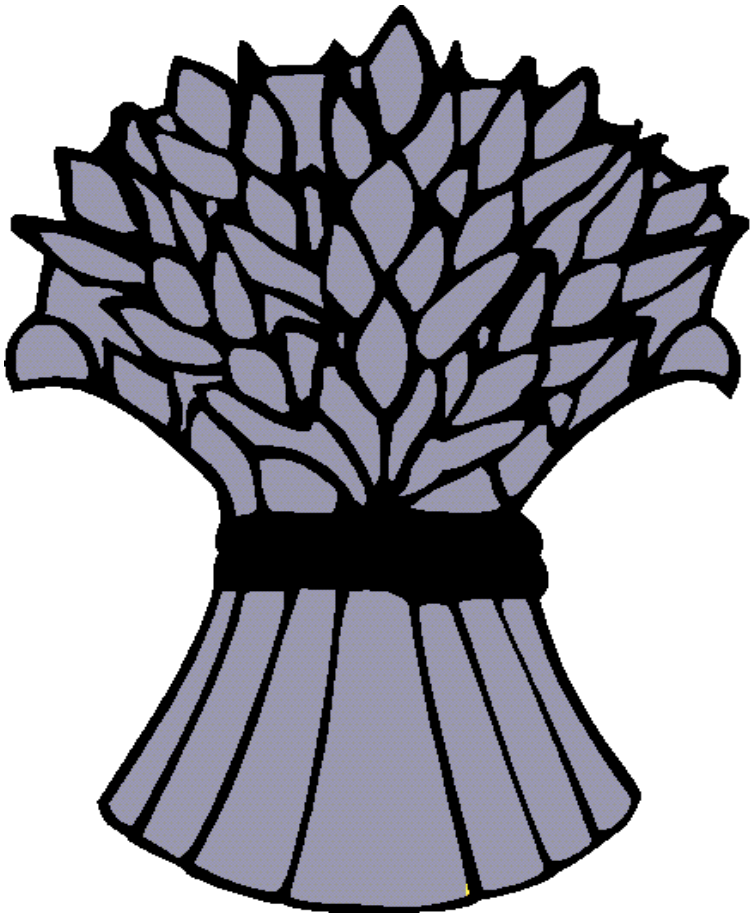
2000 National Fusarium Head Blight Forum



Holiday Inn Cincinnati-Airport
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INTRODUCTION OF A MODIFIED RIBOSOMAL PROTEIN L3 GENE AS A STRATEGY TO INCREASE TRICHOTHECENE TOXIN RESISTANCE IN PLANTS

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OBJECTIVES

The goal of our project is to identify alterations in the ribosomal target of trichothecenes, the ribosomal protein L3, which confer toxin resistance, and to explore the feasibility of increasing toxin resistance in transgenic plants by expression of engineered RPL3 genes.

INTRODUCTION

Trichothecene myctoxins act primarily as inhibitors of eukaryotic protein synthesis. Gene disruption studies indicate that production of deoxynivalenol (DON) is a virulence factor of Fusarium graminearum. The probable role of the toxin is to inhibit active defense responses of the plant by interfering with the expression of defense related proteins and to condition the host for colonization. Correlative evidence is available that toxin resistance significantly contributes to field resistance of wheat. We used yeast as a model system to explore molecular mechanisms responsible for trichothecene resistance and identified semidominant mutations in the gene encoding ribosomal protein L3. Transgenic tobacco was used as a model to test the feasibility of increasing toxin resistance in plants by introducing a modified copy of RPL3.

MATERIALS AND METHODS

In order to save expensive toxin, all yeast strains employed contain a disruption of the gene PDR5, which encodes a plasma membrane localized ABC transporter protein with specificity for trichothecenes (Adam & Lemmens, 1996). For the shuttle mutagenesis of yeast RPL3 a strain was constructed containing a deletion of the chromosomal RPL3 gene. This strain (YZGA315) is viable on galactose medium due to a plasmid allowing expression of RPL3 under control of the glucose repressible GAL1 promoter. A plasmid, which contains a wildtype copy of RPL3 and the TRP1 and ADE2 genes as selectable markers, was mutagenized by passage in the E. coli mutator strain XL1-Red (Stratagene) and by hydroxylamine-mutagenesis. Upon transformation of YZGA315, colonies were selected on glucose based medium and transferred to plates containing 100 ppm DON. Plasmid was recovered from resistant colonies, and the alterations responsible for resistance identified by subcloning and sequencing.

A tomato cDNA clone LeRPL3 was isolated from a phage lambda cDNA library and sequenced. One of the alterations identified in yeast was introduced into the gene by overlap

extension PCR. Furthermore, a c-Myc epitope was added at the C-terminus. The constructs were cloned into a binary plasmid behind the 35S promoter and introduced into tobacco by Agrobacterium-mediated transformation. Transgenic plants were characterized by Southern and Western blotting and tested for alterations in toxin resistance using seed germination, leaf disk regeneration and a gravitropism assay.

RESULTS AND DISCUSSION

Semidominant mutations in RPL3 (formerly known as TCM1) conferring resistance to the trichothecene trichodermin had been described previously (Fried & Warner, 1981), but the nature of the mutation was not determined. We have performed a random mutagenesis of yeast RPL3 and characterized 100 plasmids conferring resistance to DON. Five single amino-acid alterations in four different positions of the protein which lead to DON resistance were identified by subcloning and sequencing. In addition, we have also identified the amino-acid change in two of the original TCM1 mutants. One of the changes (W255C in ScRPL3) was engineered into the highly homologous tomato RPL3 cDNA and introduced into yeast and tobacco.

The tomato gene, without and with a C-terminal epitope tag is able to complement a yeast mutant containing a deletion of RPL3. In addition, the engineered version of the tomato cDNA also confers DON resistance in yeast. Subsequently we have introduced the wildtype and the engineered versions of LeRPL3, with and without the c-Myc tag into tobacco.

Characterization of the several transgenic plants in different assays (seed germination on toxin containing medium, regeneration of leaf disks, gravitropism response in the presence of DON) revealed that, at best, a disappointingly small increase in toxin resistance could be achieved. Analysis of protein extracts of the transgenic plants furthermore showed that, in contrast to the wild type protein, only traces of the tagged Rpl3p containing the mutation are detected. Our hypothesis is that the mutant RPL3 protein has a disadvantage during assembly into the ribosome, and that this protein is rapidly degraded. A similar phenomenon was observed in yeast heterozygous for RPL3wt/RPL3W255C. Despite being present in unstressed yeast in a low amount, the mutant form of the protein confers semidominant resistance. Pretreatment of yeast with sub-inhibitory amounts of DON leads to a rapid accumulation of the mutant form of the protein and dramatically improved toxin resistance.

In summary, we have identified several mutations in the ribosmal target that could become potentially useful in the screening of wheat germplasm for natural ribosomal resistance (Miller & Ewen, 1998) and for biotechnological approaches. Yet, our results also suggest that the prospects to achieve a marked increase in toxin resistance by simply introducing a modified RPL3 gene into wheat are not very good. Wheat contains most likely six RPL3 genes contributing to the pool of Rpl3p – a highly competitive situation.

We can only speculate whether or not an engineered Rpl3 protein - or a naturally existing variant conferring higher resistance - would accumulate in wheat tissue (with rapid synthesis of ribosomes), to give biologically meaningful resistance, as in the case of the unicellular yeast model. It is conceivable that such an effect takes place in the low concentration range of the gradient of toxin diffusing ahead of the fungus.

In an optimistic scenario, this could still be sufficient to allow adaptation, defense gene expression and containment of the spreading fungus, without leading to a prominent resistance phenotype in in vitro assays based on challenge with toxin levels blocking protein synthesis immediately. Further research is needed to answer this question.

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REFERENCES

Adam, G. and M. Lemmens: Involvement of ABC-transporter proteins in trichothecene resistance. Conference Abs B3, 8th International Congress on Molecular Plant-Microbe Interactions, Knoxville, TN, July 14-19, 1996

Fried, H.M. and Warner, J.R. (1981): Cloning of yeast gene for trichodermin resistance and ribosomal protein L3. Proc. Natl. Acad. Sci. USA 78: 238-242

Miller, J.D., and Ewen, M.A. (1998): Toxic effects of deoxynivalenol on ribosomes and tissues of the spring wheat cultivars Frontana and Casavant. Nat. Tox. 5: 234-237

REDUCED VIRULENCE OF FUSARIUM GRAMINEARUM MUTANTS DEFICIENT IN TRI101: TRANSACETYLASE ACTIVITY

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ABSTRACT

Fusarium graminearum, the causative agent of wheat head blight, produces the trichothecene deoxynivalenol (DON). DON acts as a protein synthesis inhibitor and is a significant factor contributing to the disease. However, acetylation of the C-3 hydroxyl group of a number of different trichothecenes reduces the toxicity to yeast as well as to the single-celled plant, Chlamydomonas, suggesting that the C-3 OH site is critical for toxicity. Since the TRI101 gene in F. graminearum encodes a 3-O-acetyltransferase, this gene may function for self-protection against DON and its intermediates. Disruption of the FgTRI101 was done to test if, and to what extent, this gene is involved in self-protection. The degree of virulence on wheat by mutants deficient in TRI101 activity has also been evaluated. FgTRI101 was cloned from Gz3639 into the vector pT7Blue3. The selectable marker, hygromycin B, as well as its promoter, was inserted into an EcoRI site, located approximately in the middle of the 1.4 kb DNA sequence encoding FgTRI101. Disruption was verified by both PCR and Southern hybridization. Fungal disruptants were tested on toxin-containing medium to determine if the mutants were more sensitive than the wild type strain. They were also analyzed to determine what intermediates in the pathway they synthesized. Previous work in our labs has shown that F. sporotrichioides mutants carrying a disrupted FsTRI101 gene accumulate isotrichodermol, a compound shown by us to be toxic to Chlamydomonas. However, TRI101-disrupted mutants in F. graminearum do not accumulate the C-3 hydroxylated compound and instead accumulate the C-3,8 hydroxylated compound, which has been shown to be less toxic in our Chlamydomonas toxicity tests. To test whether FgTRI101 disruptants were less virulent than wild type, wheat virulence testing in the greenhouse showed that the fungal TRI101 disruptants produced far less disease than the wild type. Our results support the conclusion, that in F. graminearum, FgTRI101 plays a role in self-protection but is not the sole mechanism. Disruption mutants are weakened in their ability to infect and cause disease in wheat, most likely because they do not produce large quantities of a C-3 OH toxic byproduct.

CHARACTERIZATION OF WHEAT PR-PROTEINS CDNA'S FOR TRANSFORMATION OF WHEAT TO ENHANCE RESISTANCE TO SCAB

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ABSTRACT

Chitinases and b-1,3-glucanases are important components of plant defense in response to attack by pathogens. To identify specific chitinases and b-1,3-glucanases involved in plant defense, a cDNA library was constructed using mRNA from wheat spikelets inoculated with conidia of Fusarium graminearum. Two chitinase and two b-1,3-glucanase clones were isolated using a rice class I chitinase and barley class II chitinase cDNA clone and a barley b-1,3-glucanase as probes. Northern blot hybridization showed that the expression of these genes is induced upon infection with Fusarium graminearum. The accumulation of transcripts for these PR-proteins is more rapid in the resistant variety Sumai 3 than its susceptible mutant during the first 24 hrs after infection. Spring wheat, 'Bobwhite', a scab-susceptible cultivar was transformed with pAHC20 vectors carrying the bar gene and the gene of interest under the control of maize ubiquitin promoter-intron. The primary transgenic lines were characterized based on PCR detection for bar gene, gene(s) of interest and western blot analyses. The integration and inheritance of the transgene and the bar gene was detected in the T1 progenies based on Southern hybridization with target gene and PCR analyses. Liberty (0.1%) painting in the young leaves confirmed the expression of the bar gene and the western blot analysis confirmed stable expression of the transgene. Several transgenic lines containing single or different combinations of PR-proteins have been identified and are being propagated.

INTRODUCTION

Plants express a wide variety of genes in response to pathogen/pest infection. Such genes are referred to as pathogenesis-related (PR) genes (Bowles 1990). The best characterized genes belonging to this group are those that encode the hydrolytic enzymes, chitinases (EC 3.2.1.14) and b-1,3-glucanases (EC 3.2.1.39). These hydrolytic enzymes inhibit the growth of many fungi in vitro by hydrolyzing chitin and b-glucan of fungal cell walls and the digestion products of chitin and b-glucan can act as signal molecules to stimulate further defense responses. Wheat scab, caused by Fusarium graminearum, can be a devastating disease that not only lower grain yield, but also adversely affects the grain quality as well, as a result of the accumulation of toxins. A cDNA library of mRNA isolated from scab-infected 'Sumai 3' a scab-resistant variety was constructed, and cDNA clones encoding chitinases and glucanases were isolated using rice and barley clones for chitinase and b-1,3-glucanase as probes.

The advancements and refinement in the plant transformation protocol for monocot crops including wheat (Zhang et al. 1999; Chen et al. 1999; Gaunt et al. 1999) have increased the efficiency of introducing foreign and agronomically important genes into wheat. Naturally occurring antifugunal genes (PR-proteins) from plants and other microrganisms are documented to enhance the level of disease resistance in wheat (Chen et al. 1999; Clausen et al. 2000). The application of plant transformation protocol to introduce environment-friendly defense gene(s) into crop plants is preferable to unsustained use of fungicides to control wheat scab. In this report we document the transfer and constitutive expression of wheat defense genes isolated and characterized from a scab-resistant cultivar for scab resistance in wheat.

MATERIALS AND METHODS

Isolation, mapping and characterization of the PR-proteins

GZ3639, a highly virulent isolate of F. graminearum, was used at a final concentration of 1.8x105 spores ml-1 to inoculate Sumai 3 (c'Funo' x 'Taiwan Wheat') a scab resistant cultivar and its mutant susceptible variety. The cDNA library construction and screening was described by Li et al. (2000). The first 60 RILs of the ITMI population and 56 F2 plants of the Ae. tauschii population were used for genetic mapping. Base maps were constructed (Li et al. 1999) for placement of chitinase and glucanase genes. The positive clones were cloned in the T- vector and the plasmid DNA from these were used as templates for sequencing. Open reading frames (ORF's) and amino acid sequences were deduced using the ORF Finder program, and homology searches were conducted using the BLAST 2.0 program of the National Center of Biotechnology Information (NCBI) at the website http://www.ncbi.nlm.nih.gov. The theoretical isoeletrical points, molecular weights, and cleavage sites of signal peptides were analyzed with "DNA & Protein Analysis Toolkit" at the website http://www.rockefeller.edu/rucs/toolkit/toolkit.html.

Wheat transformation

The plant transformation vectors harboring the wheat chitinase(s) and b-1,3-glucanase(s) genes were generated by ligating the chitinase and glucanase coding region with a maize ubiquitin promoter-intron DNA and the linearized pAHC20 vector (Christensen et al. 1992) with the selectable marker, bar gene. Immature embryos of the spring wheat cultivar, 'Bobwhite', were co-transformed with these vectors using the particle inflow gene gun. The DNA's were coated on tungsten particles and different combinations of defense genes were employed in the co-transformation experiments. The primary transformants were selected on glufosinate plates (5 mg/l) and the regenerated T0 plants were subjected to PCR detection for bar gene and the gene(s) of interest using gene specific primers. Western blot analyses were carried out using appropriate antisera. Approximately 12.5 mg total genomic DNA from each T1 plant was digested with Hind III (80 U) and probed with 32P-labelled bar gene and the transgene fragments by Southern hybridization. Liberty [0.1% (w/v)] painting was carried out on the young leaves for confirming the stable expression of the bar gene in the T1 plants.

RESULTS AND DISCUSSION

Four clones, SM169, SM194, SM233 and SM383 were identified when 400 clones of the cDNA library were screened using a mixture of the inserts of clones Chi11 (rice) and HvChtN12 (rice) as probes. SM194 and SM383 contain cDNA inserts of 956 bp and 1088 bp, respectively. SM194 and SM383 are similar to SM169 and SM233 in insert sizes, respectively. Two cDNA clones, SM289 and SM638, were isolated by screening 880 clones of the cDNA library using the barley b-1,3-glucanase cDNA clone BH72-I1 (barley) as the probe. The cDNA inserts in SM289 and SM638 are 1269 bp and 1439 bp, respectively. The details of these clones are presented in Table 1.

Southern analysis of nullitetrasomic (NT) lines with cDNA inserts of the chitinase clones, SM194 and SM383, gave identical hybridization patterns and produced about 10 bands (EcoRI digest). All the bands were localized to group 2 chromosomes of wheat. Four polymorphic and two monomorphic bands were detected by SM194 and SM383 in the Ae. tauschii parents. They were mapped to the long arm of chromosome 2D at a position between markers Xwg405 and Xpsr102, which appear to be genetically close to the centromere (Boyko et al. 1999).

Analysis of NT lines assigned the b-1,3-glucanase genes, SM289 and SM638, to group 3 chromosomes just as BH72-I1, the probe used for their isolation. Genetic mapping localized SM289 to 3BL and 3DL (Xksu933 (Glb3) and SM638 to 3DL (Xkus934 (Glb3) of wheat. These loci map very close to the position of b-1,3-glucanase loci (Glb3) detected by BH72-I1 (Li et al 1999). This result not only confirmed the presence of a b-1,3-glucanase gene cluster in 3BL, but also revealed another cluster of b-1,3-glucanase genes in 3DL of wheat.

Table1: Characteristic properties of the wheat cDNA clones

Clone name	Type of PR-protein	Size of cDNA insert (bp)	Size of polypeptide (ORF)	Molecular weight
SM194	Chitinase (type VII)	956	230 AA	24.7 kDa
SM383	Chitinase (type IV)	1082	272 AA	29.03 kDa
SM289	β-1,3-glucanase	1269	334 AA	34.88 kDa
SM638	β-1,3-glucanase	1439	334 AA	34.66 kDa

Northern blot hybridization showed that the expression of the chitinase and b-1,3-glucanase genes characterized in this study is induced in wheat spikelets upon infection with F. graminearum. The transcription profiles of these genes were different in the scab-resistant cultivar Sumai 3 and the scab-susceptible mutant indicating their role in disease resistance. The expression levels at three time points studied follow the pattern: 24 hai³48 hai>72 hai in Sumai 3, but in the mutant, the pattern generally is 24 hai<48 hai>72 hai. The transcripts of both chitinase and b-1,3-glucanase genes reached the maximum point at or before 24 hai in Sumai 3 while in the mutant, the peak values were not reached until 48 hai or later indicating a slower defense response in the mutant (Fig. 1). For SM194, a larger transcript was detected clearly at 24 hai in Sumai 3, but it was still very weak at 48 hai in the mutant. This messenger might be transcribed from another member of class VII chitinase genes. These results suggest that the mutant may be defective in a common step leading to the induction of both chitinases and b-1,3-glucanases and they are in agreement with results reported recently by Pritsch et al. (2000) who showed that several PR-protein genes were induced earlier in Sumai 3 than the susceptible (non-isogenic) genotype, 'Wheaton'.

Bobwhite embryo's were bombarded with tungsten particle coated with plasmid DNA's harboring the wheat chitinase (pAHC 194; 383) and b-1,3-glucanase (pAHC 289; 638) singly and in combination with the ubiquitin promoter driving the expression of both the selectable marker and the gene(s) of interest. The bombarded embryogenic calli were grown on glufosinate at 5 mg/l and the selection pressure was maintained till plants were regenerated. In all, 38 primary transgenic lines were raised and they were subjected to PCR analyses for the presence of bar gene. Twenty-three of the bar positive lines also showed the presence for the gene of interest by PCR. The results from five lines are shown in Fig. 2. Protein extracts from these lines reacted with appropriate antisera for chitinase and glucanase and the expected protein bands of 26.5 or 29 kDa was detected in western blots. However, the level of expression varied among different events as shown in the Fig. 3. Some lines expressed both the chitinase and the b-1,3-glucanase (lines #32, #44 and #58).

Only eight of the 23 lines were included in the current analyses, the analyses of the remaining 15 lines are in progress. Five seeds were germinated from each independent line for raising the T1 progeny. All the T1 progenies were painted with 0.1% Liberty at the stem elongation stage and checked for Liberty-resistance. The sensitive plants showed browning of tissues and clearing of chlorophyll in the painted areas of the leaf within five days, while the painted areas of the resistant lines remained green. In all, 4 out of the 8 primary transgenic lines tested were Liberty- resistant.

The inheritance of the intact transgenes was confirmed by PCR for bar gene and the gene(s) of interest in the T1 progeny. The total genomic DNA from 5 different T1 plants representative of each transgenic line were digested with the HindIII enzyme which is expected to release the 3.1-3.3 kb fragment of the intact coding region of the transgene plus the promoter. Non-transgenic and Liberty-sensitive plant was used as the negative control. In the Southern blots, DNA from transgenic plants had the expected band for bar, 383 chitinase, and 638 glucanase genes when probed with the 570 bp fragment coding region of bar gene, or the 1.1-1.3 kb fragments containing the intact coding region of the 383 and 638 transgenes respectively (Fig. 4a&b). DNA from the non-transgenic and Liberty-sensitive plants exhibited no hybridization to bar coding region fragment. No bands of 3.1-3.3 kb

size expected for the intact trasngene was detected in the non-transgenic controls, even though other bands, presumably wheat chitinase and b-1,3- glucanase genes, were detected in all lines including non-transgenic controls.

These four lines were further assayed for the stable expression chitinase and glucanase. Crude protein extracts from the 5 plants each from the representative lines showed differences in the level of expression, with a few PCR and Southern positive lines showing comparable levels of transgene expression in the T1 progeny. The highest expression levels were seen in plants T1-32-1, 32-2 and 26-5. The line 26-5 which is a singly-bombarded line with the 383 chitinase gene had the expected 26.5-kDa protein band that reacted with a barley chitinase antibody. The lines 32-1 and 32-2 that were cobombarded with the 383 chitinase and 638 glucanase genes showed strong reaction with the chitinase and glucanase antibodies and gave the expected protein products of 26.5 and 29 kDa. This confirmed the stable and constitutive expression of the transgenes in these progenies. The T2 progenies of these lines will be assayed further and bioassay with the test pathogen will be done to confirm whether these genes have enhanced the level of resistance in wheat.

REFERENCES

Bowles DJ (1990) Defense related proteins in higher plants. Ann Rev Biochem 59: 873-907

Boyko EV, Gill, KS, Mickelson-Young L, Nasuda S, Raupp WJ, Ziegle JN, Singh S, Hassawi DS, Fritz AK, Namuth D, Lapitan NLV and Gill BS (1999) A high-density genetic linkage map of Aegilops tauschii, the D-genome progenitor of bread wheat. Theor Appl Genet 99: 16-26

Chen WP, Chen PD, Liu DJ, Kynast R, Friebe B, Velazhahan R, Muthukrishnan S and Gill BS (1999) Development of wheat scab symptoms is delayed in transgenic wheat plants that constitutively express a rice thaumatin-like protein gene. Theor Appl Genet 99: 755-760

Christensen HA, Sharrock AR and Quail HP (1992) Maize polyubiquitin genes: structure, thermal perturbation of expression and transcript splicing, and promoter activity following transfer to protoplasts by electroporation. Plant Mol Biol 18: 675-689

Clausen M, Krauter R, Schachermayr G, Potrykus I and Sautter C (2000) Antifungal activity of a virally encoded gene in transgenic wheat. Nature Biotechnology 18: 446-449

Guant RS, Riley A, Barcelo P and Lazzeri PA (1999) Analysis of particle bombardment parameters to optimise DNA delivery into wheat tissues. Plant Cell Reports 19: 118-127

Li WL, Faris JD, Muthukrishnan S, Liu DJ, Chen PD and Gill BS (2000) Isolation and characterization of novel cDNA clones of acidic chitinases and. b-1,3- glucanases from wheat spikes infected with Fusarium graminearium. Theor Appl Genet (In Press)

Li WL, Faris JD, Chittoor J, Leach JE, Liu DJ, Chen PD and Gill BS (1999) Genomic mapping of defense response genes in wheat. Theor Appl Genet 98:226-233

Pritsch C, Muehlbauer GJ, Bushnell WR, Somers DA and Vance CP. (2000) Fungal development and induction of defense response gene during ear infection of wheat spikes by Fusrium graminearum. Molecular Plant-Microbe Interatction 13:159-169

Zhang L, Rybczynski JJ, Langenberg WG, Mitra A and French R (1999) An efficient wheat transformation procedure: transformed calli with longer morphogenic potential for plant regeneration. Plant Cell Reports 19: 241-250

Fig.1. Northern blot for the expression of chitinase (SM194 and SM383) and the β-1,3- glucanase (SM289 and SM638) in the scab-resistant variety Sumai 3 and its susceptible mutant.

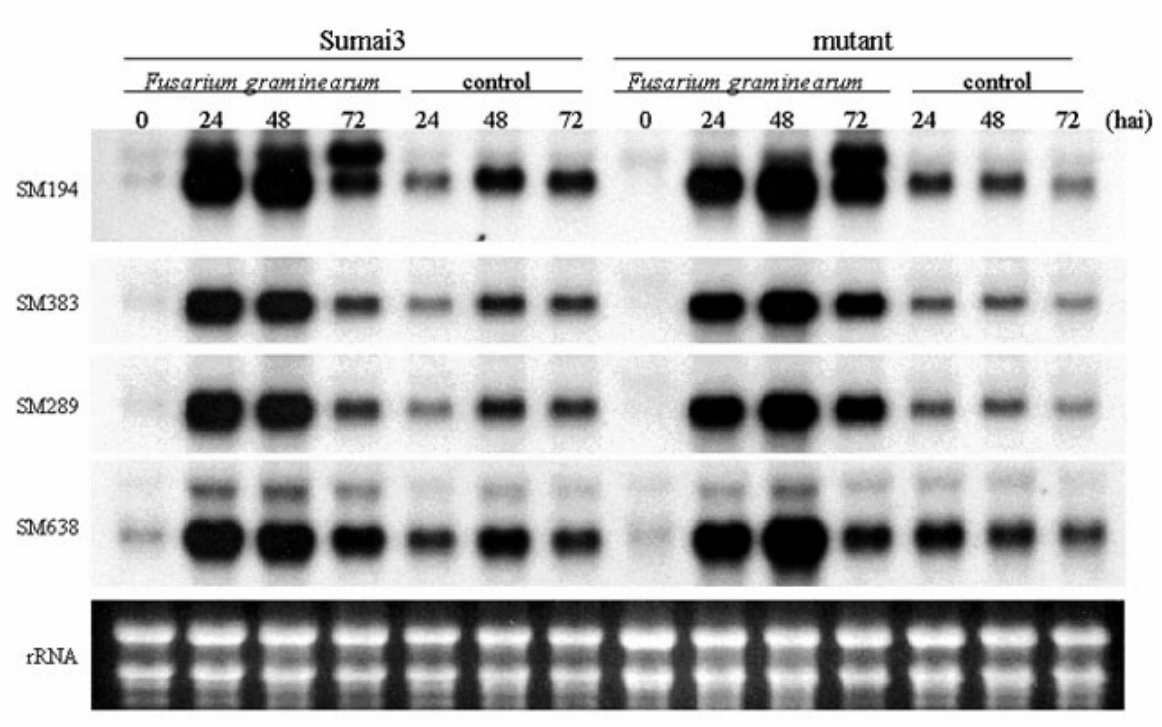
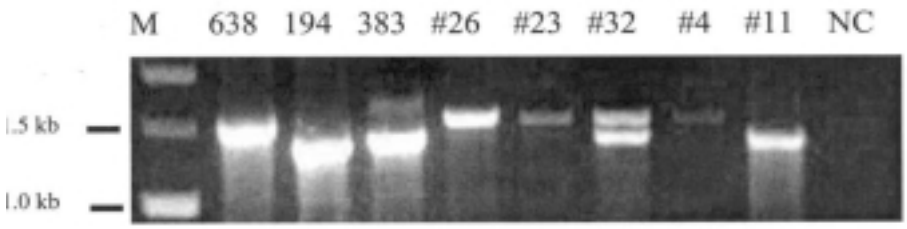
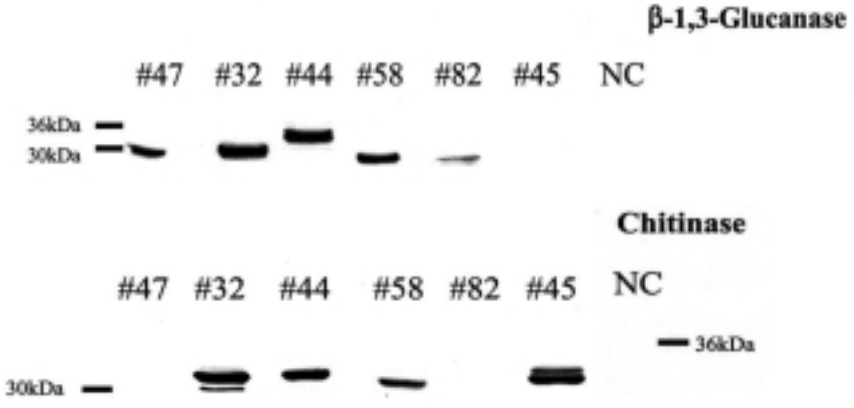


Fig. 2. PCR detection of the transgene(s) in the primary transgenic lines



#32: cobombarded with 2 gene, #26, #23 #4 & #11: with single gene insert; 638; 194; 383: plasmid controls;NC: non-transgenic plant

Fig. 3. Western blot of the putative transgenic lines expressing different PR-proteins

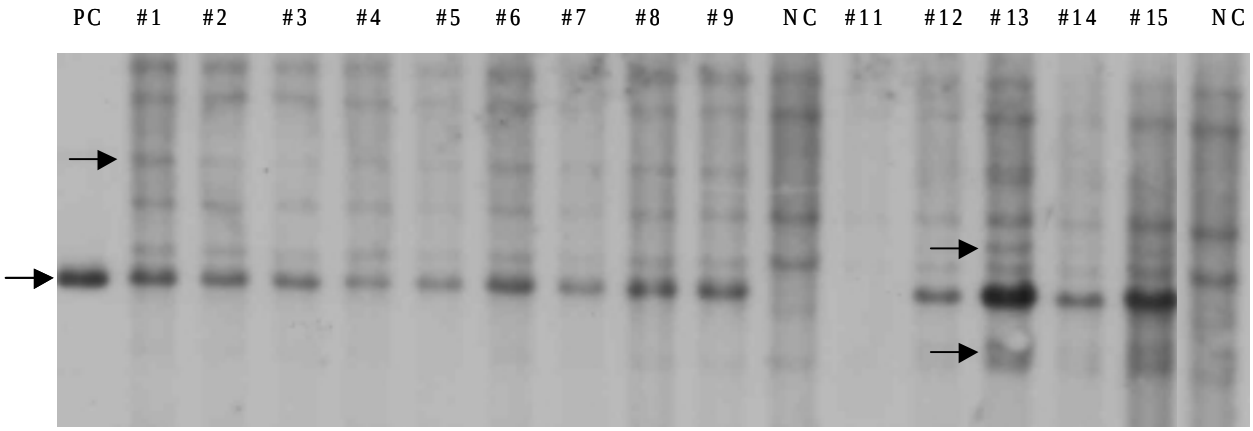


#47, #32, #58: 383:638; #82: 638:194; #45:194:383 ;#44: 289:383; NC: non-transgenic plant

194, 383: wheat chitinase; 289, 638: wheat glucanase

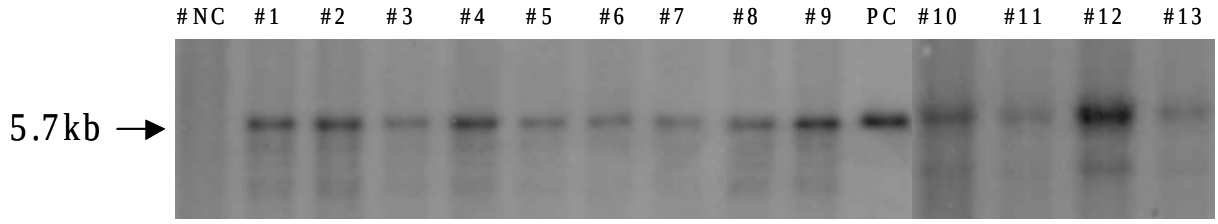
Fig. 4. Southern blot analysis of DNA from T_1 progenies of plants # 32 and # 26

a) DNA blot hybridized with 383 chitinase probe



PC: plasmid control, #1to #9: progenies of line 32, #11 to #15: progenies of line 26 and NC: non-transgenic plant. Arrows indicate the bands due to transgene.

b) DNA blot hybridized with *bar* probe



PC: plasmid control, # to #9: progenies of line 32, #10 to #13: progenies of line 26 and NC: non-transgenic plant.

MOLECULAR MAPPING OF A QTL FOR DEOXYNIVALENOL TOLERANCE IN WHEAT

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ABSTRACT

Deoxynivalenol (DON, a mycotoxin) produced by *Fusarium graminearum* in wheat grain is detrimental to livestock and humans. Significant differences in DON levels of infected grain are observed among wheat cultivars after infection with the fungus. However, genetic control of DON accumulation in wheat is not well characterized. To map quantitative trait loci (QTL) for low DON level in infected wheat grain, an amplified fragment length polymorphism linkage map was constructed using F9 recombinant inbred lines (RILs). The mapping population was derived from a cross between cultivars Ning 7840 (7 ppm of DON) and Clark (151 ppm of DON). Flowering spikes of F_{11} RILs were sprayed with conidia of *F. graminearum* in the greenhouse. The inoculated spikes were harvested at maturity, and the kernels were analyzed for DON levels with a fluorometric quantification method. One QTL for low DON level was identified, which explained about 23 and 26% of the phenotypic variance in two repeated experiments, respectively.

INTRODUCTION

Scab of wheat, mainly caused by *Fusarium graminearum*, can dramatically reduce grain yield and quality. Infected grain is often contaminated with deoxynivalenol (DON), a potent mycotoxin produced by *F. graminearum*. Because DON contamination in infected grain is a major concern for human and animal health, scab has become an especially serious disease in many wheat and barley production areas.

DON levels in harvested grain can vary greatly among wheat cultivars, ranging from a trace to more than 1000 ppm depending on wheat cultivar and environment. However, the genetic control of DON production by *Fusarium* species in wheat grain is largely unknown. Ning 7840 is a highly scab-resistant spring wheat cultivar with low DON, and Clark is a highly susceptible winter wheat cultivar with a high level of DON accumulation in infected grain. One major QTL for Type II scab resistance has been identified (Bai, et al, 1999). In this study, we use an AFLP map to identify QTL for low DON level in scab-resistant wheat cultivar Ning 7840.

MATERIALS AND METHODS

Wheat cultivars Ning 7840 (low DON) and Clark (high DON) as well as their 133 recombinant inbred progenies (Bai et al, 1999) were inoculated by spraying conidia (500 spores / mL) of a DON-producing strain of *F. graminearum* onto flowering spikes in the greenhouse at the National Center for Agricultural Utilization Research, USDA, Peoria, IL. The DON-

producing isolate “GZ 3639” was provided by Dr. R. Bowden from Kansas State University. Mung bean liquid medium was used to produce conidial inoculum (Bai et al, 1999). The inoculated plants were enclosed in an inoculation chamber for 3 days on a greenhouse bench. The plants inside the chamber were misted daily with tap water by a hand sprayer. On the fourth day after inoculation, plants were returned to their original positions on the greenhouse benches. Greenhouse temperatures averaged 25 °C during the day with a range of 19 °C to 30 °C and 19 °C at night with a range of 17 °C to 21 °C. The greenhouse test was conducted in a completely randomized block design with two replications. Each replication had 6 plants. Disease symptoms were recorded as percentage scabbed spikelets at 6, 15, and 21 days after inoculation. Visual scab ratings were analyzed on a single plant basis. DON levels were evaluated for bulked seeds from the 6 inoculated plants of each entry in each replication.

Bulked seeds from inoculated plants were ground for DON analysis. Seeds from uninoculated spikes of selected lines were also analyzed as a control. The total ground sample was extracted by shaking with 5-mL acetonitrile/water (86:14, v/v) per gram of sample for 3 h. The extract was filtered into a vial using Whatman filter paper (Whatman International, Ltd, Maidstone, England). A fluorometric quantitation method (The Fluoroquant D Test Kit, Romer Laboratories, Inc., Union, Missouri) was used to analyze DON content in harvested grain. The detection limit for DON was 1 ug g^{-1}.

Amplified fragment length polymorphism (AFLP) markers were analyzed as described previously (Bai, et al, 1999). An AFLP linkage map was constructed with MapMaker software (Lander, et al, 1987) and QTL analysis was conducted with qGene software (Nelson, 1997).

Results and Discussion

Recombinant inbred lines differed in percentage of scabbed spikelets and incidence of seed infection (Table 1). Infection not only significantly reduced harvested seed number in all inoculated plants, but also the harvested seed weight. Seed weight reduction was low in Ning7840 and some resistant lines, but high in Clark and susceptible lines (Table 1).

DON was detected in all inoculated RILs, but not the control plants. A high level of DON accumulated in Clark, and a low level of DON accumulated in Ning 7840 (Table 1). In the RILs, the correlation between percentage of scabbed seed and DON content was high (r=0.72), indicating scabby seed is a good indicator for DON content. Only 4 RILs had a level of DON less than that in Ning 7840 (7 ug g-1), and about 50% of the lines had DON levels higher than in Clark (161 ug g-1, Fig 1). Transgressive segregation indicates several QTL may be involved in DON accumulation in wheat grain. Significant variation in DON levels between the two replications was also observed, indicating that replicated DON evaluation is necessary for reliable estimates of genetic effects.

A molecular map was constructed with 568 amplified fragment length polymorphism markers (Data not shown) and was used to map QTL for low DON. When DON data from the two replications were analyzed individually, one major QTL was identified in the same location in each replicate. This QTL explained about 23% and 26% of the total phenotypic variance,

respectively. When the average DON content over two replications was log-transformed and used for further QTL analysis, the QTL for low DON was mapped in the same region as in the separate analyses and explained about 25% of phenotypic variance (Fig 2). The major QTL for low DON was located in the same region as the major QTL for Type II scab resistance reported previously (Bai, et al, 1999). Since the major QTL for low DON explained only a small portion of phenotypic variance, some other QTL may also be involved in reduced DON accumulation. Spray inoculation with a high concentration of conidia provided much more inoculum and produced more severe scab than expected in natural conditions, therefore, high disease pressure in this study may have masked the expression of QTL with minor effects on DON levels in harvested grain. DON levels in infected grain from field experiments may need to be measured for further detection of other QTL.

REFERENCE

Bai G-H, Kolb FL, Shaner GE, Domier LL. (1999). Phytopathology 89:343-348.

Lander E, Green P, Abrahamson J, Barlow A, Daley M, Lincoln S, Newburg L. (1987). Genomics. 1:171-181.

Nelson JC. (1997). Mol Breed. 3:239-245.

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Fig. 1 Frequency distribution of DON contents in infected grain of F_{11} recombinant inbred lines from the cross of Ning 7840 / Clark

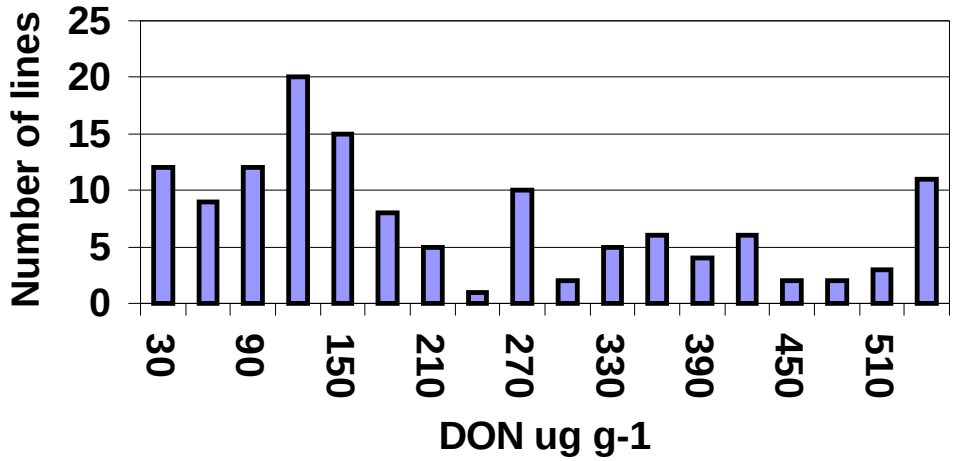
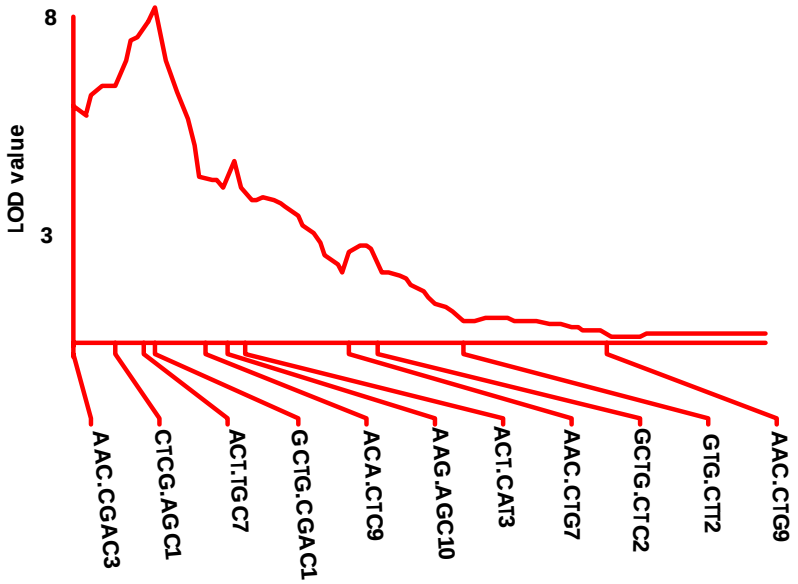


Fig. 2. LOD peak region of QTL for low DON on linkage group 7 as calculated by qGene software.



ESTABLISHMENT OF A USDA-ARS REGIONAL MOLECULAR GENOTYPING LABORATORY IN MANHATTAN, KS

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ABSTRACT

The Agriculture Research Service of USDA has set the precedent in the last century of providing leadership and services when local resources have not been available to meet national needs in crop improvement. Regional ARS laboratories have been established to improve cereal quality and rust resistance, as well as the regional germplasm repositories that distribute and characterize germplasm collected from around the world.

Molecular genotyping promises to become the next critical tool in crop breeding and the development of new germplasm. New technologies will allow molecular marker-based selection to enhance plant breeding through improved accuracy and speed of genotype identification. Gains in efficiency and novel technologies have reduced the cost of a single observation by 5 fold. Molecular breeding tools represent the technology of promise as we enter the next era of plant breeding. As markets move away from a commodity basis toward a value-defined, end product basis, plant breeders must equip themselves with gene-specific markers which give them rapid access to traits of value.

To sustain and strengthen the USDA-ARS role in solving problems of regional and national scope it is critical to incorporate molecular genotyping technology. The adoption of this technology will position the USDA-ARS to facilitate the rapid deployment critically needed genes for wheat and barley.

A regional molecular genotyping laboratory for wheat that will serve public plant breeding programs in the US is being established. Currently, many genes for important traits are currently mapped due to efforts like the International *Triticae* Mapping Initiative. These markers are waiting full utilization in current breeding programs. The laboratory will be involved in:

■ Utilizing molecular markers linked to traits of value such as end use quality and resistance to insects and to diseases like *Fusarium* head scab, rust, wheat streak mosaic virus, and Karnal bunt in agronomically relevant populations in collaboration with public pant breeding programs. The laboratory will screen populations, individuals and lines provided by breeders; determine marker genotype then report back to breeders to facilitate speed and efficiency in germplasm release.

■ Creating marker profiles of cultivars and breeding lines that are used as parents and provide this information to the plant breeders so that markers can be selected that will be

useful in a particular cross. Marker profiles will also be cross-linked to other genetic information.

This laboratory is part of the Plant Science and Entomology Research Unit of the Grain Marketing Production Research Center in Manhattan KS which has lead scientists with expertise in germplasm development, mapping, and molecular biology. The unit already has an established wheat genomics facility that is a partnership with the Wheat Genetics Resource Center of Kansas State University. The major equipment necessary (automation and analysis) for a genotyping facility has already been purchased. Yearly funding of $250,000 has been obtained a scientist, support staff, and operating costs.

GENETIC ANALYSIS OF RESISTANCE TO *FUSARIUM* HEAD BLIGHT IN COMMON WHEAT

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ABSTRACT

Fusarium head blight or scab is a devastating and economically important disease in all classes of wheat and barley worldwide. Insufficient or ambiguous knowledge concerning the amount of available genetic diversity and genetic mechanisms governing scab resistance, and lack of selectable molecular markers are constraints that greatly limit breeding efforts. Objectives of the current study are: 1) Determine the inheritance of scab resistance in three identified resistance sources; 2) Elucidate the genetic relationship between type II, III & IV resistance based on the segregation of scab severity, DON content and scabby seeds in four F_2 populations; and 3) Identify SSR molecular markers associated with type II, III and IV resistance in source W14 using F_2 population Pioneer 2684 x W14.

Three resistance sources, W14, Shaan 85 and Ernie identified in our previous studies (Griffey et al., 1998 & 1999), were crossed with susceptible soft red winter (SRW) wheat variety Madison and/or Pioneer 2684. The F_2 populations were evaluated in greenhouse tests using single floret inoculation procedures. W14 and Shaan 85 are improved type II resistance sources derived from Sumai 3 and may also possess other types of resistance. Ernie is a SRW wheat variety that lacks any of the known scab resistant sources in its ancestry (Chen et al., 2000a,b).

Two complementary genes with major effects were found to confer scab resistance in W14 and Shaan 85 based on similar segregation patterns of F_2 populations for type II, III and IV resistance characterized by disease severity, DON content and percentage of infected kernels (scabby seeds), respectively (Table 1 & 2). One to two genes were found to confer resistance in SRW wheat Ernie (Table 2). Segregation patterns observed in the four F_2 populations suggest that gene interaction is likely and may explain transgressive segregation as observed in this study and in those reported by other researchers (Buerstmayr et al., 1999 and 2000; Mesterhazy et al., 1999).

Significant positive correlations were found between disease severity (type II resistance), DON content (type III resistance), and scabby seeds (type IV resistance) based on segregation data from four F_2 populations. Highly resistant individuals with type II resistance were found to also possess type III and type IV resistance; however, about 25 % of individuals with type III and IV resistance didn't express type II resistance. Individuals with type IV resistance expressed type III resistance in most cases (Table 4).

The F_2 population Pioneer 2684 x W14 (150 individuals) was used to initiate mapping studies. Sixty-two SSR markers previously located on chromosomes 3B, 5A and 6B (Röder et al., 1998) were selected and evaluated for polymorphism between parental lines. Of the 62 SSR markers, 21 (34%) were polymorphic between resistant parent W14 and susceptible parent Pioneer 2684. Three markers, GMS389, GMS410, and GMS533, likely are associated with scab resistance and cumulatively accounted for 23, 18 and 19% of the phenotypic variance for type II, III and IV resistance, respectively in the current study (Table 3).

Correlation and regression analyses indicate that a specific association may exist between SSR markers and type of resistance (Table 3 & 4). GMS410 previously located on chromosome 5A (Röder et al., 1998) explained more of the phenotypic variance for DON production than for disease severity and scabby seeds; whereas, GMS533 explained more of the variation for disease severity and scabby seeds than for DON. GMS 389 explained more of the variation for disease severity and DON than for scabby seeds. GMS533 explained less of the phenotypic variance for resistance in the current study than in that of Anderson et al. (2001) and may be the result of multiple alleles being present at this resistance locus and/or variable linkage distances between the marker and QTL in different genetic backgrounds. This is supported by analyses of DNA polymorphism of the three markers evaluated in the current study among resistance sources Sumai 3, Funo (one of parents of Sumai 3), Shaan 85, W14, VR95B717, and SRW wheat varieties Ernie, Madison, Pioneer 2684.

Additional molecular markers, SSR and other types, will be evaluated in current and other mapping populations to identify putative QTLs associated with resistance, saturate chromosome regions associated with resistance, and develop a skeletal map. This research has a potential to identify new QTLs associated with scab resistance, provide additional markers linked to previously reported QTLs, and to identify markers that are effective across a variety of genetic backgrounds; all of which are essential for successful exploitation of marker-assisted selection.

REFERENCES

Anderson, J.A., R.W. Stack, S.Liu, B.L. Waldron, A.D. Fjeld, C. Coyne, B. Moreno-Sevilla, J. Mitchell Fetch, Q.J. Song, P.B. Cregan, and R.C. Frohberg. 2001. DNA markers for *Fusarium* Head blight resistance QTLS in two wheat populations. TAG (Accepted, in press).

Buerstmayr, H., B. Steiner, M. Lemmens, and P. Ruckenbauer. 2000. Resistance to Fusarium head blight in winter wheat: heritability and trait association. Crop Science 40: 1012-1018.

Buerstmayr, H., M.Lemmens, S.Berlakovich and P.Ruchenbauer. 1999. Combination ability of resistance to head blight caused by Fusarium culmorum (W.G.Smith) in the F1 of seven parent diallel of winter wheat. Euphytica 110: 199-206.

Chen J., C. A. Griffey, T. Pridgen, M. Chappell and J. Shaw. 2000a. Assessment and rational utilization of scab-resistant sources in the Virginia breeding program. P10-17. *In*: Proceeding of the international symposium on wheat improvement for scab resistance. May 5-11. Suzhou and Nanjing, P.R. China.

Chen J., C.A. Griffey, T.Pridgen, M. Chappell, W. Xie, and J. Shaw. 2000b. Characterization of scab (Fusarium) resistance in wheat. P91. *In*: Proceedings of the 3rd International Crop Science Congress. August 17-22. Hamburg, Germany.

Griffey C.A., J. Chen, T. Pridgen, M.Chappell and E.L. Stromberg. 1998. Assessment and selection of scab resistance in soft winter wheat. P.273-275. *In*: A.E.Slinkard (ed). Proceeding of Ninth International Wheat Genetics Symposium. Aug 2-7. University of Saskatchewan. Univ. Extension Press. Saskatchtoon, Canada.

Griffey C.A., J. Chen, T. Pridgen, M.Chappell and E.L. Stromberg. 1999. Research on *Fusarium* Head Blight in the Virginia Tech Small Grains Program. P. 95-99. In: Proceeding of 1999 Joint Research Conference of the Eastern & Southern Wheat Workers. May 2-4. Williamsburg, Virginia.

Mesterhazy A., T. Bartok, C.G. Mirocha and R. Komoroczy. 1999. Nature of wheat resistance to *Fusarium* head blight and the role of deoxynivalenol for breeding. Plant Breeding 118: 97-110.

Röder M.S., V. Korzum, K. Wendehake, J. Plaschke, M.H. Tixier, P. Leroy, and M.W.Ganal. 1998. A microsatellite map of wheat. Genetics 149: 2007-2023.

Table 1. Inheritance of scab resistance conferred by source W14 based on segregation of progeny for disease severity (%), scabby seeds (%) and DON content (ppm) in F_2 populations Madison x W14 and Pioneer 2684 x W14 assessed via floret inoculation in 1999 greenhouse tests.

	No. of Plants		Expected	χ^2	
Parents and cross	R *	S	Ratio	value	Probability
Madison x W14	64	74	7 : 9	0.2876	0.50 – 0.75
Pioneer 2684 x W14	80	126	7 : 9	1.8274	0.10 – 0.25
Madison x W14	62	76	7 : 9	0.0373	0.75 - 0.90
Pioneer 2684 x W14	115	91	9 : 7	0.0028	> 0.99
Madison x W14	83	55	9 : 7	0.6998	0.25 – 0.50
Pioneer 2684 x W14	86	120	7 : 9	0.2592	0.50 – 0.75

* R = Resistant and S = Susceptible progeny. Classification of resistant progeny was determined by the distribution and mean of the two parents, and based on the mean of resistant parents or the mean plus 0.5 to 1.5 times the standard deviation.

Table 2. Inheritance of scab resistance conferred by sources Shaan 85 and Ernie based on segregation of progeny for disease severity (%), scabby seeds (%) and DON content (ppm) in F_2 populations Pioneer 2684 x Shaan 85 and Pioneer 2684 x Ernie assessed via floret inoculation in 1999 greenhouse tests.

Parents and cross	R *	S	Expected Ratio	χ^2 value	Probability
Pioneer 2684 x Shaan 85	124	96	9 : 7	0.0012	> 0.99
Pioneer 2684 x Ernie	168	149	9 : 7	1.2342	0.25 – 0.50
Pioneer 2684 x Shaan 85	69	90	7 : 9	0.0001	> 0.99
Pioneer 2684 x Ernie	230	87	3 : 1	0.8843	0.25 – 0.50
Pioneer 2684 x Shaan 85	68	91	7 : 9	0.0289	0.75 – 0.90

*R = Resistant and S = Susceptible progeny. Classification of resistant progeny was determined by the distribution and mean of the two parents, and based on the mean of resistant parents or the mean plus 0.5 to 1.5 times the standard deviation.

Table 3. Multiple linear regression analysis for SSR markers associated with scab resistance in 150 F2 individuals from cross Pioneer 2684 x W14.

Markers	DF*	Probability			Coefficient of Determination			Favorable genotype**		
		Disease Severity (%)	Scabby Seeds (%)	DON Content (ppm)	Disease Severity (%)	Scabby Seeds (%)	DON Content (ppm)	Disease Severity (%)	Scabby Seeds (%)	DON Content (ppm)
GMS410	148	< 0.001	< 0.001	< 0.001	7.56	7.83	9.10	P	P	P
GMS389	148	< 0.001	< 0.001	< 0.001	10.53	7.35	9.50	W	W	W
GMS533a	148	< 0.001	< 0.002	0.039	9.92	6.40	3.94	W	W	W
GMS533b	148	< 0.001	0.002	0.009	10.51	6.60	4.57	W	W	W
All Makers	146	0.000	< 0.001	0.000	22.78	17.72	18.96	WP	WP	WP

* DF: Degree of freedom of error. ** W indicates W14 alleles that decrease disease severity, scabby seeds and DON content.P indicates alleles from Pioneer 2684.

Table 4. Linear correlation analysis between SSR maker data and disease data characterized by disease severity, scabby seeds and DON content in 150 F_2 individuals from population of Pioneer 2684 x W14.

				---- SSR Markers ----		
	Disease Severity	Scabby Seeds	DON Content	GMS389	GMS410	GMS533a
Scabby Seeds	0.5911**					
DON Content	0.5388**	0.8646**				
GMS389	- 0.3245**	- 0.2711**	- 0.3082**			
GMS410	0.2749**	0.2798**	0.3016**	-0.0567		
GMS533a	- 0.3150**	- 0.2529**	- 0.1984*	0.2756**	-0.055	
GMS533b	- 0.3241**	- 0.2568**	- 0.2137**	0.3077**	-0.0552	0.8089**

* Significant at P = 0.05; ** Significant at P = 0.01

EFFECTIVENESS OF MAS FOR SELECTION OF HEAD BLIGHT RESISTANCE IN SOFT RED WINTER WHEAT

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ABSTRACT

The development of molecular genome analysis tools has created an interest in the use of marker assisted selection (MAS) in applied plant breeding programs. MAS may become an important aid to plant breeders in selection of superior genotypes. Instead of using phenotypic selection for a trait, once a tight linkage has been established between the trait and the marker, the marker can be used for selection. MAS may become especially useful for traits that are difficult to assay by phenotype such as scab or head blight resistance. In this research, we will examine the effectiveness of MAS for selection of germplasm with resistance to head blight of wheat by comparing field selection with MAS using Amplified Fragment Polymorphism markers (AFLPs) in two crosses (Ning7840/Pioneer 2643 and Ning 7840/Pioneer 2684). Bai et al. (1999) have recently identified AFLP markers linked to a major QTL controlling resistance to scab present in the cultivar Ning 7840. This QTL, located on chromosome 7 B, explained up to 60% of the variation in scab resistance. AFLP markers linked to this QTL will be used to indirectly select resistance genotypes in crosses between a resistant genotype and adapted SRWW genotypes. Breeding lines will be evaluated for agronomic traits and screened for the presence of the favorable AFLP alleles. Selection progress for scab resistance using MAS will be compared with phenotypic selection. We are currently screening the wheat parental lines for AFLP polymorphism.

EXPRESSION PATTERNS OF GENES FROM A HEAD SCAB INFECTED SPIKE CDNA LIBRARY.

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ABSTRACT

The sequencing of randomly selected cDNA clones has become a rapidly growing area for the identification of genes expressed in organisms with large genomes. A cDNA library was made from wheat spikes of the variety 'Sumai 3', 24 hr after inoculation with *Fusarium graminearum*. Isolated plasmids from 864 colonies were sequenced using automated DNA analysis. Quality sequence from 799 colonies was submitted for database alignment using BLASTX and alignment analysis revealed that the library contained 580 singletons. BLASTX alignments assigned putative function to 346 of the 580 singletons. The remaining singletons were aligned with genes of unknown function or had no significant alignment to sequences in the database. Of particular interest were 30 putatively assigned defense response genes, 7 disease resistance genes, and 7 stress response genes. Nylon arrays were made of the singletons and probed with cDNA made from mRNA of Sumai 3 spikes 0, 24, and 48 hr after infection. The expression patterns will be analyzed and reported.

FINE MAPPING OF A QUANTITATIVE TRAIT LOCUS FOR WHEAT SCAB RESISTANCE USING *PST*I-AFLP

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ABSTRACT

In the previous study, we demonstrated that amplified fragment length polymorphism (AFLP) is a useful marker system for identification of wheat scab resistance QTL, and identified one major QTL for scab resistance from Ning 7840 by using *Eco*RI-AFLP. *Pst*I-AFLP markers ware reported to distribute more randomly in the corn genome than *Eco*RI-AFLP. Thus, we employed *Pst*I-AFLP markers to conduct further fine mapping in the major QTL region. The recombinant inbred lines (RILs) were derived from the cross between resistant cultivar Ning 7840 and susceptible cultivar Clark by single seed decent. The RILs were evaluated for scab resistance in the greenhouses by single spikelet inoculation. Wheat DNA from parents and 66 F_9 RILs was double-digested by *Pst*I and *Mse*I. Total 95 primer pairs were screened against the parents, and eighteen primer pairs showed a high level of polymorphism, therefore were used to analyze the RILs. Total 2,018 bands were amplified and 274 polymorphic bands were scored. Most of these markers were integrated into the existing *EcoR*I-AFLP map. The results indicated that *Pst*I-AFLP also randomly distributed in wheat genome. Addition of *Pst*I-AFLP markers not only increased genome coverage by filling the gaps in the *EcoR*I-AFLP map, but also increased the marker density. In the linkage group with major QTL, five *Pst*I-AFLP markers showed a significant association with scab resistance, and three of them explained high up to 70% of phenotypic variance in 66 RILs. Therefore, *Pst*I-AFLP is an effective tool for high-resolution mapping of scab resistance QTL in wheat.

FINDING QUANTITATIVE TRAIT LOCUS ASSOCIATED WITH FUSARIUM HEAD BLIGHT OF WHEAT USING SIMPLE SEQUENCE REPEAT MARKERS

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INTRODUCTION

Fusarium Head Blight (FHB) is a devastating disease of wheat (*Triticum aestivum*) reported since 1890. It is caused by fungus *F. graminearum* (teleomorph: *Gibberella zeae* Schwabe.). It has caused major grain losses all over the United States and worldwide. The loss is primarily due to floret sterility, poor to no seed filling, pink and shriveled tombstone kernels, low-test weight grains and presence of fungal mycotoxin-deoxynivalenol (DON) in the infected grains.

Molecular markers are powerful tools for marker assisted selection. Many markers closely linked with the disease resistance genes and quantitative trait loci (QTL) have been reported for many crops. QTL mapping will help locate genes that account for genetic variation in FHB resistance phenotypes. Restriction fragment length polymorphic (RFLP) and Amplified Fragment Length polymorphic (AFLP) markers have been used to identify FHB resistance QTLs in different genetic backgrounds. We have used Simple Sequence Repeat Markers to identify QTL associated with FHB resistance.

OBJECTIVE

Our main objective is to find quantitative trait locus associated with the FHB using two segregating populations, Ning7840 (resistant)/OH542 (susceptible) and Ning7840 (resistant)/Freedom (resistant). We are also interested in determining if the genes for resistance to FHB in Ning7840 and Freedom are similar or different.

MATERIALS AND METHODS

Two populations of $F_{2:3}$ lines developed by single seed descent method were used. These two populations were derived from a cross between Ning7840/OH542 (108 progenies) and Ning7840/Freedom (100 progenies).

Plant materials: Ten plants per family were evaluated for all the progenies of each population in the greenhouse. Seeds were sown in flats of soil in the greenhouse. Plants were vernalized for 60 days in a lighted cold room maintained day and night at 4^0C. Each germinated seed was transplanted individually. The greenhouse temperature varied from 19^0C to 30^0C during the day and 17^0C to 21^0C during the night.

The lines were also evaluated for resistance in the field at OARDC, Wooster, Ohio in 1999 for resistance to FHB. Lines were planted in a completely randomized block design with two replications each. Experimental units were 1m long and 30 cm apart (0.3 sq. feet). Patterson and Pioneer 2545 and OH542 were included as susceptible checks and Ning7840, Ernie and Freedom as resistant checks.

Inoculum Preparation: For greenhouse inoculation, fungal cultures from four aggressive *Fusarium graminearum* isolates were mixed in equal volume. The final concentration was adjusted to 10^5 conidia/ml. For field inoculations*, Fusarium graminearum* colonized corn kernels (Campbell and Lipps, 1998) were used as inoculum. The field was mist-irrigated daily throughout flowering.

I**noculation:** Hypodermic syringe inoculation technique was used for greenhouse inoculations. At anthesis, the center spikelet of each head was inoculated with a drop of freshly prepared conidial suspension (10^5 conidia/ml). Plants were maintained in a moist chamber at 100% relative humidity with temperatures ranging from 23^0C to 25^0C for three consecutive nights and then returned to the greenhouse bench.

Colonized corn kernels were spread in the field 18-21days prior to flowering. Heading dates were recorded as early, mid and late. 20 heads from each genotype were rated for %spikelet affected approximately 21 days after anthesis for severity ratings. Data was analyzed and compared with the greenhouse data.

Molecular Markers: DNA was isolated from parents and all 108 and 100 $F_{2:3}$ progenies of above two segregating populations. DNA extraction protocol perfomed was as described by Wang et al (1993). Primers for simple sequence repeat markers published by Roder et al (1996) were synthesized and screened for polymorphism among the three parents of above population. Priority was given to chromosomal regions that were found to be associated with QTL for FHB resistance in prior literature. Polymerase Chain Reaction (PCR) used was as described by Roder et al (1996) with a modification of 40 cycles of amplification instead of 45. Annealing temperature(T_M) was calculated for each primer individually based on the molecular weight. Amplified DNA products were visualized on a 4% superfine resolution agarose (SFR) run for 6-7hrs on the electrode. Ethidium bromide staining procedure was used. Primers showing polymorphism between three parents were used to evaluate all the progenies of the two populations.

Data: Inoculated heads were assessed for severity ratings as percentage of spikelet affected after 10 and 14 days in the greenhouse. FHB severity was recorded using a visual assessment scale (Stack et al, 1994, NDSU Extension) for greenhouse. Disease parameters recorded in the field were incidence, severity, visual kernel assessment scale (Stack et al, 1994, NDSU Extension), total kernel weight, percent scabby seed by weight and DON level (ppm).

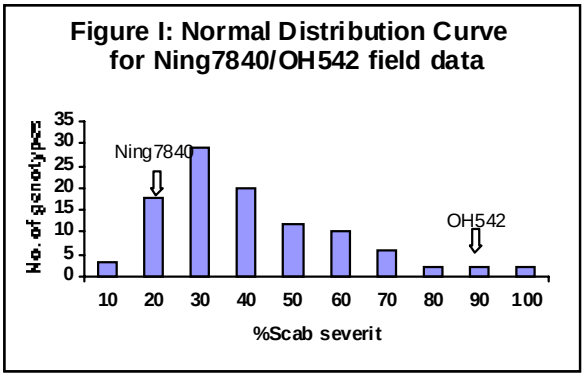
Statistical Model for Phenotypic Data Analysis: For both greenhouse and field, univariate plots for all the disease parameters were plotted independently using PROC UNIVARIATE in SAS(SAS institute version 6.03). One way analysis of variance (ANOVA) was conducted for the field and greenhouse data separately for the entire population using PROC GLM.

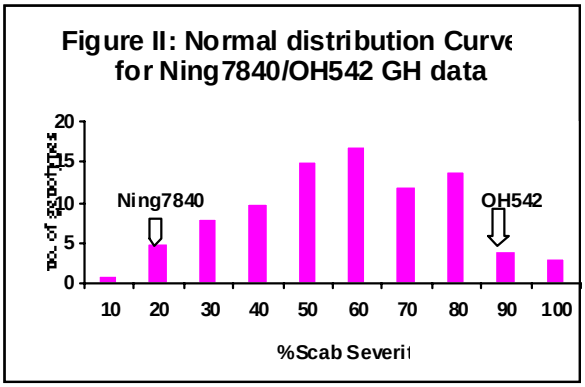
Pearson product movement correlations were calculated by PROC CORR to compare the disease severity ratings in different environments. The same procedure was also used to compare the disease severity ratings with other disease parameters from the field. In all cases, the correlation values were calculated from the means of genotypes at individual environment.

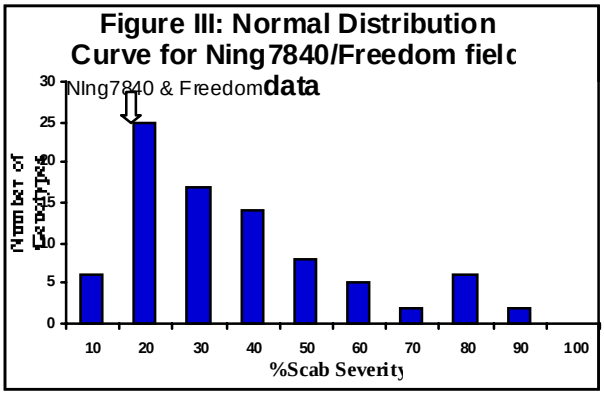
Statistical model for QTL Analysis: Quantitative trait anaysis was performed using SAS. One way ANOVA based on marker classes was conducted for each environment separately and on mean trait values over all environments using general linear model (PROC GLM) procedure for single codominant marker model for F_2 progeny. Rep and genotype within marker were considered random effect in the model. Error term was defined as gen(marker) to explain the uncontrolled variation.

