l	12.9	hl	2.6	5	1
KY03C-2170-06	60.3		25.9	l	14.8	l	14.1	l	39.2	l	6.7	l	7.7	5	0
IL05-27333	60.3		22.4	l	15.7	l	22.7	l	38.1	l	12.1	hl	9.2	5	1
PIONEER 2545	77.3	h	46.3		36.9	h	46.8	hl	63.0	h	31.0	hl	82.4	2	5
AJAX	70.1	h	49.1	h	36.4	h	51.0	h	63.1	h	37.1	h	45.7	0	6
PENZO	75.0	h	55.5	h	41.8	h	46.8	hl	66.9	h	21.5	hl	41.5	2	6
LINUS	82.1	h	59.2	h	46.5	h	65.2	h	75.8	h	20.7	hl	81.4	1	6

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 2008-09 PNUWWSN.

ENTRY	NAME	INC	SEV	IND	FDK	ISK	DON	GHSEV	#l	#h
1	ERNIE	53.4	23.9 l	14.1 l	21.6 l	34.6 l	21.7 hl	27.3	4	1
2	TRUMAN	35.3 l	20.1 l	11.3 l	18.1 l	29.5 l	8.9 l	3.5	6	0
3	FREEDOM	62.0	27.1 l	18.8	34.6 hl	45.8	21.3 hl	5.7	3	2
4	PIONEER 2545	77.3 h	46.3	36.9 h	46.8 hl	63.0 h	31.0 hl	82.4	2	5
5	P.0513A1-2-3	57.8	25.7 l	16.7 l	26.6 hl	41.5	12.5 hl	57.3	4	2
6	P.0527A1-9-15	72.1 h	35.4	28.5	44.6 hl	61.4 h	21.4 hl	34.0	2	4
7	P.0558A1-5-5	69.2 h	31.9	23.2	35.0 hl	51.5	19.6 hl	8.5	2	3
8	P.0570A1-7-6	68.7 h	39.6	29.6	33.7 hl	55.4	20.3 hl	59.3	2	3
9	P.05218A1-6-31	63.3	27.7 l	17.7 l	36.4 hl	41.7	21.4 hl	9.2	4	2
10	OH02-12686	60.5	34.6	26.1	42.5 hl	56.4	11.2 l	5.0	2	1
11	SILAS	61.6	38.4	24.0	29.9 hl	50.9	21.6 hl	4.5	2	2
12	LINUS	82.1 h	59.2 h	46.5 h	65.2 h	75.8 h	20.7 hl	81.4	1	6
13	OKIE	57.8	40.0	23.2	45.7 hl	48.4	14.8 hl	80.6	2	2
14	PENZO	75.0 h	55.5 h	41.8 h	46.8 hl	66.9 h	21.5 hl	41.5	2	6
15	AJAX	70.1 h	49.1 h	36.4 h	51.0 h	63.1 h	37.1 h	45.7	0	6
16	IL04-11003	55.4	25.3 l	14.5 l	34.5 hl	42.2	11.6 hl	15.2	4	2
17	IL04-17762	54.3	22.6 l	12.3 l	24.5 l	37.2 l	11.9 hl	43.2	5	1
18	IL05-15079	63.5	28.8	19.1	20.1 l	42.5	15.5 hl	10.2	2	1
19	IL05-27333	60.3	22.4 l	15.7 l	22.7 l	38.1 l	12.1 hl	9.2	5	1
20	IL05-27522	49.6 l	18.0 l	11.0 l	23.5 l	24.6 l	13.2 hl	13.7	6	1
21	MH06-2370	62.3	32.6	22.0	43.6 hl	47.7	12.6 hl	30.7	2	2
22	MH06-2410	53.9	21.2 l	14.3 l	25.0 l	41.3	16.6 hl	13.7	4	1
23	ML07*7571	64.3	24.2 l	16.7 l	40.6 hl	43.4	20.6 hl	7.6	4	2
24	ML07-7758	56.3	22.9 l	12.9 l	30.3 hl	33.8 l	9.7 l	2.7	5	1
25	MO 050771	50.1	20.7 l	10.9 l	22.0 l	33.4 l	20.9 hl	17.0	5	1
26	MO 041687	62.7	35.2	23.0	24.9 l	46.6	20.2 hl	42.8	2	1
27	MO 071411	54.4	19.1 l	11.0 l	24.5 l	31.4 l	15.4 hl	37.8	5	1
28	MO 071722	66.0	35.7	22.7	26.9 hl	45.0	13.8 hl	33.2	2	2
29	MO 071522	49.5 l	28.8	20.8	17.2 l	47.3	7.0 l	5.3	3	0
30	KY02C-3007-41	53.6	17.1 l	10.6 l	20.5 l	36.3 l	13.4 hl	3.4	5	1
31	KY02C-3005-25	47.8 l	15.9 l	8.2 l	8.6 l	28.7 l	8.4 l	6.2	6	0
32	KY03C-2170-24	58.1	28.3 l	14.3 l	35.1 hl	35.0 l	13.4 hl	24.2	5	2
33	KY03C-2170-06	60.3	25.9 l	14.8 l	14.1 l	39.2 l	6.7 l	7.7	5	0
34	KY02C-3007-45	52.3	16.2 l	10.3 l	19.1 l	35.6 l	12.3 hl	6.2	5	1
35	MSU Line E5024	59.0	25.1 l	18.5 l	30.0 hl	40.9	36.9 h	75.3	3	1
36	VA07W-643	66.4	23.8 l	17.4 l	24.8 l	43.4	10.8 l	3.3	4	0
37	VA06W-580	54.2	26.9 l	14.3 l	22.0 l	38.7 l	12.9 hl	2.6	5	1
38	VA07W-591	73.3 h	31.5	21.8	36.1 hl	46.9	24.7 hl	28.2	2	3
39	VA06W-578	69.5 h	32.6	24.0	36.9 hl	49.8	22.7 hl	25.2	2	3
40	VA04W-90	57.2	28.8	17.5 l	27.0 hl	42.2	16.9 hl	10.0	3	2
41	OH05-101-1	61.9	22.4 l	13.3 l	28.4 hl	36.2 l	8.9 l	23.3	6	2
42	OH05-72-6	59.3	28.5 l	14.5 l	36.8 hl	40.7	15.0 hl	23.5	4	2
43	OH05-249-32	53.2	25.7 l	15.6 l	37.6 hl	36.4 l	13.7 hl	97.2	5	2
44	OH05-152-68	56.4	32.1	20.1	41.6 hl	46.6	16.5 hl	28.0	2	2
45	OH05-164-76	63.3	24.2 l	16.3 l	29.2 hl	44.2	16.5 hl	5.4	4	2
46	OH05-200-74	57.5	22.0 l	13.8 l	36.9 hl	40.1	15.0 hl	3.3	4	2
	AVERAGE	60.3	29.1	19.3	31.4	43.9	16.8	26.1		
	MINUMUM	35.3	15.9	8.2	8.6	24.6	6.7	2.6		
	MAXIMUM	82.1	59.2	46.5	65.2	75.8	37.1	97.2		
	LSD(0.05)	14.6	12.8	10.4	38.9	14.8	25.8	.		
	# ENVIRONS	8	7	8	5	4	2	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 2008-09 NUWWSN.

NAME	INC		SEV		IND		FDK		ISK		DON		GHSEV		#l	#h
ERNIE	50.8	hl	22.5	l	14.9	l	20.5	l	33.3	l	11.6	hl	19.8	l	7	2
TRUMAN	41.3	l	15.4	l	9.4	l	12.9	l	28.1	l	9.0	hl	4.4	l	7	1
FREEDOM	56.9	hl	20.3	l	14.6	l	29.6	h	38.5	l	10.7	hl	8.2	l	6	3
PIONEER 2545	72.4	h	43.7	h	37.4	h	44.2	h	58.6	h	14.0	h	53.1		0	6
P.03615A1-4-4	67.5	h	35.7	h	26.0	h	29.5	h	51.4	h	14.2	h	11.5	l	1	6
P.04704A1-2-1-1	63.2	h	41.0	h	31.3	h	24.3	l	48.7	h	11.5	hl	28.4	l	3	5
P.053A1-6-7	63.3	h	36.3	h	29.8	h	27.4	h	51.0	h	13.8	h	21.5	l	1	6
P.0537A1-7-12	58.0	hl	22.8	l	16.1	l	17.0	l	38.5	l	9.2	hl	27.9	l	7	2
P.0128A1-22-22	52.4	hl	22.3	l	12.0	l	11.1	l	33.8	l	4.3	l	15.9	l	7	1
MOCHA	68.2	h	38.8	h	31.4	h	39.8	h	52.5	h	10.7	hl	21.9	l	2	6
SHAVER	72.0	h	42.3	h	32.7	h	36.4	h	53.0	h	8.9	hl	32.1	l	2	6
RUBIN	59.3	hl	37.9	h	27.4	h	35.4	h	49.0	h	9.0	hl	60.8		2	6
ARENA	73.9	h	37.3	h	30.9	h	36.6	h	51.4	h	14.9	h	47.1		0	6
CANON	69.4	h	35.9	h	28.5	h	31.1	h	50.4	h	7.4	hl	24.6	l	2	6
NE06469	57.8	hl	25.7	hl	18.5	l	34.4	h	41.6	h	17.1	h	50.1		3	5
NI04420	69.9	h	35.8	h	29.9	h	38.9	h	52.7	h	15.7	h	28.2	l	1	6
NI04427	70.4	h	33.8	h	27.3	h	34.9	h	49.7	h	9.4	hl	27.7	l	1	5
NE05459	67.9	h	33.9	h	26.9	h	35.9	h	50.4	h	9.4	hl	40.0		1	6
NE06471	62.0	h	30.7	h	23.8	hl	30.0	h	49.2	h	7.8	hl	28.0	l	3	6
NY03179FHB-10	55.8	hl	25.3	hl	20.5	hl	23.1	l	40.5	l	13.2	hl	31.8	l	7	4
NY03180FHB-10	52.1	hl	29.0	hl	20.0	hl	23.4	l	39.3	l	6.5	l	24.8	l	7	3
NY03179FHB-12	61.6	h	34.0	h	26.9	h	25.1	hl	47.5	h	11.8	hl	32.3	l	3	6
NYW103-21-9183	57.3	hl	31.6	h	22.4	hl	23.7	l	44.0	h	5.4	l	20.8	l	5	4
NYW103-102-9103	40.6	l	22.7	l	13.7	l	16.2	l	29.4	l	6.1	l	10.0	l	7	0
IL02-18228	35.4	l	19.0	l	12.3	l	9.9	l	25.1	l	2.3	l	35.0	l	7	0
IL04-7874	50.0	hl	25.9	hl	19.1	l	16.3	l	32.4	l	12.2	hl	25.6	l	7	3
IL04-7942	46.2	l	24.8	hl	17.3	l	19.4	l	32.3	l	8.7	hl	16.5	l	7	2
IL04-10721	52.8	hl	25.8	hl	19.0	l	16.5	l	34.9	l	7.3	hl	20.1	l	7	3
IL04-10741	43.4	l	24.5	hl	15.3	l	18.0	l	32.1	l	7.0	l	23.5	l	7	1
MD02W81-08-2	47.3	l	17.9	l	12.1	l	17.0	l	29.6	l	7.0	l	11.3	l	7	0
MD02W81-08-4	43.6	l	14.2	l	9.1	l	18.9	l	27.6	l	3.5	l	14.1	l	7	0
ACF213003B	60.1	hl	30.3	hl	22.3	hl	30.2	h	40.5	l	11.2	hl	35.0	l	6	5
ACF126103	62.1	h	29.9	hl	23.2	hl	26.7	hl	43.2	h	17.7	h	29.5	l	4	7
ACF12004	64.8	h	31.1	h	23.5	hl	35.8	h	43.9	h	8.8	hl	45.5		2	6
RCUOGTr34	42.3	l	19.7	l	12.8	l	19.6	l	33.3	l	5.8	l	28.4	l	7	0
RCUOGTr35	47.3	l	26.1	hl	15.4	l	23.9	l	36.9	l	4.3	l	32.0	l	7	1
M05-1531	53.9	hl	25.5	hl	17.9	l	23.0	l	38.2	l	4.6	l	12.3	l	7	2
B0390207	62.3	h	40.7	h	30.9	h	30.2	h	50.7	h	14.7	h	80.4		0	6
03M1539#031	49.2	hl	30.2	hl	18.6	l	17.4	l	38.3	l	9.2	hl	41.6		6	3
03M1599#0007	70.4	h	44.2	h	34.6	h	33.4	h	52.3	h	7.5	hl	67.3		1	6
MO050101	48.0	l	18.2	l	12.9	l	9.1	l	31.3	l	6.6	l	6.8	l	7	0
MO050921	42.9	l	19.3	l	11.3	l	14.8	l	27.7	l	12.8	hl	10.5	l	7	1
MO041020	52.2	hl	18.9	l	13.5	l	15.8	l	34.9	l	13.2	hl	10.9	l	7	2
MO050219	55.1	hl	22.2	l	17.1	l	15.5	l	37.6	l	12.6	hl	27.0	l	7	2
MO050144	56.2	hl	17.7	l	13.5	l	17.3	l	32.4	l	7.7	hl	12.1	l	7	2
KY00C-2059-19	70.4	h	32.3	h	25.4	h	27.9	h	45.5	h	18.4	h	42.8		0	6
KY00C-2515-02	64.0	h	37.2	h	27.4	h	35.6	h	50.8	h	13.6	h	67.4		0	6
KY00C-2059-24	68.4	h	34.0	h	26.9	h	26.9	hl	48.7	h	17.9	h	66.5		1	6
KY00C-2567-01	61.9	h	32.3	h	23.3	hl	25.1	hl	44.0	h	15.1	h	35.5	l	3	6
KY00C-2143-08	62.2	h	32.7	h	22.4	hl	27.6	h	40.9	l	9.3	hl	61.5		3	5
MSU Line E6003	40.0	l	10.3	l	6.2	l	7.9	l	23.9	l	5.2	l	19.6	l	7	0
MSU Line E7035R	67.7	h	23.0	l	18.5	l	18.7	l	40.6	l	6.1	l	12.6	l	6	1
OH04-264-58	65.1	h	28.4	hl	22.7	hl	21.0	l	42.1	h	10.0	hl	40.8		4	5
OH04-268-39	52.7	hl	27.9	hl	21.3	hl	27.6	h	40.9	l	10.5	hl	11.1	l	6	5
OH05-248-38	69.0	h	40.0	h	30.4	h	36.0	h	51.3	h	14.3	h	84.6		0	6
VA07W-580	51.7	hl	22.4	l	15.3	l	22.9	l	35.6	l	11.5	hl	55.8		6	2
VA07W-600	53.0	hl	20.2	l	14.4	l	15.5	l	34.9	l	12.4	hl	14.3	l	7	2
VA07W-672	51.2	hl	35.3	h	21.8	hl	24.3	l	44.8	h	9.7	hl	128.0	h	4	6
VA06W-558	52.9	hl	24.5	hl	16.9	l	21.0	l	33.4	l	5.4	l	17.3	l	7	2
VA06W-615	59.6	hl	26.0	hl	18.6	l	22.4	l	38.1	l	8.2	hl	14.3	l	7	3
AVERAGE	57.3		28.5		20.9		24.5		40.9		10.1		32.1			
LSD(0.05)	25.6		20.2		18.0		19.2		17.5		11.2		34.5			
# environs	8		11		11		8		6		4		2			

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

CAN HOST PLANT RESISTANCE PROTECT THE QUALITY OF WHEAT FROM FUSARIUM HEAD BLIGHT?

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ABSTRACT

Fusarium head blight (FHB) infection reduces the amount of millable grain from an infected field, reduces mill yields, and generally degrades end-use quality. In 2009, the Logan County, KY, wheat trial had extended conditions for infection with FHB resulting in extensive and uniform infection within the trial. FHB disease incidence and field grain yield were recorded. The trials were harvested and evaluated for percent of millable grain, milling yield and soft wheat quality using standard methods of the American Association of Cereal Chemistry. Four field replications of samples were weighed before and after aspiration; after aspiration the four replications were combined to form two replications for milling and baking evaluation. Cultivars differed for the amount of grain aspirated during cleaning (Cultivar F-value > 22) with Coker 9511 having the smallest loss due to aspiration (3.4% removed) and SS 8641 having the greatest aspiration removal (74.4% removed). Generally the results correlated to known resistance levels with resistant cultivars having fewer scabby or shriveled grains. The percent of aspirated seed was negatively correlated to field yield (r > -0.25*) and test weight (r > -0. 87***), and was positively correlated to field infection (r > 0.63***). Effects of infection on end-use quality varied for the cultivars and will be discussed in greater detail.

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EFFECT OF TOLERANT VARIETIES AND FUNGICIDE TREATMENT ON FHB RATING, DON CONTENT AND YIELD UNDER HIGH INFECTION PRESSURE

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OBJECTIVES

To assess Fusarium Head Blight (FHB) impact on yield reduction and deoxynivalenol (DON) accumulation in winter wheat varieties with different resistance level and different fungicide treatment. To find out if medium tolerant varieties and fungicide treatment are sufficient to reach acceptable FHB rating and keep DON content below hygienic limit.

INTRODUCTION

Food safety is nowadays priority for cereal producers and grain-processing industry. FHB causes severe yield losses and decreases baking and food quality (Mesterházy, 2003). Natural occurrence of FHB in the Czech Republic varies between years and locations. Increasing of FHB was recorded during last years in consequence of higher ratio of corn in crop rotations and frequent using of no tillage system of soil processing. A survey of *Fusarium* mycotoxin deoxynivalenol in cereals intended for human consumption was carried out in Czech Republic for eight-year period (2000-2007). Wheat samples were collected directly from farmers so as to represent most of regions in the Czech Republic. 82% of 444 wheat samples were positive for DON content and maximum levels for DON according to Commission Regulation (EC) 1881/2006 were exceeded in 3.6% of wheat samples (Stockova et al., 2008).

Breeding for resistance to FHB has a more than twenty year tradition in breeding company Selgen. Number of resistance sources was used in breeding program (in the first place Sumai3 and Nobeoca Bozu). However, high FHB resistance level was lost during selection for other important agronomic traits. Also transfer of resistance from spring wheat to winter wheat type (80 % of wheat area in the Czech Republic) could be a complication.

Similarly, the results of many research studies shows us that it is difficult to reach high resistance level and simultaneously high yield and necessary bread-making quality (Mesterházy, 2003). Our results from tests of F_4 generation randomly founded number of tolerant types with desirable other agronomic traits. Two lines were managed to new winter wheat varieties, line SG-S1800 (Sakura) (Horcicka et al., 2007) and line SG-S1875 (Simila) (Horcicka and Hanisova, 2006).

Whether this level of FHB tolerance is sufficient under high pathogen impact and how important is contribution of variety and fungicide on pathogen development, DON content and yield reduction were focused in this work.

MATERIALS AND METHODS

Nine winter wheat varieties differed into 3 groups were used: R - tolerant group (with medium resistant varieties – Sakura, Simila, Petrus), M – medium susceptible (Bohemia, Raduza, Rheia) and S - susceptible group (Darwin, Mladka, Sulamit). Varieties were sown in 3 replications each of 4 variants of treatment: 1) control – without artificial infection and fungicidal treatment, 2) infection – with artificial *Fusarium* infection, without fungicide, 3) infection + common fungicide for leaf diseases (Tango Super), 4) infection + common leaf fungicide and targeted fungicide for spike diseases (Swing Top). Variant with Tango Super (1.0 l.ha-

^{1}, active substances: epoxiconazole 84 g.ha^{-1} and fenpropimorph 250 g.ha^{-1}) was sprayed in growing stage DC 37 – 39; variant with Swing Top (1.5 l.ha^{-1}, active substances: dimoxystrobin 250 g.ha^{-1} and epoxiconazole 84 g.ha^{-1}) was sprayed 24 hours before *Fusarium* infection. The experiment was planted by small parcel sowing machine type Hege. Final parcel area was 10 square meters. Experiment was done in three years (2007-2009) and at two locations.

Inoculum with spore concentrations of 6-7x10^{6} spores/ml was prepared and each parcel was infected with 1 liter of inoculum. Inoculum contained mix of pathotypes collected in whole area of the Czech Republic by State Phytosanitary Administration and multiplied by Research Institute of Crop Production in Prague. 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 small plot harvester. Yield and DON content were evaluated. Data was statistically analyzed using ANOVA (Statgraphics XV.II).

RESULTS AND DISCUSSION

Basic Statistic ANOVA - The significant effects of genotype (variety), spike fungicide treatment, environment and interaction of genotype with the environment were found on DON content, FHB rating and yield from the ANOVA results. Analysis of the share of individual sources of variability on overall variability in the trial showed a highly predominant role of year and location. A significant source of variability was also the genotype (about 18% of variability for DON content, 55% for FHB rating and 13% for relative yield reduction). The effect of basic fungicide treatment (Tango Super) was not significant for DON content and FHB rating and results are not included. Treatment combination of Tango Super and Swing Top had also significantly positive effect on these traits but represented lower source of variation than genotype.

The role of tolerant varieties - Tolerant varieties have with strong infectious pressure significantly lower occurrence of pathogen, DON content and percentage of yield reduction in comparison with susceptible varieties (Table 1). Tolerant varieties not exceed in average hygienic limit for DON content at infection variant, however variety Simila reached boundary value of medium susceptible group. Absolute values passed beyond hygienic limit (1.25 ppm) in some samples of each tolerant variety. Medium tolerant varieties contributed to lover DON content than in susceptible ones (approximately 7 ppm). However, tolerant varieties were standalone insufficient for safety production in high infection pressure and favorable weather conditions. FHB rating below 1.3 (approximately less than 13% of spikes with visible symptoms) could leads to safe grain production although this relation can not run properly in every condition. Important is also 6-15% yield effect of tolerant varieties in compare with susceptible.

The effect of fungicide treatment – Fungicide treatment on spikes led to reduced occurence of symptoms (the evaluation was about 1 point better) and lower DON content (Table 2). Yield reduction of fungicide treated infected variant was compared with only control variant of experiment without infection and fungicide treatment. Therefore such evaluation is not exact. Impact of fungicide on DON content and FHB rating was lower than effect of genotype, but sufficient to keep the most of tolerant varieties samples under hygienic limit. Only five samples of this group were over. These results agree with Mielke and Weinert (1996) work, who found FHB rating near zero when moderately tolerant varieties were treated by fungicide. Susceptible group was over limit in spite of fungicide treatment.

Conclusions – These results showed importance of tolerant varieties as basic FHB-prevention. Medium tolerant varieties (Petrus, Sakura, Simila) could be sufficient prevention of over-limit mycotoxins production at common growing condition in the Czech Republic. Fungicide treatment into spikes could be useful when wheat growth follow corn and at no tillage technology. However type of application and environmental effects also play

very important role. Optimal timing of fungicide application should be taken into account in this experiment. Medium susceptible varieties are suitable to use together with fungicide treatment. Epidemic severity of FHB causes unacceptable mycotoxins content when these varieties are used. Additional separation of harvested grains by specific weight is the only possible means of reducing monitory levels below the hygienic limit, but this brings higher costs of wheat production. FHB susceptible varieties represent risk of production with high DON content and should be excluded from food and feed production.