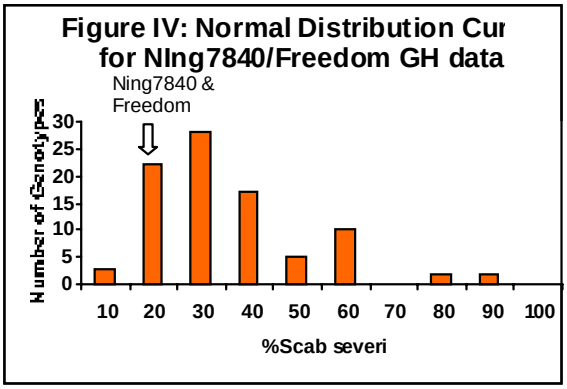
RESULTS

The tests for normality showed data for both the populations from greenhouse and field to be normally distributed. Data for other scab ratings from the field were also found to be normally distributed. Mean distribution curve for Ning7840/Freedom population and Ning7840/OH542 population for greenhouse and field are shown in figure I, II, III, IV. Field data of Ning7840/OH542 population is based on only one rep since 2nd rep was lost in the field due to mechanical failure of the planter. Greenhouse and field data were found to be moderately correlated for both the populations (r =0.37 & 0.35)









Genotypic data: Some of the SSR primers were found to be significantly associated with the trait in all the four experiments. Three other SSR primers located at 7BS and 2AS were found to be significantly associated with field FHB screening data(0.008 and 0.002 respectively) in the Ning7840/Freedom population. The test for significance was performed at 0.05 alpha level. Primers with low level of significance (0.10) are also reported in this preliminary report. All the progenies from above two populations are currently screened for a second year greenhouse testing for resistant to FHB. The final (2001) year data will help us in determining the true nature of QTL association. SSR markers significantly associated with one year greenhouse and one year field FHB disease screening are listed in Table I and II.

Table I: SSR Marker location and level of significance for association with FHB trait in Ning7840/Freedom population

SSR marker	Location	Significance level	
Xgwm493	3BS	0.05	*
Xgwm389	3BS	0.1	*
Xgwm285	3BS	0.1	**
Xgwm480	3AL	0.02	**
Xgwm296	2AS	0.003	*
Xgwm46	7BS	0.008	*
Xgwm518	6BS	0.1	***
Xgwm126	5AL	0.1	**
Xgwm186	5AL	0.1	*

Table II: SSR Marker location and level of significance for association with FHB trait Ning7840/OH542 population

SSR marker	Location	Significance level	
Xgwm389	3BS	0.05	**
Xgwm156	5AL	0.02	*
Xgwm2	3AS	0.01	*
Xgwm126	5AL	0.1	***
Xgwm533	3BS	0.03	**

* if the marker is significantly associated with the FHB screening data from field
** if the marker is significantly associated with the FHB screening data from greenhouse
*** if the marker is significantly associated with the FHB screening data from both greenhouse and field

A VISIBLE FUNGAL GROWTH APPROACH TO RAPID ANTIFUNGAL PROTEIN GENE PRETESTING

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OBJECTIVE

To develop a rapid pretest protocol for antifungal protein gene (AFP) constructs used in biolistic transformation of whole plants. The rapid system is based on a plant cell suspension culture approach to make it possible to test biolistic AFP constructs for their ability to stop or slow visible colony growth of the Fusarium Headblight fungus, *Fusarium graminearum*.

INTRODUCTION

Pre-testing of AFPs in suspension plant cell cultures is highly desirable because whole-plant transformation and headblight testing of adult transformants is expensive and time-consuming [Chen *et al.,* 1999; Smith *et al.*, 2000]. It often takes a minimum of eighteen months or more to test the efficacy of antifungal genes using whole plant transformation and adult plant screening procedures [Van de Mortel *et al.*, 1999]. In addition, individual AFPs are often not broad-spectrum and many isoforms may need to be tested. Plant suspension cell expression systems that accept a wide range of constructs are more desirable than other expression systems that require additional cloning steps. In addition some expression systems may not properly process biologically active eukaryotic antifungal proteins. Therefore a protocol using a plant suspension cell assay for pre-testing eukaryotic antifungal protein, biolistic constructs (AFPs) useful in genetic engineering of fungal disease resistant cereals [Bushnell *et al.,* 1998] is being developed. In this protocol plant suspension cell cultures transformed using biolistic constructs of antifungal genes are tested against the visible colony growth of the Fusarium Headblight fungus.

METHODS

The protocol uses Black Mexican Sweet Corn suspension cells (BMS) because they are easily transformed by microprojectile bombardment and are fast growing on MS solid and in liquid media [Murashige and Skoog, 1962]. BMS cells are ideal for protocol development because they respond to several commonly used plant promoters like the cauliflower mosaic virus promoter, maize ubiquitin promoter and others [Hilburn *et al.,* 2000]. In addition preliminary studies indicate that BMS cells can be stably transformed and subcultured for up to eight months without loss of transgene transcription capability (Table 1). Transgene viability during subculturing may depend upon the type of promoter used in a particular plasmid construct.

Plasmid and Promoter Driving GUS Expression And Cell Line Number	(~4 months after transformation)	(~8 months after transformation)	(~12 months after transformation)	(~17 months after transformation)
pKScBV GUS 13-3-1C	+	+	+	+
17-1-2A	+	+	+	+
17-1-2C	+	+	+	+
17-2-1C	+	+	+	+
19-1-2C	+	+	+	+
19-3-2C	+	+	+	+
pKUbi GUS 12-1-2D	+	+	+	+
13-1-1A	+	+	+	+
13-4-1B	+	+	+	+
16-4-1B	+	~+	~+	~+
18-2-2A	+	+	+	+
18-1-4E	+	+	+	+
19-3-2C	+	+	+	NS
pK35S GUS 12-1-2C	+	+	+	+
14-4-1A	+	+	NS	NS
13-4-1D	+	+	NS	NS
16-1-2C	+	+	NS	NS
19-2-1B	+	+	NS	NS

Table 1. Stability of GUS transformed BMS lines over time. BMS cells were co-transformed with a plasmid containing the GUS reporter gene and a plasmid containing the *nptII* gene as a selectable marker. Constructs with three differing promoters were used to drive the GUS gene. These were the Sugarcane Bacilliform Badna Virus promoter (ScBV), the maize ubiquitin promoter (Ubi), and the CaMV 35S promoter (35S). Stable lines were created and transferred weekly. Staining for GUS expression was used to determine GUS transgene stability throughout cell generations over time. A (+) designates positive GUS staining (blue product), a (~+) designates weak GUS staining, NS designates no sample available.

To develop this protocol four AFPs were chosen, all had previously documented antifungal activity in non-cereal plant systems [Datta *et al.*, 1999]. The AFPs were 1) rice chitinase [Zhu and Lamb, 1991], oat thaumatin-like protein [Lin *et al.*, 1996], and barley chitinase and barley glucanase [Leah *et al.*, 1991]. Several stably transformed BMS cell lines for each of four AFPs in constructs driven by the ScBV promoter [Tzafrir *et al.*, 1998] were created. To accomplish this, suspension cultured BMS cells were collected on cellulose filter pads, placed on solid MS media and transformed by microprojectile bombardment. The *nptII* gene (paromomycin resistance) was used as a selectable marker in co-transformation with all AFP constructs. After cell line selection, BMS-AFP lines were placed into MS liquid culture and grown in the presence of the paromomycin selector. A week before their use in the fungal growth assay procedure, BMS cell lines were transferred to liquid MS media without paromomycin [Hilburn *et al.*, 2000].

BMS-AFP suspension lines were verified for AFP mRNA transcription by RT-PCR. For preliminary study, a single BMS line, representing a single transformation event, was selected for each AFP. These BMS-AFP cultures were used to form cell "lawns" on cellulose filters. The AFP-BMS cell lawns were placed on solid MS media and centrally inoculated with a macroconidia suspension of known concentration from a virulent strain of *Fusarium graminearum.* The growth of the fungus (colony diameter) on each AFP-transformed cell line was recorded daily during an 8-day period.

PRELIMINARY RESULTS AND DISCUSSION

In replicated preliminary experiments none of the initial four AFPs, from single event BMS transformed cell lines, slowed or stopped the growth of the FHB fungus (Fig. 1). This occurred even though they transcribed mRNA for their AFP transgene. Although replicated, these results should be considered preliminary since there are more AFP-BMS cell lines from independent transformation events yet to be tested. Nevertheless the results are not unexpected since a closely related fungus, *F. solani* is well known for not responding to specific isoforms of chitinase, glucanase and other AFPs [Shewry *et al.*, 1997].

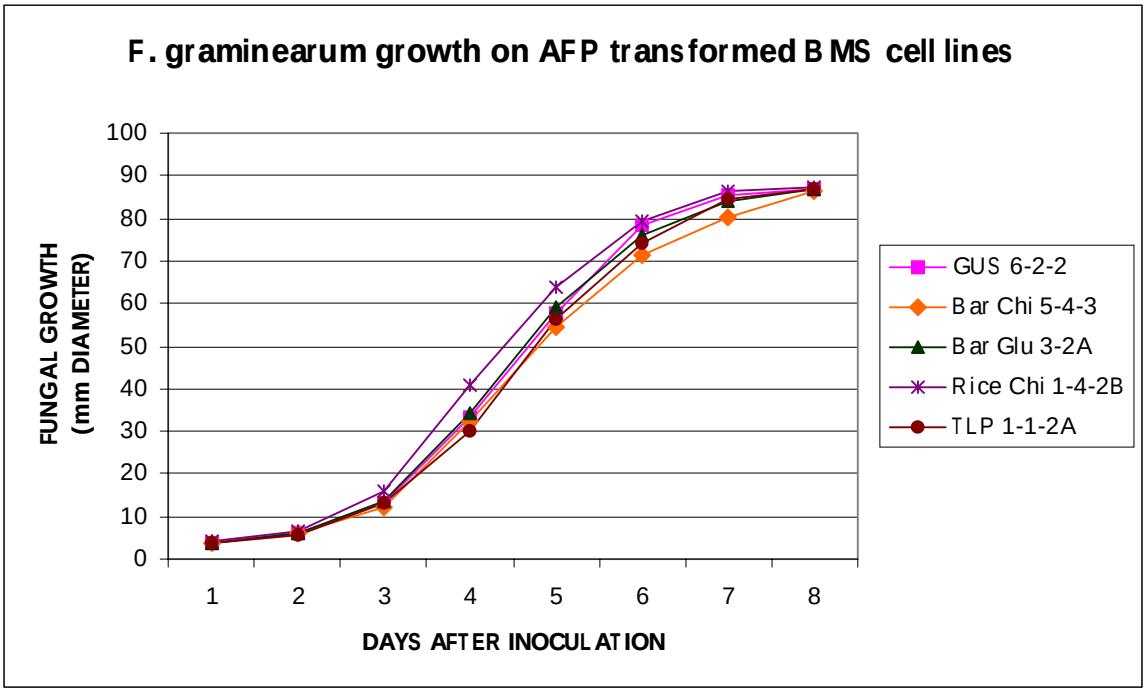

Figure 1. Growth of *F. graminearum* on AFP transformed BMS cell lines. Stable BMS suspension culture lines transcribing mRNA for their AFP were collected on filters and placed on solid MS media. These BMS cell lawns were then center inoculated with a macroconidia suspension of *F. graminearum*. Fungal growth measured as colony diameters were recorded daily, for 8 days.

A confounding factor is potential variability between AFP-BMS lines. Transformed BMS-AFP cell lines from single transformation events may differ in growth rates and in final transgene expression. Therefore more AFP-BMS lines from existing stocks and verified for transgene transcription by RT-PCR, (5 for each AFP) will be screened to verify preliminary results. In addition, *F. graminearum* grows well as saprophyte on MS media and may simply be over-growing and ignoring AFP-BMS cells by using the sucrose carbon base in the MS media as a substrate. Therefore, all future testing will be done on solid MS media minus sucrose. Preliminary experimentation revealed that BMS cells stay alive and active for up to 7 days on MS media without sucrose.

Also, it is well known that combinations of AFPs can act synergistically to halt fungi. The classic example is chitinase and b - 1,3 glucanase, which together can synergistically arrest growth of certain fungal species [Datta *et al*., 1999; Shewry *et al.*, 1997]. Therefore, mixtures of AFP transformed BMS lines will be made and tested as composite lawns against the growth of *F. graminearum.*

REFERENCES

Bushnell WR, Somers DA, Giroux RW, Szabo LJ, & Zeyen R.J. 1998. Genetic engineering of disease resistance in cereals. Canadian Journal of Plant Pathology 20:137-149.

Chen W.P, Chen P D, Liu DJ, . Kynast R, Friebe B, Velazhahan R, Muthukrishnan S, & Gill BS. 1999. Development of wheat scab symptoms is delayed in transgenic wheat plants that constitutively express a rich thaumatin-like protein gene. Theoretical and Applied Genetics 99:755-760.

Datta K, Muthukrishnan S, & Datta SK. 1999. Expression and function of PR-protein genes in transgenic plants. pp. 261-277. In: Pathogenesis-Related Proteins in Plants. S.W. Datta and S. Muthukrishnan Eds. CRC Press, Boca Raton, Florida.

Hilburn KLB, Bushnell WR, Baldridge GD, & Zeyen RJ. 2000. Toward a plant suspension cell assay for eukaryotic antifungal protein constructs used in cereal transformation. Phytopathology 90(6):S35

Leah R, H. Tommerup H, Svedsen I, & Mundy J. 1991. Biochemical and molecular characterization of three barely seed proteins with antifungal properties. Journal of Biological Chemistry 266:1564-1571.

Lin KC, Bushnell WR, Szabo LJ, & Smith AG. 1996. Isolation and expression of a host response gene family encoding thaumatin-like proteins in incompatible oat-stem rust fungus interactions. Molecular Plant-Microbe Interactions 9:511-522.

Murashige, T., Skoog, F. 1962. A revised medium for rapid assays with tobacco tissue cultures. Physiological Plantarum 15:473-497.

Smith L, Wyckoff M, Baldridge G, Zeyen R, & Muehlbauer GJ. 2000 Antifungal protein gene expression in transgenic wheat (Triticum aestivum). Agronomy Abstracts

Shewry PR & Lucas JA. 1997. Plant Proteins that Confer Resistance to Pests and Pathogens. Advances in Botanical Research 26:135 - 159.

Tzafrir I., Torbert KA, Lockhart BEL, Somers DA & Olszewski NE. 1998. The sugarcane bacilliform badnavirus promoter is active in both monocots and dicots. Plant Molecular Biology 38(3): 347-356.

Van de Mortel M, Bladridge G, Bushnell W, Somer D, Tobert K, & Zeyen R. 1999. Apparent partial powdery mildew resistance in transgenic oat genetically engineered to express rice or barley chitinases. Phytopathology 89 6:S80.

Zhu Q & Lamb CJ (1991). Isolation and characterization of a rice gene encoding a basic chitinase. Molecular and General Genetics. 226:289-296.

GENETIC TRANSFORMATION OF BARLEY WITH GENES FOR SCAB RESISTANCE

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ABSTRACT

Fusarium head blight (FHB), caused primarily by *Fusarium graminearum*, has been one of the most destructive diseases of barley since the early 1990s, resulting in huge economic losses for the growers. Of particular concern is the production of deoxynivalenol (DON), a pathogen virulence factor, which is harmful to humans and livestock. It has been proposed that increasing plant tolerance to DON could improve resistance to FHB while at the same time reducing DON accumulation in grain. *FsTRI* 101 (TriR), encodes a 3-OH trichothecene acetyltransferase that converts DON to a less toxic acetylated form while *PDR*5, an ATP-binding cassette transporter, acts as a efflux transporter, shunting DON across the plasma membrane from the interior of the cell. We have transformed the commercial malting barley cultivar Conlon with the *TRI* 101 and *PDR*5 genes with the aim of eliminating/reducing DON level in the infected grain. Ten day old callus derived from immature embryos was co-bombarded with the herbicide resistant gene, *bar*, as a selectable marker. After several rounds of selection on bialaphos medium, putative transgenic plants were regenerated. The transgenic nature of these plants were confimed by Southern analysis. Work is in progress to determine the expression of the introduced genes. Some of the regenerated plants were tetraploid. We are trying to convert transgenic tetraploid plants into diploid through conventional crossing.

IDENTIFICATION OF QTLS FOR SCAB RESISTANCE IN BARLEY

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ABSTRACT

The current epidemic of *Fusarium* head blight (scab) in the northern Great Plains has significantly reduced the availability of malting barley grown in this region. Resistant cultivars are the most economical means to control this disease. DNA markers associated with scab resistance can be used to enhance early generation selection in a breeding program. The objective of this research is to identify RFLP and SSR markers that flank genomic regions associated with scab resistance. A population of 128 F_4-derived lines from the cross Fredrickson (moderately resistant)/Stander (susceptible) was evaluated for scab resistance. 148 RFLP and SSR markers were mapped in this population. Markers associated with a major QTL for scab resistance were identified on chromosome 2.

OPTIMIZING THE EXPRESSION OF CANDIDATE ANTI-*FUSARIUM* PROTEIN GENES IN HEXAPLOID WHEAT

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OBJECTIVES

The aims of this project are to generate lines of hexaploid wheat expressing genes that encode candidate antifungal (AF) proteins, and to optimize the activities of these proteins in tissues for effectiveness against Fusarium head blight (FHB). Our short-term objective is to increase the expression of our candidate genes in wheat floret organs.

INTRODUCTION

One approach to combating FHB is the introduction of genes encoding components of the host defense response pathway and proteins having antifungal activity. Using the biolistic method of genetic transformation, we have introduced six candidate AF protein gene constructs into the regenerable cultivar Bobwhite (Table I). Two of the genes encode proteins that are intended to reduce the toxic action of deoxynivalenol (DON), and four of the genes encode pathogenesis-related proteins that have the potential to disrupt the cellular architecture of the pathogen, *F. graminearum*. All of the genes are regulated by the constitutive maize *Polyubiquitin-1* promoter/first intron (UBI, Christensen and Quail 1996). This paper describes the assessment of steady state mRNA levels in transformed wheat lines containing each of the constructs. We summarize our findings on molecular features of the AF genes that might account for their generally low expression levels and our plans to improve fungal gene expression in wheat. The engineering of two novel candidate anti-*Fusarium* genes (Table II) for expression in monocots is also discussed.

Table I. Summary of candidate genes and their expression in transgenic wheat lines

A. Candidate AF genes.

Gene	Origin	Mode of Action	# Lines	Lines	Reference
FsTRI101	*F. sporotrichioides*	DON acetyltransferase	4	4	McCormick *et al*.
PDR5	*S. cerevisiae*	DON transporter	11	4/7 tested	Balzi *et al*.
tlp-1	*T. aestivum*	membrane disruption	4	3	Rebmann *et al*
FvGlu	*F. venenatum*	glucan degradation	6	3	unpublished
FvChi1	*F. venenatum*	chitin degradation	8	4	unpublished
FvChi2	*F. venenatum*	chitin degradation	3	2	unpublished

MATERIALS AND METHODS

Qualitative RT-PCR was carried out according to Altenbach (1998), using 200-600 ng total RNA from endosperm (15 to 25 days post anthesis) or glume (15 days post anthesis), and either the RNA PCR Core Kit (Perkin Elmer) or the OneStep RT-PCR Kit (Qiagen). For semi-quantitative RT-PCR, total RNA was diluted to 5-40 ng per reaction for amplification of *tlp* and actin transcripts, and 200-600 ng per reaction for amplification of all other transcripts. Primers for the amplification of wheat actin transcripts were CACTGGAATGGTCAAGGCTG (ActA) and CTCCATGTCATCCCAGTTG (ActB). The actin RT-PCR primers were derived from three *Triticum* expressed sequence tags (GenBank accession nos. BE398871, BE425854, BE492306) and are specific to monocot actin sequences (not expected to amplify fungal, insect or human actin sequences).

To enhance the expression of several *Fusarium* AF genes, we are modifying the three nucleotides immediately upstream of the ATG start codon (start codon context), the flanking upstream nucleotides (5' leader), and the frequencies of specific codons within the coding region. Modifications (Table II) to the candidate AF genes *FsTRI101*, *TRI12*, FvGlu, and DONPep2 are being carried out using synthetic primers and PCR (e.g., see Ho *et al*. 1989).

For the transient assay, embryogenic calli from wheat cv. Bobwhite or barley cv. Golden Promise were co-bombarded with UBI::*uid*A (GUS) and either UBI::*FsTRI101*, UBI::*tlp*, or UBI::FvEndo. After incubation for up to 48 hours, RNA was isolated and semi-quantitiative RT-PCR of the AF mRNAs, GUS, and actin transcripts was carried out as described above.

RESULTS AND DISCUSSION

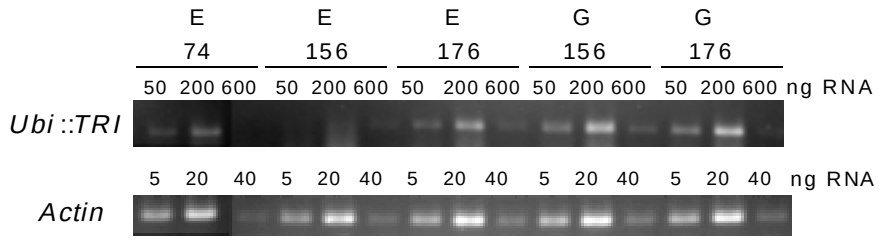
Thirty six independent wheat lines carrying one of the six antifungal gene constructs have been identified using the polymerase chain reaction (PCR) (Table I). Of these, a total of 20 transformed plants accumulated AF gene transcripts (mRNA) in endosperm, detectable by the reverse transcription-polymerase chain reaction (RT-PCR) method.

We observed plant-to-plant variations in mRNA levels for each of the candidate AF genes. In general, our RNA blot analyses (not shown) and RT-PCR experiments indicated that the expression of a *tlp* gene from wheat (Rebmann *et al*. 1992) was significantly higher than that of candidate genes derived from *Fusarium* or *Saccharomyces*, even though all of the genes

were regulated by the *Ubi* promoter/first intron. Although the levels of gene expression needed for effective action against *F. graminearum* have yet to be determined, high levels of expression are generally sought.

Semi-quantitative RT-PCR was used to distinguish among the different lines exhibiting transgene expression and to identify transformed lines expressing the highest steady-state levels of AF mRNAs (e.g., Fig. 1). Actin mRNA levels were monitored to normalize the amplification of the AF transcripts among different RNA samples. *In planta* endosperm expression levels ranged from 2- to 5-fold in independent lines expressing the same AF gene. *tlp-1* mRNA was about 10-fold more abundant than transcripts encoded by genes from *Fusarium* species. Where samples were available for testing, expression of the AF gene in glume tissues was very similar to that in endosperm. The glume is one of the first sites of *Fusarium* infection (Pritsch *et al*. 2000) under high inoculum pressure.

Fig. 1 RT-PCR detection of *TRI101* mRNA in endosperm (E) or glume (G) tissues harvested from three transgenic lines (74, 156, 175).



Our mRNA analyses indicated that the wheat *tlp-1* transcripts were significantly more abundant in wheat endosperm than were transcripts of the four *Fusarium* AF genes. To determine whether this was due to undesirable features within the fungal genes, we examined each for codon usage and consensus processing elements. The mRNAs deduced from the four fungal genes were free of known plant intron splice consensus elements, U-rich (e.g., Ko *et al.* 1998) or AU-rich segments (Haseloff *et al*. 1997; Iannacone *et al*. 1997) associated with monocot introns and transcriptional termination, and plant polyadenylation signals (Joshi 1987; Mogen *et al*. 1990). The GC content of the coding regions of the *Fusarium* AF genes ranged from 53% to 57 %, which compared favorably to the GC content of 39 non-redundant genes (containing over 11,300 codons) expressed in wheat leaves, wheat endosperm, and pollen of wheat, barley and maize. However, the *Fusarium* and monocot genes differed subtly in the usage of specific codons, and this might account for the lower accumulation AF gene transcripts of fungal origin in wheat tissues. Based on these analyses, we plan to change two of our constructs as shown in Table II.

Two new candidate AF genes, *TRI12* and DonPep2, are being modified for expression in wheat and other monocots. TRI12, a trichothecene efflux transporter from *F. sporotrichioides* (Alexander *et al*. 1999), is encoded by a 1.8 kb open reading frame. Owing to its smaller size and more favorable codon preference, the *TRI12* gene provides a promising alternative to *PDR5*. A novel peptide, DonPep2, is one of two DON antagonists that interfere with the toxic action of DON *in vitro* (Yuan *et al*. 1999). The peptide has been adjoined to the green fluo-

rescent protein for expression in plants. Modifications to *TRI12* and DonPep2 for expression in wheat are summarized in Table II.

Table II. Proposed gene modifications for enhancement of mRNA accumulation in monocots

Gene Name	Description of modification(s)
FsTRI101	start codon context from AAA to GCG with CA-rich 5' leader
FsTRI12	start codon context from AAG to GCG with CA-rich 5' leader
FvGlu	start codon context from ACT to ACC start codon context from ACT to GCC monocot codon usage
DONPep2	start codon context from ACA to GCG with CA-rich 5' leader and monocot codon usage

In order to test whether these proposed changes in 5' leader sequences or codon usage are effective in increasing the expression of fungal gene constructs in wheat, we are developing a transient expression assay. Such an assay will serve as a more rapid and tractable evaluation system than whole plant transformation. In preliminary experiments, wheat embryogenic calli were co-bombarded with UBI::*uid*A (GUS) plasmid in combination with either UBI::*tlp-1* or UBI::FvEndo plasmid. Transcripts of *uid*A, *tlp-1*, FvEndo, and endogeneous actin were readily detectable 48 hours after bombardment in our initial RT-PCR analyses, indicating that quality RNA from both introduced and endogenous genes can be isolated in suitable quantities. Barley embryogenic calli were co-bombarded with UBI::*uid*A (GUS) plasmid in combination with either UBI::*TRI101*, UBI::*tlp-1*, or UBI::FvEndo plasmid. Calli generated from embryos of barley cv. Golden Promise divide more rapidly than those from the wheat cv. Bobwhite and yield more material for bombardment. We are currently testing the suitability of barley callus tissue in transient expression assays. Assay conditions will be optimized using both wheat and barley tissues, and bombarded calli will be evaluated for reproducibility of expression. If relative expression levels of the AF genes in the transient assay correspond to those observed *in planta*, then new AF gene constructs can be evaluated within days rather than months.

ACKNOWLEDGMENTS

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REFERENCES

Alexander, NJ, McCormick SP, Hohn TM (1999) Mol Gen Genet 261: 977-984

Altenbach SB (1998) Theor Appl Genet 97: 413-421

Balzi E, Wang M, Leterme S, Van Dyck L, Goffeau A (1994) J Biol Chem 269: 2206-2214

Christensen AH, Quail PF (1996) Trans Res 5: 213-218

Haseloff J, Siemering KR, Prasher DC, Hodge S (1997) Proc Natl Acad Sci 94: 2122-2127

Ho SN, Hunt HD, Horton RM, Pullen JK, Pease LR (1989) Gene 77: 51-59

Iannacone R, Grieco PD, Cellini F (1997) Plant Mol Biol 34: 485-496

Joshi CP (1987) Nuc Acids Res 15: 9627-9640

Ko CH, Brendel V, Taylor RD, Walbot V (1998) Plant Mol Biol 36: 573-583

McCormick SP, Alexander NJ, Trapp SE, Hohn TM (1999) Appl Environ Micro 65: 5252-5256

Mogen BD, MacDonald MH, Graybosch R, Hunt AG (1990) Plant Cell 2:1261-1272

Pritsch C, Muehlbauer GJ, Bushnell WR, Somers DA, Vance CP (2000) Mol Plant-Microbe Inter 13: 159-169

Rebmann G, Mauch F, Dudler R (1991) Plant Mol Biol 17: 283-285

Yuan Q, Pestka JJ, Hespenheide BM, Kuhn LA, Linz JE, Hart LP (1999) Appl Environ Micro 65: 3279-3286

GENOMICS EFFORTS TO UNDERSTAND FUSARIUM HEAD BLIGHT IN WHEAT

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ABSTRACT

The main goal of the project is to identify and characterize genes from wheat which are involved in the host-pathogen interaction in fusarium head blight (FHB), to determine which gene pathways are modified in resistant sources and which pathways could be manipulated to play a key role in resistance. The wheat cultivar Frontana, a Type I resistance source from Brazil, has been used for the initial efforts. A suppressive subtraction hybridization (SSH) library has been made from Frontana heads inoculated at mid-anthesis with a *Fusarium graminearum* spore suspension and sampled 24 hr after inoculation. The subtraction was done with RNA from Frontana heads sprayed with water, to enrich for messages induced by the fungal infection. About 1800 ESTs have been sequenced and 1000 ESTs have been screened to select for genes with differential expression in inoculated heads. The differential expression pattern of potential candidate clones has been confirmed using Northern analysis.

We are also interested in the identification and characterization of genes from *Fusarium graminearum* which are involved in the host-pathogen interaction and in fungal pathogenicity. cDNA libraries have been constructed from *Fusarium graminearum* at various developmental stages and under different growth conditions, including mycelia growing on rich media, in Ahigh DON production@ liquid media, and on infected plant material, from asexual spore suspensions, and from developing fruiting bodies. Sequencing of ESTs from those libraries has been initiated.

PRELIMINARY CHARACTERIZATION OF WHEAT EVENTS HARBORING NOVEL TRANSGENES FOR SCAB RESISTANCE

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ABSTRACT

The wheat biotechnology program at the University of Nebraska uses an *Agrobacterium*-mediated transformation protocol to deliver transgenes to wheat. The protocol is a modification of that reported by Cheng et al. (Plant Physiol. 115:971). Transformation frequencies range from 1% to 3% on a T_0 plant in soil per explant (immature embryo) basis. The advantages of an Agrobacterium-mediated protocol over a direct DNA delivery technique include lower copy number of the foreign DNA element, lack of integration of fragmented copies, and the lower probability of plasmid vector sequences outside the gene of interest region being integrated into the genome. We have successfully utilized the system to introduced foreign genes into three spring wheat genotypes, Bobwhite, Sakha 206 and UC703. Additional genotypes are currently being evaluated.

We have introduced into wheat a number of potential fungal resistance genes as a strategy to control *Fusarium* Head Blight. These include three antiapoptotic genes, IAP, ced-9 and Bcl-xl that may provide an avenue to modulate *Fusarium* pathogenesis and one direct antifungal peptide, lactoferrin. Data will be presented on inheritance of the transgenes in wheat, along with preliminary results on disease development from greenhouse inoculations on the derived transformants.

TARGETED EXPRESSION A THIONIN GENE TO INHIBIT GROWTH OF *FUSARIUM GRAMINEARUM* IN BARLEY

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OBJECTIVES

1) Produce *Fusarium*-resistant barley through genetic transformation with specifically targeted native antifungal protein genes. 2) Develop specific promoters for lemma/palea and pericarp tissues. 3) Develop expression vectors that combine these promoters with sequences to target hordothionin and other antifungal genes to the intracellular space. 4) Examine the tissue preference and routes of *Fusarium* infection.

INTRODUCTION

F. graminearum continues to destroy much of the U.S. barley and wheat crops. The production of mycotoxins (e.g., DON) by *Fusarium* makes the harvest unsuitable for food, feed or malting. Genetic transformation with antifungal protein genes may be a viable method for introducing biochemical resistance. Low concentrations (5 micrograms/ml) of hordothionin (HTH) completely suppress germination and growth of *F. graminearum* spores (Skadsen *et al.*, 1998). However, HTH is found only in the starchy endosperm. If HTH can be placed in external tissues such as the lemma/palea and pericarp, it may provide a barrier to *F.g.*

Whereas HTH is associated with protein bodies (Carbonero et al., 1980; Ponz et al., 1983), leaf thionin is associated with the vacuole and cell wall (Reimann-Phillip *et al.*, 1989). These can be induced by fungi and have antifungal properties (Florack and Stickema, 1994). The Arabidopsis *Thi2.1* gene is inducible by pathogens, and its over-expression leads to resistance to *F. oxysporum* (Epple *et al.*, 1997). *HTH* expressed in tobacco enhanced resistance to some pathogenic fungi but not others (Carmona *et al.*, 1993; Epple, *et al.*, 1997). However, the non-seed thionins apparently do not protect field barley from *F.g.* Thionin precursors (Ponz et al., 1983) contain an N-terminal signal peptide and a 64 amino acid C-terminal acidic protein (AP). Both are processed away, leaving a mature protein of 45 amino acids.

To effectively express anti-*Fusarium* genes, it is necessary to determine the route of *F.g.* infection. In a review of *Fusarium* (Parry *et al.*, 1995), the only histological study of *F.g.* (Pugh *et al.*, 1933!) noted that hyphae penetrate wheat kernels both inter- and intracellularly. *F. oxysporum* hyphae grow through the middle lamella and collapse adjoining cells, prior to penetration (Kroes *et al.*, 1998). Penetration of wheat stem cells by *F. culmorum* involves growth through the intracellular space (Ebrahim-Nesbat *et al.*, 1991). We have conducted studies with a strain of *Fusarium* (gfp/Fusarium) transformed with the green fluorescent protein gene of jellyfish (*gfp*; Skadsen *et al.*, 1999). Soon after spores become established on the lemma, hyphae grow rapidly to the tip of the floret. Hyphae then infect the anthers and

proliferate within the florets of young florets. In older florets, hyphae rapidly proliferate on the extruded ovary epithelial hairs; the anthers support comparatively little growth. Hyphae grow downward and cover the pericarp. Hyphae also penetrate the lemma and grow directly into the pericarp within 48 h from inoculation. It is therefore essential to express antifungal genes in these exposed tissues.

Constitutive expression is being used to test the effectiveness of antifungal genes. These results will be incorporated with ongoing research, which has produced a lemma-specific promoter and several other pericarp-specific genes. Once the constitutively expressed antifungal genes can be demonstrated to resist *Fusarium*, they will be coupled with tissue-specific promoters. This may eventually allow HTH and other antifungal proteins to be produced only in these tissues. Besides HTH transformants (see Results), we have also produced transformants carrying the gene for another antifungal permatin protein, BARPERM1 (Nuutila *et al*., 1998; Skadsen *et al*., 1999) and characterized the expression of this gene (Skadsen *et al*., 2000). Our transformants have not produced BARPERM1 protein in vegetative tissues. We have located a probable cause for this (below) and will use this information to improve HTH and permatin transformation.

MATERIALS AND METHODS

Tissue-specific promoter cloning, vector construction, transformation and expression

The differential display technique was used to detect genes expressed in the lemma/palea or pericarp but not in flag leaves. Products were used to probe blots of lemma/palea, pericarp and leaf RNAs. Tissue-specific gene candidates were cloned and sequenced, and the corresponding nuclear genes were purified from a Morex genomic BAC library (Andy Kleinhofs, Washington State U.). Deduced promoter regions were subcloned and ligated to a *gfp* reporter gene (Jen Sheen, Harvard). Transient expression studies of promoter activity were conducted with the pAHC17 vector, which contains the *Ubi* promoter and first intron but no selectable marker (Christenson and Quail, 1996). We inserted *gfp* behind the *Ubi* promoter. Vectors for stable constitutive expression of *gfp* and antifungal genes were prepared from the Ubi/GUS+Ubi/BAR vector pAHC25 (Christenson and Quail, 1996) by replacement of the GUS gene. Vectors were attached to gold particles and used to transform barley through the biolistic (gene gun) approach (Wan and Lemaux, 1994), with media modifications described by Dahleen (1995). Screening of putative transformants was conducted using PCR on leaf DNA extracted by the CTAB procedure. Particle bombardments of lemmas, pericarps and leaves are being conducted with candidate tissue-specific promoters, linked upstream from *gfp*. Later, promoter/*HTH* fusions will be constructed. Stable transformants will be tested for *Fusarium* resistance. In the past, we have transformed the Golden Promise cv. However, this does not lend itself to adequate field testing because its spikes do not fully emerge from the boot. We will now transform the two-row Conlon cultivar, which has been shown to transform and regenerate (Dahleen, 2000).

HTH antibodies and Subcellular targeting of HTH

HTH-1 was inserted into a pET vector in order to produce the full-length HTH protein. This failed to produce protein in a pET/*E. coli* system. Because the *HTH-1* sequence has two

nearby start codons and a segment of 5' UTR, it was hypothesized that these may inhibit expression. *HTH-2* was produced by removing the sequence upstream of the second start codon. This produced high levels of HTH fusion protein (fused with pET thioredoxin). The fusion protein was purified and used to produce antibodies in rabbits. If effective (see Results) these will be used to confirm expression at the protein level. Our initial thionin expression vector utilized the *HTH-1* cDNA clone. All constructs were inserted into pAHC25 behind the *Ubi* promoter, after removing the GUS sequence. The 3' UTR of the *HTH-1* sequence was removed and joined with the NOS termination sequence. Because of our success with *HTH-2* in the pET system, we inserted it into the pAHC25 vector. *HTH* sequences were detected by PCR. RT-PCR was used to determine whether HTH genes were transcribed in transformants.

In order to understand the requirements for targeting HTH to the intracellular space and/or to the secretion pathway, three constructs were inserted into pAHC25, behind the HTH signal sequence. Construct 1 diverts targeting from the vacuole by inserting a synthesized KDEL ER retention signal (reviewed in Gomord and Faye, 1996) between the N-terminal signal peptide (SP) and GUS. Construct 2 contains the SP and mature protein followed by GUS. Without the AP, the HTH-GUS fusion may be directed to the intracellular space. Construct 3 contains the SP alone, followed only by the GUS sequence. The vectors were bombarded into etiolated coleoptiles. After 48 h, coleoptiles were stained for GUS activity or analyzed for secreted GUS activity (MUG assay), relative to intracellular MDH.

Route of* Fusarium *invasion

A strain of *F.g.* transformed with *gfp* was produced by Tom Hohn (Novartis, NC (formerly at USDA, Peoria, IL)). The hyphae display a green fluorescence under short-wave blue light (Skadsen *et al.*, 1998; Bushnell *et al.,* 1999). We explored several methods for visualizing the initial penetration events. Paraformaldehyde fixation and cryostat sectioning were found to be ineffective. Lemmas are infected for 6 h and then peeled into fine tissue strips, preserving conidiospore attachments. These were viewed by confocal microscopy (Keck Neural Imaging Center, Univ. of Wisconsin), but excessive "flare" prevented viewing at the epidermis. We are now experimenting with the use of tissue strips set into enclosed deep well slide chambers.

RESULTS AND DISCUSSION

A previously unknown gene, *D5*, has been cloned and found to be expressed only in the lemma/palea and embryo (Sathish et al.,1999). The promoter was used to drive the expression of a *gfp* reporter gene in a *D5*/*gfp* construct. In transient assays, expression was found in lemmas and not in leaves (Sathish, et al., 2000). As an internal control, the Ubi/GUS vector was simultaneously bombarded. GUS was expressed well in both leaves and lemmas. Several other lemma/palea- and pericarp-specific genes have been cloned. We have successfully transformed Golden Promise with *HTH-1* and have so far found five transformants that produce *HTH-1* mRNA in seedling leaves, on northern blots. However, in RT-PCR analyses, only one produced appreciable levels of *HTH-1* mRNA. We have recently bombarded embryos with *HTH-2* and have several regenerating transformants.

Subcellular targeting constructs were tested by transient expression assays. Although weak, all secreted GUS activity (MUG assay) into the apples, whereas no MDH activity was secreted (cell leakage control). The unaltered GUS control did not secrete, and had only localized GUS staining spots. In all of the non-control bombardments, the entire coleoptile turned blue after GUS staining. We are now trying to determine whether artifacts could have caused these results.

HTH antibodies were tested on western blots of developing seed proteins and purified HTH protein. They did not cross-react with purified (45 amino acid) HTH mature protein, although they did react with two seed proteins of the proper size to represent the unprocessed HTH and the AP. Two new constructs will be made to obtain a sequence that will react with mature HTH.

REFERENCES

Bushnell WR, Skadsen RW, Goff TN, Hohn TM (1999) Abs, 16th American Barley Researcher's Workshop. July, 1999, Idaho. (also in 1999 Barley Newsletter).

Carbonero P, Garcia-Olmedo F, Hernandez-Lucas C (1980) J Agric Chem 28:399-402.

Carmona MJ, Molina A, Fernandez JA, et al. (1993) Plant J 3: 457-462.

Christenson AH, Quail PH (1996) Transgenic Res 5:213-218.

Dahleen LS (1995) Plant Cell, Tissue and Organ Res. 43:267-269.

Dahleen LS (2000) Ann. Prog. Rpt.on Malting Barley, March, 2000. American Malting Barley Assoc., Milwaukee, WI. pp. 101-104.

Ebrahim-Nesbat, Apel K, Heitefuss R (1991) J Phytopath 131:259-264.

Epple P, Apel K, Florack DEA, Stiekema WJ (1994) Plant Mol Biol 26:25-37.

Florack DEA, Dirkse WG, Visser B, Heidekamp F, Stiekema WJ (1994) Plant Mol Biol 24:83-96.

Gomord V, Faye L (1996) Plant Physiol Biochem 34:165-181.

Kroes GM, Baayen RP, Lange W (1998) Eur J Plant Path 104:725-736.

Nuutila AM, Skadsen RW, O'Connor M, Kaeppler HF (1998) Cong on In Vitro Biol, 1998, Las Vegas. In Vitro 34(3): 65-A.

Parry DW, Jenkinson P, McLeod L (1995) Plant Path 44:207-238.

Ponz F, Paz-Ares J, Hernandez-Lucas C, Carbonero P, Garcia-Olmedo F (1983) EMBO J 2:1035-1040.

Pugh GW, Johann H, Dickson JG (1933) J Agric Res 46:771-797.

Reimann-Phillipp U, Schrader G, Martinoia E, Barkholt V, Apel K (1989) J Biol Chem 264:8978-8984.

Sathish P, Federico ML, Kaeppler HF, Skadsen RW (1999) Abs SP-1000. Proc Cong on InVitro Biol, 1999, New Orleans.

Sathish P, Federico ML, Fu J, Skadsen RW, Kaeppler HF (2000) 6th Internatl. Cong. on Plant Mol. Biol., Quebec, Canada. June 2000. Plant Mol. Biol. Rep. 18:2 (suppl), Abs. S03-104.

Skadsen RW, Nuutila AM, Puthigae S, Kaeppler HF (1998) Proc. Natl. Scab Forum, pp. 93-95, Oct. 1998, East Lansing, Michigan

Skadsen RW, Nuutila AM, Puthigae S, Kaeppler HF, Hohn TM (1999) Proc 32nd Barley Improvement Conf, 1999, New Orleans, Louisiana

Skadsen RW, Sathish P, Kaeppler HF (2000) Plant Sci 156:11-22.

Wan Y, Lemaux PG (1994) Plant Physiol 104:37-48.

CONSTRUCTION OF GENOMIC LIBRARIES ENRICHED WITH MICROSATELLITE SEQUENCES

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ABSTRACT

In bread wheat, Simple Sequence Repeats (SSRs) or microsatellite markers provide a higher incidence of polymorphism than other marker types such as RFLP and RAPD. SSR markers are usually developed by the following process: genomic DNA is digested with different restriction enzymes; size selected fragments are (400-700 basepairs) are ligated into a vector and used to transform *E. coli*, the clones are transferred to membranes and screened with different SSR-containing probes; clones that putatively contain an SSR are then sequenced and PCR primers are designed to the flanking regions of the SSR sequence. The construction and screening of this type of library has two notable limitations: firstly, the screening is time consuming, secondly, because of the low frequency of SSR containing clones very large numbers of clones must be screened. To overcome these problems, an elaborate method for [CT/GA]n enrichment of wheat has been used based on the marker selection method which carried out as follows:

Genomic DNA of Chinese Spring was digested with restriction enzymes *Sma*I, *BsrB*I, *Nla*IV, *Cac8*I, and *ECI136*II, and size selected on an agarose gel. DNA fragments in the 400 to 750bp range were isolated from the gel. Purified DNA fragments were ligated into the *SmaI* site of pBluescript. Ligations were propagated in bacterial strain JMG1 which combined mutations at the *dut* and *ung* loci. The presence of these two mutations permits the incorporation of uracil in place of thymidine in DNA replicated in the strain. The phagemid library was infected with VCSM13 helper phage, this permits the recovery of this library as single stranded (ss) DNA with uracil frequently substituted for thymidine. Primer extension was carried out by using the ssDNA as a template and the 5' phosphorylated $[CT]_{15}$ oligo as a primer. DNA synthesis, preceded by an optional short annealing step, is performed using *Taq* DNA polymerase. The products of this primer extension reaction were treated with T4 DNA ligase and then transformed into an *E. coli* strain XL2-Blue maintaining wild type genes at the *dut* and *ung* loci. Under these circumstances, the uracil substituted ssDNA will be restricted from growing by the host encoded uracil-N-glycosylase (the product of the *ung* locus), while the primer extended products are capable of replicating. Libraries were plated at a density of 100-200 clones per Petri plate. Random clones from both primary and marker-selected libraries were picked and cultured in a 96 well plate with LB liquid medium, after transferring to the membranes, the clones were hybridized with a $[CT]_{15}$ probe labelled with ^{32}P.

A total of 366 [CT]n containing clones were observed out of 2592 marker-selected clones. Compared with the reported frequency of 0.1% to 0.2% for [CT]n, the library enrichment for [CT]n is about 70 to 140 fold. Eighty-three positive clones were randomly selected and

sequenced. The mean repeat length of [CT]n is 14. The clones with prefect repeats =10 were 64% of the total. After being assembled on the AutoAssembler, only two clones were found to have identical sequences, the remaining clones were unique. Further screening for other motifs is in progress.

DEVELOPMENT AND PHYSICAL MAPPING OF MICROSATELLITE MARKERS IN WHEAT

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ABSTRACT

Physical mapping of BARC and BARCM primers:

Microsatellite markers, also referred as simple sequence repeat (SSR) markers, are very efficient marker system for molecular breeding. We are broadening wheat genetic marker system by identification and physical mapping of new microsatellites.

We have determined the chromosome location of 141 microsatellite markers amplified by 70 BARC and 71 BARCM primer pairs designed in the laboratory of Dr. Perry Cregan (USDA/ ARS, Beltsvilles, MD) using nulli-tetrasomic lines of Chinese Spring. Polymorphism was screened among five wheat cultivars or lines, such as CS, Opata85, Synthetic wheat W-7984 (parents of ITMI mapping population) WL711 and HD29 (parents of Karnal bunt mapping population). All BARC primer pairs were polymorphic between Opata85, and synthetic wheat W-7984, whereas all BARCM primer pairs produced monomorphism. Fifteen percent (11/70) of BARC and three percent (2/71) BARCM primers detected polymorphism between wheat lines WL711 and HD29. It was possible to assign 75 loci with 57 BARC and BARCM primer sets on wheat chromosomes (Table 1). A maximum number of loci (7) was mapped on chromosomes 5B and 7D. Six loci were placed on each of chromosomes 6B, 6D, and 7A. No markers mapped on chromosomes 1D and 4B. Sixty-eight percent of BARC and 20 percent BARCM primers were mapped on individual wheat chromosomes.

Development of new microsatellite markers using the EST database:

Rapid growth of wheat EST database provides A new resource for development of new microsatellite markers. We searched the database at Microsoft interface for di-and tri-nucleotide repeat motifs {(AC)n, (AG)n, (AT)n, (CG)n, (CT)n, (GT)n, (AAC)n, (AAG)n, (AAT)n, (ACC)n,(ACG)n,(ACT)n, (AGC)n,(AGG)n,(AGT)n,(ATT)n,(CCG)n,(CCT)n,(CGG)n,(CGT)n, (CTG)n,(CTT)n,(GGT)n, and(GTT)n}. One hundred and ten putative microsatellites were identified from 15,000 ESTs. One hundred pairs of primers were designed manually.

Out of 100 primers tested on wheat lines HD29 and WL711, 74 percent amplified a PCR product and six percent were polymorphic between these two lines. WL711 and HD29 are highly susceptible and resistant wheats to Karnal bunt pathogen respectively. Twenty-two of these primer sets were tested on nulli-tetrasomic lines of CS and twelve were mapped to individual wheat chromosomes.

Wheat EST database is expected to grow to 100,000 or more. We anticipate designing and testing a total 600 primer sets. Based on preliminary results, we expect 70 percent of the primers will amplify PCR products for grand total of 420. Based on our preliminary physical mapping data, at least 50 percent of these new markers should map as distinct loci on individual wheat chromosomes. Therefore, we expect to develop 200 new microsatellite markers from the EST database. Identifying microsatellite markers from the EST database is less time consuming and more cost effective than other methods of isolating microsatellite markers.

All the microsatellite markers developed in our laboratory along with those developed from the lab. of Dr. P. Cregan will be physically mapped using CS nullitetra lines, di-telosomic and deletion lines.

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Table 1. Physical mapping of BARC/BARCM microsatellite markers on wheat chromosomes using Chinese Spring nulli-tetrasomic set.

Chromosome*	A	B	D
1	BARC48, BARC83 BARC17, BARCM48	BARC61, BARC80 BARC88	-
2	BARC08, BARC50 BARC51, BARC48	BARC55, BARC13 BARC16, BARC18, BARCM64	BARC54, BARC08 BARC28
3	BARC57, BARC19, BARC12, BARC45	BARC77, BARC68, BARC73, BARC84, BARCM44	BARC77, BARC42, BARC71
4	BARC78, BARC52	-	BARC69
5	BARC40, RARCM32	BARC59, BARC69 BARC04, BARC16 BARC58, BARCM18, BARCM32	BARC44
6	BARC90	BARC24, BARCM26 BARCM06, BARC67 BARC67, BARCM06, BARCM67, BARCM31, BARCM68	BARC05, BARC54, BARC25, BARCM30, BARC21, BARC62
7	BARC70, BARC49 BARC29, BARCM25 BARCM04, BARCM34	BARC32,BARC90 BARC29,BARCM26 BARCM67	BARC09,BARC70 BARC26,BARC06 BARC52,BARC76 BARCM24

* Note some SSR amplified products on more than one chromosome. For example BARC48 amplified products on chromosome 1A and 2A. BARC08 on chromosomes of 1A and 1D

DEVELOPMENT OF STSS AND SNPS LINKED TO FUSARIUM HEAD BLIGHT RESISTANCE OF WHEAT USING AFLPS AND ANTIFUNGAL GENE ANALOGS

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OBJECTIVES

To design user-friendly PCR markers for scab resistance genes, using AFLP markers and sequences from genes encoding antifungal thaumatin-like proteins.

INTRODUCTION

Breeding for scab resistance currently relies mainly on phenotypic assessments of infection symptoms. Given the inherent difficulties in field-based screening, user-friendly molecular markers associated with scab resistance genes would facilitate selection. Polymerase chain reaction (PCR) based markers such as sequence-tagged sites (STSs) and single nucleotide polymorphisms (SNPs) have been applied in a number of analyses (Inoue et al. 1994; Wang et al. 1998). These allele-specific PCR markers are usually designed from previously mapped low-copy DNA markers, or built directly from sequences of known genes. Sequences of disease resistance genes of several plant species share structural motifs. These conserved domains can be used to isolate similar resistance genes from a given genome with degenerate primers (Shen et al. 1998). In previous work, AFLP markers linked to three QTL for scab resistance were identified (Bai et al. 1999). The aim of the present study was: (1) to convert the AFLP markers into STSs and SNPs, and (2) to design additional allele-specific PCR markers for scab resistance based on sequences of genes having similarity to antifungal thaumatin-like protein genes (Vigers et al. 1992; Chen et al. 1999).

MATERIALS AND METHODS

The mapping population consisted of 133 $F_{8:11}$ recombinant inbred lines (RILs) derived from 'Ning 7840'/Clark. 'Ning 7840' has strong type II resistance and Clark is very susceptible to scab spread within the spike. Disease screening methods, phenotypic data, and AFLP protocol have been described (Bai et al. 1999). AFLP fragments were recovered from polyacrylamide gels, amplified, gel purified, cloned, and sequenced. All the fragments were hybridized by Southern blotting on parental genomic DNA digested by *Eco*RI, *Hind*III, and *Hae*III to select low-copy sequences. Antifungal genes were isolated from 'Ning 7840' and Clark using degenerate primers based on amino acid motifs conserved among several thaumatin-like protein genes. All the degenerate primers contained deoxyinosine at the redundant third position of codons, and full nucleotide redundancy was used at the 3' termini. SNPs were designed on point mutations within sequences. The STSs and SNPs were integrated into an established AFLP map (Bai et al. 1999) using Mapmaker 3.0 (Lander et al.

1987) for linkage mapping, and Qgene 3.04 (Nelson, 1997) for interval analysis and regression.

RESULTS AND DISCUSSION

A total of 9 AFLP fragments linked to scab resistance were isolated from polyacrylamide gels. These fragments included 7 markers located around a major QTL, and 2 markers linked to two minor QTL (one marker per QTL). Together the three QTL explained up to 60% of the genotypic variation of scab resistance (Bai et al. 1999). Southern blotting revealed that 6 out of 9 fragments were low-copy AFLP clones. As expected, the sequence of each clone was flanked by the corresponding *EcoR*I and *Mse*I primer sequences. Based on the sequences of the AFLP markers, two types of allele-specific primers were designed. The first type was internal to the AFLP selective primers. In the second type, at least one of the direct and reverse primers included the AFLP selective primer sites. The internal primers did not give any allele-specific amplification. Instead, primers designed on the original restriction sites identified five STSs reproducing the original polymorphisms. A sixth STS amplified a fragment of the expected size in both 'Ning 7840' and Clark. These fragments revealed a single base change at position 91 (a thymine in Ning 7840 replaced a cytosine in Clark). The point mutation was used to design a SNP designated SNPmaj for its location in the major QTL region. Interval analysis showed that two allele-specific markers STSmaj and SNPmaj mapped to linkage group LG1 spanning 81.2 cM, with maximum LOD score value of 21.84 (Figure 1A). STSmaj and SNPmaj were designed from the sequences of the AFLP markers GCTG/CGAC1 and AAC/CGAC3, respectively. These AFLPs were linked to a major QTL for type II resistance (Bai et al. 1999). The allele-specific markers mapped 4.8 cM and 2.9 cM away from their respective AFLP markers (Figure 1B). Another allele-specific marker, STSmin derived from the AFLP marker CTCG/CTC4, mapped in the linkage group LG2 covering 73.6 cM (data not shown).

A number of thaumatin-like protein genes were isolated from 'Ning 7840' and Clark. Alignment of sequences identified 15 point mutations (insertions and substitutions). The allele-specific marker SNPtha, linked to the minor QTL in linkage group LG2 was derived from a 633 bp insert containing a segment of a thaumatin-like protein. The R^2 and P values of the four allele-specific PCR markers for area under disease progression curve (AUDPC) and percentage of scabbed spikelets (PSS) at 21 days after infection in F_{10} RILs are given in Table 1. STSmaj and SNPmaj located in the major QTL region explained individually 35 to 36% of the phenotypic variation of scab infection. The effects of allele substitution at STSmaj and SNPmaj are significant (0.01 to 0.001 level probability) on both AUDPC and PPS in four generations (F_5, F_6, F_7, and F_{10}). The minor QTL marked by STSmin and SNPtha on linkage group LG2 has marginal effects on scab resistance (Table 1), with LOD score value less than 3.0.

The markers STSmaj and SNPmaj can be individually multiplexed with STSmin in 25 PCR cycles. Reagent concentrations are the same for all markers, but SNPtha has a different annealing temperature. These four PCR markers should prove useful in selection for scab resistance. They will be validated in different crosses. Several other antifungal genes are being analyzed for mapping in the major QTL region.

Table 1. R^2 and P values of area under disease progression curve (AUDPC) and percentage of scabbed spikelets (PSS) at 21 days after infection for F_{10} generation of RILs.

Marker	Marker source	Allele source	AUDPC		PSS	
			R^2	P	R^2	P
STSmaj	AFLP(GCTG/CGAC1)	Clark	35	0	36.6	0
SNPmaj	AFLP(AAC/CGAC3)	Ning	35.1	0	35.2	0
STSmin	AFLP(CTCG/CTC4)	Ning	2.1	0.0996	2.6	0.019
SNPtha	Thaumatin-like protein	Clark	4.6	0.0135	4.2	0.063

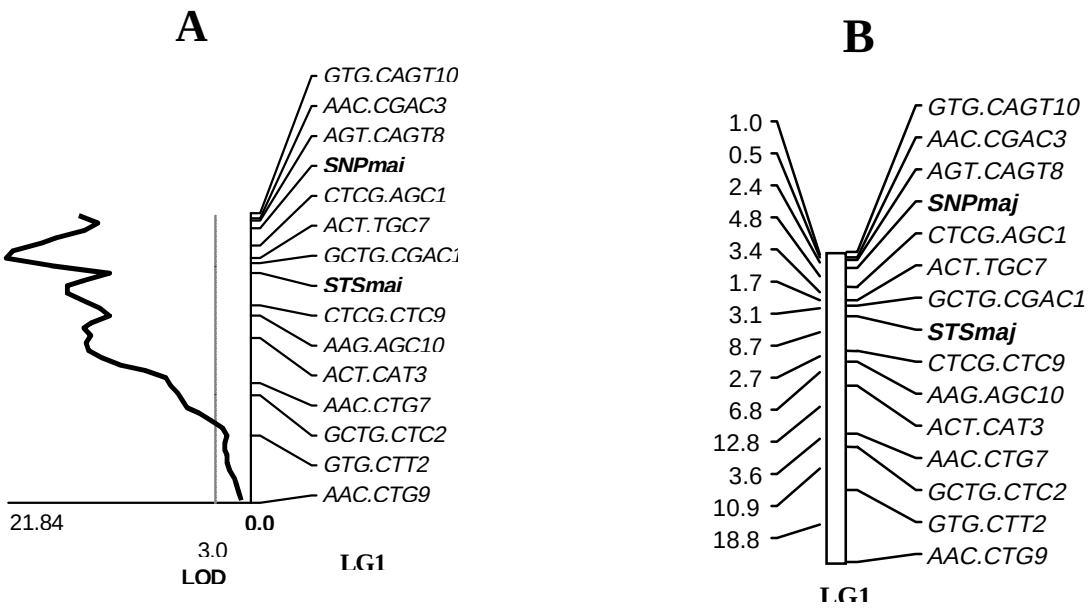

Figure 1. Interval analysis (A) and map of linkage group1 (B) carrying a major QTL for scab resistance in 'Ning 7840'/Clark recombinant inbred lines. The allele-specific markers SNPmaj and STSmaj are in bold.

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REFERENCES

Bai G, Kolb FL, Shaner G, and Domier LL (1999). Amplified fragment length polymorphism markers linked to a major quantitative trait locus controlling scab resistance in wheat. Phytopathology 89: 343-348.

Chen WP, Chen PD, Liu DJ, Kynast R, Friebe B, Velazhalan R, and Muthukrishnan S (1999). Development of wheat Scab symptoms is delayed in transgenic wheat plants that constitutively express a rice thaumatin-like protein gene. Theor Appl Genet 99: 755-760.

Inoue T, Zhong HS, Miyao A, Ashikawa I, Monna L, Fukuoka S, Miyadera N, Nagamura Y, Kurata N, Sasaki T, and Minobe Y (1994). Sequenced-tagged sites (STSs) as standard landmarkers in the rice genome. Theor Appl Genet 89: 728-734.

Lander ES, Green P, Abrahamson J, Barlow A, Daly MJ, Lincoln SE, and Newburg L (1987). MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1: 174-181.

Nelson JC. (1997). QGENE: software for marker-based genomic analysis and breeding. Mol Breed 3: 239-245.

Shen KA, Meyers BC, Nurul Islam-Faridi M, Chin DB, Stelly DM, and Michelmore RW (1998). Resistance gene candidates identified by PCR with degenerate oligonucleotide primers map to clusters of resistance genes in lettuce. Mol Plant-Microbes Interact 11: 815-823.

Vigers AJ, Wiedemann S, Robert WK, Legrand M, Selitrennikoff CP, and Fritig B (1992). Thaumatin-like proteins are antifungal. Plant Sci. 83: 155-161.

Wang DG, Fan J-B, C-J. S, Berno A, Young P, Sapolsky R, Ghandour G, Perkins N, Winchester E, Spencer J, Kruglyak L, Stein L, Hsie L, Topaloglou T, Hubebell E, Robinson E, Mittmann M, Morris MS, Shen N, Kilburn D, Rioux J, Nusbaum C, Rozen S, Hudson TJ, Lipshutz R, Chee M, and Lander ES (1998). Large scale identification, mapping, and genotyping of Single-Nucleotide Polymorphisms in the human genome. Science 280:1077-1082.

MICROSATELLITE MARKER DEVELOPMENT AND CONSTRUCTION OF A MICROSATELLITE ALLELE SIZE DATABASE OF ELITE AND SCAB RESISTANT WHEAT GENOTYPES: MEIOTIC MAPPING AT MSU AND RATIONALE FOR THE OVERALL PROJECT

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ABSTRACT

The unsuccessful search for potent scab resistance in elite U.S. wheat germplasm dictates that any new, potent resistance genes will be found in unadapted parents. Likewise, the limited number of lines amenable to transgenosis dictates that useful transgenes will also be initially available to breeders only in unadapted transgenic lines. Conventional breeding approaches for introgressing genes from unadapted parents generally requires several 'cycles' of backcrossing or modified backcrossing to dilute the frequency of undesirable donor alleles. The result is a large and costly gap between the resistance levels available to scientists, and those actually in use by growers.

Marker assisted selection is an effective means of rapid and directed elimination of unwanted donor alleles, or 'background selection' (Bernacchi et al., 1998a; Bernacchi et al., 1998b; Xiao et al., 1998). For this application, a marker system must exhibit high levels of polymorphism at loci evenly distributed throughout the genome. Co-dominant inheritance and multi-allelism are also desirable since heterozygotes are not masked and polymorphism is maximized. Finally, genetic analysis should require small amounts of DNA and must be amenable to automation to permit high-throughput genotyping. Microsatellite or simple sequence repeat (SSR) markers meet these requirements. SSR polymorphism is based on differences in the number of simple sequence repeats at loci defined by locus-specific PCR primers flanking the SSR sequence (Weber and May, 1989). Evidence in wheat indicates that SSR loci are abundant and well distributed, and the primer sequences for approximately 455 SSRs are now available including about 315 from the literature (Bryan et al., 1997; Donini et al., 1998; Korzun et al., 1999; Roder et al., 1998a; Roder et al., 1998b and others) and 141 from this project. This number of loci is inadequate to provide the genome coverage needed to optimize QTL discovery in wheat which has an estimated genome length of 3403 centiMorgans (cM) (calculated from the ITMI map on GrainGenes). This is true because it is unlikely that more than one-third of these 455 loci would be polymorphic in a given bi-parental cross. Thus, the average distance between loci would be 3403 cM / 152 loci = 22.4 cM between loci. Because marker loci are unlikely to be evenly distributed in the genome, many gaps of much greater than 22.4 cM would be anticipated. In soybean, with a genome length of about 2600 cM, a recent Genomics Whitepaper (http://129.186.26.94/Genomics/Soybean_Genomics.html) indicated the need to develop and map 1000 SSR loci in addition to the 1000 already available.

For full functionality, SSR loci should be annotated with three types of information: 1) primer sequences, 2) map position at several levels of resolution, and 3) the allelic status of relevant hexaploid and durum wheat accessions (i.e., primary sources of resistance, elite adapted breeding parents, and selected progeny). The last two types information are required for practical implementation to integrate markers into practical programs of QTL discovery and manipulation and background selection. The marker genotype data for selected progeny become part of the overall annotation database. These data help breeders avoid unnecessary marker assays with subsequent generations of background selection.

The USWBSI is funding a large scale effort to increase the number of SSR loci available in wheat and to initiate the mapping and allele databases. This poster describes the meiotic mapping underway at Michigan State University and our vision of how this combined project can acclerate deployment of scab resistance genes.

REFERENCES

Bernacchi, D., T. Beck-Bunn, Y. Eshed, S. Inai, J. Lopez, V. Petiard, H. Sayama, J. Uhlig, D. Zamir, and S. Tanksley. 1998a. Advanced backcross QTL analysis of tomato. II. Evaluation of near-isogenic lines carrying single-donor introgressions for desirable wild QTL-alleles derived from *Lycopersicon hirsutum* and *L-pimpinellifolium*. Theor. Appl. Genet. 97:170-180.

Bernacchi, D., T. Beck-Bunn, Y. Eshed, J. Lopez, V. Petiard, J. Uhlig, D. Zamir, and S. Tanksley. 1998b. Advanced backcross QTL analysis in tomato. I. Identification of QTLs for traits of agronomic importance from *Lycopersicon hirsutum*. Theor. Appl. Genet. 97:381-397.

Bryan, G. J., A. J. Collins, P. Stephenson, A. Orry, J. B. Smith, and M. D. Gale. 1997. Isolation and characterisation of microsatellites from hexaploid bread wheat. Theor. Appl. Genet. 94:557-563.

Donini, P., P. Stephenson, G. J. Bryan, and R. M. D. Koebner. 1998. The potential of microsatellites for high throughput genetic diversity assessment in wheat and barley. Genet. Resources Crop Evol. 45: 415-421.

Korzun, V., M. S. Roder, K. Wendehake, A. Pasqualone, C. Lotti, M. W. Ganal, and A. Blanco. 1999. Integration of dinucleotide microsatellites from hexaploid bread wheat into a genetic linkage map of durum wheat. Theor. Appl. Genet. 98:1202-1207.

Roder, M. S., V. Korzun, B. S. Gill, and M. W. Ganal. 1998a. The physical mapping of microsatellite markers in wheat. Genome 41:278-283.

Roder, M. S., V. Korzun, K. Wendehake, J. Plaschke, M. H. Tixier, P. Leroy, and M. W. Ganal. 1998b. A microsatellite map of wheat. Genetics 149:2007-2023.

Weber, J. L. and P. E. May. 1989. Abundant class of human DNA polymorphisms which can be typed using the polymerase chain reaction. Am. J. Hum. Genet. 44:388-396.

Xiao, J. H., J. M. Li, S. Grandillo, S. N. Ahn, L. P. Yuan, S. D. Tanksley, and S. R. McCouch. 1998. Identification of trait-improving quantitative trait loci alleles from a wild rice relative, *Oryza rufipogon*. Genetics 150: 899-909.

GENETIC ENGINEERING WHEAT FOR SCAB RESISTANCE

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ABSTRACT

Fusarium head blight (FHB) of wheat caused by *Fusarium graminearum* has resulted in serious economic losses due to reductions in yield and quality. Increasing the expression of antifungal protein (AFP) genes in wheat via genetic engineering is a promising approach for enhancing FHB resistance as well as resistance to other fungal diseases. Our objectives were to develop germplasm sources of transgenic wheat overexpressing the following AFP genes: wheat a-thionin, barley ribosome inactivating protein (RIP), barley PR-5 (thaumatin-like protein), and barley class-II ß-1,3-glucanase. Embryogenic calli of the spring wheat cultivar 'Bobwhite' were cotransformed via particle bombardment with one of the AFP genes and the plasmid pAHC25. PAHC25 carries the *bar* gene for selection on the herbicide bialophos and the reporter gene *uidA* for visual scoring of ß-glucoronidase (GUS) activity. For each AFP gene, between 700 and 1400 immature embryos were bombarded. Calli were selected and plants were regenerated on 5 mg/l bialophos for a minimum of 12 weeks. Between 80-300 plants were regenerated for each AFP gene. RT-PCR was performed on RNA isolated from T_0 and T_1 leaves and plants were found to express the wheat a-thionin, barley RIP, PR-5 and ß-1,3-glucanase AFP transgenes. Preliminary FHB disease severity data will be reported for a-thionin, barley RIP and ß-1,3-glucanase.