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Table 1: Variety means of inoculated plots (I) and plots treated with fungicide (IF) for DON content, FHB (disease severity) and relative yield reduction (% to uninfected control) in 2007-2009 experiments at two locations.

		DON (ppm)				FHB rating (1-9)*				Rel. yield (%)			
Variety		**I**		**IF**		**I**		**IF**		**I**		**IF**	
Sakura	R	0,75	a	0,44	a	1,2	a	0,5	a	98	a	104	ab
Petrus	R	0,84	a	0,38	a	1,3	a	0,9	a	98	a	98	bc
Simila	R	1,18	ab	0,74	ab	1,6	ab	1,1	ab	95	ab	99	bc
Bohemia	M	2,36	b	1,50	ab	2,4	c	1,7	bc	94	ab	100	abc
Raduza	M	2,41	b	1,60	ab	2,2	bc	1,8	cd	92	abc	99	bc
Rheia	M	3,04	b	1,75	ab	3,1	d	2,4	de	90	bcd	95	c
Sulamit	S	4,44	bc	2,71	bc	4,0	e	2,9	ef	92	abc	108	a
Darwin	S	7,70	c	4,09	c	4,4	e	3,4	fg	87	cd	98	bc
Mladka	S	11,31	d	4,21	c	5,2	f	3,7	g	83	d	97	bc
Average		**3,78**		**1,94**		**2,8**		**2,1**		**92**		**100**	

* 1= no symptoms visible

Means in the columns followed by the same letter are not significantly different from each other at P<0.05 of LSD test

Table 2: Variety group (R, M, S) means of inoculated plots (I) and plots treated with fungicide (IF) for DON content, FHB (disease severity) and relative yield reduction (% to uninfected control) in 2007-2009 experiments at two locations.

	DON (ppm)		FHB rating (1-9)*		Rel. yield (%)	
Variety group**	**IF**	**I**	**IF**	**I**	**IF**	**I**
R	0,52	0,92	0,8	1,4	100	97
M	1,62	2,60	2,0	2,6	98	92
S	3,67	7,82	3,3	4,5	101	87

* 1= no symptoms visible

** R- medium tolerant; M-medium susceptible; S-susceptible

PLANT ORGAN SPECIFIC GLYCOSYLATION OF DON IN THREE WINTER WHEAT CULTIVARS AFTER STEM BASE INFECTION WITH TOXIGENIC *FUSARIUM* SPECIES

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ABSTRACT

The ability of wheat (*Triticum aestivum* L.) to transform the *Fusarium* trichothecene B mycotoxin deoxynivalenol (DON) into non-phytotoxic DON glucosides is suggested to contribute to fusarium head blight (FHB) resistance. Recently, we found that following stem base infection with toxigenic *Fusarium* species, *Fusarium graminearum* and *F. culmorum*, DON is translocated in all plant parts including ears and grains. Mechanisms to convert DON into non-phytotoxic derivatives could also reduce damage through stem base infections with *Fusarium* spp., because DON is considered a virulence factor. Three winter wheat cultivars differing in resistance to FHB (highly, moderately susceptible and resistant) were tested for responses to soil-borne infection of the stem base with *F. graminearum* and *F. culmorum*. DON and its degradation product DON-3-glucoside (D3G) were found in the stem base, ear rachis and corresponding grains. HPLC-MS analysis of stem base, grain and corresponding ear rachis samples showed lowest levels of DON and D3G in the highly resistant cultivar, but levels did not correlate with susceptibility levels of the two sensitive cultivars. Transcript accumulation studies with real-time RT-PCR of genes associated with DON degradation and transport activities illustrated distinct differences between the wheat cultivars and between stem base and ears. Highest levels of D3G were always found in the ear rachis, in which however gene expression levels were significantly lower than in the stem base, indicating that DON degradation takes place already in the stem base and D3G is also transported within the plant. The level of D3G in stem base samples corresponded with expression levels of a gene coding for DON glucoside forming uridine diphosphate-glucosyltransferase (UGT). This study elucidates the role of DON glycosylation in cultivar resistance with regards to *Fusarium* stem base infection and translocation of mycotoxins to the ears.

DEVELOPMENT OF DURUM WHEAT GERMPLASM WITH ENHANCED RESISTANCE TO FUSARIUM HEAD BLIGHT DERIVED FROM EMMER WHEAT

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ABSTRACT

Durum wheat (*Tirticum turgidum* L. subsp. *durum*) is a unique class of commercial wheat specifically for making pasta products. Durum production has been seriously challenged by the Fusarium head blight (FHB) disease in the United States in the past decade. Although utilization of resistant cultivars is considered as an effective measure to control the FHB, the progress in development of resistant durum cultivars is limited due to the unavailability of high levels of FHB resistance in durum germplasm. We previously identified a number of cultivated emmer (*T. dicoccum*) and Persian wheat (*T. carthlicum*) accessions with increased levels of FHB resistance. These resistant tetraploid wheat accessions are currently utilized for developing durum wheat germplasm resistant to FHB. In this research, we selected five *T. carthlicum* and four *T. dicoccum* for introgression of the resistance into leading ND durum cultivars through double haploid (DH) and backcross methods. Over the past four years, we have developed 551 DH lines and 559 BC_1-derived advanced (BC_1F_5 - BC_1F_8) lines from crosses with four leading ND durum cultivars Lebsock, Ben, Maier, and Mountrail. One DH line and five BC_1-derived advanced lines have exhibited significantly improved resistance to FHB in the greenhouse and field evaluation for two years compared to their durum parents. Theses resistant lines are currently being used in a 2nd round of introgression and breeding for FHB resistance.

CHROMOSOME LOCATION OF FUSARIUM HEAD BLIGHT RESISTANCE IN 'FRONTANA' SPRING WHEAT

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OBJECTIVE

To determine which chromosomes bear resistance genes to Fusarium head blight (FHB) incidence, severity, and spread.

INTRODUCTION

Substantial resources including monetary, have been lost over the last two decades in attempts to control FHB, also known as scab, a fungal disease caused by *Fusarium* ssp in wheat (*Triticum aestivum* L.) and other small grains. The main causal agent of FHB is *Fusarium graminearum* Schwabe teleomorph *Gibberella zeae* (Schwein). The pathogen causes losses in yield, poor kernel quality, and reduced market value of the kernels due to discolored and shrunken kernels on infected plants. Kernels are also contaminated with mycotoxins, primarily deoxynivalenol (DON) that make kernels not only unpalatable but also noxious to both human and animals (McMullen et al., 1997).

Integrated disease management (IDM) is the best tool to control the disease with use of resistant cultivars as the pivotal aspect of the IDM. Schroeder and Christensen (1963) described resistance to initial infection as Type I and resistance to spread of disease within the spike as Type II.

Identifying and understanding how resistance genes work against FHB are vital for breeding endeavors. Studies to map genes for FHB resistance have been carried out in wheat. Steiner et al. (2004) and Mardi et al. (2006) mapped FHB resistance genes to 3A chromosome. Berzonsky et al. (2007) used reciprocal backcross monosomic lines (RBCM) and established that chromosomes 3A, 6A and 4D reduced *Fusarium* damaged kernels. Breeding against FHB is laborious and time consuming because FHB resistance is governed by many genes and expression is affected by the environmental conditions.

MATERIALS AND METHODS

Plant Material

Frontana and 'Chris' RBCM comprising of chromosomes 3A, 6A and 4D were employed in this study. Previously, Berzonsky et al. (2007) found that RBCM lines originating from Frontana expressed resistance to FHB while RBCM lines with a Chris background were susceptible. 'Alsen' (Frohbreg et al., 2006) and 'Choteau' were used as medium resistant and susceptible checks, respectively. The parental lines used to develop the RBCM were also included in the study (Table 1). The study was conducted under greenhouse conditions at North Dakota State University campus (46° N and 96° W) with a 16h photoperiod and temperatures were maintained between 16 and 21°C.

Inoculum and inoculation

A field isolate of *Fusarium graminearum* provided by the Plant Pathology group was used to prepare inoculum that was cultured in petri dishes on a mung bean media at 4°C for 7 days. The macroconidia were suspended in autoclaved double distilled water and a haemacytometer was used to determine the spore concentration. Point and spray inoculation techniques were used to introduce the pathogen to the plants. This was done when 50% of the spikes per plot were at anthesis.

Data Collection

Evaluations of FHB incidence, severity, and spread percentages were collected 21days after inoculation. Disease incidence and severity were determined on spray inoculated spikes using the scale proposed by Stack and McMullen (1995). Disease spread was expressed as a percentage of the number of spikelets that developed FHB symptoms beyond the initial inoculation point following a SFI to the total number of spikelets on the spike.

Experimental Design and Data analysis

The experiment was laid out in randomized as a nested block arrangement where genotypes were nested with in three chromosome groups (CG), namely 3A (CG1), 6A (CG2), and 4D (CG3). Each treatment had three replicates with eight plants per replicates. The experiment was conducted in four greenhouse seasons during 2007-2009.

Data were analyzed using a mixed model (PROC-MIX) of SAS 9.1 program (Cary, NC), and experiment-wise error was set at $p \leq 0.05$. Genotypes, chromosome groups, and inoculation method effects were considered fixed. Replicates and seasons were considered as random effects. Homogeneity test across seasons using Bartlett method ($p \leq 0.001$).

RESULTS AND DISCUSSION

Data on FHB disease incidence, severity, and spread are reported in Table 2. Using the assessment method as described by Steiner et al., (2004), type I resistance evaluation data suggest that Frontana's chromosomes 3A and 4D have genomic regions that may play a major role in governing Type I resistance. Chromosome 6A, though showed some Type I resistance level to FHB, the difference between Frontana 6A and Chris 6A was not significant. However, Frontana had much lower disease scores than all Frontana RBCM. This is probably because genes on 3A and 4D interact with genes on other chromosomes to confer resistance in Frontana.

Frontana 3A expressed reduced disease severity in CG1 following spray inoculation. This implies that chromosome 3A is involved in reducing disease severity (Table 2). Steiner et al. (2004) indicated that 3A from Frontana was associated with FHB severity explaining 16% of the phenotypic variance. Similarly to FHB incidence, Frontana had much lower disease scores than Frontana 3A. This may results from interaction (epistasis) between FHB resistance genes on 3A and genes on other chromosomes to confer resistance in Frontana. Results for CG2 illustrate that Frontana's 6A chromosome might not be involved in reducing disease severity. However, Frontana 4D (CG3) also plays a role in reducing FHB severity (Table 2). This is consistent with findings by Loffler et al. (2009); and Berzonsky et al. (2007).

In terms of disease spread, results of RBCM of the three groups (Table 2) show that Frontana 3A, 6A, and 4D had reduced FHB spread beyond the initial inoculation point. This suggests that Frontana chromosomes3A, 6A, and 4D have genes that restrict disease spread. Alsen has Type II resistance (Mergoum et al., 2007) and CG2 results between Alsen and Frontana 6A are not significantly different. Similarly, disease spread scores for Frontana 4D and Alsen in CG3 are similar. These observations suggest that 6A and 4D might be remotely involved in reducing disease spread as previously discussed by Buerstmayr et al. (1999) using a backcross reciprocal monosomic analysis involving 'Hobbit/U-136.1'.