IDENTIFICATION, CLONING AND SEQUENCING OF ESTS RELATED TO FHB RESISTANCE OF WHEAT

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ABSTRACT

One of big obstacles in fighting FHB epidemics is that little is known about the nature of FHB resistance, particularly at molecular level. Our research aims at addressing this problem and getting insight into the molecular mechanism of *F. graminearum*-wheat interaction. Our current objectives were to identify, clone, sequence and analyze ESTs related to FHB resistance by comparing the EST profiles of resistant and susceptible cultivars. Sumai 3 (resistant) and Wheaton (susceptible) were employed in this research. When the first anther spring out, a single middle floret of the spikes was inoculated with 20 ml of Fg4 isolate (70, 000 spores/ml) or water (as control). The treated plants were immediately transferred into mist chambers and cultured for 24 hours (for the treatments more than 24 hours). The inoculated spikelet and the other four spikelets immediately next to it were sampled 0, 2, 4, 8, 16, 32 and 64 hours after inoculation. The total RNA extraction was conducted using Tri reagent (Molecular Research Center Inc., Cincinnati, Ohio) according to the procedure provided by the manufacture. The RNAimage kit (GenHunter Corporation, Nashville, TN) was used for obtaining the EST profiles. The whole procedure provided with the kit was followed except that we used designed primers instead of random primers. The primers were designed according to the highly conserved “LRR” domain in the products of all known cloned resistance genes (the only exception is Pto gene). A total of 144 primer combinations were tested. Several gene expression patterns were observed: 1) constitutively expressed in Sumai 3; 2) constitutively expressed in Wheaton; 3) induced expression in *F.graminerum*-inoculated Sumai 3 and Wheaton only; 4) induced expression in *F. graminerum*-inoculated Wheaton only; and 5) induced expression in *F. graminerum*-inoculated Sumai 3 only. ESTs of the last category are most likely related to FHB resistant genes. Three such ESTs,

EST12G, *EST15AU* and *EST15AD*, were cloned with PCR-Trap cloning kit (GenHunter Corporation, Nashville, TN) and sequenced using ABI automatic sequencer. A sequence similarity-search of GeneBank data base revealed that *EST15AU* is 94% similar to part of a wheat mRNA for polypeptide elogation factor 1 beta'; *EST15AD* has three homologous regions (with 86% identity) with an EST sequence from a pathogen induced sorghum bi-color cDNA; *EST12G* is almost identical (with 99% identity) to a part of minus strand of a wheat gene for chloroplast ATP synthase CF-O subunit I and III. Confirmation of the accurate relationship of these three ESTs with FHB resistance by genetic analysis is on the way.

A MICROASSAY APPROACH TO RAPID ANTIFUNGAL PROTEIN GENE PRETESTING

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OBJECTIVE

To develop a fast (less than 3-day) pretest protocol for antifungal protein gene constructs used in biolistic transformation of whole plants.

INTRODUCTION

A microassay protocol is under development for rapidly testing eukaryotic antifungal protein (AFPs) expression constructs of potential value in genetic engineering of cereals for resistance to Fusarium headblight. An approach using plant cell suspension culture and green fluorescent protein transformed *Fusarium graminearum* was selected. This made it possible to test quickly biolistic AFP constructs for their ability to stop hyphae of *F. graminearum* from attacking AFP-transformed cell clusters.

This rapid protocol can help select promising AFP candidates from the numerous AFPs available [Datta *et al.*, 1999; Shewry and Lucas 1997]. By pretesting AFPs it may be possible to speed development of Fusarium headblight resistant wheat and barley. Currently it is very expensive and time-consuming to screen AFPs using whole-plant transformation and adult plant disease testing [Bushnell *et al.* 1998; Chen *et al.,* 1999; Smith *et al.,* 2000; Van de Mortel *et al.,* 1999]. This microassay protocol offers the advantage of eukaryotic cells in which biolistic constructs can be used. It also means that we avoid extra cloning needed for bacterial expression systems, which themselves may not properly process eukaryotic proteins. With experience the microassay protocol can be used to test large numbers of potential AFP genes in commonly used biolistic constructs.

METHODS

The protocol is based on use of a hand-held biolistic gene gun and Black Mexican Sweet Corn suspension culture cells (BMS). These were transferred to cellulose filters and placed on MS solid media [Murashige and Skoog, 1962] in Petri plates, for ease of manipulation. BMS cells were co-transformed using microprojectile bombardment. A visual marker gene set construct for upregulating anthocyanin, and one or more AFP expression constructs were used in each experiment. A Helios (BIORAD) biolistic gene gun at 140 - 160 lbs./sq. in He_2 pressure delivered the plasmid constucts to the BMS cells. One-micron diameter gold particles coated with plasmid DNA (4 ug DNA/mg gold, 1.0 ug DNA per shot) was used as the gene carrier, other variables were as described in the BIORAD Helios handgun protocol.

After 24 hours, the AFP-anthocyanin co-transformed cells were inoculated with a *F. graminearum* isolate previously transformed with an *Aequorea victoria* green fluorescent

protein (GFP) reporter gene construct by Thomas Hohn - USDA, Peoria. Inoculation was done with macroconidia placed centrally on the BMS cell filter. Thirty hours later, BMS cell filters were examined microscopically for co-transformed cells (seen as brown or red cells or cell clumps) and for GFP fungal hyphae. Interaction sites were viewed with epifluorscent UV and near-blue illumination using an Aus Jena light microscope with appropriate filters and a dichroic mirror that allowed wavelengths above 510 nm to be seen. At the edge of small fungal colonies, where individual hyphae were distinguishable, transformed BMS cells were scored for fungal hyphal contact or fungal hyphal avoidance. Singly transformed BMS cell filters (anthocyanin upregulated only) were inoculated and data from were used as the control to which all other data was compared.

AFP plasmids tested contained the Sugar cane Badna Virus (ScBV) or maize ubiquitin promoters followed by a maize alcohol dehydrogenase intron or a maize ubiquitin 1 intron, the particular AFP coding sequence and the *Agrobacterium* NOS terminator. The antifungal protein genes tested were:

- pBScBV Rchit- rice chitinase transgene [Zhu and Lamb, 1991]
- pBScBV TLP1- oat thaumatin like protein cDNA [Lin *et al.*, 1996]
- pBScBV Barchit- barley chitinase cDNA [Leah *et al.*, 1991]
- pBScBV Barglu- barley glucanase cDNA [Leah *et al.*, 1991]
- pBScBv ArabPR5- Arabidopsis PR5 cDNA [Uknes *et al*., 1992]
- pUBK Tri101-Trichothecene 3-0-acetyltransferase cDNA [Kimura *et al*.,1998]
- pAHC WIR 2- wheat thaumatin-like protein cDNA [Rebman *et al*., 1991}

The anthocyanin visual marker (pPHI687) plasmid (Bowen, 1992) contained pairs of tandem Cauliflower Mosaic Virus 35S promoters and the maize alcohol dehydrogenase intron I to drive mRNA expression of transcription factors C and R which up-regulate expression of enzymes in the BMS cell anthocyanin pathway [Grotewold *et al.,* 1994].

To check for AFP transcription, primers were made for each AFP and RT PCR was done on extracts from BMS cells, removed from individual filters 30 h after transformation.

RESULTS AND DISCUSSION

RT PCR results revealed that AFP mRNAs were transcribed in BMS cells transformed with single and/or multiple expression vectors. From previous work we estimated an 80% or better co-transformation rate for our AFP constructs and the anthocyanin marker construct.

Five cellulose filters, containing BMS cells, were used for each gene construct in each individual experiment. Experiments with single or multiple AFP containing biolistic constructs were repeated a minimum of two or three times. BMS cell filters singly transformed

with the construct that upregulated anthocyanin were used as controls. Data from each experiment, with its appropriate anthocyanin control, were pooled and statistically analyzed and shown in Table 1.

Table 1. Pooled data from experiments with single or multiple AFP-containing biolistic constructs. All experiments were repeated a minimum of two or three times. Data from each experiment were pooled and analyzed. * = A significant different from Anthocyanin control at P<0.05, using student's t test.

Antifungal Proteins Tested	Number of Fungal Encounter Sites	Percent Hyphal Contact
Anthocyanin Control	**867**	**70**
Arabidopsis PR5	694	55*
Tri 101	739	50*
Wheat WIR 2	877	50*
Anthocyanin Control	**280**	**60**
Barley Chitinase	368	57
Barley Glucanase	380	51
Barley Chit/Gluc	315	55
Anthocyanin Control	**631**	**59**
Oat Tlp1	553	51
Anthocyanin Control	**458**	**55**
Rice Chit/Tlp1 Barley Chit/Gluc	593	50
Anthocyanin Control	**469**	**61**
Rice Chitinase	425	61
Rice Chit/Tlp1	445	62

Results from this microassay protocol show that *F. graminearum* contact was repelled by the antifungal proteins Arabidopsis PR5, Fusarium Tri 101 and wheat WIR 2. However the barley chitinase, barley glucanase, rice chitinase and oat Tlp1 AFPs were ineffective in repelling *F. graminearum* contact. Also, combinations of chitinase/glucanase or chitinase/Tlp 1 were ineffective.

The BMS cell transient assay of AFP efficacy has potential as a rapid means of evaluating AFPs for later use in whole plant transformation. It also could be used with other non-biotrophic fungal pathogens of wheat and barley to test the efficacy of various AFP candidates. At present we have no strong positive control AFP, like that used by Mitra *et al.* [2000] with which to compare candidate AFPs and to optimize our microassay protocol.

REFERENCES

Bushnell WR, Somers DA, Giroux RW, Szabo LJ, & Zeyen R.J. 1998. Genetic engineering of disease resistance in cereals. *Canadian Journal of Plant Pathology* 20:137-149.

Bowen B. 1992. Anthocyanin genes as visual markers in transformed maize tissues. pp 163-177 In: *GUS Protocols: Using the GUS Gene as a Reporter of Gene Expression*. S.R. Gallagher Ed. Academic Press, San Diego.

Chen W.P, Chen PD, Liu DJ, . Kynast R, Friebe B, Velazhahan R, Muthukrishnan S, & Gill BS. 1999. Development of wheat scab symptoms is delayed in transgenic wheat plants that constitutively express a rich thaumatin-like protein gene. *Theoretical and Applied Genetics* 99:755-760.

Datta K, Muthukrishnan S, & Datta SK. 1999. Expression and function of PR-protein genes in transgenic plants. pp. 261-277. In: *Pathogenesis-Related Proteins in Plants*. S.W. Datta and S. Muthukrishnan Eds. CRC Press, Boca Raton, Florida.

Grotewold E, Drummond BJ, Bowen B, & Peterson T. 1994. The myb-homologous P gene controls phlobaphene pigmentation in maize floral organs by directly activating a flavonoid biosynthetic gene subset. *Cell* 76:543-553.

Leah R, H. Tommerup H, Svedsen I, & Mundy J. 1991. Biochemical and molecular characterization of three barely seed proteins with antifungal properties. *Journal of Biological Chemistry* 266:1564-1571.

Lin KC, Bushnell WR, Szabo LJ, & Smith AG. 1996. Isolation and expression of a host response gene family encoding thaumatin-like proteins in incompatible oat-stem rust fungus interactions. *Molecular Plant-Microbe Interactions* 9:511-522.

Kimura M, Kaneko I, Komiyama M, Takatsuki A, Koshino H, Yoneyama K, & Yamaguchi I. 1998. Trichthecene 3-O-acetyltransferase protects both the producing organism and transformed yeast from related mycotoxins. *Journal of Biological Chemistry* 273:1654-1661.

Mitra, A., Clemente TE, Dickman MD, & Baenziger PS. 1999. Enhanced resistantance in winter wheat germplasm by plant transformation. 1999 National Fusarium Head Blight Forum pp. 27 (Abstract)

Rebmann G, Mauch F, Dudler R. (1991). Sequence of a wheat cDNA encoding a pathogen induced thaumatin-like protein. *Plant Molecular Biology* 17:283-285.

Smith L, Wyckoff M, Baldridge G, R. Zeyen R, & Muehlbauer GJ. 2000 Antifungal protein gene expression in transgenic wheat (Triticum aestivum). *Agronomy Abstracts*

Shewry PR & Lucas JA. 1997. Plant Proteins that Confer Resistance to Pests and Pathogens. *Advances in Botanical Research* 26:135 - 159.

Tzafrir I., Torbert KA, Lockhart BEL, Somers DA & Olszewski NE. 1998. The sugarcane bacilliform badnavirus promoter is active in both monocots and dicots. Plant Molecular Biology 38(3): 347-356.

Uknes S, Mauch-Mani B, Moyer M, Potter S, Williams S, Dincher S, Chandler D, Slusarenko A, Ward E, & Ryals J. 1992. Acquired resistance in Arabidopsis. *The Plant Cell* 4:645-656.

Van de Mortel M, Bladridge G, Bushnell W, Somer D, Tobert K, & Zeyen R. 1999. Apparent partial powdery mildew resistance in transgenic oat genetically engineered to express rice or barley chitinases. *Phytopathology* 89 6:S80.

Zhu Q & Lamb CJ (1991). Isolation and characterization of a rice gene encoding a basic chitinase. *Molecular and General Genetics*. 226:289-296.

CREATION OF AN AFLP MAP FOR IDENTIFICATION OF SCAB RESISTANCE GENES FROM WHEAT CULTIVAR WANGSHUIBAI

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ABSTRACT

Wheat scab, mainly caused by *Fusarium graminearum,* is an important disease of wheat worldwide. Resistance genes from Sumai 3 and its derivatives Ning 7840 have been well characterized through molecular mapping approach. However, resistance genes in Wangshuibai are still a puzzle to be solved. To characterize the scab resistance genes in Wangshuibai, an amplified fragment length polymorphism (AFLP) linkage map was constructed with 104 F7 recombinant inbred lines (RILs) derived from the cross between resistant cultivar Wangshuibai and susceptible cultivar Alondra. For AFLP, DNA was digested with EcoRI and MseI restriction enzyme and corresponding primers were used for AFLP analysis. EcoR I primers were labeled with ^{33}P-?ATP and PCR-products were separated in a 5% polyacrymide gel. Total 207 AFLP primer pairs were screened for two parents, and 167 of them (80.7%) amplified scorable DNA fragments. In an average, each primer pair amplified about 8 polymorphic fragments between parents, indicating a relatively high level of polymorphism between the parents. A total of 32 informative primer pairs amplified about 410 bands segregating in the F7 RILs. About 250 markers have been mapped in 23 linkage groups covering a genetic distance of 2430 cM. The map will be further saturated with *Pst*I-AFLP and SSR markers and used to locate the QTL for scab resistance in cultivar Wangshuibai. This is first effort to map scab resistance genes from Wangshuibai, another important sources of scab resistance from China.

SSR MAPPING AND SUB-ARM PHYSICAL LOCATION OF A MAJOR SCAB RESISTANCE QTL IN WHEAT

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OBJECTIVES

To identify SSR markers for a major scab resistance QTL mapped by AFLP markers in the previous study (Bai et al., 1999), and to locate the QTL to a specific sub-arm region of the chromosome.

INTRODUCTION

Molecular marker technologies provide an accurate approach to manipulation of quantitative traits such as scab resistance. Random amplified polymorphic DNAs (RAPDs), restriction fragment length polymorphisms (RFLPs), and amplified fragment length polymorphisms (AFLPs) have been used to map scab resistance genes (Bai, 1995; Bai et al., 1999; Waldron et al., 1999). Using RAPD markers with F_5-derived recombinant inbred lines (RIL), developed by single seed descent from Ning 7840 (resistant) and Clark (susceptible), Bai found two QTL controlling scab resistance on two linkage groups. The two QTL explained 18% and 6.1% of the total phenotypic variations respectively (Bai 1995). Four years later, Bai et al. (1999) screened 133 F_9 RILs derived from the same population with AFLP markers. One major QTL for scab resistance was identified, which explained almost 60% of the genetic variation for Type II scab resistance (Bai et al., 1999); however, the chromosomal location of this QTL was not determined.

MATERIALS AND METHODS

Plant Materials: Chinese Spring and derived aneuploid lines were used for physical mapping of SSR markers associated with scab resistance QTL. Thirty-five nullitetrasomic lines (missing lines were Nulli (N) 2A Tetra (T) 2B, N2AT2D, N4BT4D, N4BT4A, N4DT4A, N5BT5A, and N6BT6D); thirty-two ditelosomic lines (missing 2AL, 4AS, 5AS, 2BS, 4BL, 5BS, 3DS, 3DL, 5DS,and 7DS); and eight deletion lines for chromosome 3BS were kindly provided by Dr. John Raupp at the Wheat Genetics Resource Center, Kansas State University.

The mapping population was a set of 133 $F_{8:11}$ RILs from the cross of Ning 7840 with Clark developed by Bai et al. (1999). The phenotypic data for scab resistance and inoculation method were described in detail by Bai et al. (1999). DNA isolation was performed with the modified SDS method.

SSR analysis

SSR primers were synthesized by Life Technologies Inc. according to sequence information published by R‘der et al. (1998).

Mapping and data analysis

The computer program MAPMAKER (Lander et al., 1987), V3.0 for the MacIntosh, was used to calculate linkage distances and integrate SSR and AFLP marker data. Algorithms used a Kosambi mapping function (Kosambi, 1944) with a LOD of 3.0. Interval analysis was performed by using Qgene software (Nelson, 1997).

Physical mapping of SSR markers: Nullitetrasomic and ditelosomic lines were used to determine the chromosome and arm location of polymorphic bands by scoring the presence or absence of specific amplified SSRs. Deletion lines related to 3BS were used to further assign the sub-arm location of mapped SSR markers after the major QTL was located on chromosome 3B.

RESULTS AND DISCUSSION

Screening and mapping of polymorphic SSR markers between Ning 7840 and Clark:

n total, ninety-three SSRs were mapped, and thirty-four were polymorphic between Ning 7840 and Clark. When the mapping data were analyzed together with the AFLP mapping data from the same mapping population, we found that Xgwm 389, a marker on 3BS, was linked to the AFLP markers tightly associated with the major QTL. All other SSRs on 3B were then analyzed. Two SSRs on 3BS, Xgwm533, and Xgwm493, and one SSR, Xgwm340, on 3BL were polymorphic between Ning 7840 and Clark. Xgwm389, Xgwm533, and Xgwm493 mapped in same linkage group. Anderson et al. (1999) also found that Xgwm533 was associated with a QTL for scab resistance in their Sumai 3/ Stoa population.

Based on LOD scores, the linkage order of the three markers was determined to be Xgwm389-Xgwm533-Xgwm493, and the linkage distances between these markers were 5.3 cM and 4.8 cM, respectively. The three SSR markers were not polymorphic between Sumai 3 and Ning 7840. Xgwm340 was not associated with scab resistance in this population and was not analyzed further.

The major scab resistance QTL on 3BS:

Based on scab resistance evaluation data in F_5, F_6, F_7, and F_{10} generations, Xgwm533, Xgwm389, and Xgwm493 were associated with scab resistance. The allelic substitution effect ranged from 25% to 43% for PSS (percentage of scabbed spikelets) and from 2.53 to 4.72 for AUDPC (area under disease progress curve). In all four generations, Xgwm533 provided the greatest differences between groups of resistant and susceptible lines. All three SSR markers showed relatively high R^2 values when scab data from the four generations were analyzed. The phenotypic data were collected in the greenhouse using needle inoculation, therefore, the three SSR markers were associated with Type II scab resistance.

In the F_{10}, Xgwm389, Xgwm533, and Xgwm493 explained 36%, 44%, and 34% of the phenotypic variation for scab resistance, respectively. Marker Xgwm533 was associated more closely with the scab resistance QTL than the other two markers.

Physical sub-arm mapping of the major scab resistance QTL on 3BS

The three markers were analyzed on 8 Chinese Spring deletion lines, 3BS-1, 2, 3, 4, 5, 7, 8, and 9. Based on presence or absence of each SSR marker on these deletion lines, the sub-arm physical location can be identified for these SSR markers. Xgwm389 amplified the same size band in Chinese Spring, 3BS ditelosomic line, Ning 7840, and Sumai 3, but this band was not detected in any of the eight deletion lines or the 3BL ditelosomic line. Therefore, Xgwm389 is located distal to breakpoint 3BS-3 (Figure 1). For SSR Xgwm533, only one band was detected in Chinese Spring and its' 3BS ditelosomic line. This band is the same size as the polymorphic band between Ning 7840 and Clark. This band was found only in deletion line 3BS-3, and it was not found in other deletion lines of 3BS. The polymorphic band between Ning 7840 and Clark amplified by Xgwm493 also was detected only in the 3BS-3 deletion line. According to these results, Xgwm533 and Xgwm493 are located between breakpoint-3 and breakpoint-8. The physical mapping of the three markers confirmed that the map order of the three markers is Xgwm389, Xgwm533, and Xgwm493.

The SSR markers were integrated into the AFLP linkage group including the major scab resistance QTL reported previously (Bai et al, 1999) (Figure 1). Relative positions of the three SSR markers were used to determine the orientation of the linkage map. In single-factor analysis of the F_{10} AUDPC data, AFLP markers GCTG/CGAC1 and ACT/TGC7 explained 49% and 48% of the phenotypic variation, respectively (Bai et al., 1999). These two markers flanked the peak region of the major QTL for scab resistance. In the integrated map, these two markers were flanked by Xgwm533 and Xgwm389. Therefore, the major QTL is also flanked by the two SSR markers. As both Xgwm533 and Xgwm493 were located between breakpoints 3BS-8 and 3BS-3, it is clear that the major QTL is located distally to breakpoint 3BS-8. The fraction length of deletion 3BS-8 is 0.78 (Endo and Gill, 1996). It is still not clear if the major QTL is located between 3BS-3 and 3BS-8, or distal to 3BS-3.

The two SSR markers, Xgwm389 and Xgwm493, which are flanking a major QTL for scab resistance, can be used directly for MAS. The linkage distance between these two markers is 10.1 cM, and Xgwm533 is located almost between them. Xgwm533 is significantly closer to the QTL than Xgwm493 and Xgwm389. If we suppose that the QTL is located in the middle of the two markers that are 10 cM apart, the probability of missing the major QTL by selecting both two markers is 0.25%. These SSRs can be detected by nonradioactive gel electrophoresis of PCR products. We analyzed each of the SSR markers twice on the mapping population. They showed high stability and repeatability. Therefore, these markers are suitable for large scale screening of breeding populations for scab resistance when polymorphism occurs between the parents. We are validating the linkage between the three SSR markers and the major scab resistance QTL and its genetic effect in different backgrounds. We are mapping other QTL with SSRs in the same population and developing other PCR based markers for all these QTL.

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REFERENCES

Anderson JA, Waldron BL, Stack RW, and Frohberg RC (1999). Update on DNA markers for Fusarium head blight resistance QTL in two wheat populations. P 19-21 In J. Wagester, R. Ward, L. P. Hart, S. P. Hazen, J. Lewis, H. Borden (eds) Proc 1999 National FHB Forum University Printing, East Lansing, MI

Bai G (1995) . Scab of wheat: epidemiology, inheritance of resistance and molecular markers linked to cultivar resistance Ph D thesis, Purdue University, W Lafayette, Indiana, USA

Bai GH, Kolb FL, Shaner G, and Domier LL (1999) . Amplified fragment length polymorphism markers linked to a major quantitative trait locus controlling scab resistance in wheat. Phytopathology 89:343-348

Endo TR, and Gill BS (1996). The deletion stocks of common wheat. J Hered 87:295-307

Kosambi DD (1944). The estimation of map distances from recombination values. Ann Eugen 12:172-175

Lander ES, Green P, Abrahamson J, Barlow A, Daly MJ, Lincoln SE, Newburg I (1987). Mapmaker: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1:174-181

Nelson, JC (1997). QGene: software for marker-based genomic analysis and breeding. Molecular Breeding 3: 239-245

Roder MS, Korzun V, Wendehake K, Plaschke J, Tixier MH, Leroy P, Ganal MW (1998). A microsatellite map of wheat genetics. Genetics 149: 2007-2023

Waldron BL, Moreno-Sevilla B, Anderson JA, Stack RW and Frohberg RC (1999) . RFLP mapping of QTL for Fusarium Head Blight resistance in wheat. Crop Science 39:805-811

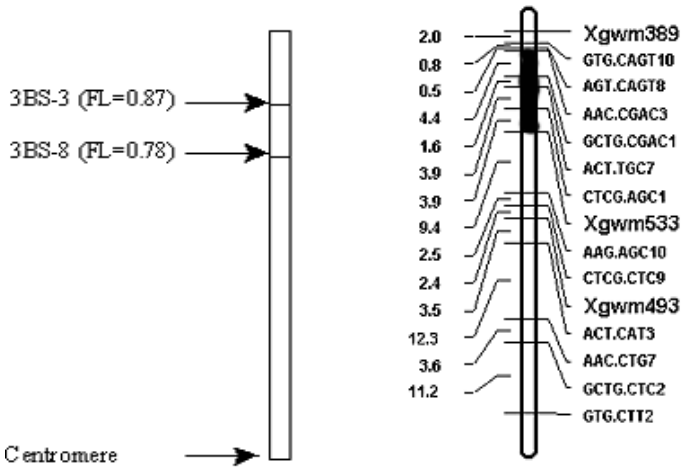

Figure1. Left: An idiogram of chromosome 3BS, arrows label breakpoints of deletion lines 3BS-3, 3BS-8 and centromere. FL means fraction length.

Right: An integrated map for microsatellite markers on chromosome 3BS and AFLP markers analyzed on the same population of Ning 7840/ Clark; distance unit between markers is cM. Dotted lines show the physical location of three microsatellite markers. Blocked bar shows putative region including the major QTL.

SEED TREATMENT WITH BACTERIAL BIOCONTROL AGENTS TO CONTROL HEAD BLIGHT

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ABSTRACT

Selected *Bacillus* and *Paenibacillus* strains isolated from South Dakota wheat foliage and residue have been shown to antagonize isolates of *Fusarium graminearum* causing FHB, as well as antagonizing *Pyrenophora tritici-repentis* which causes tanspot. Application of whole live cells of these bacterial biocontrol agents (BCAs) as well as application of concentrated ethyl acetate extracts of broth culture supernatants of the bacteria, have previously been shown to reduce symptoms of FHB and tanspot in greenhouse and field-plot trials. Another method of using these BCAs would be applying them to wheat seed before planting. By manipulating the bacteria to remove their walls and ensure their osmotic protection, cell wall deficient (CWD) forms of the bacteria such as protoplasts and L-forms can be produced. These CWD bacterial forms have been shown by other researchers to form putative intracellular associations with the cells of a variety of plants. It would be desirable to develop seed-treatment methods to reliably establish such associations between wheat plants and CWD-forms of bacterial BCAs, since it could afford protection against infection by *F. graminearum* without need for spray application of bacteria onto mature wheat plants. Alternatively, seed treatment with wall-bearing cells of bacterial BCAs could result in protection against FHB. In one greenhouse trial, soaking wheat seeds with wall-bearing cells of bacterial strain 1BE *(Paenibacillus lentimorbus)* resulted in 26% less FHB symptoms than soaking seeds with live protoplasts of the same bacterium. In a greenhouse trial involving tanspot, however, soaking wheat seeds with live protoplasts of *P. lentimorbus* 1BE resulted in 22% less FHB symptoms than soaking seeds with live wall-bearing cells of the same bacterium. More work is needed to explore the potential of seed treatment with bacterial BCAs to control FHB.

CONTROL OF FUSARIUM HEAD BLIGHT WITH BIOLOGICAL ANTAGONISTS

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INTRODUCTION AND OBJECTIVES

Fusarium head blight (scab) has been a serious concern for wheat producers in South Dakota for the past several years. Scab and low market prices are the two reasons most often cited by growers as they decrease the number of acres they plant to wheat. Fungicide alternatives for disease control are available on special year-to-year labeling options.

The objectives of this study were to evaluate the efficacy of various bacterial strains employed as biological control agents for their fungicidal properties for the suppression of Fusarium head blight (scab).

MATERIALS AND METHODS

The bacterial isolates from SDSU screened in this study were selected in laboratory assays where both live cells and ethyl acetate extracts were shown to antagonize growth of *Fusarium graminearum* isolate Fg 4. Isolates from SDSU are classified as strains of *Bacillus* or *Paenibacillus*. Two of these strains were applied to two spring wheat cultivars, Oxen and Ingot, planted at Brookings, South Dakota. The previous crop was soybean. No *Fusarium* inoculum was added to the sites nor was the environment modified with mist irrigation. Six microbial isolates were tested. Isolate SDSU #1-1BA is a *Paenibacillus lentimorbus*. All other strains are putative *Bacillus* sp. Bacterial treatments were applied and compared to an untreated control and a chemical control of Folicur (4 fl. oz./A), plus Induce non-ionic surfactant (NIS) (0.125%). Treatments were replicated four times and applied at Feekes 10.3-10.54 as a 10^4 CFU/ml suspension of whole bacterial cells in nutrient broth. Ingot typically reaches anthesis was about two days later than Oxen, but all plots were flowering on early tillers when treatments were applied. Plots were evaluated for scab and leaf disease 21-28 days after treatment, about soft dough. Yield, test weight, DON, protein, and FDK measurements were taken following harvest.

RESULTS AND DISCUSSION

The Brookings environment was particularly dry in 2000. Little scab developed as a result and significant differences were not observed among most of the variables measured (Table 1). Folicur did reduce scab numerically and leaf disease significantly from the untreated control. However, at such low levels of scab, it is difficult to interpret these data. Failure to increase yield was not a reliable measurement in 2000 because of the low levels of disease. While significant differences were reported among treatments for leaf disease, the

biological control agents were not significantly different from the untreated. The biological control agents did not approach the activity of the Folicur standard.

No product significantly reduced FDK below the level observed in the control or increased protein, yield or test weight significantly higher than the control.

Nonetheless, trends observed for scab incidence and head severity may indicate that the SDSU isolates have activity in the range of Folicur. Additional testing is needed to substantiate this possible difference. These isolates are scheduled for field screening in 2001 under mist irrigation. A mist system intended for use in 2000 was not completed in time due to problems with work completion and the environment in 2000 pushing development of the crop earlier than in most years.

The levels of scab observed in South Dakota in 2000 were lower than have been observed for several years.

Table 1. Measurements of disease and yield in HRSW Scab Biocontrol Trial – SDSU Agronomy Farm, Brookings, SD, 2000.

Treatment	FHB Inc.[b]	FHB Sev.[c]	FHB Ind.[d]	Whole Plot Disease Rating[e]	FDK	Yield	Test Weight	Protein
	%	%	%		%	bu/A	lb/bu	%
Untreated	2	14.54	0.59	4.13	1	51.76	58.39	13.76
Folicur + NIS	1.25	4.29	0.19	3.31	0.5	52.15	58.54	14.14
SDSU #1- 1BA	0.75	2.63	0.07	4.06	0.75	50.47	58.57	13.79
SDSU #2- 1 BC	1.5	5.25	0.12	4	0.75	51.51	58.63	13.81
Experimental A	2.25	12.96	0.35	4.19	0.75	51.11	58.49	13.88
Experimental B	2.25	12.38	0.41	4.19	0.88	50.54	58.67	13.64
Experimental C	1	9.75	0.2	4	0.75	49.33	58.61	13.89
Experimental D	0	0	0	4.13	0.88	50.13	57.29	13.71
LSD (0.05)	NS	NS	NS	0.4	NS	NS	NS	NS

[a] Data represents an average of disease impact on Oxen and Ingot.

[b] % of infected heads, based on a 50 head sample.

[c] % infection of blighted heads.

[d] % blighted heads x % infection on blighted heads.

[e] Subjective rating (0=green – 5=necrotic) of tissue necrosis in the plot at evaluation.

BIOCONTROL OF FUSARIUM HEAD BLIGHT IN BRAZIL

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INTRODUCTION

Fusarium graminearum Schw. (Teleomorph=*Gibberella zeae* Schw. Petch.) is the *Fusarium* species most frequently responsible for scab of wheat and barley in Brazil. This disease, also known as Fusarium head blight (FHB), is responsible for major losses which vary from 10% (Luz, 1984); to 54% (Picinini & Fernandes, 1994). At present, available and affordable control measures, such as resistant varieties, cultural practices and foliar fungicides, are only partially effective.

Only modest levels of resistance have been deployed in cultivars in commercial fields; the most widely grown cultivars are often most susceptible. Furthermore, the benefit of crop rotation as a control measure is reduced by the wide host range of the pathogen, especially on grasses (Costa Neto, 1976; Luz, 1982). Treatment with foliar fungicides remains the most important (Picinini and Fernandes,1994) and recommended (Reunião da Comissão Sul-Brasileira de Pesquisa de Trigo, 2000) tool for reducing scab in Brazil, despite its shortcomings as a control measure. The use of certain effective fungicides has been restricted in some countries because application at late developmental stages, that is, during heading and flowering, can result in chemical residues in the harvested grain.

Biological control is an additional strategy that may eventually play an important role in an integrative approach to scab management of cereals.

RESEARCH IN BIOCONTROL OF FUSARIUM HEAD BLIGHT

The chronology of publications on the biocontrol of FHB from 1988 to 2000 is listed in Table 1.

Screening of microorganisms to control wheat scab was initiated in Brazil in the 80's (Luz, 1988). At the beginning, over 300 bacteria and yeasts isolated from wheat were screened in vitro against *F. graminearum.*This work was followed by that of Perondi et al. (1990a,1990b,1996) in which microbial strains were tested for their antagonistic action against the pathogen. Potential antagonists were selected by the funnel method (Luz, 1990) which compared the effect of individual test oganisms on the radial growth of *F. graminearum*. Promising isolates were further tested in the greenhouse and in the field for their ability to control wheat scab. Individual bioprotectants significantly diminished the severity of the disease under field conditions, raising the yield of wheat between 7 and 31% when compared to nontreated plants.

The most recent published work, (Luz, 2000) showed that *Bacillus megaterium* (Embr.9790) *Bacillus subtilis* (Embr.9786) and *Paenibacillus macerans* (Embr.9770) the best isolates

significantly diminished the disease incidence and severity up to 50% and 67% respectively. The yield increase varied from 701 Kg to 818 Kg/ha.

Isolates that significantly reduced the percentage of scabby spikelets when tested under greenhouse conditions, frequently reduced disease in field trials as well, even though they may have not inhibited growth in the laboratory assays. This suggests that greenhouse evaluation for biocontrol of scab is more reliable as an early selection method than in vitro assays in large-scale screening of bioprotectants. However, since greeenhouse screening requires a substantial investment in equipment and space, in vitro assays are likely to continue to be used as an initial step in selecting potential bioprotectants.

From 1988 up to now, thousands of microorganisms have been tested for scab control in Brazil. The biodiversity of microorganisms that show potencal for managing the disease comprises the species described in Table 2.

Some other workers outside of Brazil have been investigating antagonists to control FHB (Khan et al., 1998;1999, Boeham et al., 1999; Luo & Bleakley, 1999; Schisler et al., 1999; Stockwell et al., 1997,1999,2000), under greenhouse or field conditions. Some strains have reduced the FHB severity and significantly reduced vomitoxin contamination in grains (Stockwell et al., 1997,2000).

The constraints to the application of bioprotectants to the ears of wheat and barley at flowering such as the timing of application, inoculation technology, physiological state of the organisms, spike colonization, survival of the organisms under the harsh envirommental conditions, variability of biocontrol from year to year, fermentation, formulation, and storage will be discussed. The partial control of any tactics to protect against FHB up to this moment indicates that the integration of protection measures would provide the best disease management.

(continued on next page)

Table 1. Chronology of works done on biocontrol of Fusarium Head Blight of wheat

Literature	Bioprotectants
Luz, 1988	Bacteria, Yeast
Perondi;N.L., Luz,W.C.da & Thomas,R, 1990 a,1990 b, 1996	*Bacillus subtilis* *Bacillus* spp. *Pseudomonas fluorescens* *Sporobolomyces roseus*
Stockwell, C.A; Luz,W.C. da, and Bergstrom, G.C., 1997	*Paenibacillus macerans* *Pseudomonas putida* *Sporobolomyces roseus*
Khan, N.I.,Schisler,D.A..Bochm,M.J, Lipps,P.E., Slininger,P.J. and Bothast, R.J., 1998	*Bacillus* spp.
Boehm,M.J., Khan, N.J., and Schisler, D.A,1999	Yeast, *Bacillus* sp.
Khan, N.J., and Schisler, D.A.,and Boehm, M.J.,1999	Yeast, *Bacillus* sp.
Luo, Y. & Bleakley, B. 1999	*Bacillus* spp.
Schisler, D.A., Khan, N.J. and Boehm, M.J. 1999	*Bacillus* spp.
Stockwell, C.A., Bergstrom, G.C. and Luz, W.C. da. 1999	*Paenibacillus macerans* *Pseudomonas putida* *Sporobolomyces roseus*
Luz, 2000	*Bacillus megaterium* *Bacillus subtilis* *Pantoea agglomerans* *Kluyvera cryocrescens* *Paenibacillus macerans* *Bacillus licheniformans* *Pseudomonas putida* *Sporobolomyces roseus*
Stockwell, C.A., Bergstrom, G.C. and Luz, W.C. da.,2000	*Paenibacillus macerans* *Bacillus* spp.

Table 2. Biodiversity of microorganisms for biocontrol of Fusarium Head Blight of Wheat in Brazil.

Bacillus licheniformans,
Bacillus megaterium,
Bacillus subtilis,Kluyvera cryocrescens,
Paenibacillus macerans,
Pantoea agglomerans,
Pseudomonas putida,
Pseudomonas fluorescens,
Sporobolomyces roseus,
Rhodotorula sp.

REFERENCES

Boehm, m.J., Khan, N.J., and Schisler, D.A. 1999. USDA-ARS, Ohio State University cooperative research on biologically cntrolling Fusarium Head Blight: 3. Field testing of antagonists. Pages 45-48 in: Preceedings of the 1999 National Fusarium Head Blight Forum, Michigan State University, University Printing, East Lansing, MI.

Costa Neto,J.P. da. 1976. Lista de fungos sobre gramíneas (capins e cereais) no Rio Grande do Sul. Revista da Faculdade de Agronomia. UFRGS. 1:43-78.

Khan,N.J., Schisler, D.A., and Boehm, M.J. 1999. USDA-ARS, Ohio State University cooperative research on biologically controlling Fusarium Head Blight: 2. Influence of pathogen strain, inoculum spray sequence and inoculum spray time. Pages 56-59 in: Proceedings of the 1999 National Fusarium Head Blight Forum, Michigan State University, University Printing, East Lansing, MI.

Khan,N.J., Schisler, D.A., Boehm, M.J.,Lipps, P.E., Slininger, P.J. and Bothast, R.J. 1998. Biological control of scab of wheat incited by *Giberella zeae*. Pages 45-46 in: Proceedings of the 1998 National Fusarium Head Blight Forum, Michigan State University, University Printing, East Lansing. MI.

Luo, Y. and Bleakley, B. 1999. Biological control of Fusarium Head Blight (FHB) of wheat by *Bacillus* strains. Pages 78-81 in: Proceedings of the 1999 National Fusarium Head Blight Forum, Michigan State University, University Printing, East Lansing, MI.

Luz, W.C. da. 1982. Diagnose das Doenças da Cevada. Passo Fundo – EMBRAPA-CNPT, 24p. (Circular Técnica nº. 2).

Luz, W.C. da. 1984. Yield losses caused by fungal foliar wheat pathogens in Brazil. Phytopathology 74:1403-1407.

Luz, W.C. da. 1988. Biocontrol of fungal pathogens of wheat with bacteria and yeasts. Page 348 in: 5th International Congesss of Plant Pathology, Kyoto, Japan. (Abstr.).

Luz, W.C. da. 1990. Microbiological control of Bipolaris sorokiniana 'in vitro'. Fitopatol. Brasileira 15:246-247.

Luz, W.C. da. 2000. Bioproteção da giberela do trigo. Fitopatologia brasileira 25:389 (abstr.).

Perondi, N.L., Luz, W.C. da. and Thomas, R. 1996. Controle microbiológico da giberela do trigo. Fitopatol. Brasileira 21:243-249.

Perondi, N.L., Thomas, R. and Luz, W.C. da. 1990 a. Antagonistas potenciais de *Fusarium graminearum.* In: Anais do 2º Simpósio de Controle Biológico, Brasilia, D.F., p. 128.(Abstr.).

Perondi, N.L., Thomas, R. and Luz, W.C. da. 1990 b. Controle microbiano da giberela do Trigo em campo. In: Anais do II Simpósio de Controle Biológico, Brasilia, DF. P.129(Abstr.).

Picinini, E.C. and Fernandes, J.M.C. 1994. Controle químico da *Gibberella zeae* em trigo pelo uso de fungicidas inibidores da síntese do ergoterol. Fitopatol. Brasileira 19 (Supl.):273.

Reunião da Comissão Sul-Brasileira de Pesquisa de Trigo. 1999. Recomendações da Comissão sul brasileira de pesquisa de trigo.Cruz Alta: FUNDACEP-FECOTRIGO, 90pp.

Schisler, D.A., Khan, N.J., and Boehm, M.J. 1999. USDA_ARS, Ohio State University cooperative research on biologically controlling Fusarium Head Blight: 1. Antagosist selection and testing on durum wheat. Pages 78-81 in: Proceedings of the 1999 National Fusarium Hrad Blight Forum, Michigan State University, University Printing, East Lansing, MI.

Stockwell, C.A., Luz, W.C.da., and Bergstrom, G.C. 1997. Biocontrol of wheat scab with microbial antagonists. Phytopathology 87:S94.(Abstr.).

Stockwell, C.A., Bergstrom, G.C., and Luz, W.C.da.1999. Selection of microbial antagonists for biological control of Fusarium Head blight of wheat. Pages 82-84. Proceedings of the 1999 National Fusarium Head Blight Forum, Michigan State University, University Printing, East Lansing, MI.

Stockwell, C.A., Bergstrom, G.C., and Luz, W.C.da.2000. Identification of bioprotectants for biological control of *Gibberella zeae* (in this proceedings).

INTERACTION OF 28% NITROGEN WITH FOLICUR FUNGICIDE WHEN APPLIED AT HEADING AS A TANK MIX

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INTRODUCTION AND OBJECTIVES

It is a common practice of wheat producers in the Dakotas and Minnesota to treat heading wheat with 28-0-0 nitrogen fertilizer to increase the protein in the grain and attempt to earn a premium at the elevator. This premium may only be offered in years where weather conditions favor exceptionally low protein in hard wheats. 28-0-0 is a solution of about 30# of actual nitrogen/gallon. It is a very viscous substance that has wetting properties. As such it may be used in place of other surfactants when a fungicide is applied. With the interest in applying fungicides at heading to suppress scab, growers have shown asked many questions about the compatibility of 28-0-0 and Folicur (tebuconazole), the most widely used fungicide for suppression of scab. Unfortunately, that information was not available. The Folicur label recommends the product be applied with a non-ionic surfactant (NIS) at a concentration of 0.06-0.125%.

The objective of this study was to determine the compatibility of Folicur with 28-0-0 with or without an added NIS.

MATERIALS AND METHODS

Two spring wheat cultivars, Oxen and Ingot, were planted at three South Dakota locations (Brookings, Groton, and South Shore). At the Brookings and Groton location the previous crop was soybeans, while the South Shore location was planted into corn residue. No inoculum was added to the sites nor was the environment modified with mist irrigation. Four treatments, Folicur (4 fl. oz./A) with NIS (0.125%); Folicur (4 fl. oz./A) tank mixed with 28-0-0 (28.5# actual nitrogen/A); Folicur (4 fl. oz./A) plus NIS (0.125%), tank mixed with 28-0-0 (28.5# actual nitrogen/A); compared to an untreated check. The treatments were replicated six times at all locations. Treatments were applied at Feekes 10.3-10.54. Ingot is about three days later in reaching anthesis than Oxen. At least some tillers were entering anthesis at the time of treatment. Plots were evaluated for scab and leaf disease 21(Groton) or 28 days (South Shore and Brookings) after treatment, about soft dough. Following harvest, yield, test weight, DON, protein, and FDK measurements were taken.

RESULTS AND DISCUSSION

Substantial damage was observed on the flag leaves and awns of both cultivars after treatment. By the rating date, these differences were not as apparent. Greater differences were observed between the Oxen and Ingot cultivars than between 28-0-0 treatments when compared to the untreated of the same cultivar. Mixed results were observed, with no definitive

advantage to using 28-0-0 on the crop in 2000. Nitrogen fertilizer as the carrier did not significantly alter the performance of the Folicur for scab suppression at any location. Differences were greater between the two varieties tested than among treatments.

Drought conditions caused the crop to mature very rapidly, making leaf disease evaluations difficult. However, by the end of the season the damage sustained in association with 28-0-0 was not as obvious and did not appear to result in serious losses of yield. When Folicur was applied, leaf disease was generally reduced, when only 28-0-0 was applied leaf disease ratings were similar to the untreated. Similar results were observed at Groton with leaf rust suppression across the treatments.

Yields were generally unchanged from the untreated of each cultivar, as a result of including 28-0-0 as a component of the treatment (Table 1 & 3); however, at one location yields were significantly higher when Folicur was included (Table 2). The addition of 28-0-0 did not significantly decrease the yield response realized from the typical Folicur treatment. A Folicur component always reduced leaf disease, usually significantly. Only at the South Shore location were the reductions in leaf disease insignificant. The addition of 28-0-0 did appear to increase protein content in the harvested grain, but not reliably across locations or cultivars.

At the time of this writing, not all data were available for inclusion.

Table 1. Measurements of disease and yield in HRSW Scab 28-0-0/Folicur Trial – SDSU Agronomy Farm, Brookings, SD, 2000.

Treatment **Name** (Cultivar – Application)	**FHB Inc.[a]** **%**	**FHB Sev.[b]** **%**	**FHB Ind.[c]** **%**	**Whole Plot Disease Rating[e]** (0-5)	**Yield** bu/A	**Test Weight** #/bu	**Protein** **%**
Oxen - Untreated	4	35.37	1.4	4.5	52.21	56.94	14.27
Oxen - Folicur + NIS	2.67	24	0.83	3.83	52.62	57.63	14.6
Oxen - Folicur + NIS + 28-0-0	1.33	27.75	0.72	3.92	50.6	57.13	14.88
Oxen - Folicur + 28-0-0	3.33	28.04	1.2	3.83	55.16	57.7	14.87
Oxen – 28-0-0	1.67	12.22	0.35	4.5	53.46	57.98	14.38
Ingot - Untreated	1	7.61	0.46	5	50.33	60.2	13.97
Ingot - Folicur + NIS	1.33	17.92	0.54	4.17	50.63	60.49	14.88
Ingot - Folicur + NIS + 28-0-0	0.67	20.17	0.4	4.67	49.54	60.29	15.18
Ingot - Folicur + 28-0-0	1.67	22	0.61	4.25	53.48	60.32	15.33
Ingot - 28-0-0	1	21.33	0.43	4.92	48.97	60.51	14.52
LSD (0.05)	NS	NS	NS	0.3	3.47	0.83	0.43

[a] % of infected heads, based on a 50 head sample

[b] % infection of blighted heads (head severity)

[c] % blighted heads x % infection on blighted heads

[d] Subjective rating (0=green – 5=necrotic) of tissue necrosis in the plot at evaluation.

Table 2. Measurements of disease and yield in HRSW Scab 28-0-0/Folicur Trial – Abelin Farm, Groton, SD, 2000.

Treatment **Name** (Cultivar – Application)	FHB Inc.[a] %	FHB Sev.[b] %	FHB Ind.[c] %	Leaf Disease[d] % LAR	Leaf Rust[d] % LAR	Yield bu/A	Test Weight lbs/bu	Protein %
Oxen – Untreated	7	8.4	0.88	23.88	4.77	48.33	56.41	15.92
Oxen - Folicur + NIS	8.67	18.67	1.57	5.7	0.28	56.46	57.32	15.78
Oxen - Folicur + NIS + 28-0-0	6.33	12.44	0.74	9.03	0.03	49.53	55.01	15.75
Oxen - Folicur + 28-0-0	6	14.88	0.91	7.3	0.9	52.33	56.51	15.78
Oxen - 28-0-0	5	21.53	1.2	13.3	4.52	51.13	56.46	15.65
Ingot - Untreated	3	6.13	0.28	41.98	18.47	44.36	60.21	15.8
Ingot - Folicur + NIS	2.67	8.94	0.57	3.08	0.3	53.01	60.42	16.07
Ingot - Folicur + NIS + 28-0-0	2.33	7.42	0.27	21.38	0.62	47.71	59.66	16.07
Ingot - Folicur + 28-0-0	2.33	5.44	0.21	11.47	0.38	51.96	60.43	15.92
Ingot - 28-0-0	2.33	6.81	0.26	37.25	16.78	46.93	59.57	15.65
LSD (0.05)	NS	NS	NS	11.23	4.67	4.25	1.19	NS

[a] % of infected heads, based on a 50 head sample

[b] % infection of blighted heads (head severity)

[c] % blighted heads x % infection on blighted heads (field severity)

[d] % leaf area damaged by general leaf diseases (largely tan spot) or leaf rust.

Table 3. Measurements of disease and yield in HRSW Scab 28-0-0/Folicur Trial - NE Farm, South Shore, SD, 2000.

Treatment **Name** (Cultivar - Application)	FHB Inc.[a] %	FHB Sev.[b] %	FHB Ind.[c] %	Whole Plot Disease Rating[d] (0-5)	Leaf Rust % LAR[e]	Yield bu/A	Test Weight lbs/bu	Protein %
Oxen - Untreated	12	56.76	6.3	3.88	0.4	45.22	58.38	15.73
Oxen - Folicur + NIS	6.67	50.82	3.37	3.83	0.2	44.51	59.98	15.9
Oxen - Folicur + NIS + 28-0-0	9.33	57.39	5.12	3.67	0	43.51	59.3	16.42
Oxen - Folicur + 28-0-0	8	52.86	4.51	3.67	2.4	44.98	59.12	15.5
Oxen - 28-0-0	7.33	40.83	3.39	3.83	1.8	43.76	58.95	14.68
Ingot - Untreated	3	32.88	1.41	4.38	3.2	45.53	58.32	15.68
Ingot - Folicur + NIS	5.33	49.04	2.61	4.17	0.6	44.58	59.07	16.35
Ingot - Folicur + NIS + 28-0-0	4.33	33.33	1.8	3.4	0	42.89	58.19	16.42
Ingot - Folicur + 28-0-0	6.67	44.3	3.4	4.08	1.6	45.13	58.77	16.12
Ingot - 28-0-0	4	67.42	2.63	4.08	5.6	42.43	58.27	15.37
LSD (0.05)	5.24	NS	3.12	NS	N/A	NS	NS	1.1

[a] % of infected heads, based on a 50 head sample

[b] % infection of blighted heads (head severity)

[c] % blighted heads x % infection on blighted heads (field severity)

[d] Subjective rating (0=green – 5=necrotic) of tissue necrosis in the plot at

[e] % leaf area covered by leaf rust pustules

PERFORMANCE OF VARIOUS FUNGICIDES FOR SUPPRESSION OF FUSARIUM HEAD BLIGHT (SCAB) IN SOUTH DAKOTA – 2000

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INTRODUCTION AND OBJECTIVES

Along with 14 other states, South Dakota entered three locations in the Uniform Fungicide Trial for the suppression of Fusarium head blight or scab on hard red spring wheat and one location for scab suppression studies on hard red winter wheat.

The objectives of this study were to evaluate the efficacy of various fungicides or fungicide combinations for the suppression of Fusarium head blight (scab).

MATERIALS AND METHODS

Two spring wheat cultivars, Oxen and Ingot, were planted at three South Dakota locations (Brookings, Groton, and South Shore). At the Brookings location the previous crop was soybeans, while the Groton and South Shore locations were planted into corn residue. No inoculum was added to the sites nor was the environment modified with mist irrigation. Eleven treatments were applied and compared to an untreated control. The treatments included Folicur (4 fl. oz./A), plus Induce non-ionic surfactant (NIS) (0.125%); Tilt (4 fl. oz./A) plus Induce NIS (0.125%); Stratego (14 fl. oz./A) plus Induce NIS (0.125%); BAS 500 (12.3 fl. oz./A) plus Agridex crop oil concentrate (COC) (1%); BAS 500 (6.2 fl. oz./A), plus Folicur (2 fl. oz./A) and Induce NIS (0.125%); Quadris (0.15# a.i./A) plus Benlate (0.25# a.i./A); BAS 500 (6.2 fl. oz./A) plus Agridex COC (1%); Quadris (0.125# a.i./A); Caramba (13.5 fl. oz./A); Folicur (6 fl. oz./A) plus Induce NIS (0.125%); and, Tilt (6 fl. oz./A) plus Induce NIS (0.125%). Treatments were replicated six times at all locations. Treatments were applied at Feekes 10.3-10.54. Ingot is about two days later in reaching anthesis than Oxen, but all plots were flowering on early tillers when treatments were applied. Plots were evaluated for scab and leaf disease 21-28 days after treatment, about soft dough. Following harvest, yield, test weight, DON, protein, and FDK measurements were taken. The winter wheat trial was lost due to heavy pressure form cheat grass and loss of stand due to root rotting fungi.

RESULTS AND DISCUSSION

At the Brookings location (Table 1), little scab developed and, as such, significant differences were not observed among most of the variable measured. Only Quadris, Caramba, and BAS 500 at the lower rate resulted in significantly higher yield, while most products reduced leaf disease as reported as a whole plot rating of general necrosis.

Even less scab was observed at Groton, SD (Table 2); however, leaf disease was more easily quantified. All products significantly reduced leaf diseases in general and leaf rust in

particular. These rating were based on a percent leaf are that was necrotic due to disease. No product significantly reduced FDK below the level observed in the control or increased protein significantly higher than the control. Test weight was significantly increased by Quadris and the high rate of Folicur (6 fl oz/A). All products significantly increased yield.

The highest levels of scab were recorded at the NE Research Farm (Table 3). Nonetheless, significant differences were not discernable. At this location, no significant differences from the untreated control were recorded for any of the measured variables.

The levels of scab observed in South Dakota in 2000 were lower than have been observed for several years.

TABLE 1. Measurements of disease and yield in HRSW Scab Fungicide Trial – Brookings, SD, 2000.

Treatment	FHB Inc.[b]	FHB Sev.[c]	FHB Ind,[d]	Whole Plot Disease Rating[e]	FDK Score	DON	Yield	Test Weight	Protein
Product (rate)	(%)	(%)	(%)	(0-5)	(%)	(ppm)	(bu/A)	(#/bu)	(%)
Untreated	2.5	21.49	0.93	4.75	0.75	0.09	51.27	58.57	14.12
Folicur (4) + NIS	2	20.96	0.68	4	1	0	51.62	59.06	14.74
Tilt (4) + NIS	1.5	30.92	0.83	4.38	0.67	0	51.16	58.69	14.54
Stratego + NIS	1.67	30.13	0.8	4.13	0.58	0	51.6	58.84	14.24
BAS 500 (12.3) + COC	2.17	23.35	0.9	4.33	0.67	0	51.74	58.96	14.4
BAS 500 + Folicur + COC	1.67	26.97	0.84	4.54	0.83	0	50.54	58.8	14.08
Quadris + Benlate	2.33	14.94	0.67	4.13	0.92	0.04	52.66	58.89	14.57
BAS 500 (6.2) + COC	2.17	30.38	0.89	4.67	0.58	0	49.99	58.66	14.06
Quadris	0.83	20.08	0.4	3.96	0.67	0.07	54.83	58.98	14.72
Caramba	2.17	15.65	0.76	4.08	0.67	0	54	58.77	14.56
Folicur (6) + NIS	1.83	24.53	0.78	4.63	0.75	0	50.04	58.53	14.29
Tilt (6) + NIS	1.33	12.32	0.59	4.42	0.83	0	52.75	58.91	14.22
LSD (0.05)	NS	NS	NS	0.24	NS	NS	2.46	NS	0.3

[a] Data represents an average of disease impact on Oxen and Ingot.

[b] % of infected heads, based on a 50 head sample

[c] % infection of blighted heads

[d] % blighted heads x % infection on blighted heads

[e] Subjective rating (0=green – 5=necrotic) of tissue necrosis in the plot at evaluation.

Table 2. Measurements of disease and yield in HRSW Scab Fungicide Trial - Groton, SD, 2000.

Treatment	FHB Inc.[b]	FHB Sev.[c]	FHB Ind.[d]	Leaf Disease[e]	Leaf Rust[e]	FDK	Yield	Test Weight	Protein
Product (rate)	(%)	(%)	(%)	(%)	(%)	(%)	(bu/A)	(#/bu)	(%)
Untreated	5	7.26	0.58	32.93	11.62	1	46.35	58.31	15.86
Folicur (4) + NIS	5.67	13.81	1.07	4.39	0.29	1	54.73	58.87	15.93
Tilt (4) + NIS	6	14.46	1.05	5.67	2.5	1	53.23	58.77	15.72
Stratego + NIS	4	10.99	0.61	11.88	4.45	1.08	52.45	58.76	15.72
BAS 500 (12.3) + COC	5.67	14.95	1.38	4.69	0.21	1.08	55.66	58.62	16.02
BAS 500 + Folicur + COC	5.5	10.28	0.76	8.09	1.73	1.25	50.27	58.46	15.78
Quadris + Benlate	6.83	13.48	1.23	5.91	2.03	1.33	55.05	58.52	15.81
BAS 500 (6.2) + COC	6.17	10.9	0.93	5.97	0.75	1.08	56.92	58.72	15.96
Quadris	6.17	11.81	0.96	9.72	2.27	1.33	50.41	59.26	15.8
Caramba	2.67	9.02	0.37	3.69	0.19	0.83	52.94	58.9	15.94
Folicur (6) + NIS	4.5	19.5	1.02	4.43	0.59	0.92	52.92	59.21	15.88
Tilt (6) + NIS	3.67	15.35	0.76	3.57	1.42	1	56.69	58.72	15.54
LSD (0.05)	NS	NS	NS	7.04	2.88	0.27	4.66	0.93	0.27

[a] Data represents an average of disease impact on Oxen and Ingot.

[b] % of infected heads, based on a 50 head sample

[c] % infection of blighted heads

[d] % blighted heads x % infection on blighted heads

[e] % leaf area damaged by general leaf diseases (largely tan spot) or leaf rust.

Table 3. Measurements of disease and yield in HRSW Scab Fungicide Trial - NE Research Farm, South Shore, SD, 2000.

Treatment	FHB Inc.[b]	FHB Sev.[c]	FHB Ind.[d]	Whole Plot Disease[e]	Leaf Rust[e]	FDK[f]	Yield	Test Weight	Protein
Product (rate)	(%)	(%)	(%)	(0-5)	(%)	(%)	(bu/A)	(#/bu)	(%)
Untreated	7.5	44.82	3.86	4.13	1.8	1.25	45.37	58.35	15.71
Folicur (4) + NIS	6	49.93	2.99	4	0.4	0.92	44.54	59.53	16.13
Tilt (4) + NIS	7.33	56.59	4.07	4.02	5	1.25	43.57	59.08	15.63
Stratego + NIS	9.83	56.39	5.13	4.08	1.6	1.17	44.83	59.29	15.89
BAS 500 (12.3) +COC	6.17	31.55	3.01	4.08	6.1	1.33	45.85	58.72	16.06
BAS 500 + Folicur + COC	6.67	40.24	3.02	4.21	0.6	1.25	44.74	59.48	15.69
Quadris + Benlate	6.5	54.47	3.75	3.9	2.3	1.33	41.79	59.25	15.53
BAS 500 (6.2) + COC	7	52.72	3.76	4.29	0.2	1.42	44.9	59.29	15.74
Quadris	7.5	48.06	4.23	4.15	1.7	1.42	45.7	53.69	14.66
Caramba	4	45.39	2.2	4.08	0.1	1.08	42.51	58.75	15.64
Folicur (6) + NIS Tilt (6) + NIS									
LSD (0.05)	NS	NS	NS	0.34	N/A	NS	NS	NS	NS

[a] Data represents an average of disease impact on Oxen and Ingot.

[b] % of infected heads, based on a 50 head sample

[c] % infection of blighted heads

[d] % blighted heads x % infection on blighted heads

[e] Subjective rating (0=green – 5=necrotic) of tissue necrosis in the plot at evaluation.

EFFICACY OF THE FUNGICIDE FOLICUR IN CONTROLLING BARLEY FUSARIUM HEAD BLIGHT IN GENOTYPES WITH PARTIAL RESISTANCE

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OBJECTIVES

The objective of this study is to determine if the integrated use of fungicides and resistant or moderately resistant barley genotypes reduces *Fusarium* head blight (FHB) severity and accumulation of deoxynivalenol (DON) to levels acceptable to the malting and brewing industry.

INTRODUCTION

Fusarium head blight (FHB), incited primarily by *Fusarium graminearum*, adversely affected the quality of barley grown in eastern North Dakota and northwestern Minnesota the last eight years. Quality of harvested grain was reduced because of blighted kernels and the presence of DON, a mycotoxin produced by the pathogen. Zero or low levels of DON are needed for malting barley because DON has been found to carry through malting and brewing into finished beer (Schwarz et al., 1995). Anheuser-Busch, Inc., the largest brewer in the U.S., will not purchase malt produced from barley with DON levels greater than 0.5 ppm.

Research conducted to test the efficacy of fungicides in controlling FHB and DON levels in barley has been conducted using cultivars susceptible to FHB. In a study by Pederson and McMullen (1999), they found that the fungicides Folicur, Tilt, Benlate, Mancozeb, and Quadris significantly reduced FHB severity and DON content. However, the fungicides were not successful in reducing the DON content to a level that would be acceptable to maltsters and brewers. The DON content of the barley for the most successful fungicide treatment was 17.2 ppm. Control of FHB in barley probably will require an integrated approach that includes use of cultivars with genetic resistance, proper cultural practices, and fungicides.

Minimal information is available on control of FHB using fungicides on moderately resistant barley genotypes. In a preliminary study conducted by Horsley et al. during the 1999 growing season (unpublished data), the fungicide folicur was sprayed on 13 genotypes of barley at the recommend rate and growth stage. Genotypes resistant to FHB ('Chevron', 'Svanhals', and 'Kaoto Nijo 2'), partially resistant ('MNBrite', F101-78, F103-61, F103-52, and F102-61), and susceptible ('Foster', 'Stander', 'Conlon', 'Logan', and 6B93-2978) were evaluated. The fungicide by genotype interaction was non-significant ($P>0.05$). It was determined that the interaction was not significant because the fungicide reduced the level

of FHB similarly for all genotypes. Averaged across all genotypes, fungicide application reduced FHB severity nearly 40%; yet, the fungicide main effect was not significant (P>0.05).

MATERIALS AND METHODS

Fourteen barley genotypes with different levels of FHB resistance were grown at three locations (Osnabrock, Langdon, and Carrington) in North Dakota during the 2000 growing season. Treatments were assigned to experimental units using a randomized complete block design with a split plot arrangement. Each treatment was replicated three times at each location. Whole plots were fungicide levels (0 and 4 oz. $acre^{-1}$ of folicur) and subplots were genotypes. Evaluated genotypes were either resistant to FHB (Chevron, Svanhals, and Kaoto Nijo 2), moderately resistant to FHB (MNBrite, F101-78, F103-61, F103-52, and F102-61), or susceptible to FHB (Foster, Stander, Conlon, Logan, ND15477, and 6B93-2978). Plots were not inoculated with *F. graminearum.*

The fungicides were applied using a CO_2-pressurized handheld boom sprayer operating at 40 psi, and delivering four ounces of chemical in 18 gallons of water $acre^{-1}$. Fungicides were applied when 50% of the spikes in a plot were completely emerged from the plants. Fusarium head blight severity was assessed at the soft dough stage by determining the ratio of infected kernels to total kernels on 10 spikes per row. Disease severity was expressed as percent FHB severity. At maturity, grain was harvested from each plot with a plot combine, dried, and cleaned. Grain from each experimental unit were submitted to Dr. Paul Schwarz's laboratory in the Department of Cereal Science, North Dakota State University for DON analysis. To date, DON data are not available.

Data from the experiments were analyzed as an RCBD with a split plot arrangement. Data from individual locations were analyzed separately using analysis of variance (ANOVA) and error mean squares from each location were tested for homogeneity of variance. Combined ANOVA's were done using data from locations in which error mean squares were homogeneous. Mean separation was done using an F-protected LSD (P=0.05). In the combined analyses, fungicide and genotypes will be considered fixed effects and environment a random effect.

RESULTS AND DISCUSSION

Environmental conditions at Langdon and Osnabrock, ND were more conducive for development of FHB than conditions at Fargo. Mean FHB severity was 4.8% at Langdon, 4.2% at Osnabrock, and 0.8% at Fargo. The fungicide x genotype interaction was not significant for FHB severity (P>0.05). This suggests that Folicur similarly affected FHB severity of all genotypes; however, this was not observed (Table 1). Folicur did not significantly reduce FHB severity in the resistant or moderately resistant genotypes. Thus, it appears that the integrated use of folicur and a resistant or moderately genotype may not be sufficient in reducing FHB severity to levels acceptable to the malting and brewing industry. However, we need to wait until the DON data are available before more definitive conclusions can be made. In the susceptible genotypes Drummond, Foster, Logan, and Stander, folicur gener-

ally reduced FHB severity. Conversely, slight increases in FHB severity were observed in the susceptible genotypes 6B93-2978 and Conlon when sprayed with folicur.

The fungicide x genotype interaction was significant for yield and kernel plumpness ($P<0.05$). Genotypes sprayed with folicur generally had greater yield, test weight, and kernel plumpness than unsprayed genotypes (Table 2). Much of the improvements in these traits may be due to reductions of foliar disease in genotypes sprayed with folicur (Table 1). Significant yield increases were observed only for the cultivars developed and released from upper Midwest barley breeding programs (i.e.6B93-2978, Conlon, Drummond, Foster, Logan, MNBrite, and Stander.) This suggests that factors other that foliar diseases were limiting yield in the other genotypes. Foliar disease severity data were collected at Langdon and Osnabrock. The predominant foliar disease at each location was septoria leaf blotch, incited by *Septoria spp.* Foliar diseases were not prevalent at Fargo.

Test weight of genotypes receiving and not receiving folicur was generally acceptable (> 46.0 lb/bu). Kernel plumpness of all adapted genotypes was below the minimum required by the malting and brewing industry, 65%. Thus, folicur application did not result in improvements of kernel plumpness to acceptable levels.

In the barley-growing region in the upper Midwest U.S., it costs growers about $12/acre for folicur and its application. For this cost to be recovered, a yield increase of at least 9.2 bushels/acre is needed based on a farmgate-selling price of $1.30/bushel. Based on the yield increases observed in this study, the cost of folicur and its application was recovered only when applied to the adapted cultivars 6B93-2978, Conlon, Foster, Logan, MNBrite, and Stander.

CONCLUSIONS

- Folicur application did not significantly reduce the level of FHB in resistant, moderately resistant, or susceptible genotypes.
- Genotypes sprayed with folicur generally had greater yield, test weight, and kernel plumpness than unsprayed genotypes.
- Folicur application reduced levels of foliar disease.
- Yield gains due to control of foliar diseases tended to be sufficient to cover the cost of folicur and its application in cultivars developed and released by upper Midwest barley breeding programs.

REFERENCES

Pederson, J., and M. McMullen. 1999. Evaluation of fungicides for control of Fusarium head blight (FHB) in barley. p. 244. *In* R.N. Reid (ed.) Fungicide and nematicide tests. APS Press, St. Paul, MN.