Relationships among the above FHB resistance parameters were performed. A strong relationship between FHB severity and incidence [r^2=0.94 and a Pearson Correlation of r=0.97, P<.0001] was observed. Disease spread and severity were also highly correlated (r=0.91) and so were the disease severity and *Fusarium* damaged kernels (FDK). An analysis of the association between FHB incidence and spread revealed a strong correlation (r=0.93, P<.0001). These strong relationships suggest that FHB incidence, severity and spread are under similar genetic control as indicated by some previous works (Steiner et al., 2004; Groth

et al., 1999). A negative correlation between FHB severity and plant height was observed. However, the relationship was weak [r^2=0.098 and r=0.31, P<.005)]. Therefore, tall genotypes reported to have better FHB resistance than short genotypes (Buerstmayr et al., 2000) were not confirmed by our results which indicate no relationship between FHB and plant height.

In conclusion, the results from this study indicate that 3A in Frontana is the major genomic region not only for FHB incidence (Type I) and severity but also spread (Type II). The results show also that chromosomes 4D and, to a lesser extent, 6A, play a significant role in FHB resistance types I and II.

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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. List of genotypes used in the study, their description, and known reaction to FHB.

ID	Genotype	Description	FHB Reaction
1	Frontana(3A)	RBCM†	Resistant
2	Frontana(6A)	RBCM	Resistant
3	Frontana (4D)	RBCM	Resistant
4	Chris(3A)	RBCM	Susceptible
5	Chris(6A)	RBCM	Susceptible
6	Chris(4D)	RBCM	Susceptible
	Euploid controls:		
7	Frontana	Parent (Euploid)	Resistant
8	Chris	Parent (Euploid)	Susceptible
9	Alsen	Control (Euploid)	Resistant
10	Choteau	Control (Euploid)	Susceptible

†RBCM=Reciprocal backcross monosomic line.

Table 2. Means of FHB disease incidence, severity, and spread of wheat genotypes grown under greenhouse conditions using spray inoculation method.

		FHB Incidence		FHB Severity		FHB Spread	
Genotype	CG†	Mean (%)		Mean (%)		Mean (%)	
Alsen	1	28.63	ab‡	10.82	a	20.83	b
Choteau	1	62.17	d	53.50	d	50.07	d
Chris	1	60.25	d	41.62	cd	46.12	d
Chris 3A	1	51.83	cd	40.69	cd	43.31	cd
Frontana 3A	1	27.67	a	17.70	ab	12.62	ab
Frontana	1	12.50	a	5.45	a	5.37	a
Alsen	2	17.67	a	7.76	a	27.78	bc
Choteau	2	55.83	d	41.50	cd	41.93	c
Chris	2	52.33	cd	36.24	c	38.68	c
Chris 6A	2	43.83	bcd	43.23	cd	38.26	c
Frontana 6A	2	32.92	bc	26.81	bc	26.71	b
Frontana	2	16.67	a	6.80	a	8.55	a
Alsen	3	15.50	a	8.73	a	24.61	b
Choteau	3	59.98	d	43.51	d	58.92	d
Chris	3	55.08	d	42.97	cd	44.03	cd
Chris 4D	3	53.14	cd	41.87	cd	47.99	d
Frontana 4D	3	29.08	ab	18.89	ab	23.09	b
Frontana	3	11.67	a	4.81	a	7.72	a

†CG = Chromosome group

‡Means followed by the same letter within column are not significantly different at $p<.05$

COMPARATIVE MAPPING OF THE CHROMOSOMAL REGION HARBORING THE FUSARIUM HEAD BLIGHT RESISTANCE QTL *QFHS.NDSU-3AS* IN DURUM WHEAT

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ABSTRACT

Fusarium head blight (FHB), caused mainly by *Fusarium graminearum*, has been one of the major threats to durum wheat production worldwide. A source of resistance comparable to "Sumai 3" and others in bread wheat has not been found in durum. A major FHB resistance QTL derived from *Triticum turgidum* ssp. *dicoccoides*, designated *Qfhs.ndsu-3AS*, was identified and mapped to the short arm of chromosome 3A (3AS) in our previous studies. We have saturated the chromosomal region harboring the QTL with wheat EST-derived STS (sequence tagged site) and SSR (simple sequence repeat) markers. A large portion of wheat ESTs have not been mapped to individual chromosomes. We have identified genomic regions on rice chromosome 1 and in *Brachypodium distachyon*, which are collinear with the QTL region on 3AS, using the wheat ESTs previously mapped to the QTL region. The genomic sequences of the collinear regions in rice and *Brachypodium* have been used to BLAST wheat EST pool and to identify ESTs/genes within the QTL region. A total of 813 pairs of STS primers and 42 pairs of SSR primers have been designed from tentative consensus sequences (TCs) and singletons of the ESTs identified. As a result, 56 polymorphic STS and SSR markers have been developed and 45 of them mapped to a genomic region of 232 cM on chromosome 3A. Of the 45 markers, 23 mapped to a chromosomal interval of 14.9 cM harboring *Qfhs.ndsu-3AS* in the population of 83 recombinant inbred chromosome lines (RICLs). The average map distance between maker loci was reduced from 4.9 cM in the previous study to 1.24 cM in the QTL region. Five co-segregating markers were 0.6 cM proximal to *Xgwm2*, a SSR locus closely linked to the QTL peak. Comparative analysis has identified several chromosomal intervals in the distal region of the short arm of rice chromosome 1 and a few *Brachypodium* genomic regions collinear with the chromosomal region harboring *Qfhs.ndsu-3AS* on wheat 3AS. In addition, we have been constructing a genetic map of the QTL region with a higher resolution in a large segregating population with over 1,800 F_2 individuals. This will provide a better understanding of this chromosomal region and position the FHB resistance QTL *Qfhs.ndsu-3AS* more precisely within the region. User-friendly molecular markers tagging this resistance QTL have been developed and utilized in wheat breeding and germplasm development.

SESSION 4:

PATHOGEN BIOLOGY AND GENETICS

Chairperson: Frances Trail

B-TRICHOTHECENE GENOTYPES OF *FUSARIUM GRAMINEARUM* STRAINS FROM ACROSS BARLEY PRODUCTION REGIONS AND GROWING SEASONS IN SOUTHERN BRAZIL

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ABSTRACT

Fusarium head blight (FHB) is a serious disease affecting barley in Brazil, especially for causing a significant impact to the malt industry due to mycotoxin contamination. Current information concerning the genetic diversity of Brazilian *F. graminearum* populations is limited to isolates obtained from wheat. Attempting to characterize the B-trichothecene profile of *F. graminearum* isolates from barley in Southern Brazil, fungal colonies were obtained from monitored commercial fields across 21 municipalities during the 2007 and 2008 growing seasons. Following fungal isolation from grains cultured on selective media, a total of 57 single-spore isolates were obtained. Pure fungal cultures were grown in complete liquid media for biomass production and further DNA extraction. PCR assays were conducted first using Fg16F/R primer pair target to *F. graminearum* complex, and, second, a multiplex reaction with portions of the *Tri3* and *Tri12* genes in which amplifications are predictive of Nivalenol (NIV) and Deoxynivalenol acetylates (3ADON and 15ADON). In both years, the 15ADON genotype was predominant (18/24 in 2007 and 17/33 in 2008). One 3ADON isolate was detected in each year. The NIV genotype was also detected in both years in relatively high proportions: 5/24 in 2007 and 15/33 in 2008 isolates. This was the first survey concerning trichothecene profile of regional *F. graminearum* barley populations in Brazil. The high number of NIV isolates, in a much greater proportion than those found in wheat based on our previous findings, suggest that monitoring nivalenol in barley grains may be needed due to its higher toxicological implications compared to deoxynivalenol.

STUDIES ON THE *FUSARIUM GRAMINEARUM* COMPLEX AFFECTING WHEAT IN SOUTHERN BRAZIL SUGGEST A PHYLOGENETIC SPECIES-SPECIFIC B-TRICHOTHECENE PROFILE

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ABSTRACT

Several members within the *Fusarium graminearum* species complex (*Fg complex)* have been reported in association with some host crops in Brazil. Our previous studies on a set of wheat isolates from regional populations have revealed the predominance of lineage 7 (*F. graminearum* sensu stricto) possessing a consistent 15ADON trichothecene genotype, whereas a small proportion of isolates belonged to lineage 2 (*F. meridionale*) and presented a nivalenol (NIV) genotype. We advanced our studies on three local populations of strains isolated from wheat, to test our hypothesis that multiple phylogenetic species are present and may have a species-specific B-trichothecene profile. A sample of 55 strains were obtained from symptomatic kernels collected at Cruz Alta (n=19) Ernestina (n=19) and Nonoai (n=17) municipalities during 2007 growing season. We used AFLP markers to determine the similarity among the isolates of Brazilian populations with members of the *Fg complex*. A total of 150 AFLP bands were identified in the 200–500 bp range when using three primer pair combinations (*EcoRI*-AA/*Mse*I-AT, *EcoRI*-CC/*Mse*I-CG, *EcoRI*-TG/*Mse*I-TT). Representatives of members of *Fg complex* (lineages 1 to 9) were included in the study to compare with our isolates. A multiplex PCR was used to determine the trichothecene genotypes with sequence primers targeting portions of *Tri3* and *Tri12* genes that are predictive of 15ADON, 3ADON and NIV chemotypes. Our results confirms the predominance of the 15-ADON genotypes (48/55) all grouping with lineage 7. The NIV genotype was also found (06/55) and, in agreement with our previous findings, most belonged to lineage 2. However, one NIV isolated grouped with lineage 5 (*F. acaciae-mearnsii*). The only 3-ADON, detected in Ernestina population, grouped with lineage 8 (*F. cortaderiae*). In spite of the relative low number of strains analyzed this far, we confirm that FHB in Brazil is caused by multiple phylogenetic species and suggest a species-specific B-trichothecene profile, especially for the predominant species (*F. graminearum sensu stricto*).

WITHIN-FIELD PATTERNS OF B-TRICHOTHECENE GENOTYPES IN THE *FUSARIUM GRAMINEARUM* COMPLEX AFFECTING WHEAT IN SOUTHERN BRAZIL

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ABSTRACT

In our previous studies we have shown that Southern Brazilian pathogenic populations of *Fusarium graminearum* species complex obtained from *Fusarium*-damaged wheat kernels were consistently either DON/15-ADON (lineage 7) or Nivalenol (NIV) (lineage 2) regarding to the B-trichotecene genotype. The objective of this study was to assess whether the different lineages/trichotecene genotypes are present and how they are distributed in intensively sampled wheat fields. Two fields located at two production regions in Rio Grande do Sul State, Brazil, and distant 192 km apart from each other (field A = Vacaria; B = Sarandi), were assessed at early soft dough stage showing moderate FHB incidence levels. Each field was visually divided in four sections; five georeferenced sampling points were randomly defined within a section. At each sampling point, four adjacent symptomatic heads in a 0.2m x 0.2m area were collected. A total of 80 symptomatic heads were collected in each field. In the laboratory, heads were disinfested and sections of the head were plated on selective media for recovery of fungal isolates (one isolate per head). All isolates that showed typical *F. graminearum* characteristics were purified by a single spore technique and the resulting mycelium was further grown on liquid media for biomass production. Mycelium DNA was extracted and PCR assays were conducted using Fg16F/R primer to confirm isolate identity and sequence primers targeted at *Tri3* and *Tri12* genes predictive of NIV, 3ADON and 15ADON in a multiplex reaction. In field A, 75 isolates were obtained and 15ADON (72), 3ADON (2) and NIV (1) genotypes were detected. The 3ADON and NIV isolates were found at different field sections and sampling points. In field B, from a sample of 35 isolates obtained, 15ADON was the predominant type (33/35) and two NIV types were also detected. A polymorphism detected in Fg16 primer amplifications for all NIV types were indicative of lineage 2, in agreement with our previous findings. The identity of the 3ADON type is under investigation. Our advanced population studies suggest that Fusarium head blight of wheat in Brazil is caused by distinct members of the *F. graminearum* complex that show distinct trichothecene profiles and co-occur at the field scale, where the lineage 7/15ADON genotype is predominant at the spatial hierarchies studied.

PEPTIDE TECHNOLOGIES FOR MANAGEMENT OF FUSARIUM HEAD BLIGHT

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ABSTRACT

We are developing two strategies for selection and deployment of peptides to protect wheat from infection by *Fusarium graminearum*. One strategy is based on the identification of small, combinatorially selected peptides that function as inhibitory ligand mimics for factors involved in pathogen growth and development. The three steps of this strategy include (1) selection of peptides with affinity for pathogen infectious structures, (2) assessment of impacts of affinity-selected peptides on pathogen development and, (3) delivery of bioactive peptides in susceptible tissues of host plants. We previously applied this strategy to successfully limit tomato root infection by the oomycetous pathogen, *Phytophthora capsici*, and to reduce the infection efficiency of spores produced by *Phakopsora pachyrhizi*, the fungus that causes Asian soybean rust. In our initial studies with *F. graminearum*, we have defined populations of peptides from two types of combinatorial libraries that bind to germinating macroconidia. Some of these peptides slow the rate of germ tube development. In addition to continuing assessments of peptides for inhibition, we are assessing germination of macroconidia and ascospores in relation to peptide concentration. A second strategy being pursued is the use of peptides that function as mating pheromones for *F. graminearum*. These peptides and their derivatives have been shown to inhibit germination of macroconidia. We are producing these peptides via yeast fermentation and by chemical synthesis. We are preparing to apply these peptides to plants to examine their potential for protection against infection by *F. graminearum* ascospores and macroconidia.

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AGGRESSIVENESS AND MYCOTOXIN POTENTIAL OF U.S. *FUSARIUM GRAMINEARUM* POPULATIONS IN FIELD-GROWN WHEAT AND BARLEY

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ABSTRACT

Previous greenhouse experiments have demonstrated increased toxigenic potential of certain populations of *Fusarium graminearum*. Inoculated and mist-irrigated field experiments were conducted in St. Paul, MN in 2008 & 2009 to test whether significant differences in aggressiveness and/or toxigenic potential exist between pathogen populations under field conditions and if so, whether aggressiveness and/or toxigenic potential on specific varieties or lines is correlated with specific pathogen populations. Twelve cultivars/lines of each wheat and barley were independently inoculated with three (2008) or four (2009) *F. graminearum* populations in addition to a water control. The fungal "populations" were generated by mixing conidia of 15-20 well-characterized strains that have been shown to belong to specific and genetically distinct populations (emergent (E)3ADON, E15ADON, midwestern (MW)15ADON and emergent (mix of E3ADON & E15ADON)). Experimental procedures were otherwise standard. FHB severity (SEV) in the field (proportion of infected spikelets), and visually scabby kernels (VSK; wheat only) were determined as measurements of aggressiveness. Toxigenic potential was determined by measuring mycotoxin concentrations (DON and derivatives) by GC/MS. Mycotoxin analysis was performed at the Mycotoxin Lab of the University of Minnesota (Dr. Yanhong Dong, director). Statistical analyses were performed using JMP software. For wheat, overall VSK and DON levels were much lower in 2009 than for 2008, although the same trends were observed in both years. No differences between populations were observed for VSK and SEV in wheat, and significantly higher levels of DON were again obtained for the emergent population. The added treatment in 2009 (E15ADON) also had significantly elevated levels of DON compared to MW15ADON and E3ADON. In contrast, for barley, the results from the two years were not consistent. While in 2008, there was no effect of population treatment on SEV and DON, significant population effects were evident in 2009. Inoculation with the E3ADON population resulted in significantly higher SEV than with the other three populations, while DON was significantly different between all four treatments (E3ADON > Emergent > E15ADON > MW15ADON). Overall, these latter results cannot be adequately interpreted without a third year of field experimentation. Nevertheless, we tentatively conclude at this time that the pathogen population may have an effect on FHB development and toxin accumulation in the field, which indicates that knowledge of the genetic composition of the inoculum in field trials is advisable.

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INOCULATION AND RECOVERY OF *FUSARIUM GRAMINEARUM* CHEMOTYPES FROM THE FHB NURSERY AT GLENLEA, MANITOBA IN 2008 AND 2009

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ABSTRACT

Fusarium head blight (FHB) is a serious threat to the Canadian grain industry. Most isolates of *Fusarium graminearum* Schwabe, the principal cause of FHB in North America, produce the mycotoxin deoxynivalenol (DON) and one of its acetylated derivatives, 3- or 15-ADON. In North America, a rapid shift from the 15-ADON to 3-ADON chemotype has been documented. While the 3-ADON isolates are not more aggressive than the 15-ADON isolates, they produce significantly more DON. The wheat FHB screening nursery at Glenlea, MB was inoculated with a macroconidial suspension of both chemotypes. In 2008, 2 strains producing 3-ADON and 2 producing 15-ADON were combined for increase in carboxymethyl cellulose (CMC). In 2009, the isolates were increased separately and combined in equal ratio just before inoculation. The objective of this study was to determine if isolates of 3- or 15-ADON were recovered in the same ratio as applied under each set of increase conditions. A set of 6 check cultivars/lines, planted throughout the nursery, was sampled after harvest in each year. For each check variety, 100 seeds were surface-sterilized and plated on potato dextrose agar. The first 40 isolates of *Fusarium graminearum* recovered per check were single-spored and analysed for chemotype by PCR. In 2008, the ratio of 3-ADON to 15-ADON isolates recovered from seed was on average 4:1, respectively, for all 6 checks. However, in 2009, when the isolates were applied in a 1:1 ratio of 3- and 15-ADON, respectively, the ratio of recovered isolates was 1:1. Although inoculation methods and spore concentration were unchanged between the 2 years, DON levels were much higher in 2009 (ranging from 11 ppm to 71 ppm) than in 2008 (ranging from 5 ppm to 25 ppm). This may be due to a cooler and wetter growing season in 2009 allowing for greater fungal growth and toxin production. The difference in growing conditions and chemotype frequency did not alter the relative DON rankings of the 6 lines used. Those rated as most tolerant to FHB had the lowest levels of DON, and those most susceptible had the highest DON levels in both years.

A COMPARISON OF THE AGGRESSIVENESS AND DEOXYNIVALENOL CONTENT OF CANADIAN 3-ACETYL AND 15-ACETYLDEOXYNIVALENOL PRODUCERS OF *FUSARIUM GRAMINEARUM* IN FIELD-GROWN SPRING WHEAT

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ABSTRACT

Twenty four isolates of *Fusarium graminearum* of Canadian origin half of which were 3-acetyldeoxynivalenol (3-ADON) and half 15-acetyldeoxynivalenol (15-ADON) producers were tested for their ability to cause Fusarium head blight (FHB), as measured by FHB index and production of deoxynivalenol (DON) in spring wheat. Objectives of this study were to determine (i) whether 3-ADON producers differ in aggressiveness and DON production from 15-ADON producers under field conditions and (ii) whether resistant host cultivars were stable in performance across isolates. Field tests of all isolates were conducted with three replications at each of two locations in Canada and Germany in 2008, with three host genotypes differing in FHB resistance level. Mean FHB indices and DON content were analysed. Mean FHB indices across locations ranged from 5.48 to 34.42%. The resistant host genotype showed resistance regardless of the isolate or location. The differences between mean FHB indices of 3-ADON and 15-ADON chemotypes were not significant. In contrast, DON production by the 3-ADON isolates was significantly ($P < 0.05$) higher at three locations. Acetylated forms of DON accounted for only 2.5% (3-ADON) and 0.4% (15-ADON) of the total DON concentration across the two German locations. 3-ADON isolates may produce more DON depending on location than 15-ADON producers, but their mean aggressiveness is quite similar.

FUNCTIONAL CHARACTERIZATION OF HISTONE DEACETYLASE GENES IN *FUSARIUM GRAMINEARUM*

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ABSTRACT

Fusarium head blight caused by *Fusarium graminearum* is an important disease of wheat and barley. In a previous study, the transducin-beta like gene *FTL1*, a component of a well-conserved histone deacetylase (HDAC) complex, was found to be essential for plant infection. The *F. graminearum* genome has one predicted class I HDAC gene that is homologous to yeast *RPD3*. It also contains three class II HDAC genes, FGSG_01353, FGSG_04324.3, FGSG_05636.3, that are named *FgHOS2*, *FgHDA1*, and *FgHOS3*, respectively, in this study according to their homologs in *S. cerevisiae*. Mutants deleted for these four HDAC genes were generated with the split-marker approach. Infection assays with flowering wheat heads indicated that the *FgRPD3* and *FgHOS2* genes are important for plant infection. While the *fgrpd3* deletion mutant was severely reduced in vegetative growth, the *fghos2* mutant had relatively normal growth rate. The latter produced fewer conidia and shorter aerial hyphae. On mating plates, the *fghos2* mutant was sterile. Instead of forming protoperithecia or perithecia, the mutant produced abundant sporodochia with massive amount of macroconidia. In 16 h germlings, the mutant accumulated numerous lipid droplets. These data suggested that deletion of *FgHOS2* likely affected proper regulation of subsets of genes involved in sexual reproduction, conidiation, lipid metabolism, and plant infection. Microscopic examination of plant infection defects and expression profiles of the *fgrpd3* and *fghos2* are in the progress. Data on HDAC activity assays with these mutants and genes affected by deletion for *FgHOS2* also will be presented.

TRI3, WHICH CONTROLS TRICHOTHECENE C-15 ACETYLATION, IS FUNCTIONAL IN 3ADON CHEMOTYPE

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ABSTRACT

Three different trichothecene chemotypes have been identified in U.S. strains of *Fusarium graminearum*: 3-acetyldeoxynivalenol (3ADON), 15-acetyldeoxynivalenol (15ADON), and nivalenol (NIV), although grain is typically contaminated with deoxynivalenol (DON) or nivalenol rather than the acetylated derivatives.

In DON-producing strains of *F. graminearum*, two of the trichothecene cluster genes, *TRI7* and *TRI13*, are nonfunctional as a result of multiple insertions and deletions within their coding regions, whereas in NIV-producing strains, *TRI7* and *TRI13* are functional (Lee et al., 2002). These differences, combined with the finding that TRI13 is responsible for trichothecene C-4 hydroxylation, identified the basis for NIV versus DON chemotypes in *F. graminearum*. Differences in *TRI7* and *TRI13* were used to develop PCR markers to predict DON and NIV chemotypes (Chandler et al., 2003).

PCR markers for *TRI3* and *TRI12* have been used to predict 3ADON and 15ADON chemotypes in *Fusarium graminearum* (Ward et al., 2008). In order to determine the genetic basis for these chemotypes, we looked at differences in the function of *TRI3* in 3ADON, 15ADON and NIV strains. *TRI3* controls the addition of an acetyl group at the C-15 of trichothecenes in *Fusarium sporotrichioides* (McCormick et al. 1996, Garvey et al. 2009). A group of sixty *Fusarium* strains were analyzed for production of trichothecenes in liquid culture and on rice to confirm the chemotype predicted with PCR markers for *TRI3* and *TRI12* (Ward et al., 2008). TRI3 from representative strains of each chemotype were expressed in yeast and the transformants were fed possible Tri3 substrates (15-decalonectrin, DON). Tri3 from all three chemotypes converted 15-decalonectrin to calonectrin indicating that Tri3 is functional, even in the 3ADON chemotype. DON was not a good substrate for Tri3 which supports the addition of the C-15 acetyl group earlier in the biosynthesis of 3ADON. Cell free extracts were also prepared from representative strains of each chemotype and fed 3,15-diADON. Cell-free extracts of 15-ADON and NIV strains converted 3,15-diADON to 15-ADON; cell-free extracts of 3ADON strains converted 3,15-diADON to 3ADON. The Tri8 esterase removes the acetyl group from the C-3 position in 15ADON strains (McCormick and Alexander, 2003). The results indicate that a C-15 esterase is required to produce 3ADON. Efforts to characterize this esterase are ongoing.