Schwarz, P.B., H.H. Casper, and S. Beattie. 1995. The fate and development of naturally occurring *Fusarium* mycotoxins during malting and brewing. J. Am. Soc. Brew. Chem. 53:121-127.

Table 1. Effect of folicur rate and genotype on percent Fusarium head blight and foliar disease severity of barley.

	FHB Severity†		Foliar disease	
Genotype	No folicur	Folicur	No folicur	Folicur
	----------%----------		----------score‡----------	
Chevron	1	1	4.2	2.8
Svanhals	1.2	1.3	5.7	3.3
F101-78	3.7	2.5	6.2	4.5
F102-61	1.2	1.7	6.5	3.7
F103-52	1.7	1.6	4.8	4
F103-61	5.1	3.4	5.8	3.7
Kaoto Nijo 2	3.1	3.6	5.7	3.8
MNBrite	3.7	3.5	3.8	2.2
6B93-2978	3.5	4.1	4.2	3
Conlon	3.4	4.9	5	2.7
Drummond	4.4	3.4	5.2	3.5
Foster	5.6	3.5	6.7	4.3
Logan	4.6	3.1	5.8	3
Stander	6.4	4.8	5.7	4.5
LSD (0.05)	----------ns----------		----------ns----------	

† Percent of infected kernels per spike.
‡1 = no foliar disease, 5 = severe disease.

Table 2. Effect of folicur and genotype on yield, test weight, and kernel plumpness of barley.

	Yield		Test weight		Kernel plumpness†	
Genotype	No folicur	Folicur	No folicur	Folicur	No folicur	Folicur
	bushels/acre		pounds/bushel		%	
Chevron	50.8	54	48.6	49.4	12.5	17.2
Svanhals	41.7	48.6	48.5	49.6	64.1	71.6
F101-78	61.3	62.8	51.5	52	79.9	83.9
F102-61	59.3	61.1	49.2	49.1	70.3	75.1
F103-52	46.3	51.1	49.9	50.5	74.6	72.9
F103-61	63.8	68.9	50.9	51.3	71.3	73.1
Kaoto Nijo 2	61.5	69.1	49.3	50.6	53.5	66.5
MNBrite	72.9	83.6	48.6	49.5	45.5	54.7
6B93-2978	80.8	91.5	48.4	49	43.8	42.7
Conlon	62.8	75.1	47.9	50	51.1	60.6
Drummond	75.1	84.2	48.7	50.2	49.1	58.9
Foster	75	95.7	46.6	48.2	43.6	58.5
Logan	71.1	89.7	49.5	51.1	44.3	60.3
Stander	79.8	90.8	48.7	49.9	52.5	59.1
‡LSD (0.05)	----------9.0---------		----------ns----------		----------7.6----------	

†Kernels remaining on top of a 6/64 inch slotted sieve were considered plump.
‡Calculated LSD is for comparisons of same subplot treatments in different whole plots

EFFECTS OF APPLICATION PARAMETERS ON CONTROL OF FUSARIUM HEAD BLIGHT WITH FUNGICIDES

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OBJECTIVE

To find fungicide application techniques that will improve control of Fusarium head blight in spring wheat, durum wheat, and spring barley.

INTRODUCTION

Success with fungicides in reducing Fusarium head blight (FHB) is dependent on a number of factors, including efficacy of the fungicide, appropriate timing of application, and deposition and retention of the fungicide on the grain head (Halley et al., 1999). A number of fungicides have been evaluated since the late 1970s (Wilcoxson, 1996). Folicur (tebuconazole) has shown consistency in reducing levels of FHB over a number of locations in the US in recent years (McMullen et al., 1999).

In addition to fungicide efficacy, timing of application needs to be optimal. In early studies, fungicides were applied at early or partial head emergence or at early grain fill (Milus and Parsons, 1994; Wilcoxson, 1996). Studies in Europe indicate that the flowering period is the optimum growth stage for fungicide application on wheat (Mauler-Machnik and Zahn, 1994). For barley, which flowers when the heads are still in the boot, early results in North Dakota indicate that optimum fungicide application on barley is shortly after head emergence.

Adequate deposition and retention of fungicide on grain heads also is needed for optimum FHB control. Adjuvants have been shown to have positive effects in improving fungicide deposition and retention on grain heads, resulting in enhanced FHB control (Lukach et al., 1999). Recent studies in greenhouse and field environments also have indicated that spray systems that direct the fungicide spray toward the grain head at an angle rather than vertical to the grain head provide better fungicide deposition and FHB control (Lukach et al., 1999). Our objective was to further evaluate the impact of application timing and the impact of adjuvant types and rates on fungicide effectiveness in controlling FHB.

MATERIALS AND METHODS

Application timing - Folicur fungicide was applied at various growth stages of spring wheat and barley in the greenhouse and field in 2000. ‘Grandin’ spring wheat, ‘Munich’ durum, and ‘Robust’ barley were sown separately into artificial potting mix in 6" by 12" plastic trays at a seeding rate equal to one million seeds/acre. Five trays of each crop were treated with Folicur (4 fl oz + 0.06% v/v Induce in 9 gpa) at three separate growth stages (Feekes 10.3 = one-half head emerged; Feekes 10.5 = full head emergence in barley; Feekes 10.51 =

flowering in wheat; Feekes 10.54 = kernel developing and watery ripe). Fungicides were applied with a track sprayer using two XR8001 flat fan nozzles oriented forward and backwards towards the grain heads at 30^0 from horizontal. Grain heads were inoculated at Feekes 10.51 using an atomizer with 5000 spores/ml of *Fusarium graminearum.* Plants were placed under intermittent mist for two days following inoculation.

'Russ' hard red spring wheat was planted into barley stubble that had been chiseled the prior fall, and 'Stander' barley was planted into corn stubble that had been chiseled the prior fall. Fungal inoculum came from natural sources in the barley field, while natural sources were augmented with inoculated grain spawn spread among rows in the wheat field. Four replicated plots (9' x 20') per treatment were sprayed with Folicur fungicide (4 fl oz/acre + 0.06% Induce in 18 gpa) using the same nozzle type and orientation as in the greenhouse. FHB was evaluated at soft dough stage of kernel development. Data was statistically analyzed using ANOVA.

Adjuvants - Various adjuvants were tested in conjunction with Folicur or Tilt fungicide to determine their effects on efficacy of these products. Experiments were conducted in field trials at Fargo with Stander barley and Grandin hard red spring wheat, both planted into corn stubble. Adjuvants tested included two rates of a non-ionic surfactant (Induce), two rates of an organosilicone adjuvant (Silwet), and two humectants, L64 and L88. Humectants, such as glycerol and sorbitol, are substances that enhance retention of moisture. Fungicides + adjuvants were applied with a hand held boom equipped with forward/backward flat fan nozzles, as described above. FHB was evaluated at soft dough stage of kernel development. Data was statistically analyzed using ANOVA.

RESULTS AND DISCUSSION

Application timing - Optimum growth stage for fungicide application for FHB control was at Feekes growth stage 10.51 for spring wheat and durum (Table 1). In barley, FHB field severity did not differ significantly among application timings, but was numerically lower when fungicide was applied either at Feekes 10.3 or Feekes 10.5 than at Feekes 10.54 (Table 1). Optimum timing of application resulted in 73-92% reduction in FHB field severity in spring wheat, 89% in durum wheat, and 59-63% in barley.

Adjuvants - With Folicur fungicide on barley and wheat, Induce adjuvant at 0.06% v/v consistently resulted in lower FHB field severity ratings than at 0.03% v/v, although differences were not statistically significant (Table 2). Over all of the treatments with Folicur or Tilt, the lowest FBH field severity ratings were with Folicur + 0.06% v/v Induce. A comparison of Silwet rates with Tilt indicated that the 0.03% v/v rate of Silwet gave a significantly lower FHB field severity rating than use of the 0.06% v/v rate. Results indicate that benefits with adjuvants may be rate dependent. Addition of humectants resulted in similar FHB field severity ratings as with the Induce or Silwet, but slightly lower wheat leaf disease levels than with other adjuvants.

REFERENCES

Halley, S., Pederson, J., McMullen, M., and Lukach, J. 1999. Sprayer modifications for enhanced control of Fusarium head blight with fungicides. Page 51-52 in: Proc. of the 1999 National Fusarium Head Blight Forum, Sioux Falls, SD, Dec. 5-7, 1999. Michigan State Univ.

Lukach, J., Halley, S., and Gregoire. 1999. Effect of nozzle type, application volume, and adjuvants on fungicide efficacy in control Fusarium head blight (FHB) in wheat, 1988. Fungicide and Nematicide Tests 54:334.

Mauler-Machnik, A., and Zahn, K. 1994. Ear fusarioses in wheat - new findings on their epidemiology and control with Folicur (tebuconazole). Pflanzenshutz-Nach. Bayer 47:129-155.

McMullen, M., Milus, G., and Prom, L. 1999. Uniform fungicide trials to identify products effective against Fusarium head blight in wheat. Pages 64-68 in: Proc. Of the 1999 National Fusarium Head Blight Forum, Sioux Falls, SD. Dec. 5-7, 1999. Michigan State University.

Milus, G., and Parsons, C. E. 1994. Evaluation of foliar fungicides for controlling Fusarium head blight of wheat. Plant Dis. 78:697-699.

Wilcoxson, R. D. 1996. Fungicides for control of Fusarium head blight - a review. MN. Agr. Exp. Stat. Paper 22507. 17p.

Table 1. Field severity of Fusarium head blight (FHB) following application of Folicur fungicide (4 fl oz/acre + 0.06% v/v Induce) at various growth stages, Fargo, 2000.

	% FHB Field Severity (Field Severity = Incidence x Head Severity)				
Feekes growth stage* at fungicide application	**'Grandin' HRSW** *greenhouse*	**'Russ' HRSW** *field*	**'Munich' Durum** *greenhouse*	**'Robust' Barley** *greenhouse*	**'Stander' Barley** *field*
Untreated	7	23.4	32.1	3.2	8.7
10.3	1.6	11.2	16.4	1.5	3.2
10.5-10.51	0.5	6.3	3.5	1.3	4.1
10.54	7	8.8	22.5	1.9	4.5
LSD P= 0.05	3.9	4.6	17.2	2.8	2.8

* Feekes 10.3 = head half emerged; Feekes 10.5 = full head emergence; Feekes 10.51 = early flowering in wheat; Feekes 10.54 = kernel watery ripe

Table 2. Effects of adjuvants on efficacy of Folicur and Tilt fungicides in reducing FHB in two field experiments with barley, one field experiment with spring wheat, Fargo, ND, 2000.

Cultivar	Fungicide	Adjuvant and rate	FHB Field Severity (%)	Barley Yield (bu/ac)
Stander barley	Untreated		6.1	83.6
	Folicur 4 fl oz/A	Induce 0.03% v/v	4.4	87.9
	Folicur 4 fl oz/A	Induce 0.06% v/v	3.7	90.1
LSD P = 0.05			**1.5**	**ns**
Stander barley	Untreated		9.4	84
	Folicur 4 fl oz/A	L64 1% v/v	5	87.1
	Folicur 4 fl oz/A	L88 1% v/v	4.5	86.5
	Folicur 4 fl oz/A	Induce 0.03% v/v	4.7	83.9
	Folicur 4 fl oz/A	Induce 0.06% v/v	3.8	89.5
	Folicur 4 fl oz/A	Silwet 0.03% v/v	4	86.5
	Tilt 4 fl oz/A	Silwet 0.03% v/v	4.1	82.4
	Tilt 4 fl oz/A	Silwet 0.06% v/v	6.2	86.9
	Tilt 4 fl oz/A	Induce 0.03% v/v	4.8	86.3
	Tilt 4 fl oz/A	Induce 0.06% v/v	5.7	84.1
LSD P = 0.05			**2.1**	**ns**
				% Leaf necrosis
Grandin HRS	Untreated		39.5	60.3
	Folicur 4 fl oz/A	L64 1% v/v	15.1	5.2
	Folicur 4 fl oz/A	Induce 0.03% v/v	17	8.5
	Folicur 4 fl oz/A	Induce 0.06% v/v	11.8	8.7
	Folicur 4 fl oz/A	Silwet 0.03% v/v	11.9	7.5
	Tilt 4 fl oz/A	Induce 0.06% v/v	13.1	11.8
LSD P = 0.05			**7.1**	**6.1**

UNIFORM FUNGICIDE TRIAL FOR CONTROLLING FHB IN SPRING WHEAT, ND, 2000

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ABSTRACT

A core set of seven fungicide treatments was evaluated on hard red spring wheat for efficacy against Fusarium head blight (FHB = scab) and leaf diseases at three locations (Fargo, Carrington, and Langdon) in North Dakota in 2000. A single study on durum wheat also was done with the fungicide treatments at the Carrington Research and Extension Center. The evaluation of the fungicide treatments was part of a national effort to evaluate a uniform set of treatments across multiple states and environments. The core set of treatments included Folicur (4 fl oz/acre), Tilt (4 fl oz/acre), Stratego (14 fl oz/acre), BAS 500 (12.3 fl oz/acre), BAS 500 + Folicur (6.2 fl oz + 2 fl oz/acre), and Quadris + Benlate (9.2 fl oz + 0.25 lb/acre). Folicur (tebuconazole) had a Section 18 for use in ND in 2000. Tilt (propiconazole) has a state label for heading application on wheat in ND. Quadris (azoxystrobin) is registered for wheat. Stratego(combination product of Tilt + trifloxystrobin [Flint]) was recently sold by Novartis to Bayer, and its availability status for 2001 is uncertain. BAS 500 is a strobilurin fungicide and is not yet labeled for wheat in the US. An additional fungicide, Caramba 90SL (metconazole at 13.5 fl oz/acre), was included in the evaluations in ND. Caramba is not labeled in the US and this was the first year of testing of this product. The fungicide treatments were applied at early flowering to 'Russ' hard red spring wheat at Fargo and Carrington, to 'Grandin' hard red spring wheat at Fargo and Langdon, and to 'Munich' durum at Carrington. Fungicide treatments were applied at 35-40 psi in 17-18 gpa, using flat fan nozzles oriented forward and backward at an angle 30^0 from the horizontal. Fusarium inoculum was added in the form of inoculated grain kernels to the plots in Fargo and Langdon. Wheat straw was distributed among plots at Carrington. Plots were mist irrigated at Fargo and Langdon, while sprinkler irrigation was used at Carrington. Fusarium head blight and leaf disease ratings were recorded at soft dough stage of kernel development. Disease levels were high at all three locations. Mean data across sites was analyzed using ANOVA. Results on spring wheat and durum showed that all of the fungicide treatments significantly reduced leaf disease and FHB field severity when compared to the untreated check. In the hard red spring wheat trials, all fungicide treatments resulted in significantly lower DON levels, as well. All fungicide treatments significantly increased yield (7 to 15 bu in hard red spring and 12 to 18.4 bu in durum) and test weight (up to 2.3 lb in hard red spring and up to 3.4 lb in durum). The Caramba fungicide resulted in significantly lower DON (vomitoxin) levels across all sites than other fungicide treatments and significantly higher yields than several of the core treatments. The BAS 500 treatment resulted in the lowest leaf disease rating and the highest test weight.

UNIFORM FUNGICIDE TRIAL FOR CONTROLLING FHB IN BARLEY, ND, 2000

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ABSTRACT

A core set of seven fungicide treatments was evaluated on 'Stander' six-row barley for efficacy against Fusarium head blight (FHB = scab) and leaf diseases at two locations in North Dakota in 2000. The evaluation of the fungicide treatments was part of a national effort to compare a uniform set of treatments across multiple states and environments. The core set of treatments included Folicur (4 fl oz/acre), Tilt (4 fl oz/acre), Stratego (14 fl oz/acre), BAS 500 (12.3 fl oz/acre), BAS 500 + Folicur (6.2 fl oz + 2 fl oz/acre), and Quadris + Benlate (9.2 fl oz + 0.25 lb/acre). Folicur (tebuconazole) had a Section 18 for use on wheat and barley in ND in 2000. Tilt (propiconazole) has a federal label for application to wheat and barley through flag leaf emergence stage, and a state label for heading application on wheat in ND. Quadris (azoxystrobin) is registered for wheat. Stratego (combination product of Tilt + trifloxystrobin [Flint]) was recently sold by Novartis to Bayer, and its availability status for 2001 is uncertain. BAS 500 is a strobilurin fungicide and is not yet labeled for wheat or barley in the US. An additional fungicide, Caramba 90SL (metconazole at 13.5 fl oz/acre), was included in the evaluations. Metconazole is not labeled in the US and this was the first year of testing in ND. The fungicide treatments were applied to Stander barley at early full head emergence at both locations. Fungicide treatments were applied at 35-40 psi in 17-18 gpa, using flat fan nozzles oriented forward/backward at a 30^0 angle from the horizontal. Fusarium inoculum was added in the form of inoculated grain kernels to the plots in Fargo and Langdon, and mist irrigation was applied to the plots at both locations as needed. Fusarium head blight and leaf disease ratings were recorded at soft dough stage of kernel development. FHB, DON, and leaf disease levels were higher at Langdon than Fargo. Data across sites was analyzed using ANOVA. Compared to the untreated check, all fungicide treatments significantly reduced leaf disease, which was primarily Septoria. Among fungicide treatments, the only significant difference observed was the BAS 500 + Folicur treatment having significantly lower leaf disease than the Tilt treatment. Significant differences were not observed among treatments for FHB field severity, but fungicide treatments reduced severity levels by 45 to 66.7%. DON levels were very high in these studies and were not significantly affected by fungicide treatments, although fungicides reduced DON levels up to 45.8%. Yield differences also were not statistically significantly different because of wide yield variations between the two sites, but yield improvement averaged from 10 to 20.4 bushels, with Caramba and Stratego treatments resulting in the highest yields. At Langdon, test weights were significantly improved by all but the Tilt fungicide treatment, with Folicur giving the highest test weight.

ANALYSIS OF THE 2000 UNIFORM WHEAT FUNGICIDE TRIALS ACROSS LOCATIONS

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INTRODUCTION

Identifying fungicides that reduce incidence and severity of FHB and levels of damage and mycotoxins in the grain could have immediate and wide spread benefits to growers and users of all market classes of wheat in the event of a FHB epidemic. The overall objective of the uniform wheat fungicide trials is to hasten the integration of fungicides that are effective against FHB into cost-effective and environmentally-safe wheat disease management strategies. Because most fungicides do not have a high level of activity against FHB, current emphasis has been on identifying the most efficacious fungicides. Consequently, this analysis will include only variables that are directly related to FHB.

METHODS

Plant pathologists in 15 states (Table 1) participated in the 2000 wheat uniform fungicide trials. These states represented hard red spring wheat, hard red winter wheat, soft red winter wheat, soft white winter wheat, and durum wheat production areas. The seven uniform treatments for 2000 (Table 2) included well-known fungicides that either are registered or are in the process of being registered for use on wheat in the United States. In addition to the seven uniform treatments, some cooperators included Caramba (Metconazole) which is being used for control of FHB in Europe.

All treatments were applied at flowering stage using a CO_2-powered sprayer. Details such as plot size, number of replications, spray volume, and nozzle configuration varied among the locations but were not considered to significantly affect the results. Inoculation and/or some form of overhead misting were used at some locations to promote head blight development, and these practices likely increased the incidence and severity of head blight. Disease variables included incidence (% of heads showing symptoms) and head severity (% of head area affected) measured at soft dough stage, field severity (= FHB index = incidence x head severity), and deoxynivalenol (DON) content in the grain and percentage of Fusarium-damaged kernels (FDK) measured after harvest. Cooperators analyzed results of their individual locations and provided treatment means to the authors for analysis across locations.

RESULTS

Sixteen locations across seven of the participating states reported some level of FHB (Table 3). The trial from Maryland appeared to be a good test, but a nontreated check was not included because of unusual circumstances. In order to utilize data from this location, data

for two extra, apparently ineffective treatments were averaged, and these averages were substituted for the nontreated check. Field severity data from ten locations, FDK data from four locations, and DON data from five locations were used to analyze treatment effects across locations. All fungicides significantly reduced field severity compared to the nontreated check, but none of the treatments significantly reduced the percentage of Fusarium-damaged kernels or the level of DON (Table 4).

Seven locations included the Caramba treatment and reported some level of FHB (Table 5). Data for the nontreated check at the Maryland location were calculated as described above. Field severity data from five locations, FDK data from three locations, and DON data from four locations were used to analyze treatment effects across locations. All fungicides significantly reduced field severity compared to the nontreated check, and Caramba was significantly better than Tilt or Stratego (Table 6). Stratego, BAS 500F + Folicur, Quadris + Benlate, and Caramba significantly reduced the percentage of Fusarium-damaged kernels compared to the nontreated check, and Caramba was significantly better than BAS 500F + Folicur and Quadris + Benlate. All fungicides significantly reduced the level of DON compared to the nontreated check, and Caramba was significantly better than all of the fungicides except Quadris + Benlate. Also, Caramba was the only fungicide to reduce DON levels below the threshold level of 2 ppm.

CONCLUSIONS

Caramba was the most effective fungicide against FHB among all of the fungicides ever tested in the uniform wheat fungicide trials. Caramba also was effective against foliar fungal diseases (data not shown) and therefore appears to have potential for cost-effective commercial use. Although Caramba is currently being used in Europe, the prospect for EPA registration on wheat in the United States and cost are not known at this time.

For the analyses without Caramba (Table 4), results were very similar to those reported from the 1999 analysis across locations (McMullen, et al. 1999). In 1999 and 2000, all fungicides tested significantly reduced field severity compared to the nontreated check, but none of the fungicides significantly reduced the percentage of Fusarium-damaged kernels or the level of DON.

LITERATURE CITED

McMullen, M., Milus, E.A., and Prom, L.K. 1999. 1999 Uniform fungicide trials to identify products effective against Fusarium head blight in wheat. Pages 64-68 in Proceedings of the 1999 Fusarium Head Blight Forum, Sioux Falls, SD. University Printing, Michigan State University.

Table 1. States and principal cooperators in the uniform wheat fungicide trials.	
Arkansas	Gene Milus, University of Arkansas, Fayetteville
Illinois	Wayne Pedersen, University of Illinois, Urbana
Indiana	Greg Shaner, Purdue University, West Lafayette
Iowa	Gary Munkvold, Iowa State University, Ames
Kentucky	Don Hershman, University of Kentucky, Princeton
Maryland	Arvydas Grybauskas, University of Maryland, College Park
Michigan	Pat Hart, Michigan State University, East Lansing
Minnesota	Roger Jones, University of Minnesota, St. Paul
Missouri	Laura Sweets, University of Missouri, Columbia
New York	Gary Bergstrom, Cornell University, Ithaca
North Carolina	Steven Leath, North Carolina State University, Raleigh
North Dakota	Marcia McMullen, North Dakota State University, Fargo
Ohio	Pat Lipps, Ohio State University, Wooster
South Dakota	Marty Draper, South Dakota State University, Brookings
Virginia	Erik Stromberg, Virginia Technical, Blacksburg

Table 2. Fungicide, rate, and adjuvant used in the uniform treatments in 2000.

#	Fungicide	Rate/A	Adjuvant
1	Nontreated		
2	Folicur 3.6 F	4 fl oz	0.12% Induce
3	Tilt 3.6 EC	4 fl oz	0.06% Induce
4	Stratego 2.08 EC	14 fl oz	0.06% Induce
5	BAS 500F 2.09 EC	12.3 fl oz	1% Agri-Dex
6	BAS 500F + Folicur	6.2 + 2 fl oz	1% Agri-Dex
7	Quadris 2.08 F + Benlate 50 WP	9.2 fl oz + 0.25 lb	
7.5	Caramba 90 SL	13.5 fl oz	0.06% Induce

Table 3. Locations in the 2000 uniform trials that reported some level of FHB and provided data on field severity, Fusarium-damaged kernels, or DON level and the means across all seven treatments for those variables.

Location	Field severity[a]	Fusarium-damaged kernels[a]	DON[a]
	(%)	(%)	(ppm)
Fargo, ND #1	**13.2**	**5.4**	**7.3**
Fargo, ND #2	**5.8**	2.6	**3.6**
Carrington, ND #1	**12.8**	.	**2.4**
Carrington, ND #2	**12.4**	.	3.2
Langdon, ND	**13.7**	**19.1**	.
Crookston, MN	2.4	3.6	1.2
Beltsville, MD	**14**	**24.6**	.
Aurora, NY	.	**7.6**	**11.8**
East Lansing, MI #1	**7.1**	.	.
East Lansing, MI #2	**12.6**	.	.
East Lansing, MI #3	**16.3**	.	.
East Lansing, MI #4	**11.9**	.	.
Groton, SD	1	1.1	.
Brookings, SD #1	0.8	0.8	.
Brookings, SD #2	3.7	1.2	.
Fayetteville, AR	27.1	84.3	44

[a]Values in bold font were used for analysis across locations. Other values were from locations where FHB levels were either too low or too high to be meaningful.

Table 4. Treatment means for field severity, Fusarium-damaged kernels, and DON level averaged across the locations indicated in Table 3.

Treatment	Field severity	Fusarium-damaged kernels	DON
	(%)	(%)	(ppm)
Nontreated	20.5	16.9	7.3
Folicur	9	14.5	4.6
Tilt	11.5	13.6	4.8
Stratego	12.7	12.7	6
BAS 500	8.6	14.4	5.7
BAS 500 + Folicur	10.5	14.1	6.3
Quadris + Benlate	11.2	12.9	5
LSD (P=0.05)	4.8	NS	NS

Table 5. Locations in the 2000 uniform trials that included the Caramba treatment and provided data on field severity, Fusarium-damaged kernels, or DON level and the means across all eight treatments for those variables.

Location	Field severity[a]	Fusarium-damaged kernels[a]	DON[a]
	(%)	(%)	(ppm)
Fargo, ND #1	**12.1**	**5.1**	**6.8**
Fargo, ND #2	**5.3**	2.6	**3.4**
Carrington, ND #1	**11.9**	.	**2.2**
Carrington, ND #2	**11.7**	.	**3**
Langdon, ND	**12.6**	**18.4**	.
Crookston, MN	2.4	3.3	1.1
Beltsville, MD	**13.5**	**23.5**	.

[a]Values in bold font were used for analysis across locations. Other values were from locations where the level of FHB was too low to be meaningful.

Table 6. Treatment means for field severity, Fusarium-damaged kernels, and DON level averaged across the locations indicated in Table 5.

Treatment	Field severity	Fusarium-damaged kernels	DON
	(%)	(%)	(ppm)
Nontreated	24.9	19.8	6.4
Folicur	9.3	17.1	3.5
Tilt	12.2	16	3.6
Stratego	11.3	14	4.3
BAS 500F	8.6	16.9	3.5
BAS 500F + Folicur	8.5	15.5	4.1
Quadris + Benlate	9.3	15	3.4
Caramba	5.4	10.8	1.8
LSD (P=0.05)	4.3	3.9	1.6

USDA-ARS, OHIO STATE UNIVERSITY COOPERATIVE RESEARCH ON BIOLOGICALLY CONTROLLING FUSARIUM HEAD BLIGHT: FIELD TESTS OF ANTAGONISTS IN 2000

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OBJECTIVES

Determine the effect of culture media type on the efficacy of biocontrol agents against FHB in field tests located in Illinois and Ohio and to produce antagonists in a superior cultivation medium and field test antagonists in North Dakota. Results of field studies on reducing FHB on durum wheat using microbial antagonists is presented in our associated poster.

INTRODUCTION

Research on optimizing methods for selectively isolating, mass producing and utilizing microbial antagonists effective against FHB was initiated in 1997 at the NCAUR in Peoria, IL, in conjunction with The Ohio State University. Several biological control agents remain under consideration for commercial development (Table 1; Boehm et al., 1999; Khan et al., 1999). This update provides a summary of the results obtained from a portion of our 2000 field evaluation trials conducted on the soft red winter wheat cultivars Pioneer 2545 and Freedom in OH and IL and the hard red spring wheat cultivar Grandin in Langdon, ND.

MATERIALS AND METHODS

2000 field trials of FHB antagonists on soft red winter wheat in Peoria, IL and Wooster, OH

Inoculum of 6 microbial antagonists effective against FHB was produced using two semidefined liquid culture media that differed only in respect to their carbon to nitrogen ratios (C:N) which were set at C:N 6.5 and 11. These media were selected in part because of data obtained from a 2000 field trial on durum wheat in which antagonists produced in the C:N 6.5 medium were 18% more effective in reducing FHB than the same antagonists produced in the C:N 11 medium (Schisler et al. poster, this forum). The soft red winter wheat cultivars Pioneer 2545 (susceptible) and Freedom (moderately resistant) were used in both locations because of their widespread use throughout the Midwest and to test for possible integration of biocontrol and host genetic resistance for managing FHB. Biomass was harvested from Fernbach shake flasks as described previously and applied at the beginning of wheat flowering in aqueous suspensions containing a weak buffer and wetting agent (Schisler et al., 1999). Bacterial and yeast suspensions contained 25 % fully colonized broth (~$5x10^8$ cfu/ml and ~2.5×10^7 cfu/ml, respectively). Controls were untreated plants and

plants treated with buffer/wetting agent only. Pathogen inoculum was added to plots as colonized corn kernels (*F. graminearum* 3-93 and 6-93) scattered through plots (~25-40 kernels/m^2) 2 wk prior to wheat flowering and mist irrigation provided for approximately one week after treatment application to promote FHB development. Plots were scored for disease severity and incidence. Randomized complete block designs were used in both trials (*n*=4 in Peoria; *n*=6 in Wooster).

2000 Langdon, ND, field trial of FHB antagonists on the hard red spring wheat Grandin

Biomass of the 6 FHB biocontrol agents listed in Table 1 was produced in the C:N 6.5 semidefined medium for the Langdon, ND field trial. Methods were as described above except that natural pathogen inoculum was used and the plots did not receive any mist irrigation. Controls were untreated plants and plants treated with buffer/wetting agent only. Plots were scored for disease severity and incidence. A randomized complete block design was used (*n*=6).

RESULTS AND DISCUSSION

All antagonists reduced FHB severity as compared to the buffer control in both Peoria and Wooster on Pioneer 2545 (Table 2). Yeast OH 182.9 reduced disease severity by 58% compared to the buffer control across both production media and test sites. Disease severity was very light on cultivar Freedom with few treatments differing from the controls at either field location. The C:N of the liquid culture media used to produce antagonist biomass did not have a consistent effect on the efficacy of antagonists in the field trials conducted in Peoria and Wooster (Table 2). In the Langdon, ND field trial, five of six antagonists decreased FHB severity compared to the untreated control (Table 3). Inexplicably, the buffer and wetting agent control treatment also had one of the lowest disease severity ratings, a result in contrast to our 1999 and other 2000 field trials where the buffer control had equivalent or higher disease ratings than the untreated control. As observed in previous field seasons, yeasts OH 181.1 and OH 182.9 were among the most effective antagonists.

We remain optimistic regarding the commercial development and FHB control potential of several of these antagonists. Further improvements in the level of control achieved using these antagonists will be sought via further research on optimizing media and formulations. Additional efficacy gains will also be attempted via the selection of fungicide insensitive variants of selected antagonists and their use in combination with fungicides registered for use against FHB. Lastly, improving the consistency of biological control will be sought by initiating studies to discover genes, regulatory mechanisms or other cellular processes responsible for biocontrol agent efficacy.

REFERENCES

Boehm, M.J., Khan, N.I., and Schisler, D.A. 1999. USDA-ARS, Ohio State University cooperative research on biologically controlling Fusarium head blight: 3. Field testing of antagonists. Proceedings of the 1999 National Fusarium Head Blight Forum, University Printing, Michigan State University, East Lansing, MI, pgs 45-48.

Khan, N.I., Schisler, D.A., and Boehm, M.J. 1999. USDA-ARS, Ohio State University cooperative research on biologically controlling Fusarium head blight: 2. Influence of pathogen strain, inoculum spray sequence and

inoculum spray timing. Proceedings of the 1999 National Fusarium Head Blight Forum, University Printing, Michigan State University, East Lansing, MI, pgs 56-59.

Schisler, D.A., Khan, N.I., Boehm, M.J., Slininger, P.J., and Bothast, R.J. 1999. USDA-ARS, Ohio State University cooperative research project on biologically controlling Fusarium head blight: 1. Antagonist selection and testing on durum wheat. Proceedings of the 1999 National Fusarium Head Blight Forum, University Printing, Michigan State University, East Lansing, MI, pgs 78-81.

Table 1. Antagonist strain designation and identification of bacteria and yeasts that reduce the severity of Fusarium head blight of wheat.

Antagonist	NRRL accession no.[1]	Identification
AS 43.3	B-30210	*Bacillus subtillus/amyloliquefaciens*[2]
AS 43.4	B-30211	*Bacillus subtillus/amyloliquefaciens*[2]
OH 71.4	Y-30213	*Cryptococcus* sp.(= *Torula aurea*)[3]
OH 131.1	B-30212	*Bacillus subtillus*[5]
OH 181.1	Y-30215	*Cryptococcus* sp. nov. 1[3]
OH 182.9	Y-30216	*Cryptococcus nodaensis* sp. nov[3]

[1]NRRL patent culture collection, National Center for Agricultural Utilization Research, Peoria, IL.
[2]Identification by DSMZ, Braunschweig, Germany, based on 16S rDNA sequence homologies and biochemical and physiological tests of taxonomic utility.
[3]Identification based on nucleotide sequence divergence in domain D1/D2 of large subunit 26S rDNA and on divergence in ITS 1/5.8/ITS2 rDNA. C.P. Kurtzman, personal communication.
[4]Yeast, not determined.
[5]Identification by MIDI Labs, Newark, DE, based on 16S rDNA sequence homologies and biochemical and physiological tests of taxonomic utility.

Table 2. Influence of six microbial antagonists produced in C:N 6.5 and 11 liquid media on Fusarium head blight on the soft red winter wheat

	Cultivar Pioneer 2545				Cultivar Freedom			
	Peoria, IL		Wooster, OH		Peoria, IL		Wooster, OH	
Treatment	Disease Severity, 1 %	Disease Incidence, %	Disease Severity, %	Disease Incidence, %	Disease Severity, %	Disease Incidence, %	Disease Severity, %	Disease Incidence, %
Untreated contro1	3.5 cd	23.8 bcd	4.2 c	26.4 cdefg	1.0 ab	10.8 a	2.7 de	25.8 cd
Buffer control	7.6 a	32.1 a	8.4 a	40.0 a	0.6 bc	7.9 ab	3.0 cde	24.7 d
AS 43.3 (6.5)[2]	4.7 bcd	27.1 abc	4.0 cd	27.8 cdef	0.6 bc	7.5 ab	2.2 e	22.0 d
AS 43.4 (11)	4.3 bcd	26.7 abc	4.1 cd	30.3 bcd	0.9 ab	10.0 ab	4.0 abc	30.3 abc
AS 43.3 (6.5)	4.2 bcd	24.2 bcd	5.8 b	36.1 ab	0.4 c	5.0 b	3.8 bcd	31.1 abc
AS 43.4 (11)	4.4 bcd	24.6 abcd	4.2 c	28.3 cde	1.1 a	10.8 a	5.0 a	34.2 a
OH 71.4 (6.5)	3.9 bcd	23.8 bcd	2.9 d	22.5 efg	0.7 abc	7.9 ab	3.9 abc	28.1 abcd
OH 71.4 (11)	3.3 cd	21.2 cd	4.1 cd	24.2 defg	0.8 abc	10.0 ab	2.9 cde	25.8 cd
OH 131.1 (6.5)	4.6 bcd	29.6 ab	4.0 cd	25.3 defg	0.7 abc	8.8 ab	3.8 bcd	31.1 abc
OH 131.1 (11)	4.9 bc	26.2 abcd	3.9 cd	24.2 defg	0.8 abc	10.4 a	2.2 e	21.7 d
OH 181.1 (6.5)	5.3 b	26.7 abc	4.1 cd	28.6 cde	0.8 abc	10.8 a	4.4 ab	32.8 ab
OH 181.1 (11)	3.2 cd	18.8 d	5.6 b	32.5 bc	0.9 ab	9.6 ab	3.4 bcd	27.5 bcd
OH 182.9 (6.5)	4.1 bcd	27.1 abc	2.9 cd	21.4 g	0.9 ab	10.4 ab	3.0 cde	27.8 abcd
OH 182.9 (11)	3.0 d	20.8 dc	3.4 cd	22.0 fg	0.9 ab	11.7 a	2.7 de	25.3 cd

[1]Within a column, means not followed by the same lower case letter are significantly different ($P \leq 0.05$, FPLSD).

[2]Value in parentheses indicates the carbon:nitrogen ratio of the medium used to produce biomass of the antagonist.

Table 3. Influence of six microbial antagonists produced in C:N 6.5 medium on Fusarium head blight on the hard red spring wheat cultivar Grandin in Langdon, North Dakota, 2000.

Treatment	Disease Severity,[1] %	Incidence, %
Untreated control	21.8 a	52.8 a
Buffer control	12.9 de	28.1 e
AS 43.3 (6.5)[2]	16.6 bc	39.7 c
AS 43.4 (6.5)	14.3 cd	42.2 bc
OH 71.4 (6.5)	18.9 ab	47.8 ab
OH 131.1 (6.5)	12.7 de	42.8 bc
OH 181.1 (6.5)	10.4 e	32.5 de
OH 182.9 (6.5)	12.3 de	37.2 cd

[1]Within a column, means not followed by the same lower case letter are significantly different (P#0.05, FPLSD).
[2]Value in parentheses indicates the carbon:nitrogen ratio of the medium used to produce biomass of the antagonist.

Control of Fusarium Head Blight of Wheat with Foliar Fungicides

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INTRODUCTION AND OBJECTIVES

Scab, or Fusarium head blight, has been a difficult to control throughout the world. The shift to conservation tillage methods of crop production may be why Fusarium head blight has emerged as a major disease of wheat and barley through much of the US Corn Belt and the upper Great Plains (Bai and Shaner, 1994; Dill-Macky and Jones, 2000). No single traditional disease control option (disease resistant cultivars, crop rotations, tillage to destroy residues, and fungicides) is likely to control head blight because of lack of availability, excessive cost, or negative impacts on soil conservation (McMullen 1997). Bringing Fusarium head blight under control will require multiple disease management strategies (Parry et al. 1995, Bai and Shaner 1994), coupled with greater understanding of the epidemiology of the disease.

Fungicides would provide growers with a management option when susceptible cultivars are grown, and may help protect grain yield and quality of cultivars with partial resistance under conditions particularly favorable for disease. A few fungicides have shown some efficacy against Fusarium head blight, but results are often inconsistent over locations and years (Gilbert and Tekauz, 2000; McMullen at al., 1997; Parry et al., 1995; Shaner and Buechley, 1999). Some fungicides reduce DON contamination of grain, but others may cause an increased amount of DON (Gilbert and Tekauz, 2000; Shaner and Buechley, 1997; Shaner and Buechley, 1999).

We have been participants in a uniform fungicide trial. The purpose of this cooperative study is to compare a core set of fungicide treatments at several locations throughout the Corn Belt and upper Midwest for their efficacy against Fusarium head blight of wheat and barley.

MATERIALS AND METHODS

Wheat cultivar Clark was drilled at 7-in. row spacing into disked corn stalks at two locations in Indiana during 2000. Wheat at the Purdue Agronomy Research Center (ARC) near West Lafayette was planted on 13 Oct 99; wheat at the Southeast Purdue Agricultural Center (SEPAC), near North Vernon, was planted on 6 Oct 99. Before seeding at ARC, 300 lbs./A of 10-25-25 fertilizer was broadcast and incorporated. Plots at ARC were top-dressed in early spring with 315 lbs./A of 34-0-0. Prior to planting at SEPAC, the field was fertilized with 100 lbs. of 34-0-0 and 100 lbs. of 0-0-60. Top dressing was applied in early spring as 200 lbs. of 34-0-0. Mowing alleys both parallel and perpendicular to the direction of seeding created plots 25 ft long and 10 ft (ARC) or 15 ft (SEPAC) wide. Both experiments were set up as a randomized complete block with four replications.

Disease developed from natural inoculum. Fungicides were applied with a CO_2-pressurized sprayer that delivered 40 gpa at 40 psi, with a hand-held boom that had 8 (ARC) or 14 (SEPAC) TJ 60-8002 nozzles spaced at 14 in. Fungicides were applied at flag leaf emergence (GS 37) and beginning of anthesis (GS 61) at ARC and at early boot (GS 43) and full head emergence (GS 59) at SEPAC. Intensity of foliar disease was visually estimated on a whole-plot basis. Incidence of Fusarium head blight was estimated by counting the number of blighted heads in 10 arbitrarily selected 1-ft lengths of row and expressing these counts as a percentage of the average number of heads per foot of row.

Plots were combine-harvested on 23 June at ARC and on 29 June at SEPAC. Grain was dried to 13% moisture after which yield and test weight were measured. Approximately 1500 kernels from each plot from the ARC experiment were inspected for incidence of visible scab. Grain samples from each plot at both locations were sent to Dr. Pat Hart at Michigan State University for determination of DON content.

RESULTS AND DISCUSSION

Powdery mildew, leaf rust, and Septoria and Stagonospora leaf blotches were not severe at either location. Nonetheless, most treatments except the Cornell biological control agents reduced severity compared to the untreated control. Incidence of Fusarium head blight was low at SEPAC and moderate at ARC. No treatment at SEPAC reduced incidence compared to the untreated control. At ARC, Caramba, Stratego, and Folicur + Induce, when applied at GS 61, reduced head blight incidence significantly (Table 1). Four treatments at ARC reduced the percentage of scabby kernels compared to the untreated control. Five treatments also reduced the level of DON in grain, three of which were among those that reduced incidence of scabby kernels. At SEPAC, DON levels were generally lower than those at ARC, and no treatment had less DON than the untreated control. Several treatments, however, had significantly greater levels of DON than the untreated control. These were mostly treatments that included a strobilurin fungicide with no added Tilt. The correlation between visibly scabby kernels and DON level, calculated on a plot basis for the ARC experiment, was significant, but low (R=0.41). Percentage of scabby kernels was not calculated for the SEPAC trial because the incidence appeared to be very low.

Yields at SEPAC were high, but those at ARC were mediocre. The differences in severity of leaf rust and head blight incidence would not likely account for this difference. The range in average yields at ARC was 18.4 bu/A and at SEPAC was 14.8 bu/A. Only one treatment at ARC (Caramba) yielded significantly more than the untreated control. No treatment yielded significantly more than the untreated control at SEPAC. There was no correlation between yields for the same treatments at the two locations. However, plots that received the Caramba treatment had the highest average yield at ARC and the second highest yield at SEPAC. In contrast, the Cornell biological 1 treatment had the highest yield at SEPAC but a poor yield at ARC. There were no significant differences in test weight at either location.

LITERATURE CITED

Bai, G. and Shaner, G. 1994. Scab of wheat: Prospects for control. Plant Dis. 78:760-766.

Dill-Macky, R., Jones, R. K. 2000. The effect of previous crop residues and tillage on Fusarium head blight of wheat. Plant Dis. 84:71-76.

Gilbert, J., Tekauz, A. 2000. Recent developments in research on Fusarium head blight of wheat in Canada. Can. J. Plant Pathol. 22:1-8.

McMullen, M., Jones, R. and Gallenberg, D. 1997. Scab of wheat and barley: A re-emerging disease of devastating impact. Plant Dis. 81:1340-1348.

Parry, D. W., Jenkinson, P. and McLeod, L. 1995. *Fusarium* ear blight (scab) in small grain cereals-a review. Plant Pathol. 44:207-238.

Shaner, G., Buechley, G. 1999. Effect of foliar fungicides on control of wheat diseases, 1996. Fungicide and Nematicide Tests 52:233.

Shaner, G., Buechley, G. 1999. Control of wheat diseases with foliar fungicides, 1998. Fungicide and Nematicide Tests 54:337-338.

(See table 1 on next page)

Table 1. Fusarium head blight incidence, yield, and scab severity in Clark soft red winter wheat treated with various fungicides at the Purdue Agronomy Research Center (ARC) and the Southeast Purdue Agricultural Center (SEPAC) during 2000

	ARC				SEPAC		
Treatment, rate per acre, and growth stage	FHB[1] %	Yield Bu/A	Scabby kernels %	DON ppm	FHB[1] %	Yield Bu/A	DON ppm
BAS 500 9 fl oz + MSO 1%, GS 37	5.4	53.1	2.0	2.7	2.7	86.3	1.1
BAS 500 12 fl oz + MSO 1%, GS 37	6.7	52.4	2.8	2.2	3.2	81.0	0.8
BAS 500 9 fl oz + Agridex 1%, GS 61	4.9	56.9	2.6	2.8	4.1	83.3	1.7
BAS 500 12 fl oz + Agridex 1%, GS 61	5.2	52.9	1.9	2.4	2.2	83.6	1.3
BAS 500 6 fl oz + Folicur 3.6F 2 fl oz + Agridex 1%, GS 61	5.1	55.6	1.3*	1.2*	2.9	84.5	1.2
Caramba 13.5 fl oz, GS 61	2.7*	63.1*	0.9*	0.9*	3.2	93.4	0.5
Cornell biological 1, GS 61	5.4	48.9	2.0	1.6*	2.2	94.5	0.7
Cornell biological 2, GS 61	6.3	44.7	1.2*	1.9	2.9	81.9	0.9
Folicur 3.6 EC 4 fl oz + Induce 0.06 % v/v, GS 37	5.5	51.8	1.8	2.4	2.8	79.7	0.9
Folicur 3.6 EC 4 fl oz + Induce 0.06 % v/v, GS 61	3.3*	53.5	1.1*	1.4*	2.5	92.7	0.7
Quadris 2.08 EC 6 fl oz, GS 37	6.6	54.6	2.1	2.2	2.8	84.5	1.1
Quadris 2.08 SC 9 fl oz + Benlate 4 oz, GS 61	5.2	57.1	2.0	3.1	3.1	85.1	1.3
Stratego 2.1 EC 14 fl oz + Induce 0.06%, GS 61	4.0*	55.2	1.6	1.4*	3.1	93.1	0.7
Tilt 3.6 EC 4 fl oz, GS 37	5.7	58.4	2.2	2.1	2.3	84.4	0.6
Tilt 3.6 EC 4 fl oz + Induce 0.06, GS 37	5.2	52.2	2.3	2.1	2.1	93.8	0.8
Tilt 3.6 EC 4 fl oz + Induce 0.06 %, GS 61	4.9	55.8	1.5	1.8	2.2	85.1	0.7
Untreated	6.4	55.5	2.2	2.6	2.6	86.3	0.5
LSD (0.05)	1.5	7.5	0.7	0.8	1.5	12.4	0.5

Within a column, values followed by an asterisk are significantly (P=0.05) lower (FHB, scabby kernels, or DON) or higher (yield)) than the value for the untreated control.

At ARC, GS 37 treatments were applied 28 April; GS 61 treatments were applied 10 May, except for the Cornell biological treatments, which were applied 12 May.

At SEPAC, "GS 37" treatments were actually applied at GS 43 (25 April) and "GS 61" treatments were actually applied at GS 59 (5 May).

[1]FHB = Fusarium head blight. Disease incidence (percentage of spikes showing symptoms) was rated 5 Jun at SEPAC and 6 Jun at ARC.

IDENTIFICATION OF BIOPROTECTANTS FOR CONTROL OF *GIBBERELLA ZEAE*

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OBJECTIVES

To identify microbial bioprotectants effective in controlling *Gibberella zeae* when applied to cereal spikes, seed, or crop residue, and to evaluate bio-compatible chemicals for their ability to interfere with perithecial development or ascospore release when applied to crop residue.

INTRODUCTION

There is a need for safe, affordable and efficacious biological and bio-compatible protectants in the integrated management of Fusarium head blight (FHB) caused by the pathogen *G. zeae*.

Screening of microorganisms to control FHB was initiated in Brazil over a decade ago (Luz 1988). In a glasshouse trial, an endospore-forming bacterial isolate not only reduced FHB on wheat but also reduced by 10-fold the contamination of grain with the mycotoxin deoxynivalenol (DON) (Stockwell et al., 1997). DON reduction, from a New York producer viewpoint, is the most important potential effect of bioprotectants.

We have evaluated candidate biological and bio-compatible protectants for use as a protective spray at flowering-time, and as seed and residue treatments. By these studies we intend to advance biological control of FHB of wheat closer to the ultimate goal of commercial application.

MATERIALS AND METHODS

A culture collection was assembled with organisms isolated from environmental sources in New York and 19 elite Brazilian accessions provided by Dr. Wilmar Luz of Embrapa Trigo. Emphasis was placed upon the selection of organisms which are likely to be robust under harsh field conditions. This resulted in the isolation and preservation, in 15% glycerol at -80°C, of 120 candidate biocontrol organisms from 70 different sources.

In vitro and glasshouse evaluations - A substantial portion of the biocontrol culture collection has been screened for antibiosis to *G. zeae*. Several of the more promising isolates were tested in a series of glasshouse experiments. Two of these experiments were designed to determine the optimal conditions for *G. zeae* infection and efficacy of the elite biocontrol isolate, TrigoCor 1448. The goal of additional glasshouse experiments was to evaluate other promising isolates for their ability to reduce FHB under controlled environmental conditions. Wheat heads in anthesis were sprayed to run-off with water (control) or a bacterial suspen-

sion (72 hour-old culture grown in nutrient broth with yeast extract [NBYE]) and allowed to air dry for 24 hours before inoculation with a conidial suspension (10^5 cfu/ml) of *G. zeae* macroconidia. Plants were incubated for 24, 48 or 72 hours under constant mist before being transferred to the glasshouse where they were rated for disease incidence 6 to 10 days after inoculation. Treatments were replicated 5 times.

Anthesis-time spray - Three elite biocontrol bacteria and one bio-compatible fungicide were added as treatments to the Uniform Fungicide Trial conducted at Aurora, NY. In this same trial, TrigoCor 1448 was combined with tebuconazole (4 fl oz Folicur) to determine if the combination would give enhanced FHB control over either treatment alone. Bacteria were grown for 24 hours in NBYE plus manganese (2-4 X 10^8 cfu/ml) and diluted 50% for application. Treatments were applied at flowering (Feekes 10.5.1) using a tractor-mounted sprayer. Pathogen inoculum was from natural sources supplemented by ascospores released from *G. zeae*-colonized maize kernels scattered between the plots 3 weeks prior to flowering. Plots were assessed for disease incidence, % Fusarium damaged kernels (fdk), test weight, yield and DON content. Treatments were replicated 4 times and arranged in a randomized block design.

In order to evaluate additional promising biocontrol isolates under field conditions, 10 organisms and three binary combinations were applied to small plots (two adjacent rows 36 inch in length) in a separate 'mini-plot' experiment. Treatments, applied at anthesis using hand sprayers, were replicated 5 times and arranged in a randomized block design.

Seed treatment - The bioprotectant TrigoCor 1448 was included at three concentrations (10^9, 10^{10} and 10^{11} cfu/100 lbs seed) as part of a larger field trial on the effect of seed treatments on seedling emergence, seedling weight, and grain yield of hard red winter wheat (cv. 'Crimson') with 25-32% incidence of seed infected with *G. zeae*. The bioprotectant was grown in broth (NBYE) with agitation, centrifuged and the pellet resuspended in 15 ml water. The treatments were coated onto the seeds and allowed to air-dry. Treatments were replicated four times and arranged in a randomized block design.

Debris treatment - Fourteen treatments (9 candidate biocontrol organisms, three bio-compatible chemicals, and an nontreated control) were applied to artificially-infested maize stalks and grain by immersing the plant tissue in the bacterial suspensions or chemical solutions for 3 minutes with constant agitation. Treated material was allowed to air-dry. In December 1999, the samples were placed in nylon pouches, arranged on the ground in a randomized design and allowed to overwinter under ambient field conditions. Samples were collected in Spring 2000 and were kept frozen until evaluated. Plant tissue was placed on moistened filter paper covered by an inverted plate of Komada's medium and incubated at room temperature under fluorescent UV lights for a maximum of 14 days. Perithecia were counted completely or with the use of a sampling grid. Ascospore discharge was determined by the presence of colonies of *G. zeae* on the surface of the agar.

RESULTS AND DISCUSSION

Glasshouse evaluation of biocontrol isolates

a) Timing - Incubation of wheat plants for 48 hours in a mist chamber following inoculation with a spore suspension (10^5 cfu/ml) of *G. zeae* macroconidia resulted in high incidence of infection (75% and 96%) and adequate seed set. Treatment of the wheat with TrigoCor 1448, 24 hours prior to inoculation with the spore suspension, reduced FHB incidence by 15 and 22% in the two experiments and increased 100-seed weight by 13 and 45%. TrigoCor 1448 was included as a benchmark in subsequent glasshouse evaluations of other candidate biological control organisms.

b) Isolate evaluation - Treatment with TrigoCor 1448 (average of five experiments) resulted in a 33.5% decrease in disease incidence and a 29.5% increase in 100-seed weight when compared to the nontreated control. Treatment with TrigoCor 4712 gave a 93% increase in 100-seed weight (average of two experiments). Several other isolates showed promising results as well. In the one glasshouse experiment analyzed for the presence DON toxin, treatment with TrigoCor 1448 and TrigoCor 4712 reduced the toxin content of the seed by 27 and 71%, respectively, compared to the nontreated control.

Anthesis-time spray - Two of the three bacterial isolates (TrigoCor 1448 and TrigoCor 4712) tested in the Uniform Fungicide trial at the New York location gave slight reductions in the % incidence of scabby heads and % fdk, although test weight and yield were not significantly different from nontreated. TrigoCor 1448 reduced FHB incidence and DON content 17 and 23% respectively, compared to the nontreated control. When Folicur (4 fl oz) was combined with TrigoCor 1448, % incidence of scabby heads and % fdk was the lowest and test weight the highest of any of the 12 treatments included in the trial. This combination reduced FHB incidence by 38% and DON contamination by 25% compared to nontreated wheat.

Significant effects of biocontrol agents on FHB were not demonstrated in the ‘mini-plot’ experiment, possibly due to the restricted plot size.

Seed treatment - TrigoCor 1448 at 10^{11} cfu/ 100 lbs seed increased seedling emergence but to a lesser extent than Raxil-Thiram. No treatment of scabby seed had a significant effect on seedling weight, test weight or grain yield.

Debris treatment - In nodal tissue of maize, only treatment with acetic acid (5% v/v) resulted in the complete absence of perithecia. All other treatments resulted in higher numbers of perithecia and ascospore discharge than the control (water). Internode stem pieces and kernels which were collected from the field site at later dates were in more advanced states of decomposition, but those treated with acetic acid also did not produce any perithecia when incubated under favorable laboratory conditions.

DISCUSSION

The modest success of biocontrol of FHB in the glasshouse and the field with the elite bioprotectant TrigoCor 1448 suggests the potential of bioprotectants as a component in an

integrative approach to FHB control. Combination of the bioprotectant with a chemical fungicide gave promising results. At the same time, despite excellent spray coverage and nearly perfect spray timing, no treatment reduced FHB or DON levels as dramatically as required for agriculture application. Reduction in disease incidence in biocontrol field tests falls within the range reported by other groups (Boehm et al., 1999; Luo and Bleakley, 1999). Future efforts will be focussed to gain a better understanding of the FHB biocontrol process and to improve efficacy of elite bioprotectants alone or in combination with chemical fungicides.

The complete inhibition of perithecial production by acetic acid (5% v/v) has redirected the search for effective products to control perithecial development and ascospore discharge. In future trials, infested maize debris will be treated with organic acids in a range of concentrations.

REFERENCES

Boehm, M.J., Khan, N.J., and Schisler, D.A. 1999. USDA-ARS, Ohio State University cooperative research on biologically controlling Fusarium head blight: Field testing of antagonists. pages 45-48 in: Proceedings of the 1999 National Fusarium Head Blight Forum, Michigan State University, University Printing, East Lansing, MI.

Luo, Y. and Bleakley, B. 1999. Biological control of Fusarium head blight (FHB) of wheat by *Bacillus* strains. page 60 in: Proceedings of the 1999 National Fusarium Head Blight Forum, Michigan State University, University Printing, East Lansing, MI.

Luz, W.C. da. 1988. Biocontrol of fungal pathogens of wheat with bacteria and yeasts. Page 348 in: 5th International Congress of Plant Pathology, Kyoto, Japan. (Abstr.).

Stockwell, C.A., Luz, W.C. da, and Bergstrom, G.C. 1997. Biocontrol of wheat scab with microbial antagonists. Phytopathology 87:S94. (Abstr.)

EFFECTS OF RAINFALL AND TEMPERATURE ON PRODUCTION OF PERITHECIA BY *GIBBERELLA ZEAE* IN FIELD DEBRIS IN MICHIGAN

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OBJECTIVES

As *Gibberella zeae* infects during crop flowering, knowledge of the timing of formation of perithecia in the field is important in designing novel control methods. To evaluate the timing of perithecium formation, we collected wheat and corn stubble from commercial fields year-round from 1997 to 2000. We analyzed the timing of perithecium formation relative to local temperature and rainfall.

INTRODUCTION

Gibberella zeae produces Fusarium Head Blight (FHB) on wheat, barley and corn. FHB is considered to be primarily a monocyclic disease due to the small window of susceptibility of the affected crops. *G. zeae* produces two types of spores: the sexual ascospores and the asexual conidia. Ascospores are known to forcibly discharge from perithecia forming on crop debris and become airborne. Ascospores serve as one of the main sources of inoculum of head blight; the role of the conidia in infection is unclear (Fernando *et al.*, 1997; Parry *et al.*, 1995; Sutton and Proctor, 1982). Airborne spores land on flowers, germinate and penetrate the glume stomata (Pritsch et al., 2000), and spread throughout the wheat head resulting in chlorosis of infected kernels (Parry *et al.*, 1995; Sutton, 1982). Favorable conditions for disease outbreaks coincide with extended periods of high relative humidity (RH) and warm temperatures (24 - 29°C). (McMullen *et al.*, 1997a).

Perithecium formation in *G. zeae* and other fungi favor surfaces that are exposed to direct light (El-Gholl *et al.*, 1979; Halama and Lacoste, 1992; Khonga and Sutton, 1988; Reis, 1990b). Near ultra-violet (UV) light and RH may be significant factors in triggering perithecium development in *G. zeae* (Paulitz, 1996). The perithecia of *G. zeae* are ephemeral. Under laboratory conditions, a perithecium forms and discharges its ascospores within two weeks of induction (a solution of Tween 80 is added to the surface of hyphal cultures to stimulate perithecium formation) (Klittich and Leslie, 1988; Trail and Common, 2000). Therefore, presence of perithecia closely coincides with the presence of ascospore inoculum.

MATERIALS AND METHODS

Field collection. Between June 1997 and March 2000, thirteen commercial fields that had experienced previous outbreaks of FHB were surveyed in Ingham County, Michigan. The traditional order of crop rotation is corn, soybean, and wheat, however rotation patterns often varied. Fields containing wheat or corn stubble from the previous year's harvest were sampled. A decreasing number of wheat fields were sampled in 1999 and 2000 due to wheat being dropped from the crop rotation on many farms.

Debris samples that had visible signs of fungal fruiting structures were collected monthly unless impeded by snow cover. During the period of wheat flowering, sampling was increased to two-week intervals. Eight locations were sampled along transects in each field in a diamond pattern (two locations on each side of the diamond). The length of transects were scaled to the acreage of each field. Debris at each location was examined for the presence of fungal fruiting bodies. Samples within a 25-foot radius of each location were examined and four pieces of debris (at least 3 inches long) exhibiting symptoms of fungal infestation were collected from each location. Samples were stored in plastic bags at –20° C prior to analysis. Daily weather data, from the nearest National Weather Service (NWS) station at the Lansing Capitol Airport, was obtained from the National Oceanic and Atmospheric Administration National Climatic Data Center (NOAA-NCDC) database (NOAA-NNDC, 2000). All fields were within a 60-mile radius of the NWS station.

Identification. Each corn or wheat stubble sample was microscopically examined at 70X magnification for the presence or absence of perithecia. All pieces of debris were examined, the presence of one or more perithecia positively identified as *G. zeae* was designated as a positive occurrence for that location and sample. Species identification was confirmed by perithecium wall structure, color and ascospore morphology at 400X as described by Nelson *et al*. (1983).

Data Analysis. Data was expressed as a proportion of samples with *G. zeae* perithecia per total samples collected. The proportion of samples with perithecia was evaluated against calendar days. Wheat stubble samples were not included in analyses due to the low number of perithecia in these samples.

The distribution of proportion data was normalized with the arcsine square root transformation. All analyses were performed using PROC REG of SAS version 7.0 (SAS Institute, Cary, N.C.). Single factor regression analysis was used to evaluate the effect of rainfall and temperature. A stepwise regression analysis for all variables was used to determine if combined variables increased correlation. In all cases the y-intercept of the regression was forced through 0. Variables used are defined in Table 1.

Table 1. Variables for regression analysis.

Designation	Time period
T4	Average daily temperature 1 – 7 days preceding sampling
T7	Average daily temperature 4 – 10 days preceding sampling
T14	Average daily temperature 11 – 17 days preceding sampling
R4	Average daily rainfall 1 – 7 days preceding sampling
R7	Average daily rainfall 4 – 10 days preceding
R14	Average daily rainfall 11 – 17 days preceding sampling

RESULTS AND DISCUSSION

Of the 2186 samples collected, 272 were found to have perithecia of *G. zeae* (Table 2). Over the three years sampled, the highest proportion of samples with perithecia were found during the summer months. There was a peak in the proportion of samples containing perithecia in May of 1997 and 1998, just prior to wheat flowering. In 1999, the peak occurred in July after heavy rainfall in late June and early July (approximately 7 in). Rainfall in May (near the time of wheat flowering) was minimal (approximately 1.7 in.) and may have delayed perithecium formation until conditions became more favorable in June and July. Although it is likely moisture also plays a role in perithecium formation (Paulitz, 1996), the rainfall data used in this study was collected 25 – 60 miles away from the field sites and may not adequately represent field conditions.

Approximately 83% of the total samples with perithecia were found on corn debris. Thus, corn stubble was the predominant substrate for perithecium formation of *G. zeae*. However, the disease outbreak of FHB in wheat fields was minimal in 1998 and 1999 due to hot, dry conditions during the spring (Hart, 1998; 1999). Weather conditions that decreased colonization of the wheat plants would have resulted in lower numbers of perithecia on wheat stubble.

Both T4 and T14 (Table 1) were highly correlated with the proportion of mature perithecia with each temperature variable explaining approximately 50% of the variance (Table 3). However, combining T4 and T14 into a single model did not improve the overall correlation due to the fact that the two appear to covary. Surprisingly, none of the relative humidity variables were significantly correlated with perithecial maturation. Thus, multiple regression involving temperature and relative humidity did not improve the regression coefficient beyond that explained by T14 alone. Temperatures below 9°C appeared to inhibit perithecium formation (Table 3). If data points of T14 below 9°C are removed from the analysis, the R^2

value of T14 increases to 0.73. These data closely parallel the two-week cycle of development and maturation of perithecia in the lab (Trail *et al.*, 1998). The data did not show a limiting high temperature for perithecium formation.

Table 2. Corn and wheat debris containing *G. zeae* perithecia.

Dates of collection	Average no. of fields	No. corn fields[1]	No. wheat fields	Total no. corn samples	Total no. wheat samples	No. corn samples w/ perithecia	No. wheat samples w/ perithecia
Spring 1997 – Fall 1997	10	8	2	320	80	31	1
Fall 1997-Fall 1998	11	7	4	368	312	40	24
Fall 1998-Fall 1999	9	7	2	684	200	161	6
Fall 1999-Winter 2000	8	5	2	160	62	9	0
TOTAL	**38**	**27**	**10**	**1532**	**654**	**241**	**31**

1. Corn and wheat fields are defined here as those fields containing corn or wheat debris. Depending on the time of year the debris was collected, these fields may have been planted in another crop. At each fall harvest, the fields were redefined according to the fresh debris.

In summary, our results indicate that perithecium formation is limited by average daily temperatures below 9° C, and that corn stubble may be the predominant substrate for perithecium formation in *G. zeae* in Michigan. Perithecium formation may also be linked to day length and solarization (El-Gholl *et al.*, 1979; Halama and Lacoste, 1992). Further studies that monitor on-site weather conditions and controls for factors such as plant variety, pesticide and fertilizer applications, and initial inoculum are needed to construct a predictive model for *G. zeae* perithecium development.

Table 3. Effect of temperature on development of perithecia

Condition at days prior to collection	F value	P > F	Adjusted R^2
T4	32.34	0.0001	0.5281
T14	30.53	0.0001	0.5133
T14 (9° C threshold)	50.87	0.0001	0.7348

REFERENCES

El-Gholl, N. E., Schoulties, C. L., and Ridings, W. H., 1979. *Can. J. Bot.* 57:2497-2500.

Fernando, W. G. D., Paulitz, T. C., Seaman, W. L., Dutilleul, P., and Miller, J. D., 1997. *Phytopathology* 87(4):414-421.

Halama, P., and Lacoste, L., 1992. *Can. J. Bot.* 70(8):1563-1569.

Hart, L. P. 1998. *The National Fusarium Head Blight Forum, Proceedings*. University Printing, East Lansing, MI, Michigan State University, East Lansing Michigan.

Hart, L. P. 1999. *The National Fusarium Head Blight Forum, Proceedings*. University Printing, East Lansing, MI, Michigan State University, East Lansing Michigan.

Khonga, E. B., and Sutton, J. C., 1988. *Can J Phytopathol* 10(3):232-239.

Klittich, C. J. R., and Leslie, J. F., 1988. *Genetics* 118:417-423.

McMullen, M., Jones, R., and Gallenberg, D., 1997. *Plant Dis.* 81(12):1340-1348.

National Climatic Data Center National Oceanic and Atmospheric Administration. [Online] Available http://www.ncdc.noaa.gov/ol/climate/climatedata.html#DAILY, 2000.

Nelson, P. E., Toussoun, T. A., and Marasas, W. F. O., 1983. Fusarium species : an illustrated manual for identification. Pennsylvania State University Press, University Park.

Parry, D. W., Jenkinson, P., and McLeod, L., 1995. *Plant-Pathology (United Kingdom)* v. 44(2):p. 207-238.

Paulitz, T. C., 1996. *Plant Dis.* 80(6):674-678.

Pritsch, C., Muehlbauer, G.J., Bushnell, W.R., Somers, D.A., and Vance, C.P., 2000. *Mol. Plant-Microbe Interact.* 13(2):159-169.

Reis, E. M., 1990. *Fitopatologia-Brasileira (Brazil)* 15(4):p. 339-343.

Sutton, J. C., 1982. *Can. J. Plant Path.* 4:195-209.

Sutton, J. C., and Procter, R., 1982. *Can. J. Plant. Pathol.* 4(3):254-258.

Trail, F., and Common, R., 2000. *Mycologia* 92(1):130-138.

Trail, F., Gadoury, D., and Loranger, R., 1998. *The 1998 National Fusarium Head Blight Forum.* University Printing, Michigan State University, East Lansing Mi, pp. 11-13.

EFFECT OF FUSARIUM INFECTION DURING WHEAT SEED DEVELOPMENT ON THE PRODUCTION OF DON AND SEED QUALITY

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OBJECTIVES

1) Determine the time of infection of *Fusarium graminearum* during wheat seed development and its effect on the production of DON and seed quality.