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MULTIPLEX QUANTITATIVE ANALYSIS FOR TRICHOTHECENE GENES EXPRESSION OF *FUSARIUM GRAMINEARUM* IN DIFFERENT GENOTYPES OF WHEAT SPIKES

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ABSTRACT

Wheat resistance level to mycotoxin contamination varies among genotypes, however it remains incompletely understood whether resistance to fungal invasion produces secondary effect or specific genes works on low level accumulation of the mycotoxins. We investigated trichothecene gene (*Tri* genes) expression of *F. graminearum* in the infested wheat genotypes, using newly developed multiplex quantitative PCR method.

We applied this method to analyze *Tri* genes expression dynamism at early infection stage to reveal effective wheat genotypes that suppress trichothecene biosynthesis. We analyzed the relative expression level of *Tri* genes (*Tri5, Tri6, Tri8, Tri10* and *Tri11*) per fungal cell using actin and β-tubulin as internal standards. It is a more cost-effective way to analyze *Fusarium*-wheat gene expression crosstalk than the microarray analyses and higher throughput than the real time PCR methods. FHB resistance cv. Sumai 3 and susceptible cv. Gamenya were infected 106 unit/ml spore of *F. graminearum* 132-9 (DON producer) injected to the first and second floret in central spikelet of the spike at flowering time. Infected spikes were maintained at 22°C and kept wet 48hrs for FHB initial penetration. Then, the relative humidity was kept around 60% and the three infected spikes were sampled together to extract total RNA at 5days after inoculation (DAI), 10DAI, 15DAI and 20DAI. The extracted total RNA was used for the multiplex quantitative PCR analysis with chimeric primers consisting of *Tri* gene specific sequences with a universal tail designed to amplify different size of each *Tri* gene. We quantified the expression and calculated average of the *Tri* genes expression level with triplication per one sample. Analyzed expression level of the *Tri* genes of *F. graminearum* in Sumai 3 at 5DAI was 73% higher than that at 10DAI, 15DAI and 20DAI, excepting constant level of *Tri 11*. On the other hand, no change of the *Tri* genes expression level was found in Gamenya at 5DAI was the same as 10DAI, and 54% higher than that at 15DAI, 20DAI. Comparison with the two varietal differences, *Tri* genes expression level in Sumai 3 at 5DAI was 49% higher than that Gamenya, despite that in Sumai 3 at 10DAI was lower than Gamenya. *Tri* genes expression level of *F. graminearum* varied in the infested wheat genotype, and its expression level decrease with time.

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THE NEWLY EMERGING 3ADON POPULATION OF *FUSARIUM GRAMINEARUM* IS MORE AGGRESSIVE AND PRODUCES A HIGHER LEVEL OF DON THAN THE PREVALENT 15ADON POPULATION IN NORTH DAKOTA

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ABSTRACT

Fusarium head blight (FHB) is primarily caused by *Fusarium graminearum* in North America. The fungal pathogen produces various types of trichothecenes, including deoxynivalenol (DON), 3-acetyl-Deoxynivalenol (3ADON), 15-acetyl-Deoxynivalenol (15ADON) and nivalenol (NIV). Based on the trichothecene profile, isolates of *F. graminearum* can be identified as one of the three chemotypes, i.e, 15ADON, 3ADON, and NIV. Population studies indicated that *F. graminearum* isolates with a 3ADON chemotype were rare in North America before 1998, but the frequency of 3ADON isolates has increased dramatically in Canada and the Upper Midwest of the United States in recent years. However, little information is available on the aggressiveness and DON production of the newly emerging 3ADON population in wheat genotypes with different sources of FHB resistance. In this study, we characterized *F. graminearum* isolates collected from 1980 to 2000 (old collection) and in 2008 (new collection) and found that the frequency of 3ADON isolates was very low (3%) in the old collection but it accounted for 44% in the new collection. Evaluation of fourteen 3ADON isolates and fourteen 15ADON isolates randomly selected from the collections by single-floret inoculation on three spring wheat genotypes (Grandin, Steele-ND and ND2710) showed that the 3ADON population caused a significantly higher level of disease severity and produced more DON accumulation than the 15ADON population on Grandin (susceptible to FHB) and ND2710 (with FHB resistance from Sumai 3). However, no significant differences in disease severity and DON production were observed between the two populations on Steele-ND (with moderate resistance from *Triticum dicoccoides*). The 3ADON isolates also exhibited a higher DON productivity in rice culture and produced more spores on agar media than the 15ADON isolates, suggesting a fitness advantage of the newly emerging 3ADON population over the prevalent 15ADON population. The information obtained could have a significant impact on FHB management and host resistance deployment.

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LINKING FIELD AND ATMOSPHERIC POPULATIONS OF TOXIGENIC FUSARIA

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ABSTRACT

Fusarium is arguably one of the most important fungal genera on the planet earth. Some fusaria are plant pathogens, others saprophytes, and still others producers of dangerous secondary metabolites. Many fusaria use the atmosphere to travel from one habitat to another. Their atmospheric transport is poorly understood, yet necessary to understand their ecological roles in agricultural ecosystems and evaluate risks posed by invasive fusaria in these habitats. We collected hundreds of fusaria with autonomous (self-controlling) unmanned aerial vehicles (UAVs) tens to hundreds of meters above of the surface of the earth at Virginia Tech's Kentland Farm. Partial translation elongation factor (TEF) DNA sequences were generated from a series of single-spored isolates, and BLAST queries were performed against a curated *Fusarium* TEF database and GenBank. At least 12 different species of *Fusarium* were present in samples collected from 75 different autonomous UAV flights. Most of the flight populations contained more than one *Fusarium* species, suggesting that these fungi are traveling together through the atmosphere as part of discrete assemblages. Strains of *Fusarium graminearum* collected with UAVs 40 to 300 meters above the ground during fall, winter, spring, and summer months were able to cause Fusarium head blight on a susceptible cultivar of spring wheat and produce a variety of trichothecene mycotoxins. A new framework for understanding punctuated changes in the population structure of atmospheric fusaria is being developed and tested at both local (individual farm) and regional (eastern U.S.) scales. This work aims to transform our knowledge of the atmospheric transport of microorganisms and develop new paradigms that link field and atmospheric populations of toxigenic fusaria.

ACKNOWLEDGEMENT

This material is based in part upon work supported by the National Science Foundation under award number 0919088.

AGGRESSIVENESS OF 15-ACETYL-DEOXYNIVALENOL AND NIVALENOL *FUSARIUM GRAMINEARUM* TRICHOTHECENE GENOTYPES TOWARDS WHEAT VARIETIES

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ABSTRACT

Fusarium head blight (FHB) of wheat in Brazil is caused mainly by *Fusarium graminearum* species complex members that possess a deoxynivalenol (DON) or nivalenol (NIV) B-trichotecene genotype. Our recent research showed that one acetylated form of DON (15ADON) seems to predominate over NIV across several wheat production regions. In this study we tested whether 15ADON strains have fitness advantages over NIV strains when inoculated onto two wheat varieties (Guamirim – moderate resistant and BRS194 - susceptible) of known reaction to FHB in the field. Two separate greenhouse experiments were conducted for each variety using four strains of distinct trichothecene genotype and isolated from wheat or barley (15ADON-wheat, 15ADON-barley, NIV-wheat and NIV-barley). Two inoculation methods were used for assessing 1) infection rate in excised head tissues (lemma and paella) following different incubation times and 2) rate of disease spread in the heads (mid-point inoculation). A significant difference for the infection and colonization rates among isolates was observed only for Guamirim while the disease developed similarly in BRS194, regardless of the strain type. Both rates were higher for 15ADON strains compared to NIV strains in Guamirim; head severity at 15 days after mid-point inoculation averaged 45% and 10% for 15ADON and NIV, respectively. Infection frequency on spikelet tissues was 50% and 5% for NIV-wheat and NIV-barley, respectively. Our results may help to explain the predominance of 15ADON over NIV genotypes in the field and suggest that they may play a differential role in pathogenesis depending on the resistance level of the wheat variety, which deserves further investigation.

SESSION 5:

GENE DISCOVERY AND ENGINEERING RESISTANCE

Chairperson: Steve Scofield

QUANTITATIVE TRAIT LOCI MAPPING OF FUSARIUM HEAD BLIGHT RESISTANCE IN ADVANCED BACK CROSS POPULATION (BC1F6) DERIVED FROM TUN 34 × LEBSOCK TETRAPLOID WHEAT

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ABSTRACT

Fusarium head blight (FHB) caused by *Fusarium graminearum* has resulted in significant reduction in grain yield, quality and farm income in durum wheat growing areas of North America. There are a few known sources of resistance for FHB mostly derived from the Chinese hexaploid sources like Sumai3 and Wangshuibai. Distinct sources of resistance were identified by NDSU in Tunisian pedigrees. It has been shown that Tunisian lines have no relation to Chinese genotypes and therefore can be used to enhance the resistance in durum wheat by pyramiding resistant genes from different genetic backgrounds. To expedite identification of durum lines carrying these resistant alleles in earlier generations, use of molecular markers associated with FHB is essential.

Genomic regions associated with FHB were examined in 168 progenies of tetraploid Tun34 × Lebsock advanced back cross (BC_1F_6) population. To construct the genetic map, a total of 2300 DArT markers were tested for polymorphism between parents. The polymorphic markers were assembled into linkage groups at likelihood ratio statistic (LOD) greater or equal to three and followed by assembly of a consensus map using Kosambi mapping function.

Of the total DArT markers screened for polymorphism between parent lines Tun34 and Lebsock, 379 clones (15.1%) were polymorphic. Segregation ratios were compared to expected ratios for all markers using chi-square goodness of fit test. Results indicate segregation distortion of 5.2% ($P<0.01$) for this population. Of 379 markers, 359 were assigned ($LOD\geq3.0$) into 44 linkage groups with the minimum number of three markers. Following the grouping, genetic maps were constructed. Almost all of the linkage groups except two could be assigned to durum wheat chromosomes by alignment to previous published maps.

Genomic scan using Kruskal–Wallis rank-sum test identified significant ($P\leq0.001$) putative QTL associated with FHB on chromosomes 5B, 2A, 6B, 7A and 7B. A region on chromosome arm 5BL (4cM interval) showed the highest K score and an increase in resistance to FHB due to alleles of Lebsock parent. Composite interval mapping confirmed the presence of this significant (LOD=6.1) QTL explaining 14.7% of phenotypic variation for FHB. Since this population was phenotyped at two different seasons in replicated experiments, information from these QTL can be used in marker assisted selection (MAS) to control FHB.