2) Investigate effect of disease tolerance and susceptibility on severity of seed infection

INTRODUCTION

Head scab caused by Fusarium graminaerum (Schwabe) has caused significant losses in the soft red winter wheat crop in Kentucky and in small grain crops in many regions of North America. Damage from head scab results in reductions in seed quality, emergence, and yield of wheat. In addition, losses in food-grain quality are caused by production of fungal mycotoxins, specifically vomitoxin (deoxynivalenol = DON). Relatively little information is available regarding when peak infection occurs during seed development and maturation and how these infection levels relate to the production of DON and the eventual seed germination and vigor. Considering that the seed is the delivery system for improvements in germplasm and the source for regeneration of new cultivars, it provides a vital link between the FHB research initiative and the farmer. This study will determine the effect of fungal infection during wheat seed development and maturation on the production of DON and on seed germination and vigor. This could have direct application regarding the timing of harvest for both seed and grain producers to achieve maximum seed and grain quality, as well as providing preliminary information on genetic tolerance to seed infection during seed development.

MATERIALS AND METHODS

Field plot establishment and environment

Replicated plots of four soft red winter wheat cultivars, two susceptible (Roane, Pioneer 2552) and two tolerant (Coker 9474, Pioneer 25R18) were established following corn in a chisel plowed and disked seedbed on Spindletop Farm in Lexington, KY in October of production year1999-2000. This study was conducted as part of the uniform southern scab nursery. The corn seed inoculation procedure was modeled after the method of Paulitz (1996) and inoculum was distributed among field plots on April 24. An irrigation schedule initiated on April 28, 2000 continued throughout seed development to stimulate FHB epidemic conditions. Air and canopy temperature were recorded, as well as temperature in the

developing heads of two cultivars as described in Panozzo et al., (1999). Plots were mist-irrigated twice daily until May 26. At anthesis (Feekes 10.2), spikes in each replication of each cultivar with anthers extruded in mid-spikelet were identified. At ten days after anthesis (DAA) seventy-five previously marked spikes were harvested, with harvesting continuing at four-day intervals through harvest maturity (HM, ~14 % seed moisture, fwb).

Seed Development

Fresh weight, dry weight and seed moisture were determined at each harvest for all varieties. In addition, seeds were also assigned a numerical rating as an indicator of disease severity and classified as normal=3, slightly shriveled or discolored=2, and white tombstone=1.

Seed Assessment

Floral structures (glumes, lemma, palea, caryopsis) of Pioneer 2552 were evaluated for infection at each harvest date. Ten consecutive spikelets from each spike were numbered, and the basal glume and floret in each of the ten spikelets was removed for evaluation. The ten fresh, complete florets were separated into glume, lemma, palea, and caryopsis, surface sterilized, plated on Komada medium, and evaluated for *Fusarium spp*. infection approximately fourteen days later. The remaining three varieties were evaluated for seed infection only.

Seed quality

Seed from twenty-five spikes of each harvest was submitted to laboratory of L. P. Hart, Michigan State University for analysis of deoxynivalenol (DON) as described previously (Hart and Brazelton, 1983). Standard germination, accelerated aging germination, a stress vigor test, and the conductivity test for membrane integrity were conducted according to the Association of Official Seed Analysts (AOSA, 1999) guidelines.

RESULTS AND DISCUSSION

Physiological maturity (PM, maximum seed dry weight) occurred between 40-45% seed moisture (dwb) for all varieties. Roane and P25R18 reached PM 17 and 6 days respectively before the highest *Fusarium spp*. seed infection levels (Figure 1). Similar trends were shown for P2552 and Coker 9474, with PM at 51 and 30 DAA (data not shown). Peak infection occurred between 22-28% seed moisture in three of four varieties. The average infection by *Fusarium spp.* in floral parts from seven harvests of P2552 ranged from 25% on Jun. 8 to over 90% on Jun. 16 (Fig. 2). Seed infection followed similar trends, ranging from 20-68%, and was significantly lower than other floral structures at harvests four and five.

The cultivar, P2552 was most susceptible to infection (19-67%), but this susceptibility had little impact on measures of seed quality (Table 1). Germinability of seeds from all harvests and all varieties was generally high. Weak correlations between both laboratory quality tests (standard germination (SG) and accelerated aging vigor (AA)) and seed infection percentage were observed for all varieties. Accelerated aging germination was higher than SG,

which would indicate the fungus was killed during aging at 41°C. A moderate relationship was observed for Roane and P2552 when visually assigned seed infection was correlated with actual seed infection by *Fusarium spp*.(data not shown).

Individual head and whole plant canopy temperature data taken from Jun. 9 to Jun. 26 (Figure 3) show similar minimum and maximum temperatures during cooler, rainy conditions. However, in warmer and drier conditions, temperature differences in the head exceeded those in the canopy by 3-4°C. Temperature and irrigation provided a favorable environment for infection, but no significant disease pressure was observed in any variety until early to mid June (Figure4). Significantly higher levels of seed infection were observed in P2552 for the final 3 harvests, while Roane and P25R18, exhibited intermediate seed infection levels.

The retention of high seed quality in our study with significant seed infection late in the field season suggests many of the infections were late and mostly superficial, leaving a somewhat depleted seed, but a viable embryo. The absence of significant disease pressure at flowering and throughout early development can be attributed to late placement of inoculum, delaying ascospore maturation, and resulting in minimal infection at anthesis. This study will be repeated for the 2000-2001 production year using the same experimental procedures. Inoculum will be placed in field plots earlier to stimulate more severe disease conditions in early reproductive development.

REFERENCES

Association of Official Seed Analysts. 1999. Rules for Testing Seeds. Assoc. Off. Seed Anal., Lincoln, NE.

Hart, L. P. and W. E. Braselton. 1983. Distribution of vomitoxin in dry milled fractions of wheat infected with *Gibberella zeae.* J. Agric. Food Chem. 31: 657-659.

Panozzo, J.F., H.A. Eagles, R.J. Cawood, and M. Wooten. 1999. Wheat spike temperatures in relation to varying environmental conditions. Aust. J. Agric. Res. 50:997-1005.

Paulitz, T.C. 1996. Diurnal release of ascospores by Gibberella zeae in inoculated wheat plots. Plant Dis. 80:674-678.

Figure 2. Infection of floral components over 7 harvests in Pioneer 2552.

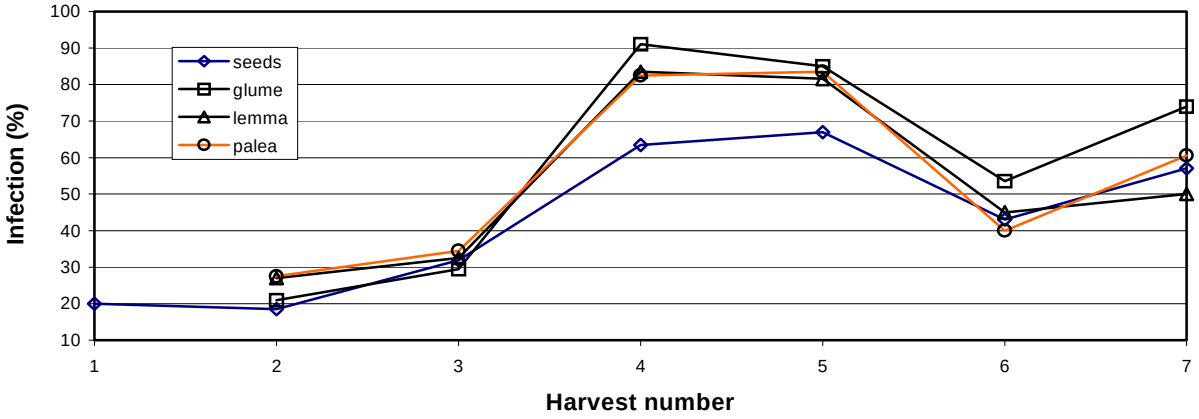
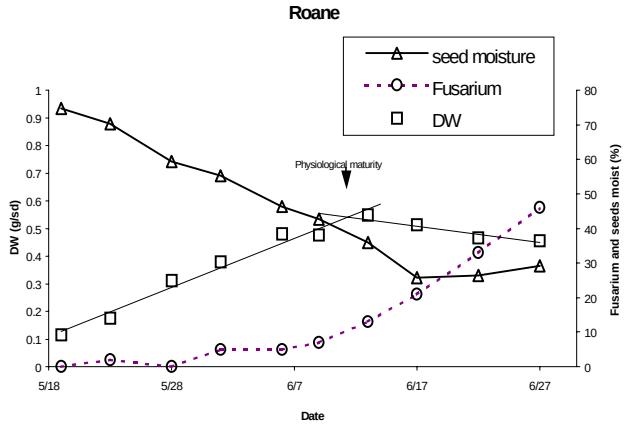


Table 1. Seed development and quality characteristics for Pioneer 2552

Date	Visual seed FHB rating score	Dry wt. (g/sd)	Seed moist. (%)	Fusarium (%) 100 sd	SG (%) 100 sd	AA (%)100 sd
8-Jun	2.6	0.641	47.5	20	91	91
12-Jun	2.7	0.79	43.1	19	91	94
16-Jun	2.3	0.779	37.9	32	88	97

Figure 1. Changes in dry weight, seed moisture and Fusarium spp. infection during seed development in two wheat varieties



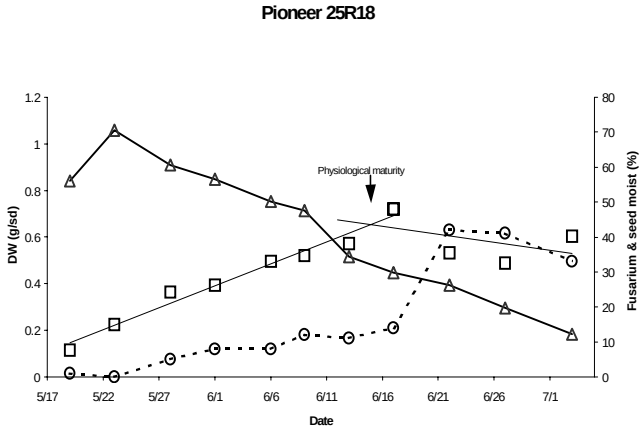


Figure 3.. Head and canopy temperature comparison in Coker 9474

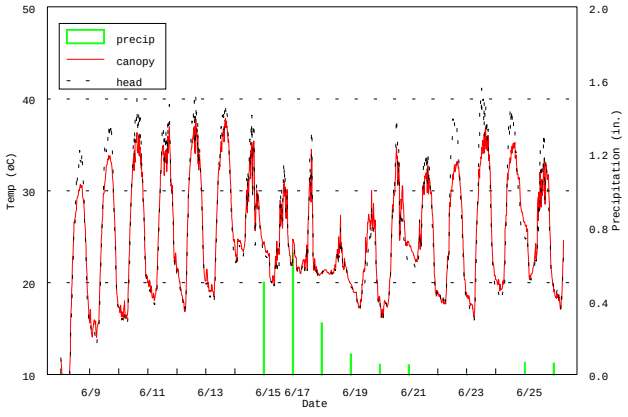
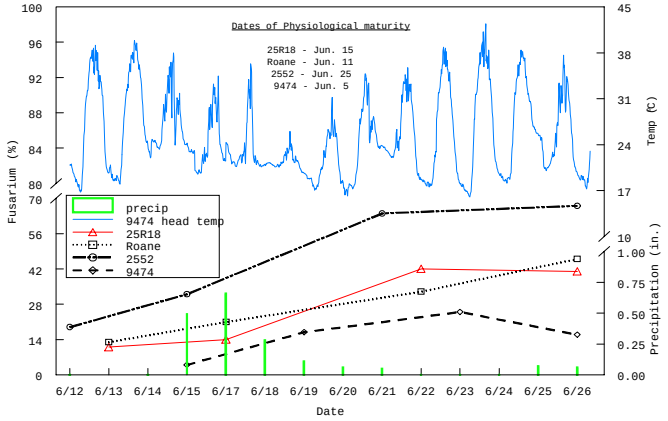


Figure 4. Relationship of temperature and precipitation to *Fusarium spp.* seed infection in four wheat varieties during seed maturation



ARE *GIBBERELLA ZEAE* SEXUAL SPORES THE CRITICAL INOCULUM FOR WHEAT HEAD BLIGHT?

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ABSTRACT

Gibberella zeae (anamorph *Fusarium graminearum*) causes scab (blight) in wheat and barley, and ear rot in corn. Since 1991, epidemics of *Gibberella* head blight have struck the Midwestern states with disastrous effects on wheat and barley growers. The fungus decreases yields and also contaminates grain with trichothecene mycotoxins that are harmful to human and animal health. To understand and control head scab, the factors and conditions that lead to epidemics must be identified. We propose that the sexual spores of *Gibberella zeae* play a important role in head blight epidemics. We will test this hypothesis by deleting critical genes required for sexual spore development (ascospores) and examine the resulting strains under field conditions for their effect on disease progression on wheat. If ascospores are the major inoculum source, than we predict that exposure of wheat to a MAT-null strain will result in significantly less disease than exposure to a wild-type strain.

DEVELOPMENT OF *FUSARIUM GRAMINEARUM* IN DETACHED SEGMENTS OF BARLEY LEAVES

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ABSTRACT

With the objective of understanding pathogenesis in tissues invaded by *Fusarium graminearum*, we are using a transformed strain of the fungus containing a constitutively-expressed gene for green fluorescent protein (GFP) in a model detached leaf system. Segments 2 cm long are cut from seedling leaves of Robust barley and placed on agar containing 60ppm benzimidazole. The segments are inoculated through a cut end with mycelium growing from 5x5mm squares of dialysis membrane. The fungus is viewed by epifluorescence microscopy in living tissues of intact segments or from sections cut from the segments either free hand with a razor blade or with a Vibratome. With these techniques, the fungus is readily visible on the leaf surface, beneath the cuticle, and beneath the epidermis in intercellular spaces of the mesophyll. In 12 separate experiments, we followed the development of the fungus for 4 days as it progressed along the segments from the inoculated cut end. By 3 days, surface hyphae advanced 2.4 mm from the cut end; intercellular hyphae, 1.6 mm; and intracellular hyphae, 0.6 mm. At 3 days, chlorosis extended 0.9 mm from the cut end. The intercellular hyphae extended well into living tissues, 1-2 days ahead of chlorosis and intracellular hyphae, confirming that the fungus initially establishes a biotrophic relationship with living leaf tissues. However, the GFP-labeled fungus became difficult to see in chlorotic tissues because these tissues became highly autofluorescent. We have not been able to visualize how the fungus enters host cells to become intracellular or determine whether the host cells are alive at the time of entry. To help see the fungus in chlorotic tissue, we have sectioned resin-embedded leaf segments and stained the sections with methylene blue/azure I, followed by basic fuchsin. The fungus stains lavender and is visible in either longitudinal or cross sections. Using both fresh and resin-embedded tissue, we will further investigate the transition from intercellular to intracellular growth. The techniques are also being used to investigate infection processes and pathogenesis in barley florets.

VARIATION IN *FUSARIUM GRAMINEARUM* ISOLATES FROM NEPAL ASSOCIATED WITH THEIR HOST OF ORIGIN

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ABSTRACT

A collection of group II *Fusarium graminearum* isolates obtained from maize, wheat, and rice from different locations in Nepal was identified using a combination of morphological and molecular criteria. The variation within this collection was analyzed using RAPD markers, IGS RFLP and PCR polymorphisms. The isolates divided into two groups, designated A and B, by RAPD analysis. Isolates in group A yielded four different PCR polymorphic markers but all of the isolates in group B yielded a single polymorphic marker. The IGS RFLP analysis was consistent with the division of the isolates into the two groups. Isolates from wheat and rice were more frequently placed in group A, with isolates from maize more evenly distributed between the two groups. Results indicate that host preference might be a factor in the division of the isolates, although the year of isolation may have had an influence as well. No geographical factors or agricultural practices could be identified that could account for the observed variation.

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PREDICTION OF FUSARIUM HEAD BLIGHT EPIDEMICS

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OBJECTIVES

Develop risk assessment models for wheat Fusarium head blight

INTRODUCTION

Nearly a century ago, the United States experienced a series of severe Fusarium head blight epidemics (Adams 1921, Atanasoff 1920, Pugh et al. 1933). Plant pathologists noted the severity of these epidemics and observed the apparent relationship between extended periods of wet weather and disease severity. For many years, Fusarium head blight was not reported to be a major problem in the U.S.; however, during the past decade many wheat production regions have experienced a series of severe epidemics that have resulted in tremendous losses to producers (McMullen et al. 1997). A disease forecasting system that could provide a reliable and timely prognosis of disease is highly desirable.

Prediction of Fusarium head blight epidemics has been attempted in Argentina where researchers used information collected over a number of years in small plots to identify weather variables correlated with disease levels and developed linear equations to predict the severity of disease (Moschini and Fortugno 1996). In North America, Francl et al. (1999) emphasized the importance of both inoculum and environment to epidemic development. De Wolf et al. (2000), used weather and disease information collected in Ohio from 1982 through 1999 to identify critical environmental periods and develop risk assessment models. We report here on the expansion of this modeling effort to include data from other wheat production regions, and discuss progress in the development of risk assessment models for wheat Fusarium head blight.

MATERIALS AND METHODS

Information was collected from records maintained at Ohio, North Dakota, Missouri, and Kansas. Weather variables included hourly temperature (EC), relative humidity (%) and precipitation (mm) observations, and researchers provided the corresponding mean disease levels from each location. The total data set consisted of 50 location-years (number of locations x the number years), and represented two wheat classes and three distinctly different environments in which wheat is grown in the U.S (Table 1).

Hourly weather data were used to create a data set of various representations of temperature, relative humidity and precipitation information from each year. Representations of weather included averages, minimums, maximums, and durations of favorable temperature, relative humidity and rainfall. Variables that combined temperature and RH into single variable were also evaluated. Each variable was summarized for two presumed critical time

periods. The first, 7 days prior to crop flowering, and the second, 10 days after initial flowering. All variables were rescaled between zero and one to facilitate the calculations of interaction terms later in the analysis. Disease intensity level or yield loss estimation (Kansas data only) was coded as a binary variable in which a location-year with >10% field severity or >15% yield loss was considered to be a major epidemic (assigned the value of 1). Location-years with less than this level of disease severity or yield loss criterion were concluded to be no or minor epidemics (assigned the value of 0).

Correlation analysis was used to identify representations of weather variables potentially associated with epidemic status. Variables with a Kendall correlation coefficient of <0.23 were dropped from the modeling process unless they were deemed to contain valuable information not already represented by other variables with greater correlation coefficients. This process reduced the number of potential independent variables from 49 to 25.

Variables identified in correlation analysis were then used to develop logistic regression models for classifying the epidemics in the location-years. Variable selection was done using a stepwise regression procedure. The stepwise procedure identified three variables useful for the prediction of epidemics. Two of the variables were summaries of the environment 7 days prior to crop anthesis, and included the duration (hours) of precipitation (DPPT7) and the duration of temperature within the temperature range of 15 to 30EC (T15307). The third variable summarized the environment 10 days after the initiation of anthesis. This variable was a combination of temperature and RH variables, and is defined as the duration of time that temperature was between 15 and 30EC and corresponding RH was >90% (TRH9010). The stepwise logistic procedure was repeated using these three variables and their interaction terms. Variables and interaction terms are defined in Table 2. In addition, logistic models with only single independent variables were evaluated to assess accuracy of the models with individual variables. All models were evaluated by cross-validation prediction accuracy (percent correctly classified observations). Errors of the models with the highest prediction accuracy were analyzed to aid in the evaluation of model performance. Sensitivity (% correctly classified epidemics) and specificity (% correctly classified non-epidemics) also were determined for all the models.

RESULTS AND DISCUSSION

Cross-validation prediction accuracy of the logistic models ranged from 62% to 84% (Table 3). Four different models all correctly classified 84% of the 50 location-years from Ohio, North Dakota, Missouri and Kansas. These models utilized differed independent variables and had different levels of sensitivity and specificity. One of these four models used only the temperature and humidity combination variable TRH9010. The other three models with 84% prediction accuracy utilized at least one interaction term (product of two or three independent variables). Each of the models incorrectly classified eight cases. The number of false positives (falsely predicting a major epidemic) and false negatives (falsely predicting minor or no epidemic) is specific to each model, but four cases were incorrectly classified by all four of the identified models. Two of these four errors were false negatives. These errors appear to be the result of favorable environmental conditions or limiting factors beyond the critical time periods used by the models. Errors were not limited to a single state or location.

Logistic models utilizing only single independent variables confirmed the importance of environment during crop anthesis as critical to the development of Fusarium head blight epidemics. Models that used only independent variables that summarized temperature (T15307) and moisture (DPPT7) prior to flowering were less accurate (Table 3). Moreover, models that utilized interaction terms between pre and post-flowering environment had prediction accuracies of less than or equal to that of the model that used TRH9010 variable alone. Analysis of model errors indicated that variables summarizing environment prior to anthesis may provide models with information about potentially limiting factors, specifically, conditions that were unfavorable for inoculum production.

In some years, the environment during time periods other than those addressed by the models may influence inoculum level and head colonization, thus further enhancing or diminishing disease development and yield losses. However, we have shown here that fairly narrow time periods around crop anthesis were very useful for predicting epidemics. The usefulness of these models for making real-time disease forecasts will depend on the availability and accuracy of weather forecasts.

The models with the highest level of accuracy as determined by this analysis will be further validated with data collected during the 2000 growing season. It is possible that one of these models or some other similar model will be used to provide wheat producers with reasonably accurate regional Fusarium head blight forecasts. During these finial stages of model validation it will be essential to develop the necessary infrastructure to operate the risk assessment model(s) and deliver reliable disease forecasts at a regional level.

Table 1. Summary of information used to develop prediction models for wheat Fusarium head blight.

State	Location	Years
Ohio	Wooster	16
Ohio	Hoytville	2
Ohio	S. Charelston	1
North Dakota	Fargo	7
North Dakota	Cando	4
North Dakota	Langdon	4
North Dakota	Carrington	2
North Dakota	Dazey	1
Missouri	Novlty	5
Missouri	Columbia	3
Missouri	Lamar	3
Kansas	Powhattan	2

Table 2. Definition of independent variables and interaction terms utilized by logistic models for forecasting wheat Fusarium head blight.

Variable	Definition
TRH9010	Duration $15 \leq T^a \leq 30$ & $RH^b \geq 90$ 10days after flowering (hours)
T15307	Duration $15 \leq T \leq 30$ & $RH \geq 90$ 7 days prior to flowering (hours)
DPPT7	Duration of rain 7 days prior to flowering (hours)
FHB1	(T15307*DPPT7)
FHB2	(DPPT7*TRH9010)
FHB3	(T15307*TRH9010)
FHB6	(T15307*DPPT7*TRH9010)

[a]Temperature (°C)

[b]Relative humidity (%)

Table 3. Cross-validation prediction accuracy for logistic models of wheat Fusarium head blight developed with information from Ohio, North Dakota, Missouri and Kansas.

No. of var. [a]	Variable(s)	Percent Correct [b]	Sensitivity [c]	Specificity [d]
1	TRH9010	84	83	84
1	FHB3	84	83	84
2	FHB1, FHB6	84	72	91
2	FHB6, DPPT7	84	67	94
7	TRH9010, T15307, DPPT7, FHB1, FHB2, FHB3, FHB6	82	78	84
2	FHB2, FHB3	82	72	88
2	FHB2, T15307	82	67	91
1	FHB6	80	72	84
3	TRH9010, T15307, DPPT7	78	72	81
1	FHB2	76	61	84
2	T15307, DPPT7	70	56	78
1	FHB1	64	44	75
1	T15307	64	33	81
1	DPPT7	62	11	91

[a]Number of variables used by the model

[b]Percentage of correctly classified epidemics and non-epidemic

[c]Percentage of correctly predicted epidemics

[d]Percentage of correctly predicted non-epidemics

REFERENCES

Adams, J. F. 1921. Observations on wheat scab in Pennsylvania and its pathological histology. Phytopathology 11:115-124.

Atanasoff, D. 1920. Fusarium-blight (Scab) of wheat and other cereals. J. Ag. Res. 20:1-32.

Francl, L., Shaner, G., Bergstrom, G., Gilbert, J., Pederson, W., Dil-Macky, R., Sweets, L., Corwin, B., Jin, Y., Gallenberg, D. and Wiersma, J. 1999. Daily inoculum levels of *Gibberella zeae* on wheat spikes. Plant Dis. 83:662-666.

McMullen, M., Jones, R. and Gallenberg, D. 1997. Scab of wheat and barley: a re-emerging disease of devastating impact. Plant Dis. 81:1340-1348.

Moschini, R. C. and Fortugno, C. 1996. Predicting wheat head blight incidence using models based on meteorological factors in Pergamino, Argentina. Eur. J. Plant Pathol. 102:211-218.

Pugh, G. W., Johann, H. and Dickson, J. G. 1933. Factors affecting infection of wheat heads by *Gibberella saubinetii*. J. Ag. Res. 46:771-797.

CROP RESIDUE MOISTURE AND *GIBBERELLA ZEAE* PERITHECIA DEVELOPMENT

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ABSTRACT

Gibberella zeae, which causes wheat Fusarium head blight, as well as corn stalk and ear rot, has been a devastating pathogen in many crop production regions of the U.S. where both wheat and corn are grown. The effects of temperature and moisture interactions on *G. zeae* perithecia development have not been evaluated nor has perithecia development been investigated on crop residues directly. Sensors used to continuously monitor crop residue moisture were adapted for use with corn residues. These sensors are being used to monitor moisture under controlled conditions. The quantity and rate of perithecia development are currently being evaluated at 15, 25 and 30EC and three moisture levels. Sensors are also being used to monitor residue moisture in a natural environment. Monitoring factors that influence the reproduction of *G. zeae* on crop residues will provide information critical to the development of reliable disease prediction systems and facilitate management recommendations.

FACTORS AFFECTING THE DEVELOPMENT OF WHEAT FUSARIUM HEAD BLIGHT

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OBJECTIVES

Develop a disease forecasting system for wheat Fusarium head blight based on the environment and inoculum level.

INTORDUCTON

Fusarium head blight (FHB) has been a severe problem in many of the wheat production regions of the United States (McMullen et al. 1997). The development of a reliable disease forecasting system would greatly increase the ability of wheat producers to make disease management and grain marketing decisions. Recent attempts to predict Fusarium head blight have emphasized the importance of both inoculum and environment to disease epidemics (Francl et al. 1999, DeWolf et al. 2000). However, the precise nature in which environment and inoculum interact during epidemic development remains unclear. Cooperators in OH, IN, SD, ND, and MB have agreed to follow a common protocol to create a forecasting model (De Wolf et al. 1999). We report here on how an infection bioassay may provide important insights into the interactions of inoculum, environment, and the resulting disease level.

MATERIALS AND METHODS

Adapted, FHB-susceptible cultivars were grown with standard agronomic practices in replicated plots at Wooster, OH (Hopewell SRWW), Fargo, ND (Norm HRSW), and Brookings, SD (Norm HRSW). The environment at each location was monitored by an automated weather station equipped with temperature, relative humidity, precipitation, and surface wetness instrumentation. Each day, 30 wheat spikes were collected from each plot by cutting the stem just above the first node. Twelve spikes per plot were placed into a dry growth chamber environment. A second group of 12 spikes was maintained in a saturated environment (100% RH) for 24 h. Following the 24 h wet treatment, these spikes were placed in the dry environment with the heads that had remained dry. Crop growth stage each day was evaluated using the remaining heads. FHB incidence and severity of both wet and dry spikes were recorded on the day of collection and after 12 days. For this report, viable inoculum during the flowering and milk stages of crop development was estimated by FHB incidence of the heads that received the additional moisture treatment. For comparison among locations and to judge epidemic intensity in each location, plot FHB incidence and severity were evaluated during the early dough stage.

RESULTS AND DISCUSSION

FHB intensity in the replicated plots at Fargo was the highest of the research locations during the 2000 growing season (Table 1). Plots near Wooster had the least amount of disease and plots in Brookings had a disease level intermediate to the Fargo and Wooster locations.

The spike bioassay indicated that Wooster had lower estimated inoculum levels than Fargo and Brookings, which were roughly equivalent (Fig. 1). Incidence at Fargo sharply increased in association with precipitation on days 186 and 188; whereas, incidence at Brookings was not strongly associated with a rainfall event.

Wetness parameters summarized from the beginning of anthesis and early milk stages of growth indicate that precipitation was similar between Wooster and Fargo and Wooster had the longest daily average wetness. The Brookings location had 43 mm less precipitation and 9 h per day of wetness duration. Relative humidity was similar at all three locations. Average temperature at Fargo and Brookings locations were equal; however, average temperature near Wooster was 5 degrees lower than the Dakota locations.

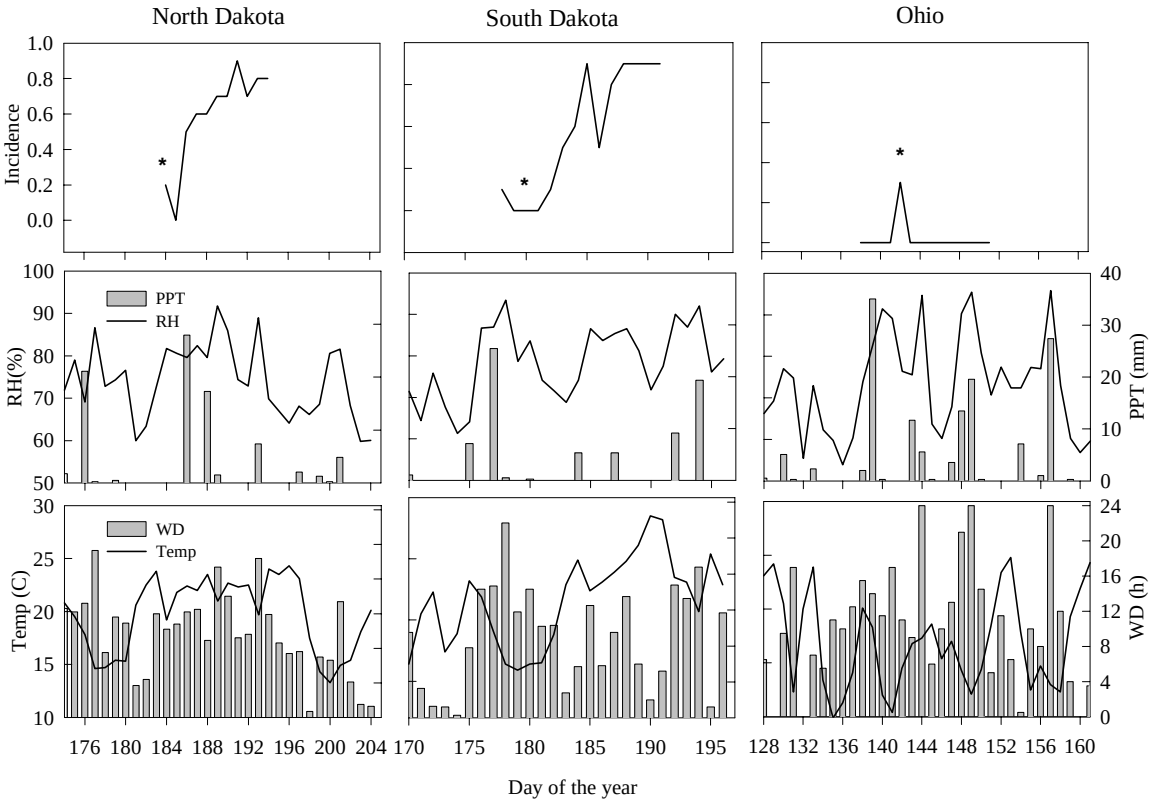
Differences in disease level between the three locations can be attributed, in part, to differences in temperature and moisture parameters, which likely influenced inoculum levels and infection periods. The low average temperature at Wooster may best explain observed low levels of estimated inoculum and disease intensity since moisture parameters were at least equivalent to the other locations. The high incidence levels on bioassays at Fargo and Brookings suggest that inoculum was abundant. Differences between the Fargo and Brookings locations in moisture levels during crop anthesis, specifically duration of surface wetness, suggest that moisture may have been a limiting factor for FHB development at Brookings.

These results demonstrate the importance of monitoring both inoculum and environment in the development of FHB epidemics. As additional information is collected, modeling fluctuations in inoculum level and infection periods based on environment should be possible. This database development is quickened by our experimental approach that utilizes multiple locations.

Table 1. Summary of environmental conditions at three research locations from beginning of crop anthesis until early milk stages of growth and Fusarium head blight (FHB) intensity in research plots at soft dough.

Variable	Location		
	North Dakota	South Dakota	Ohio
Avg. wetness duration (h)	12	9	15
Avg. temperature (C)	22	22	15
Avg. relative humidity (%)	81	80	81
Total rainfall (mm)	54	11	55
Plot FHB incidence (%)	60	38	8
Plot FHB severity (%)	31	25	4

Figure 1. Summaries of environment and incidence levels at research locations in North Dakota, South Dakota, and Ohio.



Each series of plots gives the temperature (Temp), relative humidity (RH), precipitation (PPT), wetness duration (WD), and proportional incidence level (Incidence). Viable inoculum on spikes in the field can be estimated from daily incidence because incidence values were derived from a bioassay in which wheat pikes collected from field plots were subjected to conditions favorable for infection by *G. zeae*. The * designates the beginning of crop anthesis.

REFERENCES

De Wolf, E.D., Francl, L.J. Lipps, P.E. and Madden, L.V. 1999. Role of environment and inoculum level in wheat Fusarium head blight development. Pages 87-91 in: Proceedings of the National Fusarium Head Blight Forum. December 5-7, 1999.

De Wolf E.D., Madden, L.V. and Lipps, P.E. 2000. Risk assessment models for wheat Fusarium head blight. Phytopathology 90:S19 (abstr.).

Francl, L., Shaner, G., Bergstrom, G., Gilbert, J., Pederson, W., Dil-Macky, R., Sweets, L., Corwin, B., Jin, Y., Gallenberg, D. and Wiersma, J. 1999. Daily inoculum levels of *Gibberella zeae* on wheat spikes. Plant Dis. 83:662-666.

McMullen, M., Jones, R. and Gallenberg, D. 1997. Scab of wheat and barley: a re-emerging disease of devastating impact. Plant Dis. 81:1340-1348.

A VISUAL SCALE FOR ESTIMATING DAMAGE TO SOFT RED WINTER WHEAT KERNELS BY FUSARIUM HEAD BLIGHT

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INTRODUCTION

Fusarium head blight (scab), caused by *Fusarium graminearum*, has become an increasingly important problem in the United States due to an increase in crop acreage managed using tillage practices that leave crop residues on the soil surface (3,5,6). In 1993, the losses in United States wheat production to Fusarium head blight were estimated to exceed $1 billion, and more recent epidemics have added to the relative importance of this disease (6).

Infection by *F. graminearum* causes floret sterility and poor grain fill resulting in reduced yield and test weight (1,2,4). Diseased kernels are often shriveled and may have a white or pink discoloration. Affected grain may also contain mycotoxins, including deoxynivalenol (DON, vomitoxin) and zearalone, that have detrimental affects on animal and human health (2,7). Fusarium head blight also reduces seed quality. Seed that appears unaffected may be contaminated with mycelium or conidia of *F. graminearum*, resulting in seedling blight and foot rot when contaminated seed is planted (3).

Damage caused by Fusarium head blight can be quantified by assessing head disease incidence and severity, grain mycotoxin level, and grain yields. Researchers, interested in evaluating fungicide efficacy or the genetic resistance of wheat cultivars and breeding lines, are also concerned with kernel damage. Estimations of kernel damage are also useful to grain farmers and handlers required to assess grain marketability or feed value.

A visual scale estimating the percentage of affected kernels of hard red wheat has been proposed by R. Jones and J. Jenkins, Department of Plant Pathology, University of Minnesota. This series of photographs, representing a range of damaged kernels from 0 to 50%, were 1:1 reproductions of disease and healthy kernel mixtures on a 1000 kernel count basis. This scale proved invaluable for making visual kernel assessments, but differences between soft red wheat and hard red wheat kernel characteristics were great enough to limit the usefulness of this system. Soft red wheat is generally lighter in color than hard red wheat, thus the visual differences between diseased and healthy kernels is more difficult to discern. These differences lead to the construction of a visual scale for soft red wheat.

OBJECTIVE

To develop a visual assessment scale for soft red wheat kernels affected by Fusarium head blight.

MATERIALS AND METHODS

A visual scale for soft red wheat was prepared by creating a grain sample with a known percentage of diseased kernels from the susceptible soft red wheat cv. Hopewell. Each sample contained 200 kernels. The sample was then mixed and placed in a 5 cm-diameter container. Photographs are actual size to facilitate the comparisons with other grain samples. The visual scale can be used by passing grain samples over the photographs until the percentage of affected kernels is approximated by the damage seen in the scale. Record the appropriate percentage and continue onto the next sample. It is important to take a random, uniform sample from the harvested wheat.

DISCUSSION

There is considerable variation in the types of diseased kernels in grain samples. Harvested grain, especially grain threshed using research size equipment, may retain glumes and other plant parts. Intact spikelets should be examined carefully to confirm that Fusarium head blight caused the damage. To reduce sampling error and improve mean estimates, multiple readers may be employed, although correlation among evaluators has been high.

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REFERENCES

Anderson, A. L. 1948. The development of *Gibberella zeae* head blight of wheat. Phytopathology. 38:595-611.

Arseniuk, E., Foremska, E., Goral, T., and Chelkowski, J. 1999. Fusarium head blight reactions and accumulation of DON and some of its derivatives in kernels of wheat, triticale, and rye. J. Phytopathol. 147:577-590.

Bai, G-H. and Shaner, G. 1994. Scab of wheat: prospects for control. Plant Dis. 78:760-766.

Bai, G-H. and Shaner, G. 1996. Variation in *Fusarium graminearum* and cultivar resistance to wheat scab. Plant Dis. 80:975-979.

Khonga, E. B. and Sutton J. C. 1988. Inoculum production and survival of *Gibberella zeae* in Maize and wheat residues. Can. J. Plant Pathol. 10:232-239.

McMullen, M., Jones, R. and Gallenberg, D. 1997. Scab of wheat and barely: a re-emerging disease of devastating impact. Plant Dis. 81:1340-1347.

Parry, D. W. , Jenkinson P., and McLead L. 1995. Fusarium ear blight (scab) in small grain cereals - a review. Plant Pathol. 44:207-238.

INFLUENCE OF MIST-IRRIGATION VOLUME ON THE SEVERITY OF FUSARIUM HEAD BLIGHT AND SEED CHARACTERISTICS IN SELECTED CHECK CULTIVARS AND LINES OF WHEAT AND BARLEY

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ABSTRACT

A field experiment was conducted to investigate refining mist-irrigation treatments that might improve our ability to screen for resistance to *Fusarium* head blight (FHB) in wheat and barley. Mist-irrigation was applied to barley cultivars and lines Stander (susceptible, S), Robust (S), MNBrite (resistant, R), MNS93 (R); wheat cultivars were Norm (S), McVey (moderately resistant, MR), P2375 (MR), and BacUp (R). The experimental design consisted of four separate randomized complete split-blocks with four replications. One randomized complete split-block was non-misted as a control. The mist-irrigation treatments were: non-misted, 2.0, 4.0, and 8.0 mm of water per day. Split-block treatments were inoculated versus non-inoculated. Plots were inoculated with macroconidia using a CO_2 backpack sprayer to control both concentration and timing of inoculum application. Variables measured in barley plots over the different mist-irrigation treatments included FHB severity, incidence of infection, discolored kernels, and concentration of deoxynivalenol (DON) in harvested grain. Differentiation among the barley cultivars over the four variables was consistent under no mist and at the 8 mm per day volume. The variables measured in wheat plots over the same mist-irrigation treatments included FHB severity, incidence of infection, visually scabby kernels (VSK), and concentration of DON in harvested grain. Differentiation among the wheat cultivars over the four variables was more consistent than among the barley cultivars and was most consistent under no mist or at the 2 mm per day volume. We feel the lower disease levels reflect conditions in years with low FHB severity. These preliminary data also suggest that breeders could obtain useful information regarding promising breeding lines by screening for resistance to FHB utilizing inoculated plots that would be non mist irrigated.

GIBBERELLA ZEAE POPULATION DYNAMICS: A PROGRESS REPORT

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OBJECTIVE

Increase our understanding of fungal population dynamics to enhance the reliability of disease management with fungicides.

INTRODUCTION

North Dakota had been ground zero in the majority of recent FHB epidemics; consequently, agricultural industries have been displaced and small grain producers have endured economic hardships, often so severe as to preclude another season of farming. Another moderately severe, and in some North Dakota counties extremely severe, FHB epidemic occurred during the 2000 growing season. This epidemic series can be dated back to the 1993 catastrophic FHB outbreak on wheat and barley in South Dakota, North Dakota, Minnesota and Manitoba (McMullen et al., 1997).

Research on forecasting systems has suggested that a reliable model needs to take into consideration inoculation events (De Wolf et al., elsewhere in these proceedings). Inoculation has been shown to occur on multiple occasions during an FHB epidemic (Francl et al., 1999). As part of a regional forecasting system, a heuristic forecasting scheme for FHB is based on airborne spore samples of *Gibberella zeae* (Francl et al., these proceedings).

Prediction of inoculation events is being pursued as an adjunct research project to a multi-institution forecasting model development effort. This project may eventually obviate the need for airborne spore counts. Also, populations of *G. zeae* conidia may be produced on wheat leaves and these spores may then serve as a source of inoculum. We provide here the status of research on inoculation events and report on some preliminary results from wheat leaf assays for *G. zeae*.

MATERIALS AND METHODS

Inoculation timing. Fungal spore data came from two sources. First, a Burkard 7-day volumetric spore sampler was placed in the center of a wheat stubble field to sample airborne *G. zeae* ascospores continuously. The entire tape area was observed microscopically for ascospores of *G. zeae* and approximately 50 days per year for two years are available for analysis. Second, daily inoculum levels were assayed on potted Norm wheat spikes exposed to the field for 24 h. Four spikes from each pot were clipped and put in a solution of 40-ml sterile distilled water and one drop of Tween20 and shaken vigorously for 2 min. The solution was decanted and frozen until later assessment. Spores counts were assessed by colonization on Komada's selective medium and then carrot agar for colony identification of

G. zeae by observation of perithecia. To date, 54 days have been assayed over two growing seasons.

Colony forming units per spike, efficiency of fungal recovery from spikes, and spore type (ascospore or conidium) on spikes are being investigated. Relationships among environmental variables, time of day, and inoculum will be analyzed statistically. Linear regression and correlation will be used together with a critical pathway analysis to determine the variables important to inoculation timing.

Colonization of leaves. Twenty leaves formed the basic sampling unit and there were five sampling dates in 2000. Some leaves were asymptomatic (youngest on plant) while other leaf samples showed necrotic lesions. Fifty uniform pieces were cut from these leaves and plated on Komada's medium. Some pieces were surface sterilized (0.5% NaOCl for 2 min) while others were left unsterilized. After 10-12 days, colonies showing characteristics of Fusarium were transferred to half-strength PDA for identification based on colony type and conidia morphology. Colonies were plated on carnation leaf agar as needed to resolve questionable identifications.

RESULTS AND DISCUSSION

Preliminary results on inoculation were presented in the Forum last year and at the North Central Phytopathology meeting last summer (Markell and Francl, 1999, 2001). Briefly, there was a good correspondence between the Burkard and spike bioassay sources of data. Major and moderate inoculation events seemed closely linked with rainfall in excess of 5 mm. Airborne ascospore incidence exhibited diurnal periodicity and was greatest between midnight and 10:00 AM.

A final report on inoculation timing should be completed next year. Critical factors leading to inoculation events are being researched in an ongoing attempt to predict Fusarium head blight based on environment. Either aerobiota data or model estimates appear needed for successful prediction of Fusarium head blight. A successful result here would eliminate the need for spore samplers and save much of the operating cost of the NDSU scab forecasting system.

Preliminary data on leaf colonization from the 2000 growing season in North Dakota show that various species of Fusarium can be found between the three-leaf and early milk growth stages (Table 1). Furthermore, both epiphytic and apparently pathogenic relationships can be noted, often in temporal progression. This work will be expanded in 2001 and will include the following contrasting parameters: samples from fields with and without corn, wheat, or barley residue; symptomatic (i.e., necrotrophic lesions) and asymptomatic leaves; and surface sterilized vs. unsterilized pieces.

If indeed fungal colonies routinely occur on leaves, then this avenue of pathogen movement to the spike should be investigated further. Conidiation and splash dispersal are likely to occur during extended warm, wet weather. Also, one might hypothesize that inhibition of this potential source of inoculum by a fungicide may decrease the severity of an FHB epidemic.

Table 1. Incidence of *Fusarium* species on symptomatic (necrotrophic lesions observed) and asymptomatic (youngest) wheat leaves collected from a wheat-on-wheat field on the NDSU Experimental Research Station, Fargo. Samples were collected on 1 June (#1), 9 June (#2), 19 June (#3), 26 June (#4), and 10 July 2000 (#5), sterilized in 0.5 % NaOCl for 2 min or left unsterilized, isolated on Komada's medium, and identified on half-strength PDA.

Fusarium species	Date Pathogen Detected			
	Asymptomatic leaves		Symptomatic leaves	
	Sterilized	Unsterilized	Sterilized	Unsterilized
F. acuminatum	#5	#1,#2,#5	#2,#3,#5	#1,#2,#3,#5
F. avenaceum		#5	#5	#5
F. equiseti	#2,#5	#1,#2,#3,#4,#5	#2,#3,#4,#5	#1,#2,#3,#4,#5
F. graminearum	#4,#5	#3,#4,#5	#3,#4,#5	#3,#4,#5
F. poae			#2	#2
F. sambucinum	#2,#5	#2,#5	#2,#5	#1,#2,#5
F. sporotrichioides	#5	#1,#4,#5	#2,#4,#5	#1,#2,#4,#5

LITERATURE

Francl, L., Shaner, G., Bergstrom, G., Gilbert, J., Pedersen, W., Dill-Macky, R., Sweets, L., Corwin, B., Jin, Y., Gallenberg, D., and Wiersma, J. 1999. Daily inoculum levels of *Gibberella zeae* on wheat spikes. Plant Disease 83:662-666.

Markell, S. and Francl, L. 1999. A comparative time line for dispersal, inoculum level and environmental conditions in a moderate wheat head blight epidemic. In: Proceedings of the 1999 National Fusarium Head Blight Forum. 5-7 December 1999, Sioux Falls, SD.

Markell, S. and Francl, L. 2001. Relationships among environmental variables, ascospore dispersal, and inoculum of *Gibberella zeae.* Phytopathology 91:(in press).

McMullen, M., Jones, R., and Gallenburg, D. 1997. Scab of wheat and barley: A re-emerging disease of devastating impact. Plant Dis. 81:1340-1348.

DESCRIPTION AND EVALUATION OF THE NDSU REGIONAL WHEAT DISEASE FORECASTING SYSTEM

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ABSTRACT

A wheat disease forecasting system has been implemented in the North Central region. Daily infection periods of three leaf diseases were predicted in 23 localities by computer models using environmental data from automated weather stations. Airborne spores of *Gibberella zeae* were sampled at 17 locations and heuristic forecasts for FHB were derived from these counts as well as temperature and wetness data. Information was delivered via the Internet and a toll-free telephone number. The system correctly predicted epidemics in 1999 and 2000. Operational problems were encountered in sampling efficiency and weather-related incidents. The system continues to be evaluated for biological realism, economic thresholds, and facilitation of user interaction. A quantitative FHB forecasting model may be ready to replace the present rule-of-thumb guidance as early as the 2002 season.

INTRODUCTION

Predictions of impending plant disease epidemics can increase appropriate pesticide use, agricultural productivity, and returns to management. Key elements of a successful forecasting system include accurate prediction for multiple and economically important plant diseases, timely and understandable communication, and cost effective remedies. Most existing forecasters are devoted to high-value crops because of the large reward for sound disease management in those cropping systems. Many of these systems consist of self-contained computer programs and environmental loggers positioned on-site. In contrast, few forecasters for major field crops exist despite the large aggregate amounts of pesticides applied. System development is hindered by the minimal marginal returns to added disease management expense and the relative economic inefficiency of an on-site system.

Diseases have considerably reduced wheat production in North America in the 1990's. Scab or Fusarium head blight (caused by *Gibberella zeae*) has caused billions of dollars of crop loss in a series of epidemics (McMullen et al., 1997). Leaf rust (*Puccinia triticina*), Stagonospora blotch (*Phaeosphaeria nodorum* and *Ph. avenaria*), and tan spot (*Pyrenophora tritici-repentis*) have been perennial foliar diseases amounting to perhaps 5-10% average crop loss per year (Long et al., 1998; McMullen and Nelson, 1992).

A regional forecasting system for three wheat diseases was initiated in North Dakota and Minnesota in 1999 and deployed again in 2000. The system was composed of environmental and aerobiota input data, computer and rule-based models to predict disease, compila-

tion of results, and two conduits of information delivery. This report describes the system and its evaluation and discusses the challenges faced in its operation.

METHODS

Data for infection period models came from bioassays in a field environment (Francl, 1995) and literature reports. Tan spot and Stagonospora leaf blotch infections within a 24 h period were predicted with accuracies of more than 80% by back propagation neural network models (De Wolf and Francl, 2000). Model inputs were cumulative growing degree days, daily average temperature and relative humidity, total daily precipitation, and hours of wetness. Leaf rust infection was based on hours of wetness and a minimum temperature threshold during the wet period.

The forecasting system in 1999 employed environmental data from 17 automated weather stations of the North Dakota Agricultural Weather Network (NDAWN) to provide regionally specific information. The deployed model relied on a wetness duration estimate, which a generalized regression neural network predicted from logger data input (Chtioui et al., 1999). For the year 2000, six additional NDAWN sites were selected to provide input for leaf disease models so that more wheat producers can take advantage of the system.

A foliar disease control advisory involved scouting for a 50% disease incidence threshold on either the penultimate or antepenultimate leaf. Scouting began at stem elongation and ended at the early milk growth stage. Once leaf disease reached the threshold, six to eight predicted infection periods were allowed to accumulate and a second confirmatory scouting was recommended. If disease progress was evident, a labeled fungicide spray was recommended.

A heuristic advisory for head blight infection was based on airborne spore concentration, wetness duration, and proximity of the managed crop to an inoculum source (infested residue). To estimate inoculum concentration, Burkard cyclonic flow volumetric samplers were placed near each of 17 NDAWN weather stations in fields with wheat stubble on the soil surface. Air samples were collected three times a week and examined under a microscope for sexually produced ascospores of *Gibberella zeae* and conidia of its anamorph, *Fusarium graminearum*

During the critical part of the wheat growing season, foliar infection period predictions were updated daily and head blight information was updated within 24 h of sample collection. Forecasts were provided via the Internet (www.ag.ndsu.nodak.edu/cropdisease) and a toll-free telephone message. A computer program automated the interface between the NDAWN environmental database, disease prediction models, and web pages. For each location, a summary of spore counts, system forecasts, and environment was placed on the web site after the 2000 season ended.

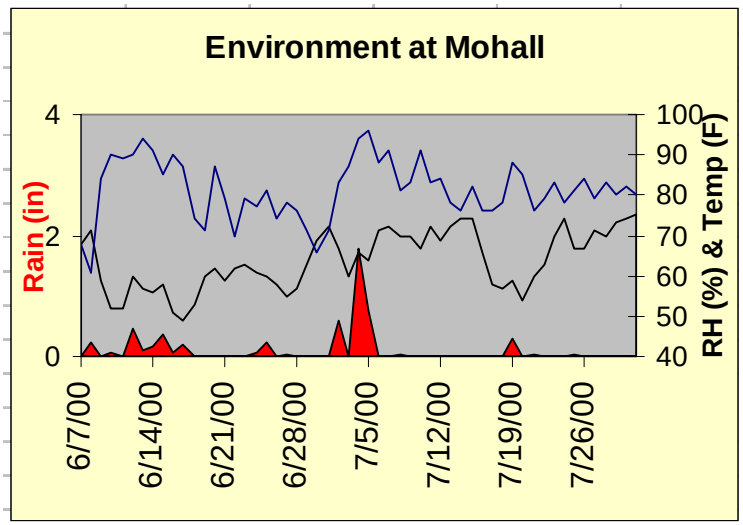
System evaluation in 1999 included a replicated field trial (n=4) on the North Dakota Agricultural Experiment Station in Fargo. The systemic fungicide azoxystrobin was applied at 120 g/ha active ingredient to the spring wheat cv. Grandin based on growth stage or according to forecast system advice. An untreated check and twice treated check were included.

Yield parameters and disease suppression were analyzed with ANOVA to judge treatment effectiveness. Similar trials were conducted in Fargo and Mohall, ND in 2000 but results are not included in this report.

After the 1999 growing season, the Burkard samplers were evaluated for sampling efficiency of *Sordaria fimicola* and *G. zeae* ascospores. Sporulating fungal cultures were placed in a container that had an opening for the machine orifice. Replicated tests (n=12) were conducted on three samplers that were powered by a regulated DC transformer.

RESULTS AND DISCUSSION

In 1999, the forecasting system correctly predicted a widespread tan spot epidemic and minor impacts due to Stagonospora blotch and Fusarium head blight. The web site received >7,000 hits from 1,408 distinct hosts. In 2000, the system web page received 15% more visitors, correctly forecasted foliar disease epidemics, and alerted growers to the presence of airborne *G. zeae* spores (Fig. 1).



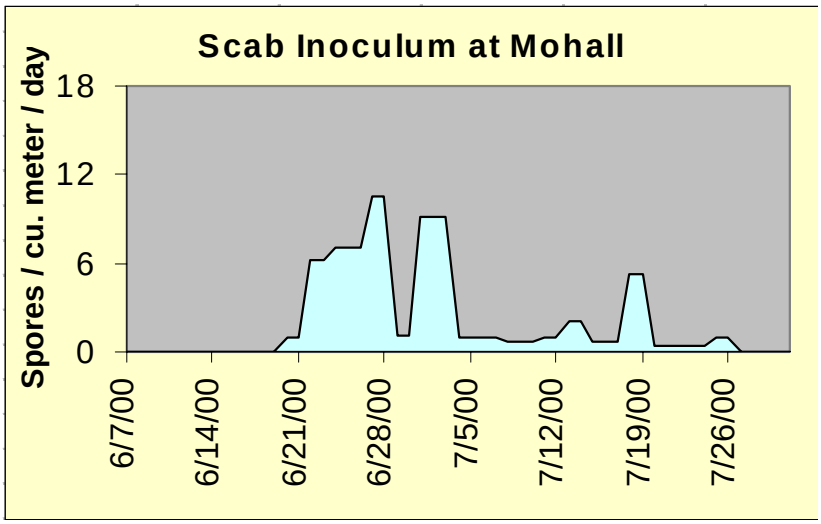


Fig. 1. Airborne spore counts and environmental summary for the Mohall, ND location. Much of the wheat crop in the area flowered around 16 July.

Fungicide trial results indicated that leaf disease was severe enough to warrant control because the forecasting system resulted in statistically significant disease suppression and yield enhancement over the control (Table 1). A single fungicide application at flag leaf emergence or heading increased protein but not yield.

Table 1. Effect of azoxystrobin fungicide application timing on disease and spring wheat yield in a replicated trial at Fargo, 1999.

Fungicide treatment	Flag leaf disease (%)	Yield (g/m2)	Protein (%)
Untreated	18 c	236 c	15.4 b
Flag leaf emergence	7 ab	244 c	15.6 ab
Heading	2:00 AM	245 c	15.7 a
Forecasting system (flowering)	9 ab	274 a	15.4 b
Heading and flowering	1:00 AM	293 a	15.8 a

During the 1999 season, two power outages prevented web page updates for 24-48 h; however, the recorded telephone message continued to provide information prior to re-initialization. On 19 June 2000, the NDSU campus was flooded. The web site was off-line for about 72 h and the phone line was down for two weeks. Also, frequent rains in both years caused problems in physically accessing the sampling sites. Timely spore counts were possible only through extended laboratory hours processing samples.

Sampling efficiency was questioned and addressed after the 1999 growing season. The Burkard cyclonic sampler was designed to spin particles within a metal cylinder until deposition into an eppendorf collection vial. However, debris collected on the walls during the season and tests showed that freshly liberated ascospores stuck to the walls of the sampling chamber. Wall adherence averaged 22% of the total sample for *S. fimicola* and 75% for *G. zeae*.

Spore sample collection in 2000 included a rinsing step to ensure better performance. The Burkard cyclonic sampler, a newly designed model, was chosen because of the potential for sample assay methods other than microscopy. For example, a PCR assay presently is being compared with results from microscopy and culturing on selective media (L. Francl, unpublished).

A user survey to assess system functionality is planned for the fall of 2000. In addition, data continue to be collected on prediction accuracy based on bioassay data and economic thresholds from fungicide trials. Fusarium head blight epidemic severity is related to multiple inoculation events and wet weather (Francl et al., 1999). A quantitative FHB forecasting model presently is under development (E. De Wolf et al., these proceedings) and incorporation into the NDSU forecasting system could happen as early as the 2002 season.

REFERENCES

Chtioui, Y., Francl, L.J., and Panigrahi, S., 1999. Moisture prediction from simple micrometeorological data. Phytopathology 89:668-672.

De Wolf, E.D. and Francl, L.J., 2000. Neural network classification of tan spot and Stagonospora blotch infection periods in a wheat field environment. Phytopathology 90:108-113.

Francl, L.J., 1995. Challenge of bioassay plants in a monitored outdoor environment. Can. J. Plant Path.17:138-143.

Francl, L., Shaner, G., Bergstrom, G., Gilbert, J., Pedersen, W., Dill-Macky, R., Sweets, L., Corwin, B., Jin, Y., Gallenberg, D., and Wiersma, J. 1999. Daily inoculum levels of *Gibberella zeae* on wheat spikes. Plant Dis. 83:662-666.

Long, D.L., Leonard, K.J., and Roberts, J. J. 1998. Virulence and diversity of wheat leaf rust in the United States in 1993 to 1995. Plant Dis. 82:1391-1400.

McMullen, M.P. and Nelson, D.R. 1992. Tan spot and five years of wheat disease survey. Advances in Tan Spot Research, L.J. Francl, J. Krupinsky, and M.P. McMullen, eds. No. Dak. State Univ. Agr. Exp. Sta.

McMullen, M., Jones, R., and Gallenburg, D. 1997. Scab of wheat and barley: A re-emerging disease of devastating impact. Plant Dis. 81:1340-1348.

PATHOGENICITY AND VIRULENCE OF EIGHT *FUSARIUM GRAMINEARUM* ISOLATES ORIGINATING IN FOUR REGIONS OF MEXICO

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INTRODUCTION

The expression of wheat host plant resistance to head blight caused by *Fusarium graminearum* Schw. varies widely, depending on environmental conditions (rainfall, temperature) and the inoculum used (age, concentration, incremental substrate, and isolates). It is important to have good control of these factors to avoid variation in the expression of resistance.

At present, a mixture of highly virulent pathogen isolates is commonly used as inoculum in screening wheat for Fusarium head blight (FHB) resistance in the belief that there are no vertical races in *F. graminearum,* as noted in the literature. There are, nonetheless, significant differences in pathogenicity among isolates that can greatly influence the measurement of resistance levels (Mesterhazy, 1997).

In our program differences in pathogenicity observed during FHB resistance evaluation made us suspect there was significant cultivar x isolate interaction. This led us to initiate the study reported here, whose main objective was to evaluate and confirm the presence of cultivar x isolate interaction.

MATERIALS AND METHODS

During the 2000 crop cycle in Atizapan, Toluca, Mexico, a trial was carried out in which four resistant (Sumai # 3, Frontana, Catbird, and Sha4/Chilero) and one susceptible (Flycatcher) wheat cultivars were inoculated with eight different *F. graminearum* isolates. The test isolates originated in Tepatitlan (isolates 3, 4, 5, 6), Jesus Maria (2), and El Tigre (1) in the state of Jalisco, and in Patzcuaro (7, 8), state of Michoacan.

The trial was planted with three replications; the cultivar was the main plot and the isolate, the sub-plot. The inoculum was increased in mungo bean medium, and its concentration adjusted to 50,000 spores/ml after growing five days. Twenty wheat spikes per plot were inoculated at flowering using the cotton method (Gilchrist et al., 1997).

Supplementary moisture in the form of mist irrigation was provided on the four rainless days. The different treatments were evaluated 35 days after inoculation by counting the number of affected spikelets per spike. Results were analyzed using categorical data analysis.

RESULTS AND DISCUSSION

Results of the analysis of variance (Table 1) showed highly significant differences at 0.001% between isolates, cultivar, and cultivar x isolate interactions. Isolate 1 from El Tigre was the most virulent, and 7 and 8 from Patzcuaro the least virulent (Table 2). The cultivar Frontana showed the best resistance to the eight isolates used (Table 3). Table 4 shows the absolute ratio of infected:healthy grains for each wheat cultivar with every isolate.

Table 1. Analysis of variance of blighted spikelets of five wheat cultivars inoculated with eight individual *Fusarium graminearum* isolates, Atizapan, Toluca, Mexico, 2000.

Source	DF	Chi Square	Prob
Cultivar	4	565.02	***
Isolate	7	178.10	***
Cultivar x isolate	28	106.38	***

Table 2. Analysis of contrast among the five test wheat cultivars.

Cultivars	2	3	4	5
1	***	***	***	NS
2		*	***	***
3			***	***
4				***

Table 3. Analysis of contrast among the eight *Fusarium graminearum* isolates.

Isolates	2	3	4	5	6	7	8
1	***	***	***	***	***	***	***
2		**	NS	NS	NS	NS	NS
3			*	**	**	**	**
4				NS	NS	*	*
5					NS	NS	NS
6						NS	NS
7							NS

Table 4. Absolute ratios of infected:healthy grains on four resistant and one susceptible wheat cultivars inoculated with eight different *Fusarium graminearum* isolates from four regions of Mexico. Atizapan, Toluca, Mexico, 2000.

Cultivars	Isolates							
	1	2	3	4	5	6	7	8
Sha4/Chil	0.194	0.098	0.096	0.103	0.093	0.135	0.085	0.090
Catbird	0.111	0.060	0.880	0.880	0.054	0.046	0.053	0.062
Sumai#3	0.090	0.046	0.079	0.055	0.053	0.065	0.059	0.054
Frontana	0.055	0.051	0.058	0.035	0.039	0.051	0.039	0.043
Flycatcher	0.210	0.127	0.131	0.132	0.131	0.084	0.108	0.082

CONCLUSION

The results confirm the presence of cultivar x isolate interaction.

REFERENCES

Gilchrist, L., S. Rajaram, M. van Ginkel, M. Kazi, and J. Franco. 1997. Characterizing *Fusarium graminearum* resistance of CIMMYT bread wheat germplasm. Fifth European Fusarium Seminar. Szeged, Hungary.

Mesterhazy, A. 1997. Breeding for resistance to Fusarium head blight of wheat. In: Fusarium Head Blight: Global Status and Future Prospects. H.J. Dubin, L. Gilchrist, J. Reeves, and A. McNab, eds. Mexico, D.F.: CIMMYT.

LOCAL GENETIC DIVERSITY OF *GIBBERELLA ZEAE* POPULATIONS FROM CORN STUBBLE, WHEAT STUBBLE AND INFECTED WHEAT HEADS

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ABSTRACT

Small-scale genetic structure of *Gibberella zeae* populations is being investigated to provide information on the epidemiology of this pathogen. Four target population complexes, one each from Michigan, Minnesota, North Dakota and South Dakota, are being characterized using amplified fragment length polymorphisms (AFLP) technology. A target complex is composed of an infected wheat field and the corn and wheat stubble fields that surround it. *Gibberella zeae* overwinters on the stubble and the colonies produce inoculum for infecting wheat heads in the subsequent year. Corn and wheat stubble was collected in June 1999 at the time of wheat anthesis in the adjacent field. This ensured that the stubble *G. zeae* populations were sampled at the time of wheat head infection. Infected wheat heads were collected two to three weeks after flowering. Preliminary results indicate that individuals do vary in their AFLP banding pattern, and populations are polymorphic. We are now increasing sample size for each population to obtain good assessments of population structure. We will report on the progress of the work in relation to the study's objectives. The first objective is to determine whether *G. zeae* populations isolated from corn and wheat stubble differ genetically. Since the timing of infection differs for corn and wheat, it is possible that they will differ genetically due to differences in the genetic structure of *G. zeae* inoculum. If these stubble populations differ genetically, then we will utilize the unique genetic features to investigate the relative importance of wheat versus corn stubble as inoculum sources in causing wheat scab. The relative importance of ascospores versus conidia as inoculum sources in causing scab will be determined from the frequency of multilocus clonal lineages within the *G. zeae* population causing scab.

AFLP LINKAGE MAP OF *GIBBERELLA ZEAE*

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ABSTRACT

A genetic linkage map of *Gibberella zeae* (*Fusarium graminearum*) was constructed by crossing nitrate nonutilizing (*nit*) mutants of *G. zeae* strains R-5470 (from Japan) and Z-3639 (from Kansas). Ninety-nine *nit+* progeny were selected and analyzed for polymorphisms using AFLP markers. Thirty-one pairs of two-base selective primers revealed 1072 polymorphic markers that mapped to 441 unique loci on nine linkage groups. The total map length of the genome from this analysis was 1036 centimorgans with an average interval of 2.3 map units between loci. Three linkage groups had high levels of segregation distortion. Selection of *nit+* recombinant progeny accounts for two of the skewed regions. One linkage group appeared to have an intercalary inversion. Loci governing trichothecene toxin amount and type (deoxynivalenol versus nivalenol) were mapped. A linkage map will be useful in population genetics studies, map-based cloning, QTL analysis, ordering genomic libraries, and comparisons with related species.

SITES OF ACTION OF TYPE II RESISTANCE TO FHB IN WHEAT: NING 7840 RETARDS SPREAD OF *F. GRAMINEARUM* WITHIN RACHIS

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ABSTRACT

Sumai 3 and its derivatives are excellent sources of genes conferring Type II resistance to Fusarium Head Blight in wheat. Type II resistance is defined as reduction in the rate of disease progression within a spike from a single point of infection. Observations are generally made on the number of visibly discolored (scabby) spikelets over a period of time. Our goal was to identify a measure of resistance that provides a higher level of precision that could be used in mapping and manipulating Sumai 3 resistance genes. Theoretically, there are several ways that a plant can reduce the rate of increase in scabby spikelets including: 1) Reduced spread from initially infected spikelet to rachis, 2) Reduced spread within the rachis, and 3) Reduced spread from within the rachis to adjoining spikelets. In addition, a plant may influence the fungus's change from a biotrophic to a necrotrophic growth habit. Ning 7840 (a Sumai 3 derivative) and a susceptible genotype (Norm) were grown in the greenhouse. Just prior to anthesis forty spikes of each genotype were single floret inoculated, then misted for 3 days. Seven and fourteen days post inoculation 20 spikes of each genotype were evaluated for the number of scabby spikelets, visible symptoms of disease in the rachis, and presence of the fungus in the rachis via a bioassay of rachis sections on PDA. The entire experiment was replicated 3 times and analyzed using Sas Proc GLM. Visible symptoms in the rachis were an accurate reflection of the presence of the fungus in both Ning 7840 and Norm, and the spread of the fungus in the rachis was significantly greater than reflected by the number of scabby spikelets. The ratio of the spread of scab in the rachis (according to visual symptoms of rachis portions infected) to the number of scabby spikelets in Ning 7840 was 2.4 and 3.4 for 7 and 14 days post inoculation (respectively), and for Norm was 2.8 and 2.2 for 7 and 14 days post inoculation (respectively). Subsequent field sampling of seven genotypes from the Uniform Scab Nursery (3 - 4 infected heads per genotype) showed 2 genotypes where the visual symptoms in the rachis were significantly greater than the presence of the fungus in the rachis according to the bioassay. In addition, in contrast to the study of Ning7840 and Norm, the presence of the fungus in the rachis was not always significantly greater than the number of scabby spikelets. Overall, the data suggest that there may be separate gene systems conferring resistance to spikelet infection vs. rachis infection. Although the visual symptoms of scab in the rachis were not significantly different from the presence of the fungus in the rachis according to the bioassay for Ning 7840 and Norm, this may not be the case for all genotypes. This poster was presented at the International Symposium on Wheat Improvement for Scab Resistance, May 5-11, 2000, Suzhou and Nanjing, China.