ACKNOWLEDGEMENT AND DISCLAIMER

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TESTING TRANSGENIC SPRING WHEAT AND BARLEY LINES FOR REACTION TO FUSARIUM HEAD BLIGHT: 2009 FIELD NURSERY REPORT

Dill-Macky, R.[1*], Wennberg, K.J.[1], Scanlan, T.C.[1], Muehlbauer, G.J.[2], Shin, S.[2], Shah, D.[3], Kaur, J.[3] and Dahleen L.S.[4]

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ABSTRACT

The 2009 field screening nursery, with 128 wheat and 208 barley plots was located at UMore Park, Rosemount MN. Trial entries and untransformed controls were submitted by the University of Minnesota (19+1 wheat), the Donald Danforth Plant Science Center (4+2 wheat) and USDA (48+1 barley). Lines with known reactions to Fusarium head blight (FHB) were also included as checks. The wheat checks used were the moderately resistant Alsen and Tom, the moderately susceptible 2375 and the susceptible cultivars Wheaton and Roblin. The barley checks were the moderately resistant line M122 and the susceptible cultivars Conlon (2-rowed), Robust and Stander. The experimental design was a randomized block with four replicates. Plots were 2.4 m long single rows. The trial was planted on May 6, 2009. All plots, except a non-inoculated Wheaton check, were inoculated twice. The first inoculation was applied at anthesis for wheat and at head emergence for barley. The second inoculation was applied three days after the initial inoculation (dai) for each plot. The inoculum was a composite of 50 *F. graminearum* isolates at a concentration of 200,000 macroconidia.ml^{-1} with Tween 20 (polysorbate) added at 2.5 ml.L^{-1} as a wetting agent. The inoculum was applied using a CO_2-powered backpack sprayer fitted with a SS8003 TeeJet spray nozzle with an output of 10ml.sec^{-1} at a working pressure of 275 kPa. Mist-irrigation was applied from the first inoculation on June 26 till July 26 to facilitate FHB development. FHB incidence and severity were assessed visually 20-21 d.a.i. for wheat and 13-14 d.a.i. for barley on 20 arbitrarily selected spikes per plot. FHB incidence was determined by the percentage of spikes with visually symptomatic spikelets of the 20 spikes observed. FHB severity was determined as the percentage symptomatic spikelets of the total of all spikelets observed in these 20 spikes. Plots were harvested at maturity on August 14 (barley) and 24 (wheat). The harvested seed from each plot was split to obtain a 25 g sub-sample, which was then cleaned by hand. The wheat subsamples were used to estimate the percentage of visually scabby kernels (VSK) and then all samples (wheat and barley) were ground and submitted for deoxynivalenol (DON) analysis. The data indicated that resistance was expressed in some of the transformed lines.

ACKNOWLEDGEMENT AND DISCLAIMER

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MOLECULAR AND GENETIC STUDIES ON FUSARIUM EAR BLIGHT DISEASE OF WHEAT

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ABSTRACT

In the UK, Fusarium Ear Blight (FEB) disease of cereal crops is sporadic and epidemic years are difficult to predict. Wheat ear infections are predominantly caused by two species, *F. graminearum* and *F. culmorum*. The former now predominates in areas where maize is frequently grown in the rotation as well as near major shipping ports. Infection by these species leads to the contamination of grain with mycotoxins. Since 2008, prior to arrival at the flour mill, each lorry load of wheat grain has to be tested for mycotoxin contamination, which is adding to farm costs.

Despite intensive investigation over the past 15 years on the molecular basis underpinning susceptibility and resistance to FEB in wheat, our knowledge on this pathosystem remains fragmentary. However, several key features have so far emerged: When infecting wheat ears, most *Fusarium* isolates produce a range of trichothecene mycotoxins including deoxynivalenol (DON) and its acetyl derivatives. Removal of DON producing ability from toxigenic *Fusarium* isolates causes reduced virulence. In wheat ears, natural resistance is (a) primarily effective post infection, (b) known to be QTL based and (c) *Fusarium* species non-specific. So far the only well characterised natural resistance mechanism is that associated with the major 3BS QTL derived from Sumai-3, which confers upon the plant the ability to convert DON to a DON-O-3 glycoside with reduced toxicity.

In this presentation, three research topics will be addressed. Firstly, we have dissected the hyphal infection process from the initial infected spikelet, through the rachis and into the adjoining spikelets of a susceptible wheat genotype. This has included a comprehensive microscopic study to locate hyphae and to characterise the responses of the neighbouring wheat cells (Brown et al., (2009) submitted). This study is now permitting the recovery of specific cell types by laser capture microscopy and tissue dissection for gene expression analysis by RT-PCR, Affymetrix microarray and 2nd generation sequencing analysis. Secondly, we are using the model *Arabidopsis* floral *Fusarium* - pathosystem (Urban et al., 2002), to identify both the pathogen and host components which either restrict or support the *Fusarium* infection process. Through a reverse genetics approach we have discovered that both NPR1 and EDS11 are independently required for floral resistance against *Fusarium*, whereas the salicylic acid (SA) and ethylene (ET) signalling pathways are either not required or have only a minimal effect on the interaction outcome (Cuzick et al., 2008, 2009). Previously, EDS11 had only been reported to be required for basal defence against virulent bacteria (Volko et al., 1998). The results arising from these *Arabidopsis* mutant analyses are in agreement with the results obtained from the wheat ear microscopic study. Thirdly, we are in the final stages of establishing at Rothamsted a Category 3 biological containment facility that combines controlled growth rooms and laboratories. This purpose built facility, will permit us to use the virus induced gene silencing (VIGS) technology based around the Barley stripe mosaic virus vector in combination with transgenic *Fusarium* strains and/or wild-type *Fusarium* isolates

of non-UK origin (none of which must be allowed to escape into the environment). The VIGS experiments done in this facility will explore the biological relevance of the genes discovered at the wheat host-pathogen interface and in the *Arabidopsis* pathosystem to *Fusarium* infection of wheat ears and leaves in susceptible and resistant genotypes.

ACKNOWLEDGEMENT

Rothamsted Research receives grant-aided support from the Biotechnology and Biological Sciences Research Council (BBSRC) of the UK. This research has also received support from a BBSRC responsive mode grant. AB and NB are supported by BBSRC studentships with Syngenta as the CASE partner.

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IDENTIFICATION OF TRICHOTHECENE TARGETS: NOVEL GENES FOR SCAB RESISTANCE IN BARLEY AND WHEAT

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ABSTRACT

The molecular mechanisms that control trichothecene mycotoxin sensitivity in plants are not well understood. To identify functional eukaryotic targets we screened the yeast non-essential deletion library (~4700 genes) with trichothecin (Tcin) and identified >100 trichothecene targets that enhance resistance when deleted from the genome. For the top resistant yeast mutants, the single gene knock-out conferred resistance to six fold higher concentration than the lethal dose for the isogenic parental strain. The largest group of resistant strains affected mitochondrial function, suggesting a role for fully active mitochondria in trichothecene toxicity. Tcin inhibited mitochondrial translation in the wild type strain to a greater extent than in the most resistant strains, implicating mitochondrial translation as a previously unrecognized site of action. The Tcin-resistant strains were cross-resistant to anisomycin and chloramphenicol, suggesting that Tcin targets the peptidyltransferase center of mitochondrial ribosomes. Tcin induced cell death was partially rescued by mutants that regulate mitochondrial fusion and maintenance of the tubular morphology of mitochondria. Treatment of yeast cells with Tcin led to the fragmentation of the tubular mitochondrial network, supporting a role for Tcin in disruption of mitochondrial membrane morphology. These results provided genome-wide insight into the mode of action of trichothecene mycotoxins and uncovered a critical role for mitochondrial translation and membrane maintenance in their toxicity. Our goal is to use the information from the yeast screen to identify mechanisms that contribute to trichothecene resistance in plants. *Arabidopsis* orthologs of the genes identified in yeast have been cataloged and scored based on both protein sequence homology and functional characterization. The genes represent a broad array of functional gene classes, including factors that influence translation, sterol synthesis, stress response, mitochondrial genome maintenance, mitochondrial morphology, lipid metabolism, ubiquitination, the unfolded protein response (UPR) pathway, mitochondrial ribosome function, and sphingolipid metabolism. Homozyous *Arabidopsis* knockout lines (T-DNA insertions) were identified using the *Arabidopsis* Information Resource (TAIR) database. These plants are currently being tested for response to mycotoxin exposure (DON and Tcin). In addition, the parental *Arabidopsis* strain (Columbia) with GFP-labeled mitochondria, endoplasmic reticulum, and chloroplast are being used to visualize the *in vivo* effect of the mycotoxins on organelle morphology and function.

CHARACTERIZATION OF FUSARIUM HEAD BLIGHT-RESPONSIVE GENES IN DIVERSE WILD AND CULTIVATED BARLEY

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ABSTRACT

Wild (*Hordeum vulgare* subsp. *spontaneum*) and cultivated barley (*Hordeum vulgare*) accessions offer varying degrees of resistance to Fusarium head blight (FHB). Integration of resistance from multiple diverse sources has the potential to extend resistance durability, ultimately helping barley producers control FHB. To identify genetically diverse barley lines carrying FHB resistance, DArT markers were used to genotype 102 wild or cultivated barley lines (80 FHB-resistant and 22 FHB-susceptible). Two major clades were identified: one comprised entirely of resistant, wild barley, the other containing resistant or susceptible, wild and cultivated barley. Multiple wild and cultivated lines, including parents of mapping populations, were selected from across these major clades for haplotype analysis. Previous GeneChip experiments have identified over 100 barley genes with significantly up-regulated transcript levels in response to treatment of *Fusarium graminearum* or DON. Forty-four of these genes, including those implicated in defense responses such as P450s, glutathione-S-transferases, and UDP-glucosyltransferases, are being sequenced from the diverse barley lines and analyzed for haplotype differences. Initial screens suggest the lack of a "golden ticket" haplotype associated with resistance. For example, analysis of a member of the UDP-glucosyltransferase gene class responsible for DON detoxification reveals multiple haplotypes, with no haplotype solely associated with all resistant or all susceptible lines.

UNRAVELING THE TRITICEAE-*FUSARIUM GRAMINEARUM* INTERACTION

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ABSTRACT

Little is known about the wheat/barley-*Fusarium graminearum* interaction and the genes and mechanisms that exhibit host plant resistance/susceptibility. We used RNA profiling to examine gene expression patterns in barley and *F. graminearum* during infection, the differential responses between resistant and susceptible barley and wheat genotypes during infection, and during the accumulation of trichothecenes in barley. Our results revealed a complex interaction between the hosts and *F. graminearum* and provided an opportunity to develop models for the interactions and identify genes that may play a role in resistance/susceptibility. We proposed that barley responds to trichothecene accumulation through two responses: one that increases the susceptibility of barley to infection through the induction of cell death responses, and another that provides increased resistance through induction of genes encoding trichothecene detoxification and transport processes. Recently, we have begun to examine the interaction between barley and the trichothecene deoxynivalenol (DON). Our results showed that DON is transported from the site of inoculation and is converted into DON-3-O-glucoside to reduce toxicity. Using a set of RNA profiling data of barley inoculated with DON, we identified a set of barley UDP-glucosyltransferases that we are functionally characterizing. Overexpression of one of the UDP-glucosyltransferases in *Arabidopsis* increased tolerance to DON.

HOST FACTORS CONTRIBUTING TO RESISTANCE/ SUSCEPTIBILITY TO *FUSARIUM GRAMINEARUM*

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ABSTRACT

Fusarium head blight (FHB)/scab caused by the fungus *Fusarium graminearum* is a destructive disease of wheat and barley. Activation of salicylic acid (SA) signaling enhances resistance to *F. graminearum* in *Arabidopsis*, and constitutive overexpression of the *Arabidopsis NPR1* gene, which is a key regulator of SA signaling, enhances disease resistance in transgenic *Arabidopsis* and wheat (Makandar et al., 2006). In *Arabidopsis*, the *PAD4* and *WRKY18* genes are two other important components of SA signaling. Furthermore, constitutive overexpression of *PAD4* and *WRKY18* enhances resistance against *F. graminearum* in transgenic *Arabidopsis*. To determine if *Arabidopsis* PAD4 and WRKY18 could be utilized to enhance FHB resistance, we have generated transgenic wheat plants which express AtPAD4 from the maize Ubiquitin (Ubi) gene promoter, and have transformed wheat with a Ubi:At*WRKY18* construct to express At*WRKY18*. Silencing factors that contribute to host susceptibility to *F. graminearum* is another approach that we have taken for enhancing FHB resistance. For example, in *Arabidopsis*, a lipoxygenase (LOX) involved in the synthesis of oxidized lipids (oxylipins) was found to contribute to susceptibility to *F. graminearum*. Experiments are underway to transform wheat with RNAi constructs to silence expression of three wheat LOX genes that exhibit homology to the *Arabidopsis* LOX. Progress on these experiments will be presented.