TEMPORAL PATTERNS OF ASCOSPORE DISCHARGE BY *GIBBERELLA ZEAE* FROM COLONIZED CORN STALKS UNDER NATURAL CONDITIONS

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OBJECTIVE

To document and characterize temporal patterns of ascospore discharge by *Gibberella zeae* from mature perithecia in corn stalk tissues under natural conditions.

INTRODUCTION

Gibberella zeae (anamorph: *Fusarium graminearum*), the predominant causal fungus of Fusarium head blight of wheat and barley, overwinters in crop residues. In large regions of north central and eastern North America, corn stalks on the soil surface are believed to be the primary reservoir of overwintered *G. zeae* and the main source of windborne ascospores for infection of cereal spikes in spring. Ascospores are forcibly discharged very short distances from perithecia and may be carried by turbulent air currents to flowering cereal spikes near or distant to the perithecial substrate. Once into the planetary boundary layer of the atmosphere (two times crop canopy height up to cloud level), ascospores are subject to vertical mixing and potential long distance dispersal. Previous investigators (Fernando et al., 2000; Paulitz, 1996) have examined patterns of ascospore capture from air within and above crop canopies, but, due to limits in experimental design, were unable to discriminate newly discharged ascospores from those redistributed locally in turbulent air or settled from higher levels of the atmosphere during periods of nonturbulence.

A comprehensive project to elucidate the aerobiology of ascospores of *G. zeae* is underway at Cornell University. A critical component of ascospore aerobiology is understanding when and under what circumstances ascospores are forcibly discharged from mature perithecia in substrates such as overwintered corn stalks on the soil surface. The time of day for peak spore discharge may be important relative to the likelihood that ascospores will be exposed to turbulence and transported into the planetary boundary layer. We are interested also in the environmental cues that affect the initiation and duration of spore discharge events. We are assessing natural patterns of ascospore discharge from a corn stalk substrate in order to formulate reasonable hypotheses about ascospore discharge that are testable by subsequent experimentation and observation. This paper reports initial results of those studies.

MATERIALS AND METHODS

Production of perithecium-bearing substrate

Fully mature dent corn stalks were collected from a production field in November 1999. Segments 2-cm long and centered on a node were cut from harvested stalks. Twenty five cut stalk pieces were placed in 1-gallon plastic jugs and soaked in 500ml distilled water for 24 hours. Jugs were plugged with non-absorbent cotton wrapped in two layers of cheesecloth. After the soaking period, the water was drained and the stalk pieces were sterilized in an autoclave for one hour with slow exhaust. Stalks were autoclaved three times over three consecutive days. Fifteen mycelial plugs from a pure culture of *G. zeae* (Gz014NY98) grown on PDA were added to each jug. Stalks were incubated at room temperature for two weeks under a 12 h photoperiod of near UV light. The stalks were shaken periodically in the jugs. Perithecia formation did not occur in the jug cultures. Fully colonized stalks then were placed over two wetted filter papers in a glass deep Petri dish and incubated under near UV light at room temperature for nine days or until perithecia were visible. Ascospore maturity was confirmed by microscopy and discharge ability was confirmed by placing agar media above the stalks.

Spore trapping experiments

A Burkard seven day recording volumetric spore trap (adjusted to sample air at 10 L/min) was used to collect discharged ascospores and record spore counts on an hourly basis.

A specially designed platform to support four corn stalk pieces bearing mature perithecia was placed in front of the orifice of the spore trap. The same stalk pieces were left in place for the duration of the experiment. Ascospores were collected on Melinex tape previously treated with a thin film of adhesive (Silicone grease and hexane in a 10:1W/V ratio). Sections of the tape were mounted in lactophenol cotton blue and scanned at right angles to the direction of rotation to determine the total number of ascospores discharged on an hourly basis. Tapes were scanned at a magnification of 400 at 2-mm intervals. Weather data (relative humidity, rainfall, wind velocity and direction, and temperature) were collected on an hourly basis using a weather station (Davis Weather Monitor II, Davis, California) located within a meter of the spore sampler. Data were collected between day of year 156 (4 June) and day of year 183 (2 July).

RESULTS AND DISCUSSION

The number of ascospores released from the corn stalks bearing perithecia and captured on Mellinex tape ranged from 0 to 3862 ascospores per hour. Typically there was a background level of 5 or more ascospores per hour during the collection period in June 1999 which was characterized by frequent rain events and cool to moderate temperatures.

There were six major events of ascospore release defined by peak counts of more than 1000 ascospores per hour. These events are summarized in Table1. These events ranged in duration from 4 to 14 hours and each was initiated and terminated during daylight hours. Peak spore release (>1000 per hour) occurred over intervals of 1 to 7 hours and the maxi-

mum discharges generally coincided with daily periods of highest temperature and lowest relative humidity. No singular weather variable was found that preceeded the five dates of major spore release in contrast to the other days of observation. The last five discharge events occurred on consecutive days during the warmest portion of the collection period. In fact, events five and six overlapped on June 26. Event five diminshed from 2372 spores in one hour down to 483 spores in the following hour immediately following a 10% mid-day rise in relative humidity. Thereafter, the ascospore counts rose again to above 1000 per hour for the next three hours (event six) in conjunction with another decrease in relative humidity. It appears that decreases in relatively humidity following periods of high humidity are somehow associated with the discharge of ascospores.

We found that most ascospores were being discharged during daylight hours when atmospheric turbulence is highest. This pattern provides the maximum opportunity for ascospores to be moved into the planetary boundary layer where vertical mixing occurs up to the cloud level and the potential for long distance dispersal is greatest. We are presently conducting experiments under controlled climatic conditions to better understand the effects of moisture, relative humidity, temperature, and light on the discharge of ascospores from mature perithecia. Our observation of peak ascospore discharge during daylight hours is in stark contrast to published reports (Fernando et al., 2000; Paulitz, 1996) of peak ascospore capture in or near inoculated plots during nighttime hours. We suggest that nighttime peaks in these studies may also be due to capture of airborne spores that settle during periods of nonturbulence. Obviously, these seemingly contradictory results need to be reconciled by further experimentation on the aerobiology of this fungus.

REFERENCES

Fernando, W.G.D., J.D. Miller, W.L. Seaman, K. Seifert, and T.C. Paulitz. 2000. Daily and seasonal dynamics of airborne spores of *Fusarium graminearum* and other *Fusarium* species sampled over wheat plots. Can. J. Bot. 78:497-505.

Paulitz, T.C. 1996. Diurnal release of ascospores of *Gibberella zeae* in inoculated wheat plots. Plant Dis. 80:674-678.

Table 1. Summary of major events of ascospore release by *Gibberella zeae* from a corn stalk substrate placed near the orifice of a volumetric spore sampler under natural conditions.

		Event		Peak discharge (>10^3 per h)	
Event No.	Day of Year	Interval	Duration	Interval	Duration
1	170	2 pm – 6 pm	4 h	2 pm – 3 pm	1 h
2	174	9 am – 7 pm	10 h	12 pm – 6 pm	6 h
3	175	6 am – 8 pm	14 h	10 am – 5 pm	7 h
4	176	9 am – 7 pm	10 h	2 pm – 5 pm	3 h
5	177	6 am – 2 pm	8 h	11 am – 1 pm	2 h
6	177	2 pm – 9 pm	7 h	2 pm – 5 pm	3 h

FUSARIUM HEAD BLIGHT: INOCULUM DETECTION, DISEASE PROGRESS, AND ENVIRONMENTAL INFLUENCES

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OBJECTIVES

South Dakota State University is part of a collaborative project studying epidemiology of Fusarium head blight (FHB) on wheat under different environments throughout the upper mid-west. The ultimate goal is to develop a disease forecasting system. Primary objectives include: 1) monitoring inoculum dynamics and disease development in relation to temperature, humidity, and precipitation at locations throughout the upper mid-west; and 2) to evaluate tools and techniques for incorporation into a useful and efficient disease forecasting system.

INTRODUCTION

It has been observed that FHB occurs at epidemic levels when hot, humid conditions and frequent precipitation have occurred at anthesis (Bai and Shaner 1994, McMullin et al. 1997, Parry et al. 1995). By investigating the relationship of FHB incidence and severity to environmental conditions, a disease forecasting system may be developed to provide producers with the capability of making better management decisions. Environmental conditions are thought to influence the FHB disease cycle, but it is not certain which factors are critical, and which are most predictive of epidemics. The objective of this study is to relate certain environmental factors to the development of inoculum, the delivery of that inoculum, and the progress of the disease after infection for a study site in South Dakota.

MATERIALS AND METHODS

Spring wheat (cv. “Norm”) susceptible to FHB was planted into strips 1.4 m by 45 m using a 7-row grain drill. Two adjacent strips were planted on each of three planting dates (3 April, 25 April, and 2 May), referred to as planting date (PD) 1, 2, and 3, respectively. Multiple dates were used to help ensure that susceptible host stage and pathogen inoculum would be present concurrently. Each planting was divided into three replicate plots. Each plot was further divided into two subplots, one sampled and one unsampled. The unsampled subplot was used to assess final disease levels for each plot.

Environmental data were continuously collected using a datalogger (Campbell Scientific Inc. model CR10X) and various instruments. Leaf wetness sensors (Campbell Scientific Inc. model 237) were used to estimate the duration of leaf wetness within the canopy. Additional sensors were constructed and deployed to detect moisture at the soil surface (Osborne and Jin, this proceeding).

Daily airborne inoculum levels were monitored during the sampling period using a Burkhard Cyclone sampler (Burkhard Manufacturing). A wash of the cyclone unit was performed daily to ensure uniform sampling. The sample and wash were plated on Komada's medium for spore enumeration. Counts were reported as CFU per day. Inoculum on wheat spikes was estimated by washing heads using protocols described by Francl et al. (1999), with some modification (sampled heads were not covered prior to sampling). On each day, five primary spikes per replicate were collected and placed in a flask with 50 ml of sterile deionized water, shaken vigorously for 60 seconds to dislodge spores, then discarded. A 0.5 ml aliquot of the wash was then spread-plated onto each of three plates of Komada's medium. Plates were then incubated 10-14 days. Colonies were described and counted after incubation. Colonies were reported as CFU per spike per day.

Daily sampling for disease progress was conducted in each planting date when plants were between late emergence (GS 59) (Zadoks et al. 1974), and mid-milk (GS 75) following protocols described by Francl (1998). In each sampled plot, 24 spikes were collected by cutting stems below the upper-most node. Each sample was divided into two subsets, one labeled "dry", the other labeled "wet". Both sets were rated for the number of infection sites, and FHB severity (% of infected spikelets). One subset of daily samples ("dry") were placed directly into large incubator (RH < 50%), with their stems submerged in floral preservative to maintain freshness of tissue. The remaining subset ("wet") was treated similarly, but was subjected to 24 hours incubation in a moist chamber (100% RH) prior to incubation in the dry chamber. After an incubation period of six days, each spike was again rated for FHB infection sites and severity. After 12 days, spikes were rated for disease severity. Final FHB incidence and severity in the plots were surveyed by sampling 100 primary spikes per rep when plants reached mid-milk.

RESULTS AND DISCUSSION

In the period of seven days prior to sampling, cool wet conditions were experienced. Temperature in the canopy averaged 17°C and a total of 15.2 mm precipitation was received from seven rain events. There was little recovery of spores by either method in the few days following this cool wet period. Though moisture was high, the cool temperatures could have slowed fungal development at the soil surface, delaying the onset of ascospore production.

The Burkhard Cyclone Sampler did not perform as well as expected, however the data were considered to be a rough estimate of airborne spore concentration. Few spores were recovered on the first six days of sampling. Spores were recovered from the samples over the next 18 days at levels between 15 and 223 colony forming units (cfu) per day (Fig. 1). Head washing also resulted in very little spore recovery during the first six days of sampling. Thereafter, recovery of spores ranged from 4 to 207 cfu per head (Fig. 2). There was strong positive correlation between the overlapping sampling periods among planting dates. There was also positive correlation between Burkhard spore recovery and head washing spore recovery for PD 1 and PD 2 (r = 0.54 and 0.58, respectively).

Precipitation, canopy temperature, leaf wetness, and soil wetness for the anthesis periods of each planting date are summarized in Table 1. During susceptible periods, PD 1 and PD 2 were of similar temperature and received similar precipitation. Planting date 3 was warmer,

received much less precipitation during anthesis, and also had lower soil and leaf wetness duration. Soil wetness duration appeared to relate to airborne ascospore levels (Fig 1). Peaks in soil wetness duration were often followed by increased airborne ascospore levels though calculated correlation was negligible. Table 2 details the final disease ratings for all plantings. Planting dates 1 and 2 had moderate levels of disease. Inoculum and moisture levels were greater during anthesis periods of PD 1 and 2 than for PD 3, which would account for the higher incidence and severity. Moderately low canopy temperature may have slightly inhibited infection and FHB development during anthesis of PD 1 and 2. Dry conditions and reduced inoculum levels during anthesis of PD 3 probably resulted in reduced disease incidence. Severity was also lower in PD 3, again due to the drier conditions present.

Table 1. Environmental conditions over susceptible periods in each planting date.

Plant. Date	Time period (susceptible)	Avg. canopy temp (oC)	Precip. (mm) / events	Leaf wetness duration (hrs)	Soil wetness duration (hrs)
1	DOY 173-179	19	33 / 2	10.5	14.3
2	DOY 178-183	18	26 / 2	14.6	20.3
3	DOY 184-188	24	1-May	7.5	9.9

Table 2. Final disease ratings.

	Plant Date 1		Plant Date 2		Plant Date 3	
	Incidence %	Severity %	Incidence %	Severity %	Incidence %	Severity %
Rep 1	39	26	50	26	13	15
Rep 2	34	25	28	22	13	20
Rep 3	28	26	36	27	13	19
PD Mean	34	26	38	25	13	18
Overall: Disease Incidence = 28% Disease Severity = 23%						

Total incoming solar radiation varied from 4 to 17 MJ $m^{-2}d^{-1}$ (Fig. 1). It was observed that peaks in solar radiation corresponded to peaks in airborne inoculum estimates, though calculated correlation was not large (r = 0.49). Daily solar radiation may have affected airborne inoculum due to light requirements of the fungus, rapid changes in relative humidity, or increased spore escape from the boundary layer by way of increased convection of air away from the soil surface.

Disease progress was measured in terms of disease incidence (infected heads / total heads sampled) and disease severity (infected spikelets / total spikelets). There was almost always greater disease incidence in sample sets subjected to 24 hours of high moisture incubation than in those with no additional moisture, as expected. Little correlation was observed between airborne inoculum (Burkhard data) and disease incidence on those samples given

no additional moist incubation as read at six days post-sampling. Shifting the Burkhard data to compensate for any lag between airborne inoculum and initial infection (i.e. shifting the Burkhard data ahead 1, 2, 3, or 4 days) did result in increased correlation coefficients relative to the 6 day-post readings of samples, in some cases. For PD 1, with no shift, r = (-)0.39; with 2 day shift, r = 0.36; 3 day shift, r = 0.66; and 4 day shift, r = 0.74. For PD 2, with no shift, r = 0; with 2 day shift, r = 0.71; with 3 day shift, r = 0.59. Such pattern, however, was not found for PD 3.

The increase in correlation following shifting suggests that estimates of airborne inoculum may precede disease development by up to several days. This lead time would be very valuable for application of disease forecasting models to management decisions. There was weak to moderate correlation between inoculum levels found on heads and disease incidence at 6 days post-sampling for PD 1, 2, and 3 (r = 0.61, 0.22, and 0.39, respectively). Shifting the data generally did not improve correlation in these relationships.

Shifting of some of the data (i.e. airborne inoculum estimates) to compensate for lag periods proved useful. This indicates that this type of data may be more useful in forecasting systems in the future. Estimates of airborne inoculum did not relate well to infection sites on detached heads. This may suggest that the presence of inoculum is simply one requirement for infection. If inoculum fails to contact the susceptible head, or conditions are unfavorable for infection, disease may be reduced, or fail to develop. Head washing was shown to be a more reliable estimate of inoculum levels than spore trapping. High correlation among head washing data (overlapping portions of planting dates) showed consistency in the method, and the correlation of spores per spike to daily disease incidence showed relevance of the head washing method.

REFERENCES

Bai, G. and G. Shaner. 1994. Scab of wheat: Prospects for control. Plant Dis. 78:760-766.

Francl, L. 1998. Development of Fusarium head blight in relation to environment and inoculum. p. 1-3. *In* Proc. National Fusarium Head Blight Forum, East Lansing MI. 26-27 Oct. 1998. U.S. Wheat and Barley Scab Initiative, East Lansing, MI.

Francl, L., G. Shaner, G. Bergstrom, J. Gilbert, W. Pedersen, R. Dill-Macky, L. Sweets, B. Corwin, Y. Jin, D. Gallenberg, and J. Wiersma. 1999. Daily inoculum levels of *Gibberella zeae* on wheat spikes. Plant Dis. 83:662-666.

McMullen, M., R. Jones. and D. Gallenberg. 1997. Scab of wheat and barley: A re-emerging disease of devastating impact. Plant Dis. 81:1340-1348.

Parry, D.W., P. Jenkinson. and L. McLeod. 1995. *Fusarium* ear blight (scab) in small grain cereals-a review. Plant Pathol. 44:207-238.

Zadoks, J.C., T.T. Chang. and C.F. Konzak. 1974. A decimal code for the growth stage of cereals. Weed Res. 14:415-421.

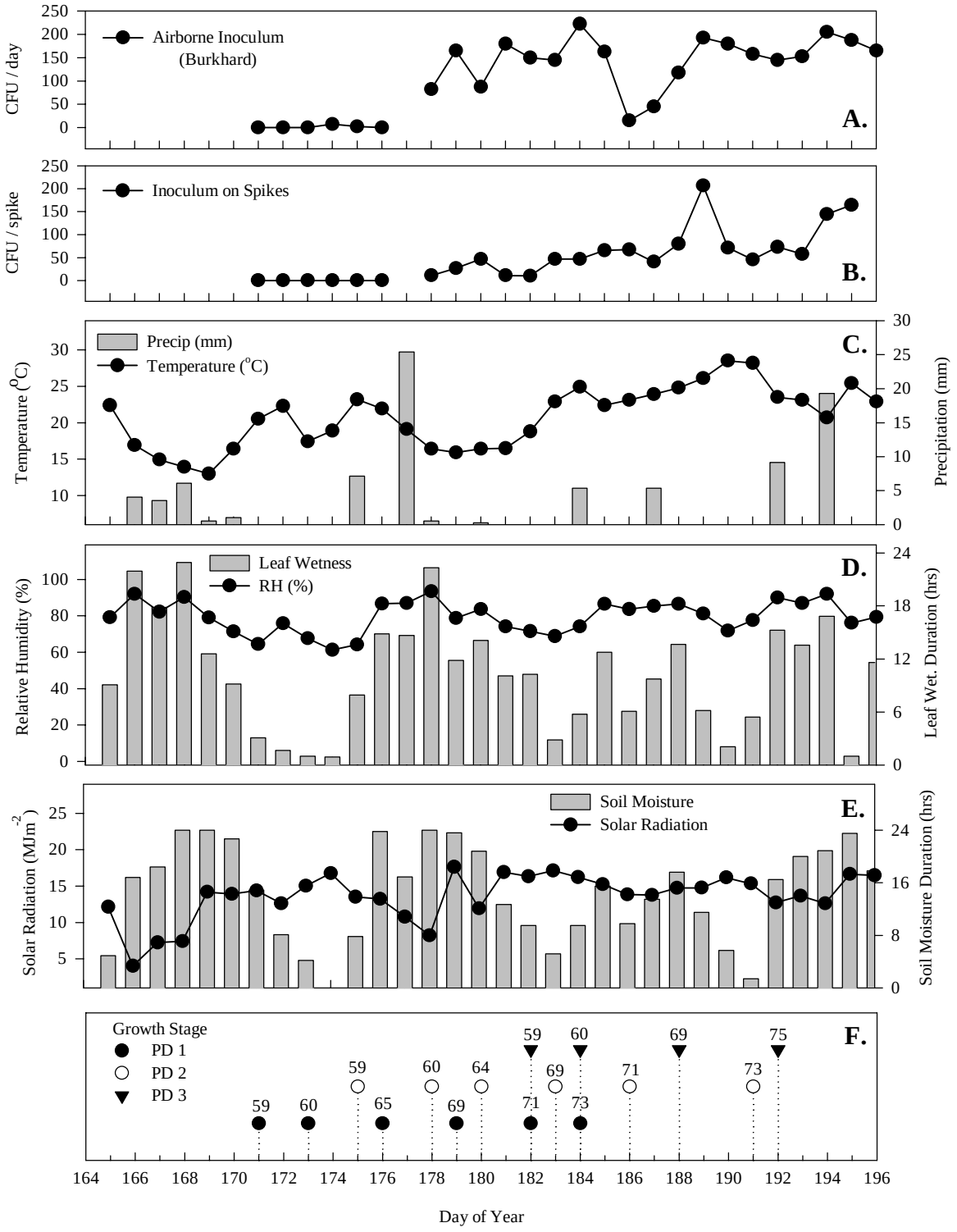

Fig. 1. Inoculum and environmental data over susceptible periods. (A): Airborne inoculum; (B): Inoculum on sampled spikes; (C): Precipitation and canopy air temperature; (D): Leaf wetness duration and relative humidity in the canopy; (E): Soil moisture duration and solar radiation; and (F): Growth stage (Zadoks) for each planting date.

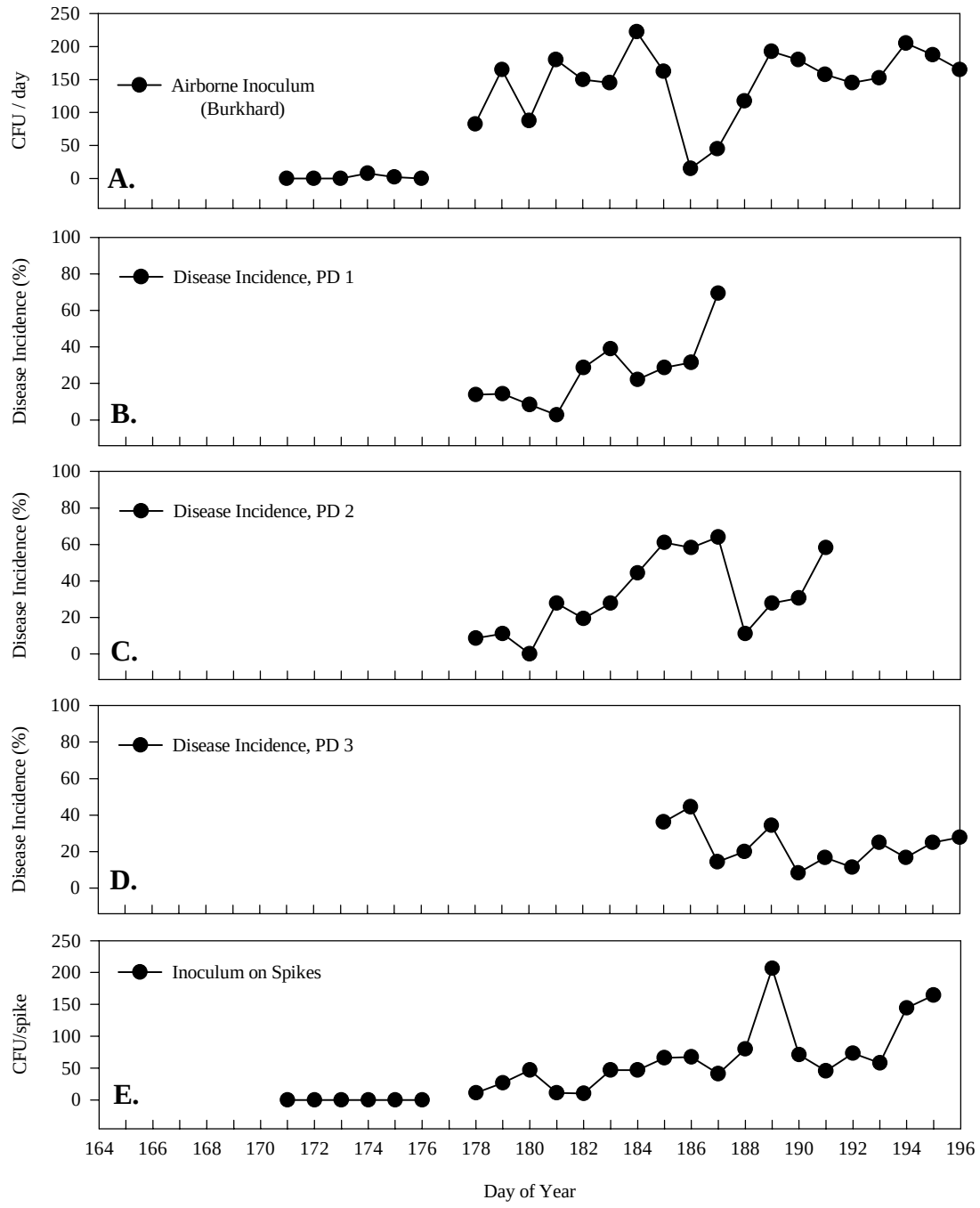

Fig. 2. Disease incidence on sampled spikes (no additional "wet" incubation) at six days post-sampling for all planting dates (B, C, and D) with Burkard Sampler estimates of airborne inoculum levels (A) given as 'cfu/ml' of sample volume (3 ml total). Inoculum estimates from detached heads (wash procedure) are given as 'cfu/spike' (E).

A SENSOR FOR MONITORING WETNESS AT THE SOIL-AIR INTERFACE

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INTRODUCTION AND OBJECTIVES

Development of epidemiological models useful to plant disease forecasting involves gathering information about environmental factors including temperature, vapor pressure, and precipitation. One aspect of the environment that has been difficult to study is moisture at the soil-air interface. The microclimate at the soil-air interface has many influences including air and soil temperature, vapor pressure of the air, precipitation, soil characteristics, and vegetation. Soil-surface moisture, often associated with dew formation in the canopy and precipitation events, is presumed to be one of the critical environmental factors affecting the development of *Gibberella zeae*. The objective of this project was to develop and test an instrument that would directly measure moisture at the soil-air interface. The instrument must be able to provide continuous measurements without altering moisture levels in the measurement area. It must also be compatible with data logging equipment. The information will be critical in understanding the effects of soil wetness on inoculum production under field conditions.

MATERIALS AND METHODS

Sensor Development. Sensors were constructed to assess soil surface wetness by measuring resistance across a plane between two electrodes. Electronically, the sensor consisted of an AC half-bridge circuit, encased in epoxy resin, with exposed wires for contacting the soil surface. Internal resistors, along with the variable resistance of the exposed sensing elements, compose the half-bridge circuit, and allow for the determination of resistance across the two exposed wires. The circuitry is similar to that found in commercial wetness sensing grids, integrating a reference resistor (R_r = 1 kW), with a fixed resistor (R_f = 100 kW) in series with the sensing elements (R_s = variable resistor). The fixed and reference resistors were encased in the instrument frame (11.5 cm by 11.5 cm by 2.3 cm) (Fig. 1). The exposed electrodes consisted of 18-gauge galvanized steel wires. An excitation voltage (V_x) was supplied to the instrument by an external source; in this case, a CR10X datalogger (Campbell Scientific, Inc.). This excitation voltage is compared ratiometrically to a voltage (V_s) measured at a point between the reference resistor (R_r) and the sensing elements (R_s + R_f). The ratio of these voltages (V_s/V_x) is equal to the ratio of the reference resistor (R_r) to the sum of all resistors ($R_r + R_s + R_f$) (see Eq. 1). To find the resistance across the sensor, the equation is solved for R_s (see Eq. 2). When there is no free moisture between the sensing elements, the circuit is open and resistance is infinite. When moisture is present, the circuit closes and resistance across the sensing elements can be measured. Resistance is higher when moisture levels are low, and approaches zero when free water is present. Excitation voltage must be supplied to the sensor in AC form, as ion polarization will occur otherwise, and sensors will decay.

Eq. 1: $V_s/V_r = R_r / (R_r + R_s + R_f)$

Eq. 2: $R_s = R_r / (V_s/V_x) – R_r – R_f$

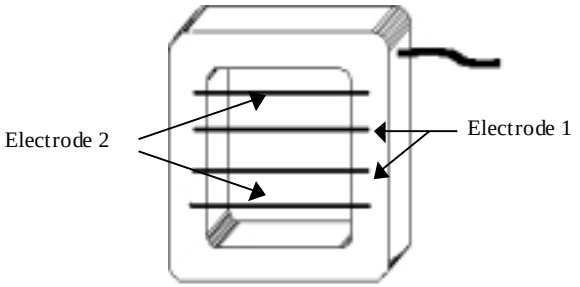

Fig. 1. Soil-surface wetness sensor. Polyurethane frame with 18-gauge galvanized steel wires (electrodes 1 and 2).

Laboratory testing and calibration. Initial testing was conducted by placing the sensors on synthetic sponges. The sponge and sensor were then placed under a desk lamp to speed drying times. Sensor output was recorded using the CR10X datalogger. Several readings were taken from the dry sponge at ten minute intervals, then water was added to the capacity of the sponge. Readings were taken every ten minutes until the sponge was very dry. Several repetitions of this procedure were conducted for each sensor. The data were plotted for each repetition and the results of each run were compared among reps and sensors.

Further testing was done using a thin layer (5 mm) of sieved soil mix (silty loam soil, vermiculite, and peat) in a small plastic container. A known weight (50 g, oven-dried) of soil was used to facilitate calculation of moisture content after addition of water. The sensor was placed atop the soil layer, and the entire assembly was placed on a digital balance. A specific weight of water was added to the soil using a misting sprayer to evenly wet the soil. Every ten minutes, the resistance across the sensor was recorded as well as the weight of the remaining water. This procedure was repeated several times with each sensor.

Measuring wetness duration and inoculum production. The soil surface moisture sensors were integrated into an automated weather station placed in field plots. Five sensors were placed at various locations throughout the plot area. Sensors were located either under the canopy, or between plots on bare soil. Measurements were taken every 2 minutes, and averaged over 30-minute intervals using the CR10X datalogger. Soil surface moisture duration in hours per day was estimated based on the period of time that sensor output was below a threshold value. When sensor output was above the threshold level, the soil was considered dry at the surface.

Airborne inoculum levels were monitored using a Burkhard Cyclone Sampler (Burkhard Manufacturing). Inoculum on wheat spikes was estimated by washing heads using protocols described by Francl et al. (1999), with some modification (sampled heads were not covered prior to sampling). Both methods are described in detail elsewhere in this proceeding (Osborne and Jin, this proceeding). Data were reported as colony forming units (CFU) per day, or per spike.

RESULTS AND DISCUSSION

Laboratory calibration. The sensors performed well in laboratory tests. In sponge trials, there was significant variability among sensors (P = 0.03), likely due to variation in components or construction (each sensor was hand-made). For individual sensors, there was no significant difference among reps, indicating consistency over time. In thin soil layer trials, individual sensors consistently indicated the point at which the surface was completely dry by a marked increase in sensor resistance, usually on the order of 100 kW or more over a ten minute period. Different sensors did not always have similar output values for specific water content, but each was consistent over reps. Visual or tactile estimates of surface-moisture compared favorably to sensor output on the sponge and on thin soil layer. As the substrate dried, sensor measurements resulted in a three-part response curve (Fig. 2).

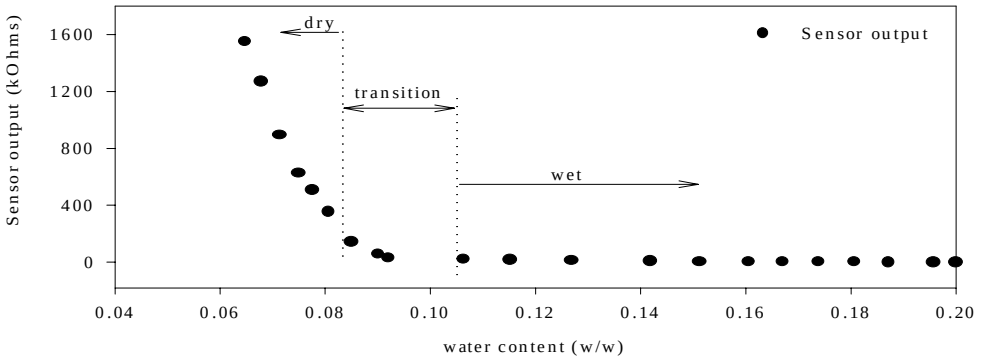

Fig. 2. Thin-soil layer calibration. Sensor output plotted against water content in the microcosm. Visual comparisons are indicated by arrows and dashed dividers.

Sensor output was very low (0 to 100 kW) when the soil was visibly wet. Output was very high (10^3 to 10^4 kW) when the soil was visibly dry on the surface. The transition period was short in laboratory tests where the drying process was increased using a heat lamp, but should be different over time for substrates with different physical characteristics. Sandy soil, for example, dries more quickly than clay soils. Therefore, the sensor response in sandy soil would be more rapid, and the transition period would be shortened. Though the sensor output varied in response to substrate differences, one could determine the wet/dry threshold for each particular application. A sensor which indicates wet or dry surface conditions and afford calculation of wetness duration would be adequate for many epidemiological modeling applications.

Effects on inoculum under field conditions. Soil surface wetness measurements were highly variable among sensors at certain times. This was expected due to the placement of the sensors in varying levels of canopy cover and at different locations with respect to drainage. Sensors in the lower portion of the field indicated wet conditions for longer periods of time than sensors on higher ground. This matched visual observations of the field. Sensors under

canopy coverage tended to indicate moisture longer than those on the bare soil. During long periods without precipitation (periods greater than 4 days), wet/dry indications from all sensors agreed 86% of the time for the 48 hours preceding precipitation. Four sensors agreed an additional 9% of the time. For the period of 48 hours following major precipitation events (5 mm or more), all sensor indications agreed 57% of the time, and four of five agreed an additional 12% of the time. This indicates that the sensors exhibit a degree of uniformity which is satisfactory under field conditions, with the intended application.

For purposes of comparing inoculum detection and soil surface moisture, data from all sensors were averaged to approximate wetness level for the plot area (Fig. 3a). Airborne inoculum levels (as estimated by the Burkhard sampler) appeared to increase as soil moisture duration increased (Fig. 3b). Decreased inoculum appeared to correspond to lower soil moisture duration. When compared to head washing data (Fig. 3c), increased spore recovery appeared to lag peaks in soil moisture duration by one day. Reduction in numbers of spores recovered also lagged reductions in soil moisture duration by one day. These preliminary results indicated that the sensors could serve as a useful tool for estimating soil surface moisture for applications such as disease forecasting, where duration of wetness may be a critical factor in the development of inoculum. To further test this idea, controlled studies are being conducted to monitor perithecial development, and spore discharge under various wetness regimes.

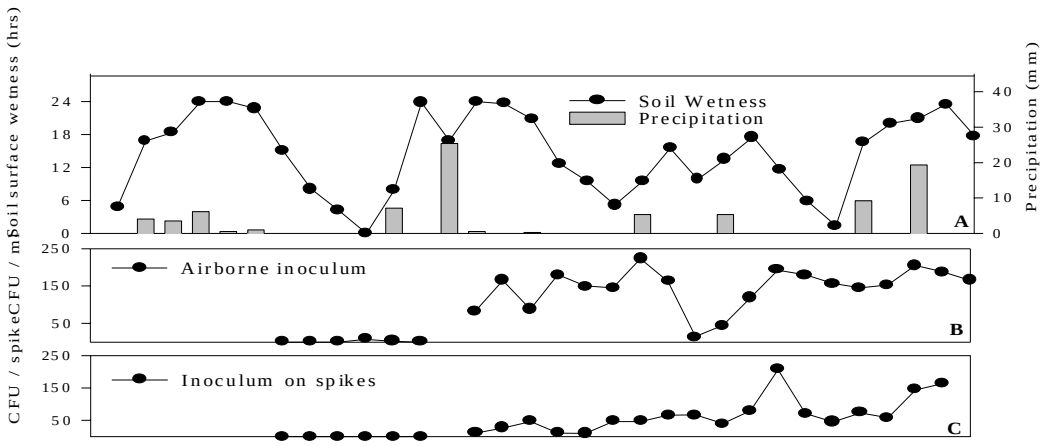

Fig. 3. Environmental parameters and inoculum measurements. (A) soil wetness duration and precipitation; (B) airborne inoculum collected by Burkhard spore collector; and (C) inoculum washed from detached spikes.

REFERENCES

Francl, L., G. Shaner, G. Bergstrom, J. Gilbert, W. Pedersen, R. Dill-Macky, L. Sweets, B. Corwin, Y. Jin, D. Gallenberg, and J. Wiersma. 1999. Daily inoculum levels of Gibberella zeae on wheat spikes. Plant Dis. 83:662-666.

MEASURING DIFFERENCES IN THE ABILITY OF STRAINS OF *FUSARIUM GRAMINEARUM* TO SPREAD WITHIN WHEAT HEADS

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ABSTRACT

We have been characterizing isolates of *Fusarium graminearum* that differ in their ability to spread, cause disease and accumulate mycotoxins in inoculated wheat heads. Identification of isolates that differ genetically for virulence and toxin production will allow the subsequent isolation of genes that are important for controlling for these traits. Knowledge of the determinants of disease spread may suggest novel strategies for disease control. Isolates of *Fusarium graminearum,* representing 8 genetic lineages of this species complex and other *Fusarium* species that cause FHB have been tested. Pathogenicity tests have been conducted on two cultivars of wheat (Norm and Pioneer 2375) very susceptible to the disease. Plants were inoculated at early to mid-anthesis by placing a 10 µl inoculum droplet containing approximately 10^4 conidia into the fifth spikelet from the base of the head. Two weeks after inoculation, disease symptoms (necrosis and/or bleaching of the spikelet) were recorded for 10 spikelets per head including the inoculated spikelet as well as 5 spikelets above and 4 below the point of inoculation. Glumes from the rated spikelets were then plated on mung bean agar medium to detect the presence of the fungus and the remainder of the spikelet, in some instances, was tested for the presence and level of mycotoxin. Symptom expression, the presence of the fungus and mycotoxin concentration were recorded for each spikelet from ~20 inoculated heads. Distribution of symptoms, fungal colonization and mycotoxin accumulation differed significantly among isolates.

SPATIAL PATTERNS OF FUSARIUM HEAD BLIGHT IN NEW YORK WHEAT FIELDS DURING THE EPIDEMIC OF 2000

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INTRODUCTION

Spatial pattern analysis can be a useful tool in understanding the epidemiology of plant diseases including the nature of inoculum sources (Madden, 1989; Madden and Hughes, 1999). There was a widespread epidemic of Fusarium head blight (FHB) in New York winter wheat in June 2000. We assessed spatial patterns of disease incidence in four distant winter wheat fields in order to gain preliminary insights about probable sources of inoculum for spike infection.

MATERIALS AND METHODS

In each of four fields, the incidence of spikes with symptoms of FHB was assessed in 60 quadrats of size 0.093 m^2 (1 ft^2), 20 spikes per quadrat, during the late dough stage. All 60 quadrats were sampled along a single row transect, at 1.5 m intervals, in fields 1,2 and 3. In field 4, samples were assessed in six rows of 10 quadrats each. The six rows were within 41 m of each other. Within rows, the quadrats were 4.5 m apart. The BBD (Beta Binomial Distribution) program (Ver. 1.3) (Madden and Hughes, 1994) was used to calculate the index of dispersion and fit the binomial and beta-binomial distributions to disease incidence data for each field.

RESULTS AND DISCUSSION

Mean incidence of FHB was less than 1% in the first three fields, but was four times higher in field 4. The index of dispersion was significantly different from its expected value assuming randomness for field 4 only. The binomial distribution adequately described the incidence of FHB in fields 1, 2 and 3, but the beta-binomial was a better descriptor than the binomial in field 4 (Table 1).

The pattern of FHB incidence in fields 1,2 and 3 appeared to be completely random. This is consistent with the hypothesis of a mainly external initial inoculum source of airborne ascospores of *Gibberella zeae*, and little or no in-field source of inoculum from crop residues clustered on the soil surface. Corn residues were not observed in fields 1, 2 or 3. In field 4, spikes affected by FHB appeared to be somewhat aggregated within particular quadrats. Since the window of spike susceptibility is brief, it was assumed that only primary inoculum present at the time of anthesis contributed to observed symptoms. A very few small remnants of corn stalks from a corn crop two years earlier were still visible on the soil in field 4. Perithecia of *Gibberella zeae* are produced on corn residue left on the soil surface for up to two years after the crop has been harvested (Khonga and Sutton, 1988). The observed

aggregation of FHB in field 4, together with the observed corn residue, suggests that at least a portion of the inoculum for spike infection was derived from within-field sources.

We consider these results as preliminary circumstantial evidence that inocula from sources external to wheat fields as well as from residues within wheat fields contribute to FHB epidemics in New York.

REFERENCES

Khonga, E. B., and Sutton, J. C. 1988. Inoculum production and survival of *Gibberella zeae* in maize and wheat residues. Can. J. Plant Pathol. 10: 232-239.

Madden, L. V. 1989. Dynamic nature of within-field disease and pathogen distributions. Pages 96-126 in: Spatial components of plant disease epidemics. M. J. Jeger, ed. Prentice-Hall International, Inc, Englewood Cliffs, NY.

Madden, L. V., and Hughes, G. 1994. BBD - Computer software for fitting the beta-binomial distribution to disease incidence data. Plant Dis. 78: 536-540.

Madden, L. V., and Hughes, G. 1999. Sampling for plant disease incidence. Phytopathology 89: 1088-1103.

Table 1. Characteristics of winter wheat fields sampled for Fusarium head blight in 2000 and statistics describing the pattern of disease.

Field	Cultivar	Previous crop		Beta-binomial parameters[c]			
		1998	1999	D[a]	P_{lrs}[b]	p	θ
1	Caledonia	wheat	oat	0.8	1	0.0525	0
2	AC Ron	corn	pea	1.11	0.55	0.06	0.0046
3	Caledonia	corn	pea	0.86	1	0.0417	0
4	unknown	corn	soybean	1.77*	0.0006	0.2375	0.0408

[a] Index of dispersion. D > 1 suggests aggregation of disease incidence. * indicates $P < 0.001$ for a test of whether D differs from its expected value for a random pattern of disease incidence.
[b] Probability associated with a likelihood ratio test of whether the beta-binomial distribution is a better descriptor of the observed disease incidence than the binomial distribution.
[c] Moment estimates of the parameters of the beta-binomial distribution.

INFLUENCE OF LOCAL VERSUS REGIONAL FACTORS ON INCIDENCE OF SEED INFECTION BY *FUSARIUM*

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INTRODUCTION

A widespread epidemic of Fusarium head blight affected winter wheat grown in western New York in 1996. We investigated whether seed harvested from fields in close proximity had more similar incidences of infection by *Fusarium* than did seed from fields that were more distant.

MATERIALS AND METHODS

One hundred seeds each from 23 seedlots, harvested in 1996 and representing four adapted winter wheat cultivars (Cayuga, Geneva, Harus, NY Batavia), were assayed for infection by *Fusarium* spp. using the freezing blotter method (Limonard, 1966). Geographic coordinates for each field were converted to planar coordinates by the Corpscon software program (Ver. 5.x, U.S. Army Corps of Engineers). Fields were on average 70 km apart (median = 62.8 km; range 1 m to 179 km). Out of the 253 possible pairwise comparisons of distances between fields, 247 could be classified within the mesoscale [10 to 200 km apart, *sensu* Francl et al. (1999)] level.

Spatially referenced (by planar coordinates) counts of the number of *Fusarium*-infected seeds were analyzed by the spatial analysis by distance indices (SADIE) software program (Perry et al., 1996). SADIE is a form of spatial correlation analysis that is conditioned on the existing heterogeneity of counts within a specified area. The SADIE index I_a is a measure of the spatial association. Values of $I_a > 1$ would indicate that fields with similar levels of seed infection are closer together than expected by chance alone. Significance testing is done by permuting the observed data set under the hypothesis that there is no association among fields in seed infection level. For this study, 5,967 randomizations were done.

RESULTS AND DISCUSSION

Percent seed infection by *Fusarium* spp. ranged from 0 to 24 (mean 8.4). The evidence for associations among fields in terms of seed infection by *Fusarium* was not strong ($I_a = 1.569$, $P = 0.0508$). Results therefore support the hypothesis that winter wheat fields are spatially random at the mesoscale with respect to *Fusarium* seed infection. Therefore, site-specific conditions at the local scale (100 m to 50 km) are of greater importance in determining the actual levels of seed infection than overall regional weather patterns. For example, two of the fields in the analyzed data set were only 1 m apart, yet percent seed infection in one (5%, cultivar Cayuga) was three times that in the other (18%, cultivar NY Batavia). Differences in flowering date, cultivar resistance to *Fusarium* or local inoculum variability may significantly impact seed infection levels.

Large-scale weather patterns affect the overall mean seed infection incidence in a given region, but the range of seed infection incidence in any year is more strongly influenced by local variables.

REFERENCES

Francl, L., Shaner, G., Bergstrom, G., Gilbert, J., Pedersen, W., Dill, M. R., Sweets, L., Corwin, B., Jin, Y., Gallenberg, D., and Wiersma, J. 1999. Daily inoculum levels of *Gibberella zeae* on wheat spikes. Plant Dis. 83:662-666.

Limonard, T. 1966. A modified blotter test for seed health. Neth. J. Plant Pathol. 72:319-321.

Perry, J. N., Bell, E. D., Smith, R. H., and Woiwod, I. P. 1996. SADIE: software to measure and model spatial pattern. Aspects of Appl. Biol.:95-102.

THE BETA-BINOMIAL DISTRIBUTION DESCRIBES THE INCIDENCE OF SEED INFECTION BY *FUSARIUM GRAMINEARUM* AMONG SEEDLOTS IN A RREGION

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INTRODUCTION

In cereal seed health assessments, an individual seed is either infected by *Fusarium graminearum* or not. If repeated samples are drawn from a well mixed seedlot, the incidence of seed infection within such samples is expected to follow a binomial distribution. The mean incidence of infection is described by the binomial parameter, *p*. If a number of different seedlots are assayed for the same pathogen, the probability of seed infection can be expected to vary from lot to lot for any of a number of reasons, including local environment, inoculum availability, and the relative susceptibilities of cultivars to seed infection. That is, *p* may vary across seedlots. A common way of accounting for nonconstant *p* is to assume that *p* itself is a variable with a beta distribution. If *p* has a beta distribution, the resultant mixture distribution is a beta-binomial distribution (Madden and Hughes, 1995). Here we demonstrate that the beta-binomial distribution is a superior (to the binomial distribution) and adequate descriptor of cereal seed infection incidence by *Fusarium graminearum* across several seedlots from a production region.

MATERIALS AND METHODS

The binomial and beta-binomial distributions were fit to seven separate data sets on the incidence of seed infection by *Fusarium graminearum* in three cereals (Table 1). Data sets 1 to 3 are on the infection of wheat seed in Kansas by *F. graminearum* (Love and Seitz, 1987). Summaries of data sets 4 to 6, on the infection of barley and oat seed in Manitoba by *F. graminearum*, were previously published (Clear et al., 1996).

The binary form of the index of dispersion (D) (Madden and Hughes, 1995) was calculated. Values of D > 1 suggest that the variance in seed infection incidence among lots is greater than that expected assuming seed infection incidence across seedlots is distributed binomially (a phenomenon called overdispersion). The quantity has a distribution (N-1 degrees of freedom) under the null hypothesis that seed infection incidence is randomly distributed, where N is the number of seedlots (Madden and Hughes, 1995).

The BBD software program (Ver. 1.3) (Madden and Hughes, 1994) was used to calculate D and to fit the binomial and beta-binomial distributions to data sets. A likelihood ratio test assessed whether the beta-binomial provided a better fit to the data than the binomial distribution.

RESULTS AND DISCUSSION

All data sets were overdispersed (Table 2). Therefore, seed infection incidence by *F. graminearum* varies across seedlots in a manner that cannot be explained by binomial variation only. Extra-binomial variation may be due to cultivar differences in seed infection, differences in inoculum availability, flowering date or to site-specific environment. The beta-binomial distribution was a significantly better descriptor than the binomial distribution of seed infection incidence for all seven data sets examined (Table 2, Fig. 1).

The parameters of the beta-binomial distribution, estimated from a relatively small random sampling of seedlots, are a concise summary of the regional incidence of cereal seed infection by *F. graminearum*. Moreover, a distributional approach to describing seed infection incidence allows one to estimate probabilities associated with seed infection incidences not in the empirical data set.

REFERENCES

Clear, R. M., Patrick, S. K., Platford, R. G., and Desjardins, M. 1996. Occurrence and distribution of *Fusarium* species in barley and oat seed from Manitoba in 1993 and 1994. Can. J. Plant Pathol. 18: 409-414.

Love, G. R., and Seitz, L. M. 1987. Effects of location and cultivar on Fusarium head blight (scab) in wheat from Kansas in 1982 and 1983. Cereal Chem. 64: 124-128.

Madden, L. V., and Hughes, G. 1994. BBD - Computer software for fitting the beta-binomial distribution to disease incidence data. Plant Dis. 78: 536-540.

Madden, L. V., and Hughes, G. 1995. Plant disease incidence: distributions, heterogeneity, and temporal analysis. Annu. Rev. Phytopathol. 33: 529-564.

Turechek, W. W., and Madden, L. V. 1999. Spatial pattern analysis of strawberry leaf blight in perennial production systems. Phytopathology 89: 421-433.

ACKNOWLEDGEMENT

Randy Clear of the Canadian Grain Commission supplied the data on seed infection incidence in Manitoba and Quebec cereals.

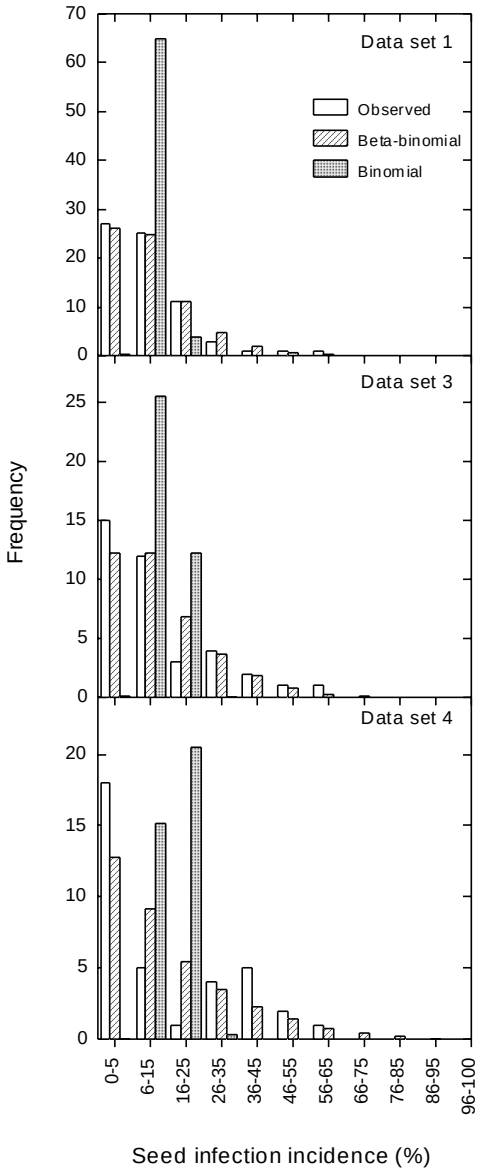

Figure 1. Fit of the binomial and beta-binomial distributions to seed infection incidence by *Fusarium graminearum* across cereal seedlots.

Table 1. Data sets used to assess the fit of the binomial and beta-binomial distributions to *Fusarium graminearum* seed infection incidence.

Data set	Source	Crop	Year	No. of seedlots	No. of cultivars	No. of locations
1	Kansas	wheat	1982	23	2	12
2	Kansas	wheat	1983	24	2	12
3	Kansas	wheat	1982	36	18	2
4	Manitoba	barley	1993	78	?	78
5	Manitoba	barley	1994	45	?	45
6	Manitoba	oat	1993	30	?	30
7	Quebec	wheat	1989	31	9	31

Table 2. Results of fitting the binomial and beta-binomial distributions to seed infection incidence by *Fusarium graminearum*.

Data set	D[a]	P_{LRS}[b]	$\hat{p}$[c]	$\hat{\theta}$[c]	P_{χ^2}[d]
1	39.89	< 0.0001	0.1537	0.4955	0.161
2	4.51	< 0.0001	0.0276	0.0525	n.d.
3	8.13	< 0.0001	0.3847	0.0718	0.605
4	41.49	< 0.0001	0.4215	0.6365	0.007
5	13.81	< 0.0001	0.4379	0.2558	n.d.
6	41.03	< 0.0001	0.1443	0.5769	0.090
7	2.42	< 0.0001	0.0425	0.156	0.002

[a] Index of dispersion. Values of D>1 indicate that the variance in seed infection incidence is greater than that expected if seed infection incidence is binomially distributed. A test was used to determine whether D differs from its expected value assuming seed infection incidence follows a binomial distribution (Madden and Hughes, 1995). In all seven instances $P < 0.001$.

[b] Significance level for a likelihood ratio test of whether the beta-binomial distribution fits the data better than the binomial distribution (Turechek and Madden, 1999).

[c] Maximum likelihood estimates of the parameters of the beta-binomial distribution.

[d] Probability associated with a goodness-of-fit test of the beta-binomial distribution. $P > 0.05$ indicates a significant fit of the beta-binomial to the data at a=0.05, for example. n.d. means that it was not possible to do a goodness-of-fit test.

SAMPLING SPORES OF FUSARIUM GRAMINEARUM

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INTRODUCTION AND OBJECTIVES

In order to develop effective control measures for Fusarium head blight, it is necessary to understand the epidemiology of the disease. One important epidemiological question is how weather influences production and release of inoculum, infection, and symptom development. This study is part of a 4-state collaborative study (North Dakota, South Dakota, Indiana, Ohio) aimed at generating a body of comparable data over diverse environments to answer these questions. Here we report results of studies conducted in Indiana during the 2000 growing season.

MATERIALS AND METHODS

Inoculum levels were monitored at two sites at the Purdue University Agronomy Research Center (PARC) during the 2000 wheat season. At one site (Field 53), wheat cultivar Clark was sown into disked corn residue. At the other site (IPM), wheat cultivar INW9513 was sown following soybeans in a plot that was adjacent to a plot that had corn residue from the previous crop year on the soil surface. The distance from the corn residue to the edge of the wheat plot (where inoculum was monitored) was about 3 m.

Burkard cyclone spore samplers were operated at both sites. Sampling began in field 53 on 1 May, when wheat was in the early boot stage (GS 44) and on 8 May in the IPM field when heads were 50% emerged (GS 55). The samplers drew in 16.5 L of air per minute. Each day at 1100 hours, the Eppendorf tube into which airborne particles were collected was replaced in each sampler.

Spores were recovered from the Eppendorf tube by adding 1 ml sterile water, shaking the tube on a Vortex mixer for 1 minute, and spreading 0.3 ml of the suspension over Komada's medium in each of two petri plates. The plates were placed in an incubator programmed to maintain 25 °C during a 12-hr photoperiod and 20 °C during a 12-hour dark period each day. After 1 week, colonies of Fusarium graminearum were counted on each plate. A sample of the remaining spore suspension was examined under a hemacytometer for conidia or ascospores of Gibberella zeae.

Beginning 12 May, when wheat in field 53 was in mid anthesis (GS 65) and heads in the IPM field were three-fourths emerged (GS 57), 5 heads were collected each day at about 1030 hours. In the laboratory, the 5 heads were placed in 50 ml of sterile water that contained 2% Tween 20. They were shaken vigorously for 1 minute, then 1-ml samples were plated onto each of 5 petri dishes containing Komada's medium. Plates were placed in the incubator described above. After 1 week, colonies of Fusarium graminearum were counted on each plate.

On 30 May (GS 77) scab incidence was estimated by counting the number of blighted heads in each of ten 60-cm lengths of row. Incidence was estimated again on 6 June (GS 79), but the samples were 30 cm long.

A Campbell weather station was operated in field 53, at the same location as the Burkard sampler. This unit recorded various weather parameters at 30-min intervals.

RESULTS

Spores of G. zeae were recovered by both samplers from the beginning of the sampling period until early June, when sampling ceased (Figs. 1 and 2). At both sites, there were days when no propagules were recovered. From days 131 (9 May) through day 138 (17 May), there was general agreement in the number of spores sampled at each site. From day 139 (18 May) through the end of the sampling period (28 May), however, there was little agreement between data from the two sites. Peaks in spore numbers in field 53 occurred when few spores were recovered at the IPM field. There was an irrigated head blight nursery about 100 m to the southeast from the spore sampler in field 53. The peak in spore numbers seen on days 142 and 143 in field 53 but not in the IPM field may have been an effect of moisture provided to corn residue by the irrigation during a period when no rain fell. Despite this discrepancy during the latter part of the sampling period, there was a significant correlation between spores collected at both sites ($R=0.39$, $P=0.04$).

During the first half of the spore sampling experiment, rain fell at least every 2 or 3 days. During this time, the number of spores collected in field 53 and the amount of rainfall were not significantly correlated ($R=0.51$, $P=0.06$). Spores were collected on every day during which rain fell. However, spores were also collected on days with no rain. The same pattern was seen for spores collected in the IPM field for the full duration of the experiment, and for this data set the correlation was significant ($R=0.71$, $P=0.002$). We also examined the relation between number of spores collected and the number of hours that relative humidity was less than 75% each day. There was no correlation at either site. A more thorough analysis of the effect of weather on number of spores in the air will be conducted when data from several cooperating states have been pooled.

The numbers of spores recovered each day from heads collected at the two sites were correlated ($R=0.48$, $P=0.02$). At both sites, there was no correlation between the number of spores recovered by the Burkard sampler and the number of spores recovered from heads during a given 24-hour period. However, if the daily pattern of spores collected by the Burkard sampler is compared with the number of spores collected from heads, there is a rough concordance for the data from field 53 (Fig. 1). When we compared the number of spores from heads on a given day against number of spores recovered by the Burkard unit on the previous day, the correlation was significant for the field 53 data ($R=0.61$, $P=0.001$), but not for the IPM field data.

Scab incidence in field 53 was 2.9% on 30 May (GS 77) and 6.4% 1 week later. Scab incidence in the IPM field was 4.4%. Incidence of scabby kernels in the grain harvested from field 53 was 2.2% and the level of DON was 2.6 ppm.

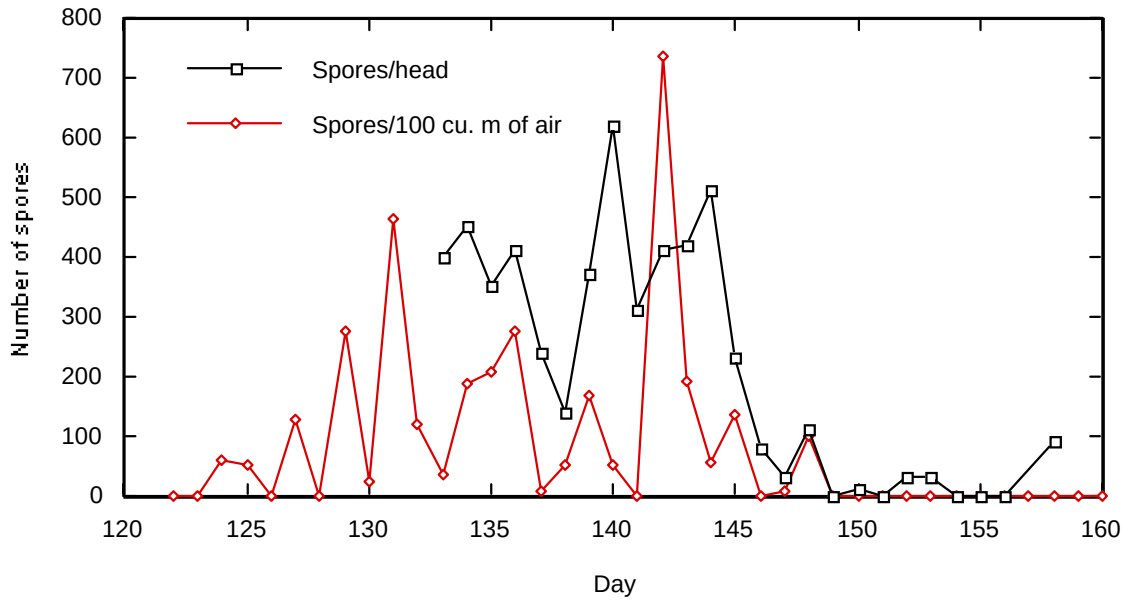

Figure 1. Number of spores of Gibberella zeae collected each day in field 53 of the Purdue Agronomy Research Center, beginning at 1100 hours, with a Burkard volumetric spore sampler or recovered from heads.

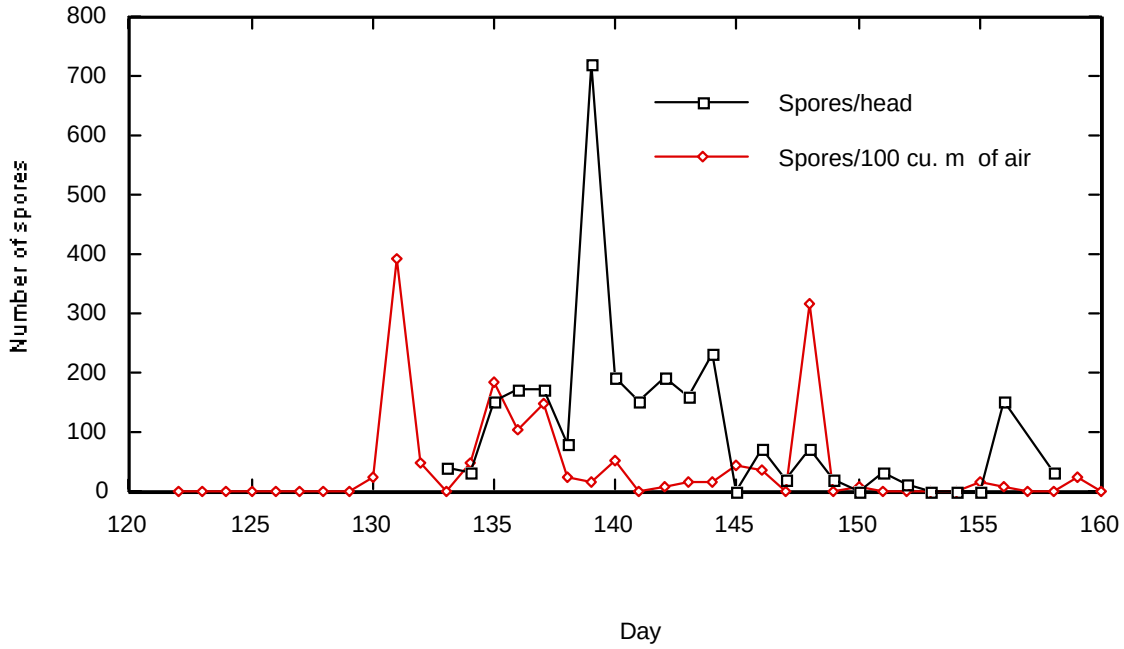

Figure 2. Number of spores of Gibberella zeae collected each day in the IPM field of the Purdue Agronomy Research Center, beginning at 1100 hours, with a Burkard volumetric spore sampler or recovered from heads.

DISCUSSION

There were no periods of sustained rainfall from the time of wheat anthesis through grain filling, in contrast to what occurred during the same wheat developmental period in 1996, when a major head blight epidemic occurred. Nonetheless, the Burkard volumetric spore samplers collected spores of F. graminearum on many days during anthesis and early grain filling. Spores were also recovered directly from head washings during this period. The incidence of head blight was light to moderate, as was the incidence of scabby kernels. This suggests that weather conditions may have been limiting for infection rather than for production of inoculum.

There was a weak correlation between spores collected by the Burkard samplers at two locations separated about 1 km apart. This suggests that local conditions as well as general weather conditions influence production and release of spores.

There appears to be a large discrepency between the number of spores recovered from heads and the number of spores collected from a volume of air during a 24-hour period. We will look at this relation further by using wind speed data to estimate the volume of air intercepted by a wheat head during the course of a day.

Limited inferences about the influence of weather on spore release and dispersal can be made from one year's data. A 4-state collaborative study, now in its second year, will provide a greater number of data points from which to draw inferences about the effect of weather on production and dispersal of inoculum, infection, and disease development

FUSARIUM HEAD BLIGHT IN BARLEY IN ONTARIO IN 2000

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OBJECTIVES

The objectives of this study was to identify the percent of kernels infected with *Fusarium* species in spring barley cultivars included in the Ontario Spring Barley Performance trials, and from a nearby commercial field of variety Chapais, with a high level of disease, to check the pathogenicity of three isolates of *F. graminearum* collected from barley on winter wheat seedlings, and to evaluate fungicide efficacy against FHB in spring barley, using the fungicides with known response against FHB in wheat.

INTRODUCTION

Fusarium head blight (FHB) epidemics are common in Ontario wheat, and corn, but have been rarely reported in barley (*Hordeum vulgare*). FHB is observed frequently in barley grown in the Red River Valley of Western Canada and North Central USA, while in Eastern Canada FHB was observed for the first time on barley in 1986 (R. Clear, personal communications), but there is no published information. This year, visible FHB symptoms on spring barley were noticed as tan or brown florets in the spike. In harvested grain, Fusarium damaged kernels (FDK) were also observed. Tekauz et al. (2000) noted that FHB in barley may result from a combination of factors such as a fundamental shift in the pathogen population, new varieties being grown in the region, and environmental conditions promoting disease. According to Campbell et al. (1999) DON contamination in Eastern Canada was particularly severe in recent years (1996, 1997, 1998), when some barley samples contained up to 8-9 mg/kg of toxin. After artificial inoculation with *Fusarium graminearum* in Ridgetown, ON, in 1997 (Schaafsma, and Tamburic-Ilincic, 1997) mean DON level across entries were higher in barley then in wheat (3.98 ppm, vs 2.23, respectively), as well as percent of seeds infected with *F. graminearum* (49.46, vs 15.81, respectively).