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MAPPING OF MRP GENE AS A CANDIDATE FOR QTL '*QFHS.KIBR-2DS'* TO REDUCE DON ACCUMULATION IN WHEAT GRAINS

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ABSTRACT

The QTL '*Qfhs.kibr-2DS'*, which reduces *Fusarium* DON accumulation in wheat grains was reported on 2DS chromosome of bread wheat cv. Gamenya and Nobeokabouzu-komugi. The gene for multi drug resistance-associated protein (MRP) located on 2DS was highly expressed during FHB spreading, and it could explain the low level of DON accumulation for the QTL. The MRP was tracked down as a candidate gene constituting the *Qfhs.kibr-2DS* (Handa et. al 2008). The wheat BAC clones of Chinese Spring (CS) were screened for the MRP genes on homoeologous chromosome 2A, 2B and 2D, and their genomic sequences were analysed. Based on the ORF sequences, the full length cDNA for Gamenya (*MRP-D.g*) and Sumai 3 (*MRP-D.s*) were isolated from FHB infected spikes by PCR. The specific primer set for 4.7kb *MRP-D.g* was designed to confirm chromosomal location of the cDNA clone by using 118 lines of DH population (Sumai 3×Gamenya). It was mapped on the expected position of *Qfhs.kibr-2DS* on the chromosome 2DS as an MRP allele. Then, seven sets of 2D genome specific primers for the *MRP-D* were designed to examine their allelic variation among wheat germplasms. So far two types of the MRP alleles were identified; Chinese wheat type (ex. Sumai 3 and CS) and the others (ex. Gamenya and Nobeokabouzu-komugi). The *MRP-D.g* isolated from Gamenya which has the QTL to reduce DON accumulation was confirmed to be located on the locus of *Qfhs.kibr-2DS*.

ACKNOWLEDGEMENT

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GENETIC MANIPULATION OF SUSCEPTIBILITY TO *FUSARIUM* HEAD BLIGHT

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ABSTRACT

Site-specific mutation of genes in the model plant *Physcomitrella patens* has identified several mechanisms responsible for susceptibility to *Fusarium graminearum* (the causal agent of Fusarium Head Blight or FHB) and to *Fusarium*-derived toxins, such as DON, DAS and ZON. A major point of control is the programmed cell death (PCD) pathway. Infection with *F. graminearum* or treatment with DON or DAS causes a stereotypical PCD that is associated with the production of reactive oxygen species (ROS), nuclear condensation and DNA fragmentation, and the induction of protease and nuclease enzyme activity and gene expression. Inhibition of PCD through the disruption of genes required for PCD or through the overexpression of anti-PCD genes suppresses sensitivity to toxins and inhibits susceptibility to *Fusarium*. This suggests that PCD is an important target for the pathogen and for pathogen-derived toxins and indicates that inhibition of PCD in the host plant may be a useful strategy for controlling FHB and DON contamination. A second effective approach to reducing infection by *Fusarium* is to induce immunity in the plant. Pre-treatment of *Physcomitrella* or wheat plants with chitosan induces a resistance response that is effective against FHB. The importance and utility of this response has been demonstrated in *Physcomitrella*, where overexpression of individual components of the induced response (particularly nucleases and peroxidases) confers enhanced resistance to FHB infection. Moreover, gene knockouts of the CEBiP chitosan receptor are no longer able to mount an effective induced response, suggesting a key role for this receptor in mediating chitosan-induced immunity. A recent screen for yeast mutants with altered sensitivity to tricothecene has identified several novel cellular targets for this toxin. We are currently creating the corresponding knockouts for these genes in *Physcomitrella* so that their contribution to FHB susceptibility and infection can be assessed *in planta*. Finally, we have recently shown that exposure to toxins, as well as FHB infection, is associated with ER-stress and the Unfolded Protein Response (UPR). Suppression of this stress pathway through genetic or chemical means provides an additional and potentially useful approach to controlling this disease.

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IDENTIFYING AND CHARACTERIZING BARLEY GENES THAT PROTECT AGAINST TRICHOTHECENES

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ABSTRACT`

Our overall goal is to identify genes that play role in resistance to Fusarium Head Blight (FHB) and to develop and test transgenic wheat carrying these genes. In particular, we are interested in identifying genes that protect barley and wheat from the effects of trichothecenes. Previously, we conducted a large array of RNA profiling experiments during *Fusarium graminearum* infection of barley and inoculation with the trichothecene deoxynivalenol (DON). We identified a set of potential resistance genes that respond to trichothecene accumulation. The potential resistance genes encode a proline-rich like protein, a Bowman-Birk type trypsin inhibitor, a NB-ARC domain containing protein, a cysteine synthase, a NF-X1 zinc finger protein, and UDP-glucosyltransferases. We are using virus-induced gene silencing assays to functionally test these genes for their role in FHB resistance/susceptibility in wheat. The NF-X1 gene functions as a negative regulator of trichothecene-induced defense response in *Arabidopsis*. Wheaton and Bobwhite inoculated with VIGS-NF-X1 constructs exhibited statistically significant reduction in disease severity during the early stages of disease development compared to the empty vector VIGS control lines ($P<0.05$). From our RNA profiling experiments, we identified nine barley UDP-glucosyltransferases and cloned five full-length cDNAs for testing in yeast. We identified a barley UDP-glucosyltransferase gene that exhibits DON resistance based on the yeast assay. As a proof of concept, we generated transgenic *Arabidopsis* over expressing the barley UDP-glucosyltransferase and tested these plants for their ability to grow on media containing DON. After 4 weeks of growth on DON-containing media, the wild-type seedlings were albino and had ceased growing. Shoot and root growth were not inhibited in the UDP-glucosyltransferase overexpression lines grown on media containing 10, 15 and 20 ppm of DON, demonstrating that overexpression of UDP-glucosyltransferase in transgenic *Arabidopsis* protects plants from the deleterious effects of DON. Currently, we are developing transgenic wheat plants upregulating this UDP-glucosyltransferase gene.

EFFORTS TOWARD DISSECTING 2H- FHB QTL WITH TRANSPOSONS IN BARLEY

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ABSTRACT

Breeding of barley varieties resistant to FHB has been given a high priority in many areas where wheat and barley are grown, but it represents a daunting challenge for breeders due to the complex nature of resistance. It is known that the genetic basis of resistance to FHB is quantitatively inherited. Molecular mapping studies in barley indicate that two QTLs on chromosome 2H and 6H have a large effect on low kernel discoloration and could be used for marker-assisted selection for FHB resistance. It has been observed that lines having the QTL on 2H had 40% less head blight than lines that lacked this QTL, therefore warranting its detailed characterization. The maize *Ac/Ds* transposon system is an effective approach for gene identification and cloning in heterologous species. Using this system, single-copy *Ds* insertion lines (TNPs) were generated in barley to identify, tag, and determine genes and their function. Our recent successful demonstration in barley of *Ds* transposition at significant frequencies over multiple generations in addition to the preference of *Ds* to re-insert near the original site of excision and into genic regions facilitates saturation mutagenesis. Plants with single *Ds* insertions (TNPs), mapping near genes of interest, are important vehicles for gene identification through re-activation and transposition of *Ds*. Mapping and bioinformatics analysis of *Ds* flanking sequences indicate that the vast majority of *Ds* insertions (88%) are in genic regions. Our data indicate that "transposon walking", the sequential re-activation of *Ds*, can be used to identify QTLs and members of clustered gene families. We are saturating FHB related QTL regions with maize *Ds* elements to facilitate identification and characterization of genes associated with FHB resistance in the 2H-QTL. *Ds* elements in TNP lines mapped on chromosome 2H were re-activated by crossing with *Ac*TPase-expressing plants. New *Ds* transpositions have been identified by Southern blotting and *Ds* tagged genes are being cloned using inverse PCR. This effort of saturation mutagenesis with *Ds* transposons will lead to a better understanding of FHB resistance and the candidate genes that display this quantitative variation.

ASSOCIATION MAPPING OF QTL FOR FUSARIUM HEAD BLIGHT RESISTANCE IN WHEAT

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ABSTRACT

Wheat Fusarium head blight (FHB) is an important wheat disease worldwide. To identify new quantitative trait loci and validate previously reported QTLs for wheat resistance to FHB spread within a spike (type II), association mapping was conducted using a collection of 149 Asian accessions from China (120), Japan (26) and Korea (3) and 213 US elite breeding lines from three major hard wheat nurseries (SRPN, NPRN, ORPN) and two soft wheat nurseries (UESRWWN and USSRWWN) and elite breeding lines from Oklahoma State University, OK. FHB was evaluated by injecting 1000 conidiospores into a central spikelet of a spike and measuring the proportion of symptomatic spikelets (PSS) in a greenhouse of Kansas State University, Manhattan, KS. In general, Asian accessions had a relatively higher type II resistance than that of U.S. accessions. A total of 261 genome-wide SSR markers including these linked to known QTL for FHB resistance were used to analyze the population. Structure analysis clearly separated the Asian and US accessions into two groups. Separated analysis on each group identified three (Asian group) and four subgroups (US group). Simulation tests selected mixed model and K model for association computation of Asian group and U.S. groups, respectively. Eighteen markers/alleles showed significant association with FHB resistance in Asian population. Three previously reported QTLs on 3BS, 3BSc, and 5AS were validated in Asian population. Four marker alleles for 5AS QTL linked to FHB susceptibility in the Asian group suggested most of Asian accessions in this study may lack the resistance allele on 5A. Marker Xgwm276 on 7A was significant associated with FHB resistance in the Asian group, which has not been reported previously. Twelve accessions (8% in Asian group) with the Xgwm276-110 allele had a mean PSS of 0.14 that is lower than these accessions with Xgwm533-159 allele (PSS= 0.21). In the U.S.A. population, 18 alleles from 17 markers were associated with FHB resistance. Two previously reported QTLs on 3BS (Xgwm493, Xbac102) and 4D (Xbarc98, Xwmc473, Xgwm608) were validated. However, Xgwm493 and Xbarc102 showed FHB susceptible effect. Among all 17 significant markers, two markers Xcfa2263-140 (2A) and Xgwm320 -274 (2D) showed the largest effect on FHB resistance with a mean of PSS of 0.38. Therefore, the QTL on 2A and 2D are likely new QTL for FHB resistance in U.S. accessions. The results not only validated previously reported important QTL, but also discovered some new QTL. Some QTL in US lines may be different from Asian sources. Therefore, association mapping is an effective approach to study FHB resistance in wheat.

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OTHER PAPERS

THE REDESIGNED US WHEAT AND BARLEY SCAB INITIATIVE WEB SITE

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ABSTRACT

The US Wheat and Barley Scab Initiative (USWBSI) web site (http://www.scabusa.org) is an integral part of the USWBSI. The site is an important information resource for all aspects of the Initiative, including Research, News, Forums, Literature, and Contact Information. The site includes several publicly searchable databases that provide information on all Projects, Grants, Institutions, Documents, Committees, and Contacts associated with the Initiative. Due to the significant role the web site plays in the USWBSI it is continually being updated and improved to provide the latest information and services for the community. Last year a complete redesign of the web site was performed. In addition to a more intuitive general web interface, several new features and services were added for the community. These web-based applications include a photo library, discussion board, calendar of events and important dates, document management system, and many more. The system was built using the Xoops (http://xoops.org) web portal system to aid in development and maintenance. This poster provides an overview of the new features and site and solicits ideas and suggestions for future improvements to the site.

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