MATERIALS AND METHODS

Forty six spring barley cultivars from the Ontario Spring Barley Performance trials were evaluated for percent of kernels infected with *Fusarium* spp. Eighteen of them were two-row feed barley, while twenty eight were six-row feed barley. After harvest, the seed was surface sterilized in a 3 % sodium hypochlorite solution for three minutes, air dried and placed on acidified potato dextrose agar (PDA). These were incubated for seven days under light on a 12:12 hr light/dark cycle, at room temperature. *Fusarium* spp. colonies were then transferred to carnation-leaf agar (CLA), and incubated as above. The identification was done according to Nelson et al. (1983), and Burgess et al. (1988).

Two varieties of spring barley (Chapais- six-row barley , and Morrison- two-row barley) were grown in a randomized complete block design with four replications. The fungicides applications were made at 50 % anthesis for each variety. Deoxynivalenol (DON) content was estimated using competitive ELISA test (Sinha and Savard, 1996). Individual spikes were collected from a commercial field of Chapais barley on the Elora Research Station then dried and threshed in bulk. Seeds were treated as previously described to obtain Fusarium colonies.

Pathogenicity tests were conducted on Knop medium (1 g KNO3; 0.12 g KCl; 0.25 g KH_2PO_4; 0.25 g $MgSO_4$ x H_2O; in trace FeCl x 6 H_2O; 15 g agar, and 1 L distilled water) in glass tubes under laboratory conditions, according to Levic and Tamburic (1996). Subcultures of the three isolates of *F. graminearum* from spring barley varieties (Chapais- isolate from the commercial field, AC Stephen, and C231-041), were grown on PDA medium at room temperature for 1 week. Plugs of inoculated PDA (4x4 mm) were placed on the medium, then the surface sterilized kernels from three wheat varieties (AC RON- highly susceptible, Harus-susceptible, and Freedom-moderate resistant) were placed 2 cm above. Controls were fungus-free. Fourteen-day-old seedlings were evaluated using scale from zero (no symptoms) to five (the mycelium covered seed, roots discoloured, and seedling growth stopped).

RESULTS AND DISCUSSIONS

Fusarium spp. colonies were isolated from sixteen cultivars of spring barley from Ontario Performance trial (34.8%). Differences in susceptibility to *Fusarium* were found among cultivars, based on percent of seed infected with *Fusarium* spp. For the group of barley varieties tested, two-rowed types had less FHB than six-rowed types. These results are consistent with those of Tekauz et al. (2000). AC Sirius, AC Stephen, and C231-041 were the most susceptible genotypes to FHB. *F. graminearum* was the predominant species (68 %), followed by *F. sporotrichioides* (10 %), *F. poae* (8 %), *F. equiseti* (6 %), *F. verticillioides* (4 %), *F. subglutinans* (2 %), *and F. proliferatum* (2%). The same *Fusarium* species have been identified from wheat, and corn crops grown across Ontario (1996-2000). There was correlation between percent of *Fusarium* spp. and *F. graminearum* isolated from infected seed (r= 0.967). Thirty eight percent of the seed from the commercial field of Chapais barley was infected with *F. graminearum*.

Morrison (two-row barley) responded better to protection by fungicides than did Chapais (six-row barley). Mean DON content was (1.8 versus 6.5 ppm). The highest rate of FOLICUR, reduced DON level significantly on the variety Morrison.

The *F. graminearum* isolates from barley crop, were pathogenic on the three wheat varieties tested (Table 3). The differences in the wheat genotype's reactions to barley isolates which were similar to their reactions to wheat isolates (DAOM178148).

Table 1. (previous page) Fusarium head blight reaction of 46 spring barley cultivars after naturally infection at Elora Research Station, Guelph, Ontario, 2000.

Treatment #	Cultivar	Percent seed infected	Percent seed infected by F. graminearum
1	AC KINGS	0	0
2	AC PARKHILL	1	1
3	AC SIRIUS	9	7
4	AC STERLING	0	0
5	ALMONTE	0	0
6	BELMORE	0	0
7	FORMOSA	0	0
8	MORRISON	0	0
9	SUNDERLAND	0	0
10	VIKING	0	0
11	CM96503	0	0
12	T123-172	0	0
13	T125-053	0	0
14	T169-055	0	0
15	T193-198	1	0
16	96/1110	0	0
17	96/1114	0	0
18	AB168-11	0	0
19	AC ALMA	2	1
20	AC HAMILTON	0	0
21	AC LEGEND	1	1
22	AC STEPHEN	9	5
23	AC WESTECH	0	0
24	ACCA	1	0
25	BRUCEFIELD	0	0
26	CHAPAIS	5	5
27	FOSTER	2	1
28	GRANT	1	0
29	LEGER	0	0
30	MYRIAM	0	0
31	NELLYGAN	0	0
32	OAC BAXTER	3	2
33	OAC KIPPEN	0	0
34	SANDRINE	0	0
35	VIVIANE	0	0
36	AB186-3	0	0
37	ABI89	0	0
38	CM862.6	1	0
39	CI62-120	0	0
40	C166-050	0	0
41	C229-004	0	0
42	C231-041	7	5
43	OBS4065-125	3	2
44	OBS4065-157	1	1
45	OBS4181-43	3	3
46	OS94-544	0	0
Total		50	34

Table 2. Fusarium head blight control in spring barley (Chapais and Morrison) with foliar application of fungicides. Ontario, 2000.

Treatments	Rate product/ha	Chapais DON (ppm)	Morrison DON (ppm)
Control		6.5	2.3
FOLICUR 432 F	289 mL	6.8	1.8
FOLICUR 432 F	364.6 mL	5.9	1.3
TILT 250 EC	500 mL	6.7	1.6
Mean		6.5	1.8
LSD (P=.05)		1.5	0.8

Table 3. The pathogenicity tests of *F. graminearum* isolated from barley on three winter wheat cultivars AC RON, Harus, and Freedom, using scale from zero (no symptoms) to five (the mycelium covered seed, roots discoloured, and seedling growth stopped).

Isolate	AC RON	Harus	Freedom
Control	0	0	0
DAOM178148	4.8	4.61	3.72
# 22	3.99	2.75	3.64
# 42	5	4.5	2.42
Chapais	4.55	4.82	4.3
Mean	4.59	4.17	3.52
LSD (P=.05)	0.5	1.52	1.01

REFERENCES

Burgess, L., Liddell, C., Summerell, B. 1988.Laboratory manual for Fusarium research. Sydney. pp. 156.

Campbell, H., Choo, T. M., Vigier, B., Underhill, L. 1999. Mycotoxins in eastern Canada=s barley and oats. Canadian Workshop on Fusarium Head Blight. Winnipeg, Manitoba, November 28-30, 1999. 108-109.

Levic, J., and Tamburic, L. 1996. Studies of *Fusarium proliferatum* and *Fusarium moniliforme* isolated from soil, maize root and stalk, and determination of their pathogenicity. Zastita bilja. Belgrade. 47 (1) 215: 71-85.

Nelson, P., Toussoun, T., Marasas, W. 1983. Fusarium species. Ann illustrated manual for identification. The Pennsylvania State University Press, University Park, pp. 193.

Schaafsma, A., and Tamburic-Ilincic, L. 1997. Susceptibility of winter barley breeding lines to Fusarium Head Blight in artificially inoculated, misted plots. Pest Research Management, report # 106. (electronic).

Schaafsma, A., and Tamburic-Ilincic, L. 1997. Susceptibility of winter wheat varieties to Fusarium Head Blight in artificially inoculated, misted plots. Pest Research Management, report # 119. (electronic).

Sinha, R.C., and Savard, M.E. 1996. Comparison of immunoassay and gas chromatography methods for the detection of the mycotoxin deoxynivalenol in grain samples. Can J Plant Path 18:233-236.

Tekauz, A. McCallum, B, and J. Gilbert. 2000. Review: Fusarium head blight of barley in western Canada. Can. J. Plant Pathol. 22. 9-16.

THE MECHANISM OF FORCIBLE DISCHARGE OF ASCOSPORES IN *GIBBERELLA ZEAE*

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ABSTRACT

In *Gibberella zeae*, ascospores are forcibly discharged from perithecia forming on debris and are believed to form the primary inoculum of head blight disease. The mechanism of forcible discharge of ascospores has not been well studied in any ascomycetous fungus. We are using physiological and genetic studies to elucidate this mechanism. It is clear that osmotic pressure builds up within the ascus and serves as the force behind discharge. We have identified several factors that are likely involved in this increase in pressure. In addition, the use of insertional mutagenesis to isolate discharge minus mutants is in progress. Analysis of discharge minus mutants will provide insight into this mechanism. This work should identify novel targets for control of *Gibberella zeae*.

AFLP MARKERS INDICATE LITTLE DIVERGENCE BETWEEN U.S. CORN BELT POPULATIONS OF *FUSARIUM GRAMINEARUM* (*GIBBERELLA ZEAE*)

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ABSTRACT

In 1999, we surveyed wheat-scab populations from five states within the U.S. Corn Belt. We isolated *Fusarium* samples either directly from scabby wheat heads, or from individual seeds within contaminated seed-lots. We have examined 42 polymorphic AFLP loci for 71-75 randomly selected *Fusarium* isolates from each tested population. The percentage of thesse isolates that we identified as *Fusarium graminearum* (*Gibberella zeae*) ranged from a low of 79% (56/71) from an Illinois seed-lot, to 100% (71/71) from a Kansas wheat head sampling. Divergence among *F. graminearum* populations from these five states was low. Pairwise G_{ST} values ranged from only 0.013 to 0.040, and indicate high rates of effective migration (*Nm*) between populations. We observed a positive, but statistically insignificant, correlation ($r = 0.46$, $p = 0.18$) between inter-population geographic separation and G_{ST} values. These data provide support for the hypothesis that regional populations of *F. graminearum* within the U.S. Corn Belt are part of a single, largely panmictic, metapopulation.

DIAGNOSTIC VOMITOXIN (DON) SERVICES IN 2000-2001

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INTRODUCTION

Fusarium Head Blight (FHB) is an important problem for American agriculture. Resolving the FHB problem involves cooperative efforts, including analytical assays for vomitoxin in the new wheat and barley varieties. In 2000, the US Wheat and Barley Scab Initiative provided grants, for diagnostic vomitoxin (DON) services, to 4 laboratories in Michigan, Minnesota, and North Dakota. The following provides an insight into the methods, quality assurance and number of samples processed by each laboratory.

MATERIALS AND METHODS

The 3 laboratories in Minnesota and North Dakota all use the method of Tacke (1), followed by GC/EC or GC/MS quantitation. Michigan used the Neogen Veratox system.

MICHIGAN
Lab Director: L. Patrick Hart, Ph.D., Dept. of Botany & Plant Pathology, Michigan State University, East Lansing, MI 48824, Phone: 517-353-9428, Fax: 517-353-5598, e-mail: hartl@pilot.msu.edu
Method: Water extraction and DON quantitation with the Neogen Veratox kit
Sample Types: Wheat and barley
Intralab Quality Assurance: Wheat Pool (2.2 ppm DON)

MINNESOTA
Lab Director: Weiping Xie, Ph.D., Dept. of Plant Pathology, University of Minnesota, 495 Borlaug Hall, 1991 Upper Buford Circle, St. Paul, MN 55108, Phone: 612-625-2751, Fax: 612-625-9728, e-mail: weipingx@puccini.crl.umn.edu
Method: Acetonitrile: water extraction, silylation and DON, 15 A-DON, 3 A-DON quantitation by GC/MS, plus a screen for 8 other trichothecenes.
Sample Types: Wheat and barley
Intralab Quality Assurance: Wheat Pool (12.9 ppm)

NORTH DAKOTA
Lab Director: Howard H. Casper, Ph.D., Dept. Vet. and Micro. Science, North Dakota State University, Fargo, ND 58105, Phone: 701-231-7529, Fax: 701-231-7514, e-mail: hcasper@ndsuext.nodak.edu
Method: Acetonitrile: water extraction, silylation and DON, Nivalenol, 15 A-DON, quantitation by GC/EC. Full screens for 17 mycotoxins can also be done by GC/MS.
Sample Types: Wheat and barley
Intralab Quality Assurance: Wheat Pool (1.8 ppm DON), Barley Pool (3.2 ppm DON).

NORTH DAKOTA
Lab Director: Paul B. Schwarz, Ph.D., Dept. Cereal Science, North Dakota State University, Fargo, ND 58105, Phone: 701-231-7732, Fax: 701-231-7723
e-mail: nubarley@badlands.nodak.edu
Method: Acetonitrile: water extraction, silylation and DON quantitation by GC/EC
Samples Types: Barley and malt products
Intralab Quality Assurance: Barley Pool (3.4 ppm DON), Wheat Pool (2.0 ppm DON)

PROFICIENCY CHECK SAMPLES
In August of 2000, H. Casper collected wheat and barley samples from local sources and distributed these samples on a monthly basis. A wheat sample and a barley sample was sent on each occasion and the data was collected from each laboratory within one week. Each laboratory did the DON analyses in their normal fashion. These check samples allowed each laboratory to evaluate the accuracy and precision of their system.

RESULTS AND DISCUSSION

Information from the 4 laboratories, pertaining to quality assurance, number of samples analyzed and proficiency check samples is listed in Tables I, II and III.

Table I. Intralab Vomitoxin Quality Assurance (QA) Data for May – November, 200

1.	MICH – P. Hart	QA: Wheat Pool;	n = 56,	Ave = 1.6 ppm,	cv = 7%
2.	MINN – W. Xie	QA: Wheat Pool;	n = 25,	Ave = 12.9 ppm,	cv = 11%
3.	ND – P. Schwarz	QA: Wheat Pool;	n = 38,	Ave = 2.0 ppm,	cv = 12%
		QA: Barley Pool:	n = 48,	Ave = 3.4 ppm,	cv = 18%
4.	ND – H. Casper	QA: Wheat Pool;	n = 59,	Ave = 1.8 ppm,	cv = 8%
	" "	" : Barley Pool;	n = 59,	Ave = 3.2 ppm,	cv = 7%

Table II. Estimated Vomitoxin Assays for 2000 – 2001.

			ppm DON					
Lab	**Method**	**Grain**	**Aug. 00**	**Sep. 00**	**Oct. 00**	**Nov. 00**		**Ave.**
MICH – P. Hart	Elisa	Wheat	10.8	1.5	0.6	7.2		5
MINN – W. Xie	GC/MS	Wheat	11.4	0.6	0.6	5.6		4.6
ND – H. Casper	GC/ECD	Wheat	10.6	0.8	0.6	5.8		4.5
ND – P. Schwarz	GC/ECD	Wheat	13.2	0.8	0.3	4.2		4.6
Ave.			11.5	0.9	0.5	5.7		
MICH – P. Hart	Elisa	Barley	2	6.6	7.9	14.5		7.8
MINN – W. Xie	GC/MS	Barley	2	5.6	7.4	14.7		7.4
ND – H. Casper	GC/ECD	Barley	2	5.7	7.8	14.2		7.4
ND – P. Schwarz	GC/ECD	Barley	1	4.4	9.5	10		6.2
Ave.			1.8	5.6	8.2	13.4		

Table III. Interlab Vomitoxin Proficiency Check Samples.

			ppm DON					
Lab	**Method**	**Grain**	**Aug. 00**	**Sep. 00**	**Oct. 00**	**Nov. 00**		**Ave.**
MICH – P. Hart	Elisa	Wheat	10.8	1.5	0.6	7.2		5.0
MINN – W. Xie	GC/MS	Wheat	11.4	0.6	0.6	5.6		4.6
ND – H. Casper	GC/ECD	Wheat	10.6	0.8	0.6	5.8		4.5
ND – P. Schwarz	GC/ECD	Wheat	13.2	0.8	0.3	4.2		4.6
Ave.			11.5	0.9	0.5	5.7		
MICH – P. Hart	Elisa	Barley	2.0	6.6	7.9	14.5		7.8
MINN – W. Xie	GC/MS	Barley	2.0	5.6	7.4	14.7		7.4
ND – H. Casper	GC/ECD	Barley	2.0	5.7	7.8	14.2		7.4
ND – P. Schwarz	GC/ECD	Barley	1.0	4.4	9.5	10.0		6.2
Ave.			1.8	5.6	8.2	13.4		

The data in Table I shows that the intralab coefficient of variation for the 4 labs varies from 6 to 16% on the control pools that were analyzed with the test samples. The interlab proficiency check samples demonstrated that the 4 laboratories are getting similar results. The ELISA kit (2) provided a reasonable intralab coefficient of variation and the overall data was not significantly different from the chromatographic techniques. In the FHB campaign for

2000-2001, we estimate that ~23,000 samples will be processed by the 4 laboratories for ~42 principal investigators in ~16 states.

The interlab proficiency check samples will be continued in the FHB campaign for 2001-2002. Each laboratory will be evaluating means of refining their analytical techniques.

REFERENCES

Tacke, B. K., and H. H. Casper. Determination of deoxynivalenol in wheat, barley, and malt by column and gas chromatography with electron capture detection. J. AOAC Intl. 79:472-475.

Hart, L. P., H. Casper, O. Schabenberger, and P. Ng. 1998. Comparison of Gas Chromatography and Enzyme Linked Immunosorbent Assay for Deoxynivalenol in Milled Fractions of Naturally Contaminated Wheat. J. Food Protec. 61:1695-1697.

DON LEVEL IN GRAIN FROM WHEAT INOCULATED WITH *F. GRAMINEARUM* IS NOT CORRELATED TO THE DON PRODUCING POTENTIAL OF INDIVIDUAL CULTURES

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ABSTRACT

A collection of twelve cultures of *F. graminearum* isolated from corn and wheat were previously tested for production of deoxynivalenol (DON). The isolates produced a range of low (<1ppm) to high (>20) DON levels under standard test conditions. (*Wolf-Hall and Bullerman. 1998. J.Food Mycol. 1:171-180.*) Some researchers have claimed that DON is a pathogenicity factor in Fusarium Head Blight (FHB). We used this set of isolates to test that hypothesis. Isolates were tested for disease causing ability using methods used previously (*Stack, 1989. Can. J. Pl. Path. 11:137-142*). The isolates were grown on half-strength PDA under ambient room lighting for two weeks; conidia were washed from plates and suspensions adjusted to 50k/ml. Droplets (10µl) were placed into single spikelets on flowering wheat heads. After 3.5 weeks of incubation, individual heads were scored for FHB severity on a 0-100% scale. Plants matured naturally and inoculated heads were harvested and threshed. Grain was examined for tombstone kernels and was analysed for DON and other mycotoxins by GC-MS. The cultures varied in disease-causing ability. Three isolates were weakly pathogenic while four others were highly pathogenic, comparable to standard tester isolates; the remaining isolates were intermediate. FHB severity and tombstone kernels in grain were highly correlated to each other but neither was correlated to the toxin production potential previously determined for these cultures. The DON level in the grain was most highly correlated to the percent of tombstones and was not correlated to the isolate toxin potential determined previously. This study does not support the hypothesis that DON plays a major role in FHB disease development. These results agree with a similar study recently reported elsewhere (*J. Gilbert et al. 2000. Proc. Int. Symp. Wheat Improv. for Scab Resist., Suzhou and Nanjing, China. p218-223.*).

RGON: A REGIONAL STRATEGY FOR FUSARIUM HEAD BLIGHT IMPROVEMENT

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ABSTRACT

In the hard winter wheat region of the Great Plains, Fusarium head blight (FHB) is a sporadic disease. Drought is more common than rain at flowering, however, within the region, rain at and after flowering often occurs in some parts of the region leading to severe FHB in localized areas. Hence the need to identify lines with FHB tolerance is needed, but most plant breeding programs have insufficient plant pathology support and financial support to actively pursue FHB tolerance.

The Regional Germplasm Observation Nursery (RGON) is a USDA coordinated nursery that screens early generation experimental lines for a number of diseases, insects, and abiotic stresses. Every public and private wheat breeding effort in the Great Plains submits between 10 and 40 lines for this collaborative screening effort. The USDA-University of Nebraska wheat improvement effort, with support of US Scab Initiative efforts, proposes testing this nursery for FHB tolerance. The advantages of screening this nursery are: 1) all of the germplasm that is developed and eventually released in the hard winter wheat region will be screened for FHB tolerance, 2) public and private programs have equal access for entering lines into the nursery and for accessing the data, 3) germplasm within the RGON often has Chinese (used to enhance noodle and steamed bread quality) and eastern European (the genetic basis form much of the Great Plains winter wheats) parents from regions where FHB tolerance has been identified, 4) lines that show promise in the initial screen can be further tested for confirmation of the initial data, and 5) the identified germplasm is freely shared.

DETECTION OF QTL LINKED TO FHB RESISTANCE IN SUMAI 3-DERIVED LINES

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ABSTRACT

During the past decade *Fusarium* Head Blight (FHB) has caused severe grain yield and quality losses of wheat in the Northern Great Plains. Given the complexity of breeding for FHB resistance, molecular markers associated with this trait will be valuable in accelerating efforts to breed new resistant varieties. The objective of this study was to identify quantitative trait loci (QTL) for FHB resistance in wheat (*Triticum aestivum* L.) using a set of lines obtained by several cycles of crossing to North Dakota adapted genotypes and deriving their resistance from Sumai 3. Microsatellite markers spanning the wheat genome were used to screen parents and derived lines. Polymorphisms for parental alleles were compared to disease scores for Type II resistance. The probability of linkage between markers and introgressed resistance genes was calculated using a binomial probability formula. Two markers were significantly associated with FHB resistance QTL: Xgwm533 and Xgwm274. (This poster was presented at the 2000 ASA Meetings, Minneapolis, MN, November 5-9, 2000).

TOWARD TRANSFERRING SCAB RESISTANCE FROM A DIPLOID WILD GRASS, *LOPHOPYRUM ELONGATUM*, INTO DURUM WHEAT

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INTRODUCTION

Scab, also known as Fusarium head blight (FHB), pink mold, and tombstone scab – caused by the fungal pathogen *Fusarium graminearum* – is responsible for extensive damage of wheat in humid and semi-humid regions of the world. We earlier found a diploid wild grass, *Lophopyrum elongatum* (2*n*=2*x*=14; EE genome) – a wild relative of wheat, to be an excellent source of resistance to FHB. We therefore hybridized this grass with some commercial durum cultivars and raised BC_1 and subsequent generations. Chromosomal constitution of the advanced hybrid derivatives and their resistance to FHB are described.

MATERIALS AND METHODS

Two durum wheat lines [Langdon, and a Langdon disomic substitution line 5D(5B)] were hybridized as female parents with *Lophopyrum elongatum* following the protocols established earlier (Jauhar and Peterson 1996; 2000a). BC_1 plants were produced from the F_1 hybrids by crossing them to the recurrent durum parent except in the case of Langdon 5D(5B)-derived F_1s, which were backcrossed to Langdon. Advanced backcross generations were produced by subsequent backcrossing.

Fusarium screening was conducted on lines which had seed fertility of 80% or greater according to Jauhar and Peterson (2000a). FHB scores were taken according to the guidelines established by Stack and McMullen (1994).

Cytogenetic studies were conducted on lines showing good resistance (33% or less). Both somatic and meiotic chromosomes where characterized by conventional staining and fluorescent genomic *in situ* hybridization (GISH) as described in Jauhar et al. (1999). Fluorescent GISH was conducted according to Jauhar and Peterson (2000a) except that *L. elongatum* genomic DNA was used as the labeled probe.

RESULTS AND DISCUSSION

We produced several advanced hybrid derivatives from durum × *L. elongatum* crosses. Some of these derivatives were cytologically characterized. The hybrid material with 80% or higher seed fertility was screened for scab resistance. Figure 1 shows the meiotic chromosomes of PRE-17E(127), a BC_1F_2. This hybrid derivative with several chromosomes of the grass parent (Fig. 1B) exhibited good resistance over multiple screenings (Table 1), and was therefore used as a common parent to several advanced lines developed and screened for scab resistance. Efforts were focused on stabilizing the chromosomal constitution and retain scab resistance in several promising hybrid derivatives.

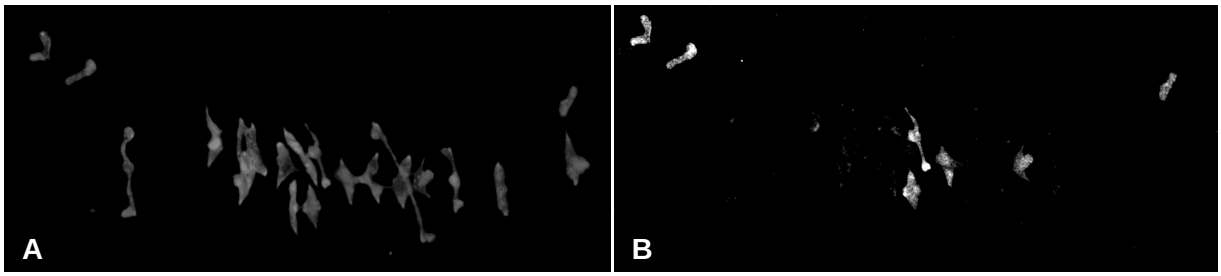

Figure 1. GISH of PRE-17E(127) – a selected hybrid derivative that showed good resistance to FHB. This line was the progenitor of all experimental lines screened in this study. **A.** PI counterstain showing 18II + 3I. **B.** Same cell as A probed with total genomic *L. elongatum* DNA labeled with biotin-14-dATP and detected with FITC. *L. elongatum* chromosomes (4 II + 3I) are clearly visible.

Table 1. Fusarium Head Blight Screening Results from Advanced Hybrid Derivatives.

		Percent infection of plant (average of three spikes)								
Cross / Line	**Plant No.**	**0-7**	**>7-15**	**>15-21**	**>21-33**	**>33-50**	**>50-67**	**>67-80**	**>80-100**	**Mean**
GHBC98-7[a]	4	3	1							7.92
GHBC98-14[a]	13	1	4	3	2	1	1		1	28.62
GHBC98-15[a]	15	3	6		2	3	1			22.02
GHBC98-16[a]	1				1					33.00
GHBC98-17[a]	6		1	2	1	1			1	38.00
GHBC98-18[a]	6	1		1	3	1				28.61
GHBC98-34[a]	16	2	7	3	4					15.98
GHBC98-68[a]	1		1							12.33
GHBC98-76[a]	11	1	7	3						13.76
PRE-17E(127)[b]	12		2	3	5			1	2	30.08
B-6[c]	10	1	6	1	1		1			17.60
B-7[d]	9	2	4	1	2					13.33
Langdon	9		1	1			1	2	4	68.33

[a] Unique crosses between PRE-17E(127) and Langdon [(Langdon/*L. elongatum*//Langdon)F_2/Langdon].

[b] Selected plant showing good resistance to FHB (Jauhar and Peterson, 1998) [(Langdon/*L. elongatum*//Langdon)F_2], derived from line B-6.

[c] BC_1 [Langdon/*L. elongatum*//Langdon] having good fertility, this is the BC_1 parent of PRE-17E(127).

[d] BC_1 [Langdon/*L. elongatum*//Langdon] derived lines from this plant are still in progress.

A general observation was that with the loss of *L. elongatum* chromatin, resistance is also lost. Line B-6 and B-7 both have good levels of resistance but as they were progressively backcrossed and selfed, resistance was lost (Table 1). However, as attempts were made to stabilize the lines through selfing and selection, some resistance is maintained. Figure 2A

shows meiosis of a line from Table 1 that had four *L. elongatum* chromosomes (univalents). This line scored 2% infection. Figure 2B is the selfed progeny from the previous plant having one *L. elongatum* chromosome present (as a univalent) and scored 21% infection. These observations support the assumption that FHB resistance is multigenic with genes occurring on different chromosomes. It is interesting that some of the monosomic addition lines of durum with a single grass chromosome (Fig. 2B) showed considerable resistance, although lower than when multiple grass chromosomes were present. However, several lines scored the same or even worse than the Langdon check. These lines have yet to be characterized cytologically, although some preliminary chromosome work has shown these lines to have the normal durum complement.

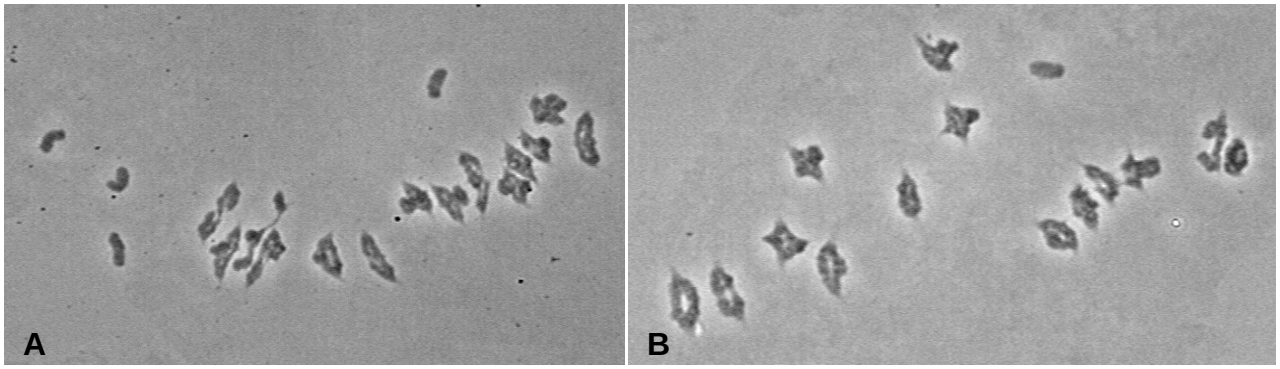

Figure 2. Meiotic chromosomes (under phase contrast) of advanced derivatives that had low infection of FHB. **A.** GHBC98-14 selection from Table 1 which had low infection (2%). This cell has 14 II of durum and 4 I from *L. elongatum*. **B.** Line GHBC98-14-14 selfed progeny from plant in Figure 2A, 14 II of durum and 1I from *L. elongatum*. It is interesting that even with a single grass chromosome this particular plant had a high level of resistance (only 21% infection).

Thus far, we have not been able to obtain disomic addition lines of durum with a pair of grass chromosomes, although we have selfed monosomic addition lines. Earlier, we have shown integration of *L. elongatum* chromatin into the durum genome (Jauhar and Peterson 2000b). Whether these integrations are maintained or whether new integrations have occurred has to be determined. We have not been able to obtain any 28-chromosome hybrid derived durum with an acceptable level of scab resistance. Nevertheless, our studies show that wide hybridization holds considerable promise for breeding scab resistance into wheat. Chromosome-mediated alien gene transfers will continue to play an important role in the germplasm enhancement of wheat (Jauhar and Chibbar 1999).

ACKNOWLEDGEMENTS

We would like to thank Dr. Robert Stack of the Plant Pathology Department, North Dakota State University for supplying the *Fusarium* isolates used for screening our hybrid derivatives. We also thank Dr. James Miller Plant Pathologist in the Cereals Unit, UDSA for his help with screening.

REFERENCES

Jauhar, P.P., and T.S. Peterson. 1996. *Thinopyrum* and *Lophopyrum* as sources of genes for wheat improvement. Cereal Res. Comm. 24: 15-21.

Jauhar, P.P., and T.S. Peterson. 1998. Wild relatives of wheat as sources of Fusarium head blight resistance. Proc. of the 1998 National Fusarium Head Blight Forum, Michigan State University, East Lansing, October 1998. pp. 77-79.

Jauhar, P.P., A.B. Almouslem, T.S. Peterson, and L.R. Joppa. 1999. Inter- and intra-genomic chromosome pairing in haploids of durum wheat. J. Hered. 90: 437-445.

Jauhar, P.P., and R.N. Chibbar. 1999. Chromosome-mediated and direct gene transfers in wheat. Genome 42: 570-583.

Jauhar, P.P., and T.S. Peterson. 2000a. Hybrids between durum wheat and *Thinopyrum junceiforme*: Prospects for breeding for scab resistance. Euphytica 109: 1-10.

Jauhar, P.P., and T.S. Peterson. 2000b. Progress in producing scab-resistant germplasm of durum wheat. Proc. International Symposium on Wheat Improvement for Scab Resistance, Nanjing Agricultural University, Nanjing, China, pp. 77-81.

Stack, R.W., and M.P. McMullen. 1994. A visual scale to estimate severity of Fusarium Head Blight in wheat. *NDSU Extension Service,* PP-1095.

GREENHOUSE BASED EVALUATION OF ASIAN AND ITALIAN WINTER WHEAT GERMPLASM FOR TYPE I RESISTANCE TO FUSARIUM HEAD BLIGHT

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INTRODUCTION

Fusarium graminearum Schwabe (teleomorph *Gibberella zeae* (Schwein.), also known as scab, is a devastating disease of wheat and barley in warm and humid regions of the world. In addition to reductions in grain yield, kernel color at harvest, and test weight, associated deoxynivalenol (DON) accumulation in the grain prevents it from being marketed. Host resistance has long been considered the most practical and effective means of control (Schroeder and Christensen, 1963; Martin and Johnston, 1982), but breeding has been hindered by a lack of effective resistance genes and by the complexity of the resistance in identified sources (Mesterházy, 1997). No source of complete resistance is known, and current sources provide only partial resistance, often in unadapted types. The identification of different sources of resistance and their incorporation into adapted wheat varieties is critical to the continued improvement of Fusarium head blight resistance in winter wheat. Mesterházy, (1995) identified five different types of resistance including reduced incidence (type I), reduced spread of the pathogen in the head following initial infection (type II), kernel retention (type III), low toxin accumulation (type IV) and tolerance (type V). Type II resistance, measured by point-inoculation in a single central floret is the most common type of resistance available and several sources have been identified and widely used in breeding programs. Good sources of type I resistance are fewer in number, partly because of the lack of precision and control associated with its evaluation and the potentially confounding effects of spread in the head subsequent to inoculation.

OBJECTIVES

This research was designed to evaluate a greenhouse-based technique for assessing type I resistance to *Fusarium graminearum.* A secondary object of this study was to complete a replicated verification of type II and III resistances in Asian and Italian germplasm.

MATERIALS AND METHODS

Germplasm identified for evaluation of type I resistance was Asian and Italian winter wheat accessions, previously evaluated at Missouri in the greenhouse (type II) and field (scab index) for reaction to artificial inoculations with *Fusarium graminearum.* Forty-two accessions were evaluated. Type II and scab index data for these accessions are provided on the website of the National Scab Initiative at .

Disease Resistance Screening: Forty vernalized seedlings of each accession were planted in the greenhouse for evaluation of type I and type II resistance. For each type of resistance, accessions were planted as two reps of 10 plants with replications separated in time by two weeks.

Type II Evaluation: At first anthesis, plants were inoculated with 10µL of a macroconidial suspension of *Fusarium graminearum* concentrated to 50,000 macroconidia/mL. Inoculum was placed in a single central floret using an Oxford 8100™ repeat dispensing syringe. For all inoculations, a single isolate was used which had been previously determined to be the most aggressive Missouri isolate on our most resistant cultivar, Ernie. Plants were incubated in a mist chamber (100% relative humidity) for 72 h post-inoculation to promote disease development and then returned to the greenhouse bench. Ratings for Type II resistance (disease spread in the spike) were made at 21 d after inoculation.

Type I Evaluation: At anthesis, heads were inoculated with a macroconidial suspension of *Fusarium graminearum* concentrated to 50,000 macroconidia/mL. Inoculum was sprayed directly on the head using a Pulmo-Aide nebulizer as the power source and an atomizer (model 163, DeVilbiss Sunrise Medical, Somerset, PA 15501-0635, USA). Inoculum was delivered to each head, spraying one side and then the other. Plants were incubated in a mist chamber (100% relative humidity) for 72 h post-inoculation to promote disease development and then returned to the greenhouse bench. At 10 d post-inoculation heads were rated for symptoms of Fusarium head blight. Total spikelets in the head were recorded followed by the number of spikelets in the head showing disease. The type I rating for each head was determined as the number of spikelets with disease divided by the total number of spikelets on the head. Ratings were taken again at 21 d post-inoculation to determine the scab index (incidence x severity) for the head. The type I rating (10 d) was taken as a measure of incidence. The 21-d rating (total number of infected spikelets/total spikelets in the inoculated head) provided an estimate of severity on the inoculated head. These data were compared to field based scab index data.

RESULTS AND DISCUSSION

Table 1 provides information on country of origin, improvement status and disease resistance data for Asian and Italian accessions with low scab reactions following two cycles of greenhouse and one cycle of field screening. Fusarium head blight index is the mean of the ratio of infected spikelets/total spikelets in the inoculated head, expressed as a percent. The field-based scab index is calculated as the product of incidence (mean percentage of plants in a 3 ft row, showing disease symptoms) and severity (mean percentage of the head showing disease symptoms). All lines had good kernel retention and quality under inoculation.

Significant differences among cultivars for greenhouse-based incidence are apparent from data collected to date. The experiment is currently ongoing and data collection is not yet complete. Final data will be presented at the 2000 Scab Forum in Cincinnati in December.

REFERENCES

Martin, R. A., and H. W. Johnston. 1982. Effects and control of Fusarium diseases of cereal grains in the Atlantic Provinces. Can J. Plant. Pathol. 4:210-216.

Mesterházy, Á. 1997. Methodology of resistance testing and breeding against *Fusarium* head blight in wheat and results of selection. Cereal Res. Commun. 25(3/2): 631-637.

Mesterházy, Á. 1995. Types and components of resistance to *Fusarium* head blight of wheat. Plant Breeding 114:377-386.

Schroeder, H. W., and J. J. Christensen. 1963. Factors affecting resistance of wheat to scab caused by *Gibberella zeae.* Phytopathology 53:831-838.

Table 1. Disease resistant data for Asian and Italian accessions having low scab reactions after two cycles of greenhouse and one cycle of field screening.

Missouri ID	Accession	Origin	Improvement Status	Greenhouse Type II	Field Scab Index
				----------%	----------
4	Cltr 5087	China	Cultivated	10	9
5	Cltr 7159	China	Landrace	9	36
12	Cltr 8299	Italy	Landrace	10	48
51	Cltr 9400	China	Landrace	15	53
52	Cltr 9401	China	Landrace	12	53
70	Cltr 9428	China	Landrace	13	76
71	Cltr 9429	China	Landrace	7	14
77	Cltr 9445	China	Landrace	6	28
91	Cltr 9488	China	Landrace	8	90
92	Cltr 9490	China	Landrace	9	20
93	Cltr 9506	China	Landrace	12	15
94	Cltr 9507	China	Landrace	10	36
102	Cltr 9521	China	Landrace	11	45
122	Cltr 10198	China	Cultivated	13	15
124	Cltr 10205	China	Cultivated	13	34
126	Cltr 10216	China	Cultivated	15	12
128	Cltr 10264	China	Cultivated	13	45
147	Cltr 10335	China	Cultivated	16	35
151	Cltr 10353	China	Cultivated	11	2
193	Cltr 10491	China	Cultivated	14	48
198	Cltr 10504	China	Cultivated	7	80
202	Cltr 10509	China	Cultivated	4	32
206	Cltr 10520	China	Cultivated	14	38
209	Cltr 10524	China	Cultivated	14	35
217	Cltr 10574	China	Cultivated	5	32
242	Cltr 10623	China	Cultivated	15	42
244	Cltr 10627	China	Cultivated	9	30
295	Cltr 10783	China	Cultivated	10	48
348	Cltr 11155	China	Landrace	8	43
355	Cltr 15162	Italy	Cultivar	16	29
368	PI 94576	Italy	Landrace	13	70
371	PI 118726	China	Breeding	7	70
419	PI 132858	Italy	Cultivar	10	39
433	PI 155271	Japan	Cultivar	7	36
451	PI 157593	South Korea	Cultivar	9	27
463	PI 157910	Italy	Cultivar	9	60
473	PI 174639	Italy	Landrace	8	66
Checks	Ernie	Missouri	Cultivar	20	14
	Sumai 3	China		15	-
	Wangshuibai	China		10	24
	Patterson	Indiana	Cultivar	83	52

BROADENING THE GENETIC BASE FOR SCAB RESISTANCE THROUGH A CIMMYT/NATIONAL SCAB INITIATIVE PARTNERSHIP

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INTRODUCTION

Fusarium graminearum Schwabe (teleomorph *Gibberella zeae* (Schwein.), also known as scab, is a major disease limiting production of wheat and barley in warm and humid regions of the world. From the first description of scab in 1884 by W. G. Smith in England, epidemics were soon reported in many parts of the world. In a recent as yet unpublished review, Bob Stack reports that by 1917, scab had been recognized in 31 states of the United States, throughout many parts of Europe, in Russia and in Japan. Although present throughout much of the 20th century, scab has become an increasingly important problem in the north-central region of the United States because of the increased emphasis on conservation tillage, increased corn acreage and/or rotations with corn, the lack of effective cultural and/or fungicide control , and the lack of effective sources of genetic resistance. In addition to reduced kernel density and color at harvest, associated deoxynivalenol (DON) accumulation in the grain prevents it from being marketed.

Host plant resistance has long been recognized as the most practical and economical means of controlling scab in both wheat and barley, however, breeding has been hindered by a lack of genetically diverse, effective resistance genes. No source of complete resistance is known and current sources provide only partial resistance.

The narrow genetic base for scab resistance globally is clear from the widespread use of a limited number of good sources of resistance predominantly found in spring wheat. Although several sources of resistance are known, Sumai 3, developed at the Suzhou Institute of Agricultural Science in Jiangsu Province from the cross of two moderately susceptible lines, Funo and Taiwanxiaomai, has been most widely used. It is characterized by: high levels of stable resistance, low incidence, reduced spread and low toxin levels in grain colonized by the pathogen. Despite its relatively poor agronomic type, Sumai 3 has proven to have among the best combining ability of sources known and therefore, has been extensively used in breeding programs in China. Currently, there are more than 120 derivatives of Sumai 3 in production in China occupying a significant proportion of the 30 million hectares of wheat under cultivation. Narrowing the genetic base globally is the fact that Sumai 3 has been introduced into and widely used in Japan, Mexico, Canada, the United States and Europe.

OBJECTIVES

The National Wheat and Barley Scab Initiative's Germplasm Introduction and Evaluation programmatic area has as its focus, the broadening of the genetic base of scab resistance through four main objectives. These include: conducting an aggressive, world-wide search

for new sources of resistance through systematic screening of national wheat and barley germplasm collections; global acquisition of germplasm with known sources of scab resistance; global exchange of germplasm through the international nursery system; dissemination of information on resistance through internet databases.

The acquisition and testing of known sources of resistance is being conducted through a collaborative agreement between CIMMYT and the National Scab Initiative. Through this effort, CIMMYT has proposed to: provide agronomically suitable scab resistant germplasm to US collaborators through pre-breeding activities using synthetic wheats and major U.S. cultivars, conduct a world-wide search for and acquisition of suitable scab resistant wheat and barley germplasm and to make this germplasm available to U.S. Wheat and Barley Scab Initiative scientists, and to test germplasm through the International Nursery System.

MATERIALS AND METHODS

Targeted screening of accessions from geographical areas where environmental conditions are conducive to scab development or where scab resistance has been identified has been ongoing for the past 3 years under initiative funding. In addition to winter wheat screening being conducted at the University of Missouri, Columbia, and by Dr. Paul Murphy at North Carolina State University, spring wheat accessions are being screened by Dr. Yu Jin (South Dakota State University), durum wheat accessions are being evaluated by Dr. Elias Elias (North Dakota State University), and barley accessions by Dr. Brian Steffenson (University of Minnesota) Initial geographical areas targeted have included Asia (China, Japan, South Korea), Eastern Europe, Italy and South America. Screening protocols vary with program but in general measure incidence, spread, kernel quality and DON levels in germplasm maintained in the National Small Grains Collection.

Researchers at CIMMYT are working on incorporating genetic resistance for scab into commercially grown varieties. Specifically, they are working to identify and combine low incidence, and reduced spread, with genes for kernel retention and low DON accumulation. Sources of resistance include genetic sources from Brazil, Japan, Argentina, China and Romania coupled with promising sources identified in CIMMYT's wide crossing program. CIMMYT varieties combine good levels of scab resistance with resistances to other biotic stresses including resistance to Septoria leaf blotch and stripe rust and good agronomic type. Through the collaborative agreement, CIMMYT will enable U.S. breeders to access these improved sources of resistance currently being developed in Mexico.

CIMMYT scientists will also facilitate the acquisition of germplasm possessing either different types or sources of resistance from programs in China, South America and Europe. Lines to be acquired will be identified by either CIMMYT or U.S. Scab Initiative scientists. Secondary distribution rights will be acquired by CIMMYT who will then facilitate the introduction of this promising germplasm into U.S. breeding programs involved with the National Scab Initiative.

RESULTS AND DISCUSSION

Results from spring and winter wheat as well as from durum wheat and barley will be presented in other papers in this forum. For spring and winter wheat and for barley, results of these evaluations have been posted on the National Scab Initiative web site at . Where these sources are genetically different, their utilization will help to broaden the genetic base for resistance. As transgressive segregation is a relatively common phenomenon when different sources of resistance are crossed, their combination may also enhance the levels of resistance in breeding programs.

U.S. Wheat and Barley Scab Initiative scientists visited CIMMYT's wheat and barley breeding programs in September 2000. After visiting the nurseries at Toluca, a number of lines from both the bread wheat and wide-crossing programs were identified for introduction into the US. These lines (Table 1) have been shipped to the U.S. Wheat lines will be quarantined in Missouri and then distributed to interested scientists in the spring. Durum and barley lines were introduced into the U.S. through an import permit issued to Dr. Elias.

Table 1. Scab resistant wheat and barley germplasm from CIMMYT breeding programs introduced into the United States in November 2000 as part of the CIMMYT/National Scab Initiative partnership facilitating global germplasm exchange.

CROP	CROSS/PEDIGREE
BREAD WHEAT	
	CATBIRD
	NG8675/CATBIRD
	MILAN/SHA7
	CHUM 18//JUP/BJY
	NS73/PCI//B143.241.2/3/NING 8647
	MIAN YANG 81-5//PC B084.985/JIANZIMAI
	PC B084/JIANZIMAI//8744
	SHANGAI
	GOV/AZ//MUS/3/DODO/4/BOW
	RECURRENT SELECTION 1
	NG 8675/NING 8645
	SODAT/SUM 3//NING 820/3/NING 8626
	NG8201/KAUZ
	JIAN85.11//SUZHOU 7906/NING 8249
SYNTHETIC HEXAPLOID DERIVATIVES	
	TURACO/5/CHIR3/4/SIREN//ALTAR 84/ AE. SQUARROSA (205)/3/3*BUC
	BCN//DOY 1/AE. SQUARROSA (447)
	MAYOOR//TK SN1081/AE. SQUARROSA (222)
	OPATA/5/CPI/GEDIZ/3/GOO//JO69/CRA/4/AE. SQUARROSA (223)
	MAYOOR/5/CS/THINOPYRUM CURVIFOLIUM //GLEN/3/ ALD/PVN/4/ CS/LE. RACEMOSUS//2*CS/3/CN079
	CS/TH.CUR//GLEN/3/ALD/PVN/4/CSS/LE.RACEMOSUS//2*CS/3/CN079
	BCN*2//CROC 1/AE. SQUARROSA (886)
	MAYOOR
	CROC 1/AE. SQUARROSA (205) /5/BR12*3/4/IAS55*4/CI141223 /3/IAS55*4/EG. AUS//IAS55…
	BUC//RUFF/AE. SQUARROSA/3/MIAZ
	SABUF/5/BCN/4/RABI//GS/CRA/3/AE. SQUARROSA (190)
	CHIRYA.1
	LCK59.61/AE.SQUARROSA (313)
	CS/LE.RA//CS/3/PVN
DURUM WHEAT	
	CHAIKA_1/TILO
	LABUD/NEHAMA//SRN/VIC-U
	SCOOP_1/LOTUS_1
	SRN_1/6/FGO/DOM//NACH/5/ALTAR 84/4/GARZA/AFN//CRA/3/ GGOVZ39417/GEDIZ/FGO//GIA/3/CNDO/8/DUKEM_1
	ZEGZAG/ALTAR 84//DIPPER_2
BARLEY	
	TOCTE//GOB/HUMAI10/3/ATAH92/ALELI
	PENCO/CHEVRON-BAR
	ZHEDAR#1/SHYRI//OLMO
	ATAH92/GOB
	CANELA/ZHEDAR#2
	MNS1
	SVAANHAALS-BAR/MSEL//AZAF/GOB24DH
	ZHEDAR#11/4/SHYRI//GLORIA-BAR/COPAL/3/SHYRI/GRIT/5/ARUPO/K8755//MORA

While in China for the International Symposium on Improving Scab Resistance in Wheat, Dr. Lucy Gilchrist and Dr. Anne McKendry identified several lines that potentially possess either different sources or different types of scab resistance. Sources include those developed through somaclonal variation observed from wheat embryo culture of the susceptible commercial cultivar Ningmai 3 (Ning 895004, Ning 894037 and Shangkang1) and resistance lines derived from wide crosses with Roegneria kamoji, R. ciliaris, and Leymus racemosus (e.g. Zhonge 3 – derived from R. Kamoji and Yangmai 158). CIMMYT will facilitate the introduction of these lines into the U.S. in December, 2000. Along with these sources, elite lines that combine one or more sources of resistance will also be introduced from breeding programs in China and Romania (Table 2). Again, these lines will be quarantined in Missouri and subsequently distributed to scientists participating in the National Scab Initiative under terms of secondary distribution acquired by CIMMYT. A second round of collections of advanced lines is now underway. Access to these and other lines should significantly contribute to the efforts to broaden the genetic base for scab resistance in the U.S. and through testing of U.S. lines in the International Scab Nursery, facilitate the exchange of scab germplasm globally.

Table 2. Scab resistant germplasm from China and Romania introduced into the United States in November 2000 as part of the CIMMYT/National Scab Initiative partnership facilitating global germplasm exchange.

Origin	Cultivar/Descriptor
Introductions from China	
	Ning 89401
	Ning 894037
	Ning 96242
	Ningmai 9
	Mutant AT 1
	Mutant AT 2
	Yangmai 158
	Yangmai 9
	Emai 6
	Shengkang 1
	Zhonghua 1
	Sumai 2
	85004/Mexico 354
Advanced Lines from Nanjing Agricultural University	
	SB 107
	SB 108
	SB 109
	SB 110
	SB 111
	SB 114
	SB 115
	SB 116
Advanced Lines from Romania	
	Fundulea 01 R
	Fundulea 183 P5
	Fundulea 483
	Fundulea 143-T3-103
	Turda 95
	Turda 195
	Turda 2317-90

EVALUATION OF YUGOSLAVIAN WINTER WHEAT GERMPLASM FOR RESISTANCE TO FUSARIUM HEAD BLIGHT

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INTRODUCTION

Fusarium graminearum Schwabe (teleomorph *Gibberella zeae* (Schwein.), also known as scab is an increasingly important problem in the north-central region of the United States because of the emphasis on conservation tillage, (Wilcoxson et al., 1988; Bai and Shaner, 1994), rotations with corn (Windels and Kommedahl, 1984), the lack of effective cultural and/or fungicide control (McMullen et al., 1997) and the lack of effective sources of genetic resistance. In addition to reduced kernel density and color at harvest, associated deoxynivalinol (DON) accumulation in the grain prevents it from being marketed. Host resistance has long been considered the most economical and effective means of control (Schroeder and Christensen, 1963; Martin and Johnston, 1982), but breeding has been hindered by a lack of effective resistance genes and by the complexity of the resistance in identified sources (Mesterházy, 1997). No source of complete resistance is known, and current sources provide only partial resistance, often in genetic backgrounds with inferior agronomic type. The identification of different sources of resistance and their incorporation into adapted wheat varieties is critical to the continued improvement of Fusarium head blight resistance in winter wheat. Research funded by the National Wheat and Barley Scab Initiative has led to the systematic evaluation of resistance to scab in winter wheat accessions from targeted geographical regions of the world where resistance has been identified or where environmental conditions are conducive to scab development. Accessions from China, Korea, Japan, Italy and Brazil have been screened and a number of promising sources of resistance have been identified (McKendry et al. 1999). Eastern Europe was targeted as a region where scab has been a problem and approximately 2,000 winter wheat accessions from Yugoslavia and the Balkans were identified in the USDA National Small Grains collection for evaluation.

OBJECTIVES

The purpose of this research was to evaluate, under greenhouse and field conditions, approximately 1,000 Yugoslavian accessions for resistance to *Fusarium graminearum.*

MATERIALS AND METHODS

In the fall of 1999, 1006 accessions representing winter wheat landraces, breeding lines, cultivars and cultivated genotypes from Yugoslavia were acquired from the USDA-ARS Small Grains Collection at Aberdeen, Idaho.

Disease Resistance Screening - Greenhouse

Vernalized seedlings (4 per accession) were planted in the greenhouse. At first anthesis, plants were inoculated with 10µL of a macroconidial suspension of *Fusarium graminearum* concentrated to 50,000 macroconidia/mL. Inoculum was placed in a single central floret using an Oxford 8100™ repeat dispensing syringe. For all inoculations, a single isolate was used which had been previously determined to be the most aggressive Missouri isolate on our most resistant cultivar, Ernie. Previous research had also determined that this Missouri isolate was more aggressive in causing disease than similar isolates acquired from Indiana, Michigan, Ohio and Virginia. Plants were incubated in a mist chamber (100% relative humidity) for 72 h post-inoculation to promote disease development and then returned to the greenhouse bench. Ratings for disease spread in the spike were made at 21 d after inoculation. At maturity, heads were harvested, kernels were counted and evaluated for the degree of shriveling and the presence of tombstone kernels. Seeds were counted and each was given a value on a 5 point scale as follows: 1 (sound): 2 (slightly shriveled): 3 (moderately shriveled): 4 (very shriveled): 5 (tombstone). Lines meeting the following criteria for resistance are currently being progeny tested to verify resistance. Concurrently, Dr. Paul Murphy at North Carolina State University conducted a similar greenhouse screening of a subsample of approximately 500 accessions from this group. The aim of this joint screening was to expedite verification of resistance in this collection. Plants were identified for further evaluation that had low spread in the head (mean spread $\leq$ 2 spikelets), and good kernel quality relative to an uninoculated head. Resistant check cultivars included Sumai 3, Ning 7840, Ernie, Roane Wangshuibai and Futai 8944. Susceptible checks were Patterson, and Coker 9663.

Disease Resistance Screening - Field

Accessions were planted as head rows in the field at the Agronomy Research Center near Columbia, MO. Plants were sprayed at 75% heading with a macroconidial suspension concentrated to 50,000 macroconidia/mL. Head rows were maintained under overhead mist irrigation through heading and evaluated for scab incidence 7-10 d post inoculation and severity18 - 21 d after inoculation. A field scab index was determined as incidence * severity.

RESULTS AND DISCUSSION

Table 1 provides information on the accession, improvement status, and resistance data for accessions identified as having some level of potentially useful resistance in both the Missouri and North Carolina evaluation programs. Data are presented for accessions that had either reduced spread in the spike following greenhouse inoculations, a low scab index in the field (determined as the product of incidence and severity) or both. Of note are accessions that had both low spread in the head and a field scab index $\leq$ 30% (PI 221346, PI 345022, PI 350033, PI 350089, PI362463, and PI 362676). Kernel quality scores, collected in the greenhouse, ranged from 1 – 3.5 in this set of accessions. It is important to note that often, those with a kernel quality score of 2 –3 (indicating some shriveling of the seed) were only marginally worse under inoculated head than in the uninoculated head. Much of the Yugoslavian material was late in the greenhouse and grain fill was often affected. Kernel

quality data from the Missouri screening will be presented at the scab forum in December 2000 for comparative purposes.

Resistance identified in a further 200 accessions screened only at Missouri is being verified and data will be presented on these lines at the 2000 Scab Forum. The majority of accessions with low scab reaction were landraces. Of 237 accessions being re-evaluated at Missouri, 209 are landraces, 12 are breeding lines, 9 are cultivated and 7 are cultivars. Progeny evaluations of each of these accessions are ongoing. Field evaluations of accessions being re-screened will be completed during the summer 2001 season.

Field and greenhouse evaluations of the remaining 1000 accessions from the Balkans will be completed during the 2000/2001 season.

Table 1. Fusarium head blight resistance data for Yugoslavian winter wheat germplasm with low scab reactions simultaneously screened at the University of Missouri and North Carolina State University in 2000.

		Greenhouse Type II		Kernel quality (NC State)		Field Data	
Accession	Improvement Status	Missouri	NC State	Inoculated	Un-inoculated	Scab index	BYDV
		----------%----------		----------1 to 5 ----------		----------%---------	
PI 221341	Cultivated	25	16	1	-	27	20
PI 221346	Cultivated	18	16	1.7	-	28	20
PI 316425	Breeding	26	25	3	-	10	25
PI 345022	Landrace	17	7	1.3	-	30	30
PI 345106	Landrace	14	13	3	-	34	30
PI 345108	Landrace	16	25	2.2	-	21	10
PI 345163	Landrace	21	15	1	-	27	40
PI 350021	Landrace	25	19	1	-	29	60
PI 350027	Landrace	10	25	3	-	38	10
PI 350033	Landrace	7	15	1	-	27	50
PI 350036	Landrace	17	8	2	-	38	10
PI 350058	Landrace	19	9	1.3	-	42	20
PI 350089	Landrace	10	7	2	-	27	20
PI 362434	Landrace	22	15	3.2	3	27	-
PI 362450	Landrace	28	8	2.5	2.5	15	10
PI 362459	Landrace	34	13	3.3	1.5	14	50
PI 362463	Landrace	14	7	3	3	3	50
PI 362477	Landrace	22	6	2.8	2.5	10	40
PI 362512	Landrace	12	24	2.5	1	35	45
PI 362541	Landrace	9	32	2.5	2	16	50
PI 362552	Landrace	14	4	2.5	2.5	66	50
PI 362565	Landrace	19	17	2.5	2	34	30
PI 362676	Landrace	12	10	2.9	2	27	50
PI 374476	Landrace	27	17	3.1	1.5	29	25
PI 374481	Landrace	17	19	3.1	3	38	30
PI 378265	Landrace	25	22	2.7	-	18	-
PI 378277	Landrace	12	3	2.4	2	32	-
PI 378319	Landrace	10	7	3.2	3	45	-
PI 378320	Landrace	7	13	3	3	57	-
PI 378323	Landrace	7	9	2.5	-	76	-
PI 378331	Landrace	7	5	3.5	2	38	-
Checks	Ernie	20	18	2	-	14	-
	Roane	20	23	3.2	-	15	
	Futai 8944		11	1.7	-	-	-
	Sumai 3	15	-	-	-	-	-
	Wangshuibai	11	-	-	-	24	-
	Coker 9663	-	89	4.2	-	-	-
	Patterson	83	-	-	-	52	

REFERENCES

Bai, G., and G. Shaner. 1994. Scab of wheat: Prospects for Control. Plant Dis. 78:760-766.

Martin, R. A., and H. W. Johnston. 1982. Effects and control of Fusarium diseases of cereal grains in the Atlantic Provinces. Can J. Plant. Pathol. 4:210-216.

Mesterházy, Á. 1997. Methodology of resistance testing and breeding against *Fusarium* head blight in wheat and results of selection. Cereal Res. Commun. 25(3/2): 631-637.

McKendry, A. L., K. S. Salzman, and S. Liu. 1999. Evaluation of Asian, Italian, and Brazilian winter wheat germplasm for types II and III resistance to Fusarium head blight. Proceedings of the 1999 National Fusarium Head Blight Forum, Sioux Falls South Dakota, December 5-7, 1999. pp. 129-132.

McMullen, M. P., B. Schatz, R. Stover, and T. Gregoire. 1997. Studies of Fungicide efficacy, application timing, and application technologies to reduce Fusarium head blight and deoxynivalinol. Cereal Res. Commun. 25(3/2): 779-783.

Rubiales, D., C. H. A. Snijders, P. Nicholson, and A. Martin. 1996. Reaction of tritordeum to *Fusarium culmorum* and *Septoria nodorum*. Euphytica. 88:165-174.

Schroeder, H. W., and J. J. Christensen. 1963. Factors affecting resistance of wheat to scab caused by *Gibberella zeae.* Phytopathology 53:831-838.

Wilcoxson, R. D., T. Kommedahl, E. A. Oxmon, and C.E. Windels. 1988. Occurrence of Fusarium species in scabby wheat from Minnesota and their pathogenicity to wheat. Phytopathology 78:586-589.

Windels, C.E., and T. Kommedahl. 1984. Late season colonization and survival of *Fusarium graminearum* group II in corn stalks in Minnesota. Plant Dis. 68:791-793.

ALIEN GENETIC DIVERSITY FOR WHEAT IMPROVEMENT: FOCUS ON SCAB RESISTANCE

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INTRODUCTION

Because of their diversity and global distribution, accessions of the primary gene pool diploid wheat relative, *Aegilops tauschii,* (2n=2x=14, DD) syn. *Ae. squarrosa, Triticum tauschii,* constitute a unique source of novel genetic variability for bread wheat, providing among other things resistance to several factors that reduce the crop's productivity in developing countries. Due to stress screening constraints, *Ae. tauschii's* winter habit, and its tendency for grain shattering, we have hybridized available accessions indiscriminately with elite *T. turgidum* cultivars, producing 800 synthetic hexaploids (SH; 2n=6x=42, AABBDD) to date, with several involving a unique *Ae. tauschii* accession. We report here on the current status of scab resistance in these SH wheats, in their advanced, free threshing derivatives upon hybridization with elite but scab susceptible bread wheats (BW), and on the development of a doubled haploid mapping population involving a BW/SH stable advanced line with resistance to multiple scab types (Types I-IV) and to several other biotic constraints. Finally, the potential of some tertiary pool species for scab resistance is briefly addressed.

MATERIALS AND METHODS

- 800 SH wheats derived from crosses of 51 *T. turgidum* cultivars and 438 of the 490 *Ae. tauschii* accessions in the wide crosses working collection at CIMMYT.

- Advanced bread wheat/SH derivatives.

- 64 doubled haploids of a mapping population involving the resistant BW/SH line (Mayoor//TKSN 1081/*Ae. tauschii* (222) and the susceptible BW cultivar 'Flycatcher'.

- Intergeneric amphiploids, backcross I self-fertile intergeneric derivatives, and alien disomic addition lines in wheat.

- 174 A genome hexaploids derived from durum x A genome diploid combinations (2n=6x=42, AAAABB)

Location: CIMMYT station, Toluca, Mexico (19° 17'N, 99° 39'W, 2640 m above sea level).

Plot size: Unreplicated hill plots except for BW/SH advanced derivatives in two 2.0 m rows spaced at 15 cm between rows in 90 cm beds.

Disease inoculation: Fusarium head scab isolates were obtained from Toluca, Patzcuaro, and El Tigre, Mexico. A spore concentration of 50,000/ml of water and the cotton inoculation method were used (a tiny, inoculum-permeated tuft of cotton is placed in the floret by opening the glumes of a spikelet in the middle of the spike with a pair of tweezers. The spike is then covered with a glassine bag to prevent damage). Ten, randomly selected spikes of each entry were inoculated.

Disease evaluation: Fusarium head scab (Type II: Spread) disease scoring was done 30 to 35 days after inoculation. The inoculated spikes were harvested, percentage of spikelets infected with scab evaluated, and scab scores of the inoculated spikes averaged. Results and Discussion

RESULTS

Primary gene pool

Resistance in synthetic hexaploid wheats: The SH wheats (*T. turgidum* x *Ae. tauschii*) most resistant (less than 15% infection) to *Fusarium graminearum* (Type II) are presented in Table 1. Resistant BW check Sumai 3 scored around 15% or slightly less, while the moderately susceptible BW check 'Flycatcher' always had over 20% infection and the durum wheat 'Altar 84' over 40%. After three cycles of testing the advanced BW/SH scab resistant entries were selected for Type II resistance. These derivatives also possessed resistance to leaf rust, stripe rust and *Septoria tritici*. Each scab resistant entry selected had a disease score of less than 15% across each test year. Sumai-3 averaged 12% over the three test years (Delgado et al. 2000).

The most promising entries from the BW/SH combinations were further tested for the other three scab categories (I, III, IV). Four were found to possess combined resistance to all four types of scab (Table 2). These are currently being used in bread wheat breeding at CIMMYT and in the collaborative activity with the US Scab Initiative.

The combination Mayoor//TK SN 1081/*Ae. tauschii* (222) and several of its sister lines exhibit superior scab resistance across its four categories and also possess resistance to *S. tritici*, *N. indica,* and *H. sativum* (Mujeeb-Kazi et al. 2000). One line was crossed with 'Flycatcher' (susceptible to all the above stresses), and the F_1 seed used to produce 150 doubled haploids (DH) for molecular mapping/phenotyping. A partial batch of 51 DHs were tested for Type II and a 24:27 resistant:susceptible segregation was observed, indicating a 1:1 frequency. The F_1 of the above cross was completely resistant for Type II infection.

Tertiary gene pool

Tertiary pool species hold promise for providing additional genetic diversity for scab resistance (Table 3). Of high priority at this stage are crosses of wheat x *Th. bessarabicum* and their backcross derivatives, where the *ph* locus is involved to promote the introgression of alien genes.

Durum wheat improvement. Several diploid (2n=2x=14, AA) accessions combined with elite durum cultivars yielded AAAABB hexaploids, after their AAB F_1 hybrids were colchicine doubled. In the initial screening only 3 of the 174 hexaploids exhibited Type II promise with mean infection scores between 13.5 to 15.0%. These will be evaluated further. A novel batch of B genome hexaploids have been produced that may have potential for scab resistance. These are AABBBB and result from durum x *Ae. speltoides* combinations.

Another strategy in place is attempting to incorporate the resistant D genome diversity into the A genome via homoeologous exchange facilitated by the *ph1c* genetic durum stock 'Capelli'. Cytological evidence from F_1 hybrids validate A and D genome chromosome pairing since 7 bivalents are commonly observed. The univalents are identified as B genome chromosomes due to their C-banded giemsa stained sites.

CONCLUSIONS

- *Ae. tauschii* is a valuable source of genetic diversity for resistance to Fusarium head scab in bread wheat. The resistance is distributed over several accessions.

- Synthetic hexaploid wheats derived from *T. turgidum x Ae. tauschii* crosses express moderate levels of diversity for scab resistance equivalent to resistance levels in the best bread wheat cultivars.

- This resistance has been transferred to elite-but-susceptible bread wheat cultivars.

- Some advanced BW/SH derivatives possess resistance to scab Types I-IV, coupled with resistance to other important biotic stresses.

- The most promising line— the multiple disease resistant Mayoor//TK SN1081/ *Ae. tauschii* (222)—has been crossed with Flycatcher (susceptible) and a DH population developed from the F_1 progeny for molecular mapping for several stresses and for phenotyping.

- Tertiary pool diversity for scab identified in some *Thinopyrum* and *Leymus* species is being introgressed into bread wheat using cytogenetic transfer protocols associated with *ph* manipulation and molecular diagnostics.

- Durum improvement is being addressed via AAAABB, AABBBB hexaploids and by D genome to A genome homoeologous transfers.

REFERENCES

Delgado, R., A. Cortés, L.I. Gilchrist, and A. Mujeeb-Kazi. 2000. Status of alien genetic diversity for scab resistance in bread wheat. Amer. Soc. Agron. 92nd. Ann. Meetings, Minnesota, p. 98.

Mujeeb-Kazi, A., L.I. Gilchrist and R. Delgado. 2000. New synthetic hexaploids and a set of bread wheat/ synthetic hexaploid derivatives as sources for scab resistance. Annual Wheat Newsletter. 46:81.

Table 1. Promising D genome synthetic hexaploids screened for head scab (Type II) at Toluca, Mexico.

Germplasm pedigree	1999	2000
YUK/*Ae. tauschii* (217)†	11.4	11.8*
68.111/RGB-U//WARD/3/FGO/4/RABI/5/*Ae. tauschii* (629)	11.9	10
68.111/RGB-U//WARD/3/FGO/4/RABI/5/*Ae. tauschii* (878)	12.4	13.1
68.111/RGB-U//WARD/3/FGO/4/RABI/5/*Ae. tauschii* (882)	11.1	13.6
SORA/*Ae. tauschii* (884)	12.9	13.5
68.111/RGB-U//WARD/3/FGO/4/RABI/5/*Ae. tauschii* (890)	11.4	14.1
CETA/*Ae. tauschii* (895)	10.8	13.2
GAN/*Ae. tauschii* (180)	10.7	10.9
LCK59.61/*Ae. tauschii* (313)	11.5	12.2
SCOOP 1/*Ae. tauschii* (358)	12	13.9
YUK/*Ae. tauschii* (217)	11.4	11.8
TRN/*Ae. tauschii* (700)	13.4	13.7
DOY1/*Ae. tauschii* (333)	11.1	13.9
DVERD_2/*Ae. tauschii* (1027)	14.6	11.7
MAYOOR//TK SN1081/*Ae. tauschii* (222)	11.7	5.7
FLYCATCHER (Mean across years)		33.8
SUMAI-3 (Mean across years)		12
ALTAR 84		40.8
* Percentage score means from 10 spikes tested. † *Ae. tauschii* accession in wide crosses working collection.		

Table 2. Some promising BW/SH derivatives tested in Toluca for the various Scab resistance categories (Type I to IV) and grain finish over 2 years in Toluca.

Lines	Type I* 1998-1999	Type II* 1998-1999	DON (ppm)	Test weight losses (%)	Grain (0-5)†
TURACO/5/CHIR3/4/SIREN//ALTAR 84/*Ae. tauschii* (205)/3/3*BUC CASS94Y00034S-24PR-2B-0M-0FGR-0FGR-0FGR	8	9.9	0.6	5.3	2
BCN//DOY1/*Ae. tauschii* (447) 0FRG	9.6	10.1	1	2.6	1
MAYOOR//TK SN1081/*Ae. tauschii* (222) CASS94Y00009S-18PR-3M-0M-0FRG-0FRG-0FRG	7.3	9.9	1.2	6.1	1
MAYOOR//TK SN1081/*Ae. tauschii* (222) CASS94Y00009S-50PR-2B-0M-0FRG-0FRG-0FRG	4.1	11.7	1.2	6.5	1
SUMAI # 3 (resistant check)	3	12.9	0.3	38.6	3
FRONTANA (moderately resistant check)	11.6	22.4	2	7.7	2

* = Percent damage

† = Grain 0 = Excellent (no differences in appearance with fungicide protected grain).

Table 3. Scab screening (Type II) of promising intergeneric amphiploids and backcross I fertile combinations at Toluca, Mexico.

Germplasm pedigree	2000
Amphiploids	
CS/*Th. elongatum* (2n=8x=56)	6.2*
CS/*Th. scirpeum* (2n=10x=70)	7.5
CS/*Th. bessarabicum* (2n=8x=56)	6.5
Th. elongatum /GOSHAWK (2n=8x=56)	15.2
Backcross I Self Fertiles	
CS/*Th. curvifolium* //PAVON (2n=8x=56)	14.8
CS/*Th. scirpeum* //CIANO 79 (2n=8x=56)	5.2
CS/*Th. scirpeum* //PAVON (2n=8x=56)	10.6
* Percentage score means from 10 spikes tested.	

FUSARIUM HEAD BLIGHT REACTION OF DURUM WHEAT LINES CONDITIONED BY CHROMOSOME SUBSTITUTIONS FROM *TRITICUM TURGIDUM* L. VAR. *DICOCCOIDES*

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ABSTRACT

Fusarium head blight (FHB) is a serious disease problem on durum wheat (*Triticum turgidum* L. var. *durum*). As far as has been reported to date, the resistance to FHB available in hexaploid wheat sources has not successfully been transferred to durum — a tetraploid wheat. Wild emmer (*Triticum turgidum* L.var. *dicoccoides*) is a wild tetraploid wheat that possesses many interesting traits including some unique disease resistances. In the 1980's, USDA geneticist L.R. Joppa produced a set of disomic substitution lines derived from 'Langdon' durum, each with a different pair of chromosomes from *T. t. dicoccoides* substituted for the corresponding durum chromosomes. We tested these lines for FHB response by inoculation with *Fusarium graminearum* under controlled conditions in several repeated experiments. One of the lines, LDN(DIC-3A), was significantly less susceptible and another, LDN(DIC-2A), was significantly more susceptible in all trials when compared to the 'Langdon' durum parent, which itself showed an intermediate FHB reaction. Several other substitution lines were significantly more or less susceptible than 'Langdon' in some experiments. Since each line differs by an entire chromosome pair, the results suggest that FHB resistance genes are on several different chromosomes in durum. Chromosome 3A is not among those which have been identified in recent papers as a site of resistance genes in hexaploid wheat. (This poster was presented at the 1999 American Phytopathological Soc. Annual Meeting in Montreal, Canada. This abstract in slightly different form was published in Phytopathology 89:S74)

INHERITANCE OF RESISTANCE TO FUSARIUM HEAD BLIGHT IN SPRING WHEAT F-1 HYBRIDS

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ABSTRACT

Fusarium Head Blight (FHB), caused by *Fusarium graminearum*, has occasioned serious economic loss in spring cereals in the north-central United States. As a result, resistance to FHB has become a high priority for spring wheat breeding. In replicated greenhouse experiments, we tested the FHB reactions of reciprocal F-1 hybrid wheats from seven crosses between four FHB-resistant parents and four susceptible adapted lines. Resistant parent lines were removed by one, two, or three breeding cycles from the Chinese line “Sumai3”, the original resistance source. For all crosses the FHB reaction of the F-1’s were intermediate between the resistant and susceptible parents (Table 1). The reciprocal F-1’s did not differ from each other. In development of hybrid wheat requiring FHB resistance, both parents will likely have to carry resistance if Sumai3 derived resistance sources are to be used. (This poster was presented at the International Symposium on Wheat Improvement for Scab Resistance, Suzhou and Nanjing, China, May 5-11, 2000. The paper is in those Proceedings p.94-97.)

Table 1. Fusarium Head Blight reaction of resistant and susceptible parents and reciprocal F-1 hybrid spring wheat lines inoculated with *Fusarium graminearum*.

Genotype	FHB incidence	FHB severity (%)	Visual FDK (%)
RESISTANT PARENT	0.45	11.1	0.8
F-1 (female=RESISTANT)	0.53	28.3	3.6
F-1 (female=SUSCEPTIBLE)	0.53	30.5	2.9
SUSCEPTIBLE PARENT	0.74	63.2	11.1
FLSD(.05)	0.12	10.4	1.9

INHERITANCE OF SCAB RESISTANCE IN SAPPORO HARU KOMUGI JUGO

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ABSTRACT

A spring wheat cultivar, Sapporo Haru Komugi Jugo (PI 81791) originated from Japan, has shown consistent resistant response to scab or Fusarium head blight (FHB) in various tests under greenhouse and field environments. Inheritance of FHB resistance was investigated in a cross using Wheaton as the susceptible parent. Backcross F_2 (BCF_2) families were evaluated in the greenhouse for their response to point-inoculation. BCF_2 plants with low disease severity were harvested and F_3 plants were re-evaluated for FHB reaction to confirm the resistance in F_2 plants. Frequency distribution of the number of heterogeneous resistant and homogenous susceptible BCF_2 families suggested that three dominant genes may be involved in FHB resistance in Sapporo Haru Komugi Jugo.

FUSARIUM HEAD BLIGHT RESISTANT SOURCES OF SPRING WHEAT IDENTIFIED FROM THE USDA COLLECTION

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INTRODUCTION

The use of resistant cultivars in wheat will be one of the major components in managing Fusarium head blight (FHB). Breeding for resistance is, however, hindered by a lack of adequate resistant sources. Identifying additional sources of resistance and incorporating new resistances are critical for diversifying the current resistance gene pool and for enhancing the level of resistance. Since 1998, we have begun to characterize variations for FHB resistance in the USDA National Small Grain Collection, focusing on spring wheat accessions from regions where FHB has historically been problematic. This report summarizes the methodology, resistant selections made from previous years, and observations of rust reactions of these selections.

MATERIALS AND METHODS

In the past three years, we have been working towards the development of a germplasm evaluation and enhancement scheme from which reliable data can be derived while maintaining the efficiency of screening operation and the accessibility of the selected germplasm. The scheme is illustrated (Fig. 1) and described as following.

1. Entries were planted into single 4-foot row plots in a field nursery. The nursery was inoculated with Fusarium-colonized corn grains at a weekly interval for four consecutive weeks beginning at the early jointing stage of plant development. Ground was maintained moist through irrigation to promote perithecial development. Entries at anthesis were tagged and inoculated with a conidial suspension (50,000-75,000 conidia/ml) using a sprayer. A second spray-inoculation was applied seven days later. The nursery was mist-irrigated following a schedule of 3-min misting with 30-min recess between 8:00pm and 9:00am during the course of inoculation. We used this intense inoculation protocol to generate high disease pressure throughout the evaluation period to select for high levels of resistance and to prevent disease escape due to late tillering or differences in heading dates. ND 2710, BacUp, Wheaton and Sonalika (with different levels of resistance/ susceptibility and maturity) were used as checks and check-to-entry ratio was 1:37.

Disease severity (% of infected spikelets) and incidence (% of infected spikes) were recorded 14 to 20 days after the first spray inoculation on 20 spikes/plot, depending upon the disease development. Entries (or plants within an entry) with a low disease index (severity*incidence) were selected for further testing. In addition to selections based on FHB index, entries with good seed setting were selected. Selections from PSN were increased in an off-season nursery in New Zealand.

2. Field selections were evaluated in the greenhouse in the fall and again in the spring by spray and point inoculation. These evaluations were designed 1) to verify the resistance of field selections, and 2) to characterize the resistance types. Approximately 30-50 plants were evaluated in each of the two greenhouse seasons. Plants were grown in pots and inoculated at anthesis. Inoculated plants were incubated in a mist-chamber for 48 hours. Although selections were made to derive the entries of the Elite Germplasm Nursery (EGN) for next year, the selection pressure was minimal at this stage in order to avoid eliminating genotypes with good field resistance.

3. Greenhouse evaluations of PSN selections were used to derive entries for the EGN of next year. Entries were planted in 4-foot rows and replicated three times in a randomized complete block design. In 2000, 160 lines selected from 1998 and 1999 PSNs were tested in EGN with a check-to-entry ratio of 1:26. Nursery management (tagging of flowering stage, inoculation and irrigation) and check varieties were the same as that of PSN. Fusarium head blight incidence and severity were recorded. All entries were harvested for scoring seed infection, yield (weight per row), and volume-weight. Selected entries with low or moderate FHB reactions were tested for DON using bulked seed (among reps). Selections from EGN will be evaluated again in the next year's EGN. Entries of EGN were also evaluated for stem rust and leaf rust in field rust nurseries.

4. Five to six most elite selections from EGN were entered into the Uniform Regional Scab Nursery for spring wheat. This allowed evaluations of the elite selections over multiple locations, and provided the accessibility to individual programs for crossing.

RESULTS AND DISCUSSION

Evaluation data (FHB index converted into a 0-9 scale) from the 1998, 1999, and 2000 PSNs were reported in the GRIN database (USDA-ARS, National Genetic Resources Program, Germplasm Resources Information Network, www.ars-grin.gov/cgi-bin/npgs/html/desc.pl?65066). Lines with low FHB reactions (disease index <40% and tombstone <40%) were considered as elite selections (Table 1). Lines with intermediate FHB reactions (disease index <55% and tombstone <55%) are given in Table 2. Lines with low disease index but high tombstone percentage (>55%), or vise versa, were also listed in the intermediate group. In the elite selection group (Table 1) the correlation coefficients were highly significant between disease index and %tombstone (r = 0.83) and between disease index and DON content (r = 0.74). The correlation coefficient between %tombstone and DON content was also significant (r = 0.68). Disease index and %tombstone was not correlated in the intermediate group (Table 2). Some of the selections possess resistance to leaf rust and/or stem rust under natural rust infections (Table 1 & 2).

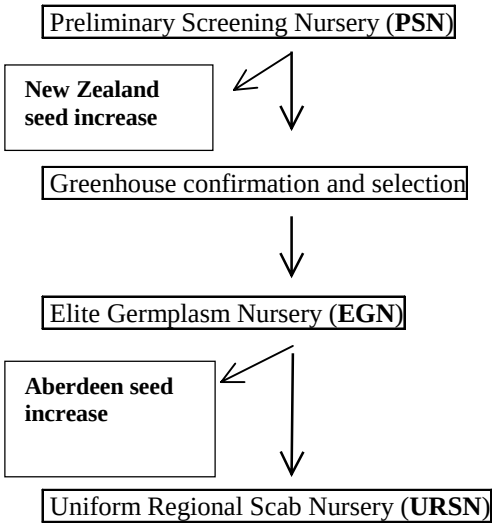

Figure 1. A scheme used for identifying Fusarium head blight resistance in spring wheat.

Table 1. Spring wheat germplasm selections with low FHB reaction from the 2000 Elite Germplasm Nursery.

Accession	ID	Origin	Improv. Status	FHB index (%)	Tombstone (%)	DON (ppm)	Leaf[d] rust	Stem[d] rust
PI 382167	16-52-9	Brazil	breed. mat.[e]	10.9	23.3	5.5	tR	60MS
PI 382161[a]	Tokai 66	Brazil	cultivar	11.2	16	1.9	90S	80S
PI 349478[b]	193C	Switzerland	landrace	11.5	10	4.4	-[g]	-
PI 382154	Nyu Bai	Japan	landrace	11.9	15	1.5	90S	80S
CItr 5103	274	Argentina	landrace	13.1	19	16.5	90S	90S
PI 350768[b]	69Z108.42	Austria	landrace	14.3	21.7	6.1	tMS	0
PI 382140[b]	Abura	Brazil	cultivar	15.1	38.3	3.3	10MS	0
PI 214392	Colotana 266/51	Brazil	cult. mat.[f]	15.7	30	16.3	-	-
PI 382153	Nobeoka B.	Japan	landrace	18.1	13.8	4.8	90S	60S
PI 462151	S. C. W. No. 3	China	cult. mat.	20	18.8	4.2	60S	80S
PI 81791[a]	Sapporo H. K. J.	Japan	cultivar	20.5	21.7	18	30MS	30S
PI 294975	Artemowska	Bulgaria	cult. mat.	24.5	20	11.4	-	-
PI 163429		Argentina	cult. mat.	24.9	30	12	90S	60S
PI 345731	Tezanos P. P.	Argentina	cultivar	30.4	20	12	80S	tMS
PI 264927	220	Greece	landrace	31.9	16.7	5.6	80S	50MS
PI 185380[b]	Prodigio I.	Italy	cult. mat.	32.2	27.5	11.2	90S	0
PI 192660	Prodigio I.	Italy	cult. mat.	34.5	22.5	16.4	90S	10MR
PI 285933	Chudoskaja	Poland	cult. mat.	34.7	26.7	21.8	80S	80S
PI 362437	III/14-B	Yugoslavia	landrace	35.5	33.3	8.9	90S	60S
PI 351743	Cluj 49-926	Romania	cultivar	39.1	26	9.1	90S	50MS
CItr 11215	Belgrade 4	Yugoslavia	cult. mat.	39.6	35	9.2	80S	70MS
CItr 17427	16-52-2	Brazil	breed. mat.	39.7	33.3	19.4	10MR	70S
PI 104131[b]	Excelsior	Argentina	cultivar	40.6	21.7	7.6	90S	70MS
PI 519798	PF 79782	Brazil	breed. mat.	40.7	27.5	14.6	40MS	0
PI 256958	Academia 48	Romania	cultivar	42.4	20	11.7	70S	60S
	ND 2710--ck	USA	breed. mat.	14.2±2.	22.8±8.8	5.4		
PI 596533	BacUp--ck	USA	cultivar	25.5±7.	30.8±5.5	7.7		
PI 469271	Wheaton--ck	USA	cultivar	86.9±1.	93.3±5.4	24.3		
PI 478282	Sonalika--ck	India	cultivar	87.1±2.	76.4±9.5	37		

[a] Lines were tested in the 2000 Uniform Region Scab Nursery for spring wheat.

[b] Lines will be tested in the 2001 Uniform Region Scab Nursery for spring wheat.

[c] DON of bulked seed (among reps) was tested by Dr. Paul Schwarz at North Dakota State University.

[d] Observations on leaf and stem rust reactions were based on natural infection in field rust nurseries.

[e] Breeding material.

[f] Cultivated material.

[g] Not tested.

Table 2. Spring wheat germplasm selections with intermediate FHB reaction from the 2000 Elite Germplasm Nursery.

Accession	ID	Origin	Improv. status	FHB index (%)	Tombstone (%)	Leaf rust	Stem rust
PI 519434	PF 82192	Brazil	breed. mat.	15.3	53.3	30s	10MS
PI 519790	274-1-118	Uruguay	breed. mat.	19.3	40.0	60S	5S
PI 434987	Estanzuela Y.	Uruguay	cultivar	19.6	58.0	30S	70S
PI 182583	Chuko	Japan	landrace	19.9	78.8	80S	80S
PI 182561	SinChunaga	Japan	cult. mat.	20.8	86.7	90S	60S
PI 351256	Japon 2	Japan	cult. mat.	21.2	41.7	30S	80S
PI 182568	Norin 34	Japan	cultivar	21.3	46.7	100S	50S
CItr 12002	Renacimiento	Uruguay	cultivar	24.9	41.7	90S	90S
PI 411132	Gogatsu-K.	Japan	cultivar	27.6	77.5	0	40MS
PI 382144	Encruzilhada	Brazil	cultivar	29.6	45.0	40S	90S
PI 182586	Norin 43	Japan	cultivar	30.0	50.0	30S	80S
PI 182565	Haya K.	Japan	cult. mat.	31.8	53.3	5MR	80S
CItr 12021	Centenario	Uruguay	cultivar	32.2	41.7	90S	30S
PI 351816	Froment D. J.	Switzerland	cult. mat.	33.1	70.0	90S	60S
PI 337151	Magnif 100	Argentina	cultivar	34.8	46.7	90S	90S
PI 351898	B 130	Switzerland	breed. mat.	37.9	70.0	40MS	80S
PI 83729	Magyarovar 81	Hungary	cultivar	38.3	46.0	70S	70MS
PI 263422	Forlani	Yugoslavia	cultivar	39.6	56.7	70S	70MR
PI 182591	Norin 61	Japan	cultivar	40.1	66.7	-	-
PI 272348	Lontoi	Hungary	cultivar	40.2	26.7	90S	80S
CItr 13136	Rio N.	Brazil	cultivar	42.8	50.0	70S	70S
PI 264998	628	Greece	landrace	43.7	30.0	30S	10R
PI 57364	CItr 7175	China	landrace	45.0	55.0	-	-
PI 185383	3084	Argentina	cult. mat.	45.4	50.0	90S	70S
PI 584926	Pantaneiro	Brazil	cultivar	47.0	45.0	90S	0
PI 168727	Bahiense	Argentina	cultivar	47.5	25.0	90S	50S/0
PI 210869	4207-50	Brazil	breed. mat.	48.5	56.7	10R	10MR
PI 192219	Hatvani	Hungary	cultivar	48.8	36.7	90S	20MS
PI 520498	Jacui	Brazil	cultivar	49.0	43.3	5MR	30S
PI 185843	Surpresa	Brazil	cultivar	49.2	42.5	80S	20S
PI 351187	T. V. S.	Switzerland	breed. mat.	49.5	26.7	80S	80S
PI 203083	Wabian	Paraguay	cult. mat.	51.1	32.5	10MR	20MR
PI 163428		Argentina	cult. mat.	51.8	46.7	-	-
PI 352000	Z.89.37	Switzerland	breed. mat.	52.2	26.7	90S	30MS
PI 344467	Oncativo I.	Argentina	cultivar	53.1	38.8	10MS	10R
PI 192634	Trintecinco	Brazil	cultivar	53.7	41.7	90S	10MR
PI 233207	Odesskaja 13	Ukraine	cultivar	54.3	32.5	60S	40MS
PI 349534	533B	Switzerland	landrace	54.3	26.7	90S	60S
PI 214394	Colotana 1085/50	Brazil	breed. mat.	54.7	30.0	-	-
PI 264940	111a	Greece	landrace	55.3	41.7	5R	5R
PI 351476	Vaulion	Switzerland	cultivar	55.8	25.0	90S	50MS
PI 344465	Laureano A. L.	Argentina	cultivar	56.0	36.7	5MS	40S
CItr 2492	Manchurian	China	landrace	57.0	25.0	-	-
PI 184221	BR 5480	Yugoslavia	landrace	57.5	45.0	70S	50MS
PI 192229	Gran C. U.	Romania	landrace	57.7	31.7	80S	30MS
PI 584934	Whestphalen	Brazil	cultivar	57.8	41.3	tR	tR
PI 352118	62 FF 70	Switzerland	breed. mat.	58.1	50.0	5MR	70MS
PI 351993	Z.88.54	Switzerland	breed. mat.	58.4	30.0	70S	30MR
PI 168716	Klein Condor	Argentina	cultivar	58.9	35.0	70S	60MS
PI 352062	Vivela Mar	Argentina	cult. mat.	58.9	50.0	70MR	70S

Table 2. (continued)

Accession	ID	Origin	Improv. status	FHB index (%)	Tombstone (%)	Leaf rust	Stem rust
PI 362043	Arnaut De T.	Romania	cultivar	59.6	23.3	90S	30MS
PI 344454	Buck Austral	Argentina	cultivar	60.0	28.8	10MR	50S
PI 192498	IV C…	Argentina	cultiv. mat.	61.8	47.5	60S	80MS
PI 185216	3111	Argentina	cultiv. mat.	61.9	50.0	10MR	60MS
PI 113949	Stepnjachka	Ukraine	cultivar	63.0	38.3	30S	30MR
PI 168653	Klein C	Argentina	cultivar	63.2	46.7	20S	70MS
PI 113948	Kooperatorka	Ukraine	cultivar	64.3	26.3	50MS	40MS
CItr 14371	8475-59	Brazil	breed. mat.	65.6	36.7	80MS	10MR
PI 559677	Ljutseune 76	Yugoslavia	cultiv. mat.	69.3	50.0	90S	80S
PI 349448	1882B	Switzerland	landrace	71.3	43.3	80S	60MS
PI 57363	163	China	landrace	72.2	41.7	90S	70S
PI 184512	H 51	Argentina	breed. mat.	76.7	33.3	20MR	40MR
PI 192647	Granadero	Argentina	cultivar	77.0	48.3	60S	40MS
PI 349447	1882A	Switzerland	landrace	77.3	20.0	-	-
PI 351649	263.25-2	Switzerland	breed. mat.	78.3	46.7	90S	40S

GEOGRAPHICAL DISTRIBUTION AND PEDIGREE ANALYSIS OF FUSARIUM HEAD BLIGHT RESISTANT SELECTIONS FROM THE USDA SPRING WHEAT GERMPLASM COLLECTION

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INTRODUCTION AND OBJECTIVES

Spring wheat selections with low and intermediate Fusarium head blight (FHB) reactions reported by Zhang et al. (this Proceeding) represented a diverse pool of germplasm. The objective of this research was to analyze the geographical distribution and pedigree of the resistant selections. Information obtained from such analysis may help in identifying potential new gene pools for FHB resistance.

MATERIALS AND METHODS

A total of 1405 accessions of spring wheat with diverse origin (Table 1) were evaluated in the Preliminary Screening Nurseries (PSN) in 1998 and 1999. Nursery management, inoculation techniques, and selection criteria are described by Zhang et al. (this Proceeding). Selections of PSN were evaluated in the Elite Germplasm Nursery (EGN) in 2000. This report analyzed the geographical distribution and pedigree of the resistant selections from EGN based on available information in GRIN (USDA-ARS, National Genetic Resources Program, Germplasm Resources Information Network).

RESULTS AND DISCUSSION

Geographical distribution of the FHB resistant selections. Table 1 lists the geographical distribution of the spring wheat germplasm evaluated and the frequency of selections. Selections from South America contributed to 43.6% of the total selections, followed by Europe (39.4%). While resistance from Asian germplasm has been recognized and used in breeding worldwide, only 16.5% of the total selections were from Asia. The results suggest that South America and Europe may possess FHB resistant gene pools, which may have been under-exploited.

Resistance lines were identified from most of the countries tested (Table 1). In Asia, resistance better or equal to Sumai 3 was not identified in test collections from China, while some selections from Japan were better than or equal to reported Japanese resistant lines. In Europe, resistance from Austria, Bulgaria, Greece, Hungary, Poland, Romania, Switzerland, Ukraine, Yugoslavia in addition to Italy was identified. Among the European countries, Romania ranked the first in terms of ratio of number of selected lines to the number of tested, Switzerland ranked the first in the number of resistant lines. The highest number of elite selections (2 lines/country) was from Italy, Romania, and Yugoslavia. In South America,

Argentina ranked the first for the ratio of selected lines to the number of lines tested, whereas the highest number of elite selections (6 lines) was from Brazil. The fact that resistant lines were identified from various countries may indicate that diversity of FHB resistant sources exists, and systematic search and characterization based on the country of origin may prove beneficial.

Pedigree analysis of the resistant selections. Of the 94 resistant selections, eighteen were landraces (Table 2). Pedigree information was obtained on 42 cultivars or breeding materials. Pedigree analysis in those 42 lines revealed that Frontana (Fronteira/Mentana) appeared as the primary or secondary parent of nine selections, Shinchunaga in four selections, and Mentana in two selections (Table 3). Resistant selections from this study, including Tokai 66, Surpresa, and Centenario, were parents of several other selections. Selections of landraces and cultivars released prior to 1940 are given in Table 2. Although pedigree of a landrace is not clear, the landraces may provide the most diversity for potentially new genes for FHB resistance. Similarly, old cultivars may be more valuable for providing potentially new resistance genes.

REFERENCES

Zhang, X., Y. Jin, R. Rudd, T. Hall, J. Rudd, and H. Bockelman. 2000. Fusarium head blight resistant sources of spring wheat identified from the USDA collection. (This proceeding)

Zhang, X., Y. Jin, R. Rudd, J. Rudd, and H. Bockelman. 1999. Screening of spring wheat scab resistance from the USDA germplasm collection. P 140-142. *In* Proc. 1999 National Fusarium Head Blight Forum. Sioux Falls, SD. Dec. 5-7, 1999.

Table 1. Number of spring wheat lines tested and selected from each country for low and intermediate Fusarium head blight reaction.

Country	Accessions tested	Selections			Composed of total selections (%)
		Elite	Interm.	% selected	
Asia	**149**	**4**	**12**	**10.3**	**16.5**
China	73	1	4	6.8	
Japan	76	3	8	14.5	
Europe	**887**	**11**	**26**	**4.2**	**39.4**
Austria	174	1	0	0.0	
Bosnia and Herzego	22	0	0	0.0	
Bulgaria	12	1	0	8.3	
Czech Republic	28	0	0	0.0	
Greece	43	1	2	7.0	
Hungary	32	0	3	9.3	
Italy	123	2	2	3.3	
Poland	45	1	0	2.2	
Romania	29	2	2	13.8	
Switzerland	291	1	11	4.1	
Ukraine	26	0	3	11.5	
Yugoslavia	62	2	3	8.1	
South America	**369**	**10**	**31**	**11.1**	**43.6**
Argentina	130	4	14	21.5	
Brazil	179	6	12	12.3	
Paraguay	9	0	1	11.1	
Uruguay	21	0	4	19.5	
Others	30	0	0	0.0	
Total	**1405**	**25**	**69**	**6.7**	**100.0**

Table 2. Fusarium head blight resistant selections of landraces and cultivars released or received by National Small Grains Collection (NSGC) before 1940.

Accession	ID	Group	Status	NSGC received year
CItr 5103	274	Elite	Landrace	1916
PI 264927	220	Elite	Landrace	1960
PI 349478	193C	Elite	Landrace	1970
PI 350768	69Z108.42	Elite	Landrace	1970
PI 362437	III/14-B	Elite	Landrace	1971
PI 382153	Nobeoka Bozu	Elite	Landrace	1973
PI 382154	Nyu Bai	Elite	Landrace	1973
CItr 11215	Belgrade 4	Elite	Cultivated	1929
PI 81791	Sapporo Haru K.	Elite	Cultivar	1929
PI 104131	Excelsior	Elite	Cultivar	1934
CItr 2492	Manchurian	Intermediate	Landrace	1904
PI 57363	163	Intermediate	Landrace	1923
PI 57364	CItr 7175	Intermediate	Landrace	1923
PI 182583	Chuko	Intermediate	Landrace	1949
PI 184221	BR 5480	Intermediate	Landrace	1949
PI 192229	Gran Commune Ungerese	Intermediate	Landrace	1950
PI 264940	111a	Intermediate	Landrace	1960
PI 264998	628	Intermediate	Landrace	1960
PI 349447	1882A	Intermediate	Landrace	1970
PI 349448	1882B	Intermediate	Landrace	1970
PI 349534	533B	Intermediate	Landrace	1970
CItr 12002	Renacimiento	Intermediate	Cultivar	1936
CItr 12021	Centenario	Intermediate	Cultivar	1938
PI 113948	Kooperatorka	Intermediate	Cultivar	1936
PI 113949	Stepnjachka	Intermediate	Cultivar	1936
PI 132856	Mentana	Intermediate	Cultivar	1939
PI 83729	Magyarovar 81	Intermediate	Cultivar	1930

Table 3. Fusarium head blight resistant selections with known resistant parent in their pedigrees.

Accession	ID	Origin	Known resistant parent
PI 584934	Whestphalen	Brazil	Frontana, Tokai 66
PI 214392	Colotana 266/51	Brazil	Frontana
PI 345731	Tezanos Pintos Precoz	Argentina	Frontana
CItr 14371	8475-59	Brazil	Frontana
PI 214394	Colotana 1085/50	Brazil	Frontana
PI 352118	62 FF 70	Switzerland	Frontana
PI 434987	Estanzuela Young	Uruguay	Frontana
PI 519790	274-1-118	Uruguay	Frontana
PI 520498	Jacui	Brazil	Frontana
CItr 12470	Frontana	Brazil	Mentana
PI 344465	Laureano Alvarez Laah	Argentina	Mentana
PI 182591	Norin 61	Japan	Shinchunaga
PI 182568	Norin 34	Japan	Shinchunaga
PI 182586	Norin 43	Japan	Shinchunaga
PI 411132	Gogatsu-Komugi	Japan	Shinchunaga
CItr 13136	Rio Negro	Brazil	Surpresa, Centenario

A PROTOCOL FOR MARKER-ASSISTED SELECTION OF A FUSARIUM HEAD BLIGHT RESISTANCE GENE DERIVED FROM SUMAI 3

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OBJECTIVES

Develop a marker-assisted selection protocol for an FHB resistance gene in wheat

INTRODUCTION

Screening for Fusarium head blight (FHB) resistance using greenhouse and field-based screening are made difficult by quantitative inheritance, laborious screening methods, and environmental effects. Selection for molecular markers linked to resistance genes may be a more effective means of screening. We have identified quantitative trait loci (QTL) for *Fusarium* head blight (FHB) resistance in two wheat populations (Waldron et al., 1999; Anderson et al., 2001). The most significant QTL for FHB was located on the short arm of chromosome 3B and designated *Qfhs.ndsu-3B*. The best markers in this region explain 25 to 42% of the variation for FHB resistance in the Sumai/Stoa and ND2603/Butte 86 recombinant inbred populations, respectively (Anderson et al., 2001). Moreover, the selection for this region results in a significant skewing of the populations toward more resistant types (Anderson et al., 2001, Fig. 1).

MATERIALS AND METHODS

Plant materials were from the U of M spring wheat breeding program and consisted of 8,829 F_3:F_4 headrows derived from 256 families. The 71 parents of families believed to be segregating for Sumai 3-derived FHB resistance genes were screened with 3 SSR markers that flank *Qfhs.ndsu-3B* [gwm533, gwm493 (Röder et al., 1998) and BARC87 (Q. Song and P. Cregan, USDA-ARS, Beltsville, MD] (Fig. 2). A 0.5 mm^2 segment of leaf tissue from five plants each of 3,370 lines were collected in the field just prior to the jointing stage. Leaf tissue from the five plants was bulked, placed in an eppendorf tube on ice and stored at -20ºC. After selection in the field for rust resistance and other agronomic traits, 870 of the 3,370 lines were screened for their allele type at each of the SSR markers. PCR amplification was as described by Röder et al. (1998) except 35 cycles of amplification were used instead of 45 in a reaction volume of 10µl. Visualization of fragments was by electrophoresis in 5% polyacrilimide gels and silver staining according to the protocol of Bassam et al. (1991) or using a LiCor sequence analyzer following manufacturer instructions.

For those lines homozygous for the presence of *Qfhs.ndsu-3B* a single plant was harvested. For those lines heterogeneous for the presence of *Qfhs.ndsu-3B*, three plants were harvested and five seeds from each plant were germinated on filter paper in the lab. Coleoptiles

were harvested from these seedlings and tested for their allele type at the three SSR loci. To speed the process of analyzing these samples, we developed a protocol for using a 96-pin clone replicator to disrupt tissue during DNA extraction. The full protocol is listed below:

Harvest approximately 1/2 to 3/4-inch of tissue from leaf tips, directly into microtiter plate (square microtiters are 650µl polystyrene, Whatman cat. no. 7701-1651) on ice. Tissue is rolled up to fit in wells. Length depends on width (age) of leaf. Freeze tissue at -20ºC until ready to extract.

Prepare another sterile microtiter plate with 200 µl 5X TE in each well.

Add 200 µl 0.25 M NaOH directly to wells containing leaf tissue with multi-channel pipette.

Grind tissue in NaOH with clone replicator for 2-3 minutes, until tissue is sufficiently lysed. Older tissue generally requires more grinding. Green color in the liquid is a good indicator of sufficient grinding.

Immediately transfer 15 µl of crude extract to plate containing 5X TE, using multi-channel pipette.

DNA is diluted 1:5 into ddH_2O directly for PCR. Further dilution may be required (1:10 or 1:20) for amplification with some primers, in cases where troubleshooting is necessary.

Results and Discussion: Our early generation FHB screening involves point inoculations in the greenhouse of 5 plants from each of the selected headrows. This is followed by a repeat screening of those genotypes that were moderately resistant or better. The selected lines are then advanced to field screening nurseries at 2-3 locations. Of the 870 lines screened with the SSRs, 300 were homozygous containing *Qfhs.ndsu-3B*, 157 were heterogeneous, and 413 were homozygous missing the QTL. After marker-assisted selection, we were able to discard more than 400 lines that did not contain this QTL. We expect a high proportion of the 457 lines heterozygous or homozygous for presence of the QTL to have some FHB resistance.

Using the LiCor DNA analyzer, we estimate our costs for the marker screening at approximately $0.90 for the first SSR run on a genotype, including all consumables and labor. This is reduced to about $0.50 per datapoint for additional SSR on the same genotype as a result of savings in the tissue collection and DNA extraction protocols. Using the DNA extraction protocol given above, one person can prepare PCR-ready DNA from leaf tissue from at least 400 lines in one day. The most time-consuming part of the DNA extraction process is the collection of leaf tissue into the microtiter plates.

Our experience with field and lab collection of leaf tissue leads us to believe that saving and germinating remnant seed in the lab is a more efficient procedure. The drawback is that remnant seed must be saved, which we normally don't do with our headrow selections. This will require a common identifier for each headrow and remnant seed envelope.

We plan to test the effectiveness of this FHB screening procedure using a portion of the heterozygous F_4 individuals identified in this research. F_4:F_5 lines near-isogenic for the chromosome 3BS QTL have been isolated. One hundred of these lines (50 homozygous with the QTL and 50 sib-lines without the QTL) plus checks will be tested under greenhouse conditions and in field FHB nurseries. The FHB reaction of these lines with and without the QTL will be compared to judge the effectiveness of this selection procedure. We are also backcrossing this QTL in advanced lines that do not contain this gene.

REFERENCES

Anderson, J.A., R.W. Stack, S. Liu, B.L. Waldron, A.D. Fjeld, C. Coyne, B. Moreno-Sevilla, J. Mitchell Fetch, Q.J. Song, and R.C. Frohberg. 2001. DNA markers for Fusarium head blight resistance QTLs in two wheat populations. Theor. Appl. Genet., in press.

Bassam, B.J., G. Caetano-Anolles, and P.M. Gresshoff. 1991. Fast and sensitive silver staining of DNA in polyacrylamide gels. Analytical Biochem. 196: 80-83.

Röder, M.S., V. Korzun, K. Wendehake, J. Plaschke, M.-H. Tixier, P. Leroy, and

M.W. Ganal. 1998. A microsatellite map of wheat. Genetics 149:2007-2023.

Waldron, B.L., B. Moreno-Sevilla, J.A. Anderson, R.W. Stack, and R.C. Frohberg. 1999. RFLP mapping of QTL for *Fusarium* head blight resistance in wheat. Crop Sci. 39:805-811.

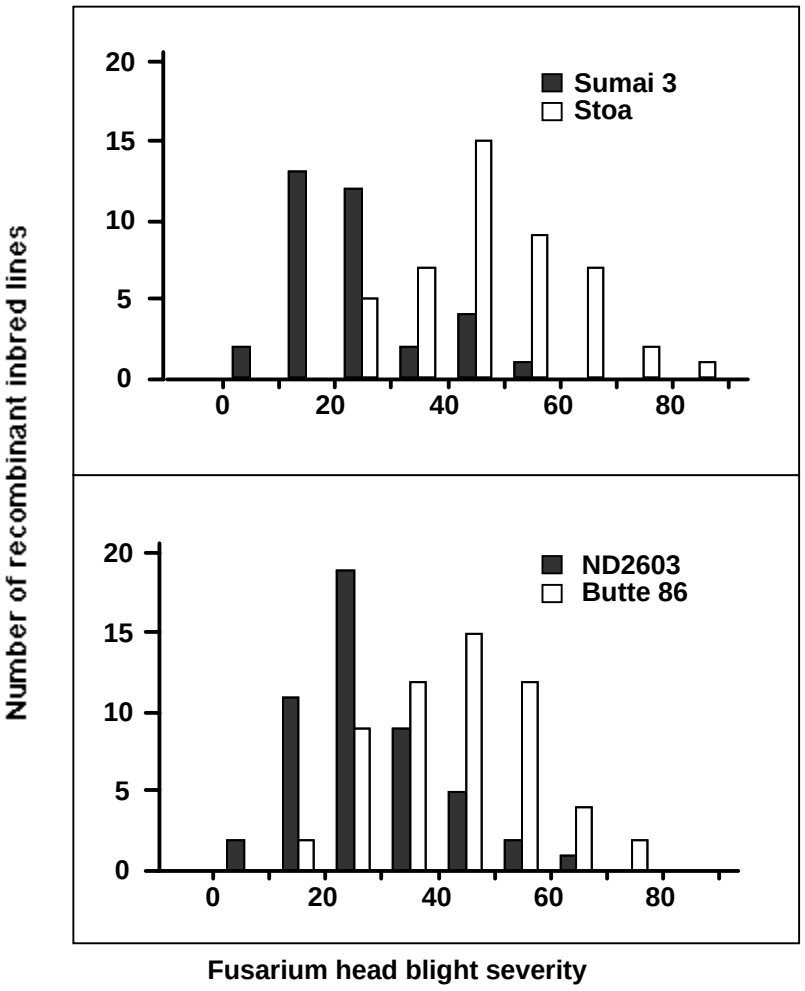

Figure 1. Histograms of Fusarium head blight severity for RI lines with resistant or susceptible parent alleles in the *Qfhs.ndsu-3BS* QTL region. Only those genotypes homozygous for this interval are included, bound by markers *Xgwm493* and *Xgwm533*.

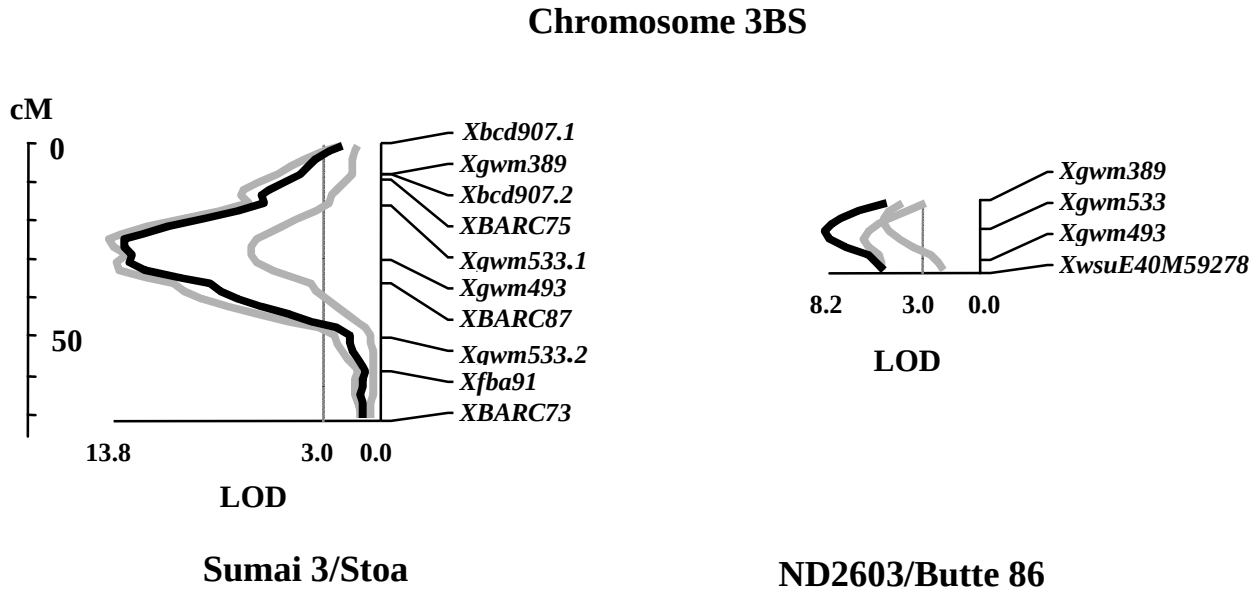

Figure 2. Interval analysis of data for chromosome 3B for Fusarium head blight resistance in the Sumai 3/Stoa and ND2603/Butte 86 recombinant inbred populations. The dark contour in each map represents the mean of the two experiments. The two lighter colored contours represent individual experiments. The two maps are aligned at the *Xgwm493* locus. *Xgwm389* and *Xgwm533* also were mapped in both populations.

DEVELOPMENT OF FHB-RESISTANT CULTIVARS FOR THE MID-SOUTH

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ABSTRACT

Two complementary approaches are being pursued at the University of Arkansas to develop FHB-resistant cultivars for the Mid-south. Following a severe outbreak of FHB in 1991, the breeding program initiated a crossing program between adapted soft wheat genotypes and 22 FHB lines from CIMMYT and Eastern Europe. These populations were advanced as bulks for 5 years. Then lines were developed using pedigree selection and were evaluated in headrows for their adaptability and resistance to other diseases. F_7 lines representing 50 populations were advanced and evaluated for FHB resistance in an inoculated screening nursery along with resistant and susceptible checks in the field in 2000. Selected lines with good adaptability and high grain yield are being tested in a replicated inoculated nursery in 2001. Four of the highest yielding lines were entered in the Uniform Winter Wheat Scab nursery. All four lines have a different resistant parent in the pedigree. In addition to field test, the selected lines will be tested in the greenhouse for Type 2 resistance. During the winter of 1999-2000, crosses were made in the greenhouse to pyramid resistance genes using F_3 Arkansas breeding lines with resistant parentage. The F_3 lines were derived from a number of adapted southern soft wheats crossed to two germplasm lines and Ernie. The F_3 lines were crossed to an adapted genotype with an alternate resistance Type. Lines from crosses between adapted genotypes and FHB resistance sources are being selected for both agronomic traits and resistance to release resistant cultivars adapted to the Mid-south as soon as possible. To develop adapted germplasm lines with high levels of FHB resistance as well as more durable types of resistance to leaf rust, stripe rust, and leaf blotch, sources of these resistances (primarily CIMMYT spring wheat cultivars and lines) were crossed in 1995 to two adapted cultivars (Agripro Mason and Pioneer 2684) with short vernalization requirement (to facilitate multiple generations per year) and photoperiod sensitivity (to confer wide adaptation). Filial, backcross (BC), and topcross (TC) populations from these crosses were grown as space-planted bulks for three seasons, and selections were made for heading date, plant type, yield potential, visual grain quality, and resistance to leaf rust, FHB, and leaf blotch. In 1999, 120 heads were selected from 117 of the best F_4, BCF_3, and TCF_3 populations and grown as headrows in 2000. After selection for agronomic traits and stripe rust resistance during 2000, 548 lines were advanced to greenhouse and multi-location testing, including two locations in Louisiana in collaboration with the LSU breeding program. During the summer of 2001, the best lines will be made available to breeders and intercrossed within the Agripro Mason and Pioneer 2684 gene pools to combine resistances.

THE NEED FOR UNIFORMITY IN DESIGNATING TYPES OF SCAB RESISTANCE

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ABSTRACT

Schroeder and Christensen (Phytopathology 53:831-838, 1963) defined two types of resistance to scab in wheat: resistance to initial infection and resistance to the spread of infection within a plant. These types have subsequently been designated respectively as types 1 and 2, types I and II or types a and b. The two types have been widely accepted, although the mechanisms of resistance are unknown. Several additional types of resistance have been postulated but without agreement among laboratories on either definition or on the sequence of numbering or lettering. For example, type 3 (sometimes designated III or c) has been used variously to designate resistance that limits trichothecene toxin accumulation (Wang and Miller, J. Phytopathol. 122:118-125, 1988), kernel infection (Mesterhazy, Pl. Breeding 114:377-386, 1995), and reduction in kernel quality (McKendry et al, 1999, National Fusarium Head Blight Forum). Additional postulated types include resistance due to insensitivity to trichothecene toxins (which is sometimes combined with reduced toxin accumulation), and tolerance (yield maintenance in presence of disease). Often the definition of resistance is based on a postulated mechanism of resistance as, for example, resistance attributed to the ability of plants to degrade toxin, leading to reduced amount of toxin accumulation (Wang and Miller, J. Phytopathol. 122:118-125, 1988).

Factors that contribute to confusion among postulated types of resistance include: (1) differences among laboratories in the ways disease development, toxin accumulation, and kernel yield and quality are measured; (2) the need to deduce the amount of some postulated types of resistance from two measured qualities as, for example, disease severity and yield reduction to determine tolerance, or relative amounts of toxin and yield loss to deduce plant insensitivity to toxin; (3) differences in objectives among laboratories; e.g. a focus on mechanisms of resistance can lead to postulated types of resistance that are not feasible to measure routinely in breeding for resistance; (4) uncertainty about the role of trichothecene toxins in pathogenesis; and (5) limited available information on the physiology and (in most cases) the genetics of resistance.

Is it possible to reconcile such factors given how little we know about the mechanisms of scab resistance? The scab community needs to make a concerted effort to develop a practical, meaningful list of postulated resistance types for use by all.

ASSESSMENT AND REACTION OF *TRITICUM AESTIVUM* GENOTYPES TO *FUSARIUM GRAMINEARUM* AND ITS EFFECTS ON TRAITS RELATED TO GRAIN YIELD AND QUALITY

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RESEARCH OBJECTIVES

This is the final report of a three-year study conducted over four environments. The objectives are twofold. 1) Identify wheat genotypes, commonly grown in Virginia and Mid-Antlantic region, which consistently perform well under Fusarium Head Blight (FHB) epidemics. 2) Discern which disease assessment parameter(s) consistently correlate well with yield and test weight loss in order to save time in precise assessing losses and resistance of genotypes.

INTRODUCTION

Fusarium graminearum (Schwabe), inciting the disease known as *Fusarium* head blight, is a major pathogen of wheat in the Mid-Atlantic States. Since its discovery in the United States, the disease has pressed south and east until at present it is an annual threat for growers of winter wheat in Virginia. (Canadian Grain Commission, 2000). One of the greatest factors contributing to the increasing geographic distribution of FHB is the use of conservation tillage in the southeastern states. Virginia has been noted as a leader in this region, with 46.2%, or 110,880 acres of winter wheat grown under conservation tillage schemes in 1999-00 (VFBN, 2000).

FHB epidemics can be devastating to growers, as was the case in 1998. Yield losses for SRW wheat averaged 13.5 Bu/Ac when compared to the six year average. The economic loss from this single FHB epidemic was an estimated 8.5 million dollars (Griffey et al., 1999).

It is apparent that there are differences among genotypes not only in infection levels but also in yield response to the disease. From a producer's standpoint, it would be beneficial to know which varieties grown in the region have the least yield and quality reduction under high disease pressure. From a breeder's view, it would be helpful to know which parameters of disease assessment correlate best with losses in yield and test weight. Identification of the most predictable, reliable and feasible assessment parameter would allow breeders to focus on a specific disease assessment parameter, therefore making field ratings less time consuming.

MATERIALS AND METHODS

Twenty (1997-98) and thirty (1998-99; 1999-00) SRW wheat genotypes were grown in replicated 100 ft^2 plots using a randomized complete block design with two treatments. In the

third year of field tests two locations (Blacksburg, VA and Warsaw, VA) were utilized in order to procure an additional test site, study possible environmental differences, and test two methods of inoculation (conidial suspension versus scabby corn kernels). Replications 1-3 comprised the inoculated block and replications 4-6 the non-inoculated control throughout the three years.

Planting density was determined based on 1000 kernel weight with a target density of 24 seeds per row foot. All seed was treated prior to planting with Batan® (1.5 oz/100 lbs), Gaucho® (2 oz/100 lbs), and Captan® (3 oz/100 lbs). Pre-plant fertilizer application included 25N-60P-90K (1997-98), 25N-100P-100K (1998-99), 25N-50P-100K (1999-00 Blacksburg), and 30N-40P-60K (1999-00 Warsaw). Harmony Extra® herbicide (0.5 oz/acre) was applied once per year as needed in early spring. Spring nitrogen was applied at a rate of 60 lbs/acre (1997-98; 1998-99) and 75 lbs/acre (1999-00) with the application of Harmony Extra® at growth stage 30.

Treated plots were inoculated twice in Blacksburg, first when head emergence was complete and again at 50% flowering, using a conidial suspension of 1L/100 ft^2 at 50,000 spores/ml. The inoculated plots in Warsaw, VA received colonized corn at a rate of 454 g/ 125 ft^2, at booting stage. After inoculation, all field plots received overhead irrigation from 8-9:30 A.M. and again from 6-7:30 P.M., unless conditions deemed irrigation not necessary. Scab incidence and severity were measured at fourteen and twenty-one days post-inoculation. Grain yield, test weight, 1000 kernel weight, DON toxin content, and percentage of scabby seeds were measured post-harvest. All data was analyzed using Agrobase software (correlation analysis, LSD, and ANOVA).

RESULTS AND DISCUSSION

Differences in inoculation methods. During the final year of this trial (1999-00), two environments were utilized to test differences in inoculation method. Over all years, conidial suspension was used as inoculum in Blacksburg, VA. Colonized maize kernels were used as inoculum at the Warsaw, VA location as it was postulated that inoculation method could effect test results. This theory is based upon the concept that inoculum from colonized maize kernels will mimic natural infection, which originates from crop debris. Conversely, conidial suspensions are sprayed directly onto spikes at flowering in accordance to growth stage of each genotype.

By controlling inoculation timing and inoculum concentration, it is obvious that applying conidial suspension ensures more uniform and predictable infections. Over all environments using conidial suspension, there was little correlation between plant height and FHB infection. Precise timing of the application of a conidial suspension minimizes the avoidance mechanism associated with traits such plant height. When assessing correlation values of plant height with the two inoculation methods, there was a large discrepancy between plant height and inoculation method. Plant height was highly correlated with all FHB disease measurements when colonized maize kernels were used, but was not correlated in any environment where conidial suspension was applied. These results suggest that infection avoidance of taller genotypes can be reduced and true resistance can be determined using a conidial-suspension inoculation method.

Parameters for assessing resistance. Correlation analysis was performed over four test years between yield, test weight, and disease assessment parameters which include visibly scabby seed, FHB severity, FHB incidence, FHB index, and toxin concentration. In three of the four environments, all assessment parameters were correlated with yield, and all assessment parameters were correlated with test weight over all environments. The 1998-99 test year was the only one in which several disease assessment parameters were not correlated with yield. In this year, FHB incidence, FHB severity, and FHB index were not highly correlated with yield. However, percentage of scabby seed and yield were highly correlated in all test environments, and this parameter provided the most consistent method of measuring type IV and V resistance of genotypes.

It is the desire of producers to have a method of disease assessment that can be utilized prior to harvest (field assessment). This has brought our attention back to field assessment parameters, which include FHB severity, FHB incidence, and FHB index. Of these three assessment methods, FHB index is the most consistent in predicting yield and test weight losses. This consistency is based upon the fact that FHB index is obtained by combining two independent parameters (FHB severity and incidence) associated with disease development and spread. When one considers the time and effort involved in disease rating, FHB severity may be the most feasible measurement of potential yield and test weight loss. Over three of the four test years, severity was highly correlated with yield and over all four years was highly correlated with test weight. Considering that correlation of severity with yield and test weight was nearly as high as that with index and the reduction in time spent assessing disease using only severity, this assessment method appears more practical than using an index value.

Interestingly, toxin accumulation was correlated with both yield and test weight over all environments. This correlation suggests that those genotypes, which are prone to higher yield and test weight loss, are also more likely to accumulate toxins. This observation is positive in that those genotypes with type IV and V resistance also seem to possess higher levels of type III resistance. This contradicts Mesterhazy (1999), who found no correlation between tolerance and toxin accumulation and first proposed separating these resistance mechanisms.

A concern in creating an environment that is ideal for *Fusarium* growth is the proliferation of other diseases, such as *Stagonospora nodorum* and root rot, that may affect similar traits and confound the effects due to scab. Excessive lodging can also occur in heavily irrigated plots. For this reason, correlation of lodging with yield and test weight was also considered. The correlation values for root rot with yield and test weight were not significant. Glume blotch and yield were significantly correlated in 1998-99, but glume blotch did not show a significant correlation with test weight. The significant correlation with yield infers that *Staganospora nodorum* can significantly impact and confound the assessment of type V resistance to scab and, therefore, must be precisely distinguished and accounted for in field trials. Mesterhazy et al. (1999) indicated that *Staganospora nodorum* could be controlled in field studies with an application of Bayleton to plants in the boot stage. Lodging also showed a significant correlation to yield and test weight. However, lodging effects can be controlled by application of a growth regulator (Cerone®). At both locations, application of

the prescribed chemicals in 1999-00 trials prevented Glume blotch and lodging and, therefore, data on these two factors was not ascertained.

Genotype reaction and response to FHB epidemics. Significant differences exist among tested SRW wheat genotypes with respect to yield, test weight, FHB severity, FHB index, percentage of scabby seed, and DON concentration. Analysis of variance and LSD indicate that there is a continuous distribution of genotypes rather than easily definable classes. For each parameter, a statistically distinct grouping has been established for genotypes that performed well over multiple environments. In addition, losses were determined for all genotypes, and those that performed well over multiple environments will be discussed.

Test weight and yield losses are the basis for type IV and V resistance, as described by Mesterhazy (1999). None of the genotypes in our study performed in the top 20 percent in regards to yield loss over more than two environments. Those genotypes in the top 20 percent included Freedom, Ernie, NY 87048W-7388, Roane, Agripro Foster, and IL-1549. Of these, NY87048W-7388, Freedom, and P92823A1-1-4-4-5 were in the top 30 percent with regards to yield loss over three environments. With regards to test weight loss, Roane and NY 87048W-7388 were in the top 20 percent over three environments and Freedom was in the top 20 percent over two environments. The above mentioned genotypes may provide breeders with useful germplasm for type IV and V resistance, as Mesterhazy (1999) has reported that related individuals seem to carry similar type IV and V resistance. In addition, the newly released cultivar Roane will provide producers with a more tolerant genotype to offset future FHB losses.

In analyzing parameters for assessing resistance, only those genotypes that have statistically low values for disease parameters will be mentioned. It was concluded that FHB severity and FBH index were the best in-field measurements, with FHB severity being the least time consuming of the two. Over four environments, Ernie was the only genotype with significantly low FHB severity. Roane, P92823A1-1-4-4-5, Freedom, NY 87048W-7388, and Agripro Patton showed low FHB severity over three of four environments. Ernie was again the only genotype with low FHB index values over four environments, with P92823A1-1-4-4-5 and Agripro Patton having low values over three of four environments. Percentage of visibly scabby seed may provide a quick method for breeders and producers to separate genotypes with regard to yield and test weight loss. No genotype showed low scabby seed values over four environments; however, Roane and P92823A1-1-4-4-5 showed low scabby seed over three of four environments. DON toxin data was also correlated with yield and test weight loss over four environments. More importantly, toxin level is employed in grading of wheat post-harvest and can lead to significant price deductions. Coker 9803, VA 96W-326, and NY87048W-7388 exhibited low toxin values over two of four environments.

REFERENCES

Canadian Grain Commission. 2000. Brief history of Fusarium head blight.

Griffey, C.A., J. Chen, E.L. Stromberg, and T. Pridgen. 1997. Assessment and Selection of Scab Resistance in Diverse Wheat Resources in Virginia. p. 35. *In*: Proceedings 1997 National Fusarium Head Blight Forum. 10-12 Nov. St. Paul, MN.

Griffey C.A., J. Chen, T. Pridgen, M. Chappell, and E. Stromberg. 1999. Research on *Fusarium* Head Blight in the Virginia Tech Small Grains Program. p. 95-99. *In*: Proceedings 1999 Joint Research Conference of the Eastern Wheat & Southern Small Grain Workers. 3-4 May, Williamsburg, VA. Univ. Printing Services, Virginia Tech., Blacksburg, VA 24061.

Mesterhazy, A., T. Bartok, C.G. Mirocha, and R. Komoroczy. 1999. Nature of Wheat Resistance to *Fusarium* Head Blight and the Role of Deoxynivalenol for Breeding. Plant Breeding 118: 97-110.

Virginia Farm Bureau Newsletter. 1999. Virginia farmers ahead of nation in conservation tillage. .

Virginia Farm Bureau Newsletter. 2000. Wheat harvest shrinks. .

REPRODUCIBILITY OF RESULTS FROM FIELD AND GREENHOUSE EVALUATIONS OF RESISTANCE TO FUSARIUM HEAD BLIGHT ON WINTER WHEAT

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ABSTRACT

The Fusarium Head Blight (FHB) winter wheat regional nursery consisted of 28 entries in 1999 and 29 entries in 2000. In both years, entries were screened under eight environments including field evaluation nurseries in eight different states in 1999 and field nurseries in six different states plus two greenhouse tests in 2000. The FHB index of individual entries from each environment was correlated with index values from the other seven environments in a given year and with the index values for entries when averaged across all eight environments. In both years, correlations of values from individual environments with the overall average were significant (average *P* value = 0.0078, range = 0.0982-0.0001). However, r^2 values were medium (average r^2 = 0.4248, range = 0.0980-0.6034) indicating significant environmental effects on the reaction of entries. Correlations among the environments were frequently not significant (average *P* = 0.2053, range = 0.0001-0.9406) and r^2 values were usually very low (average r^2 = 0.1582, range = 0.0002-0.4454). These data indicate that there can be extreme variability in results between two environments. As an example, line VA96W-326 had the lowest index rating of any entry at the Maryland field location in 2000 but the highest index value in the greenhouse nursery in Kentucky during the same year. Apparently, there are large environmental influences, including procedural and rating differences among locations, which can affect FHB ratings between two environments. Therefore, caution should be used when basing conclusions on data from only one environment. Wherever possible, multiple years and/or environments should be used to determine the reaction of an entry to FHB.

FUSARIUM HEAD BLIGHT RESISTANCE IN WHEAT CULTIVARS NING7840 AND FREEDOM

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ABSTRACT

Fusarium head blight (FHB) caused by *Fusarium graminearum* Schwabe is a destructive fungal disease of wheat (*Triticum aestivum* L.) in humid and semi-humid areas of the world. Highly FHB resistant cultivars such as Ning 7840, exhibit resistance that limits disease spread to inoculated florets and is conditioned by two or more genes. Development of cultivars with multiple resistance genes can be facilitated by determining the allelism/linkage of resistance genes from various sources and identification of molecular markers for those genes. FHB resistance from Chinese cultivar Ning 7840 was backcrossed into FHB susceptible cultivar Clark. BC_3F_5 plants with different phenotypes for FHB resistance were characterized and developed into four lines: a FHB susceptible line (L1) and three different FHB resistant lines (L2, L3, and L4), each having a unique FHB resistance gene determined by segregation analysis. The effect of the resistance of L2 is small and not significantly different from L1in some tests. Four recombinant inbred populations were developed by single seed descent from crosses of L1 X L2, L1 X L3, L1 X L4 and L1 X FHB-resistant cultivar Freedom. Populations were characterized for resistance to spread of FHB in spikes by single floret inoculation at anthesis with *Fusarium graminearum* in third or fourth spikelet from tips of spikes. Lines were evaluated in F_5, F_6, and F_7 generations in two greenhouse trials and one field trial. Lines were classified resistant or susceptible based on the percentage of infected spikelets 25 days after infection. The populations segregated in a 1:1 ratio of resistant and susceptible lines suggesting that there is a single FHB resistance gene segregating in each population and resistance in Freedom is conditioned by one gene. Differences for mean disease spread were found to be significant between L1, L2, L3, and L4. Freedom and L4 were not significantly different for mean disease spread. Several repeatable RAPD and SSR polymorphisms have been identified for these populations using bulked segregant analysis.

EVALUATION OF YUGOSLAVIAN WHEAT GERMPLASM FOR RESISTANCE TO FUSARIUM HEAD BLIGHT OF WHEAT

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INTRODUCTION

Fusarium Head Blight (FHB), or head scab caused by *Fusarium Graminearum* Schwabe., has been a huge threat to wheat production(*Triticum aestivum*) worldwide. The disease causes severe grain loss, which is primarily due to floret sterility, poor to no seed filling, or shriveling of grains resulting in low-test weights. Economic loss is further amplified by the presence of a fungal mycotoxin, deoxynivalenol (DON), which is harmful to humans and animals when consumed.

OBJECTIVE

The purpose for screening Yugoslavian wheat germplasm was to identify new sources of resistance. Resistant could be used as parents in the Ohio State Wheat Breeding Program to increase the level of resistance in the advanced breeding lines.

MATERIALS AND METHODS

Plant materials: Winter wheat genotypes were selected from GRIN database of the National Plant Germplasm System in 1998. Basis of selection was country of origin (Yugoslavia) and the improvement status (breeding lines and cultivars). 210 Yugoslavian winter wheat accessions were selected. Twenty seeds per genotype were sown in flats of soil in 1998 and 1999 in the greenhouse. Plants were vernalized for 60 days in a lighted cold room maintained day and night at 4^0C. Each germinated seed was transplanted individually into a styrofoam cup and filled with soil. Plants were watered twice a day. The greenhouse temperature varied from 19^0C to 30^0C during the day and 17^0C to 21^0C at night.

The lines were also evaluated for resistance in the field at Wooster, Ohio in 1998 and 1999 for resistance to FHB. Lines were planted in a completely randomized block design with two replications each in both years. Experimental units were 1m long and 30cm apart (0.3 sq. feet). Patterson and Pioneer 2545 were included as susceptible checks and Ernie and Freedom as resistant checks.

Inoculum Preparation: For greenhouse inoculation, fungal cultures from four aggressive *Fusarium graminearum* isolates were grown on malt extract agar by a regular single spore transfer method (Stack, 1989). Cultures were grown at 25^0C under continuous fluorescent light. Inoculum was prepared from these plates as described by Mesterhazy (1964). Conidia were harvested by flooding plates with sterile distilled water followed by a gentle

scraping of the top layer of the culture. The mixture was strained through sterile cheesecloth. The conidial suspension from four different isolates was mixed in equal volume. The final concentration was adjusted to 10^5 conidia/ml. For field inoculations, *Fusarium graminearum* colonized corn kernels (Campbell and Lipps, 1998) were used as inoculum. Colonized corn kernels were broadcast over the soil surface. Perithecia developed on the corn kernels in the field and ascospores served as inoculum. The field was mist irrigated daily throughtout flwering.

Inoculation: Hypodermic syringe inoculation technique as described by Bai et al.(1986) was used for greenhouse inoculations both the years. At anthesis, the center spikelet of each head was inoculated with a drop of freshly prepared conidial suspension (10^5 conidia/ml). Plants were maintained in a moist chamber at 100% relative humidity with temperatures ranging from 23^0C to 25^0C for three consecutive nights and then returned to the greenhouse bench.

Colonized corn kernels were spread in the field 18-21 days prior to flowering. Heading dates were recorded as early, mid and late. 20 heads from each genotype were rated for %spikelets affected approximately 21 days after anthesis. Data was analyzed and compared with the greenhouse data.

Data: Inoculated heads were assessed for disease severity as percentage of spikelet affected after 10 and 14 days in the greenhouse. FHB severity was recorded using a visual assessment scale (Stack et al, 1994, NDSU Extension). Disease parameters recorded in the field were incidence, severity, visual kernel assessment scale (Jones and Jenkins, U. of Minnesota), total kernel weight, percent scabby seed by weight and DON level (ppm).

STATISTICAL ANALYSIS

For each environment and each location, univariate plots for all the disease parameters were plotted independently using PROC UNIVARIATE in SAS (SAS institute version 6.03).

One way analysis of variance (ANOVA) was conducted for the field and greenhouse data separately for all genotypes using PROC GLM. Each location-year combination was treated as a separate variable. The data from the above four experiments were then combined. Data for 120 genotypes that were common to all the four experiments was then analyzed separately. The sources of variation in the model for combined data consisted of genotype, location, year, year*location, genotype*location terms in the model. The error term was defined by genotype*year*location. Pearson product movement correlations was calculated by PROC CORR to compare the disease severity ratings in different environments and different years. The same procedure was also used to compare the disease severity ratings with other disease parameters from the field. In all cases, the correlation values were calculated from the means of genotypes at individual environment and location. Dunnett's one tailed T test was used to select the lines that were significantly different than the susceptible check 'P2545' at 0.005, 0.01 and 0.05 level of P-value.

RESULTS

Yugoslavian winter wheat genotypes were evaluated for resistance to FHB in 1998 and 1999 in both greenhouse and field. The mean disease severity for each experiment is listed in Table II. Tests of non-normality or univariate procedure revealed that the mean scores of all the accessions were normally distributed for all the traits except visual kernel rating data in both the years. One way ANOVA revealed that the variation among 210 winter Yugoslavian wheat accessions was highly significant for all the traits measured, however, rep differences were not significant for the field years for all the traits except incidence. The genotype, location, year, year*location and genotype*location effects were significant for disease severity data for the combined analysis of four experiments. Mean FHB severity for each of the experiment is shown in figure I, II, III and IV. Correlation coefficient values calculated from the means are listed in Table I, II and III.

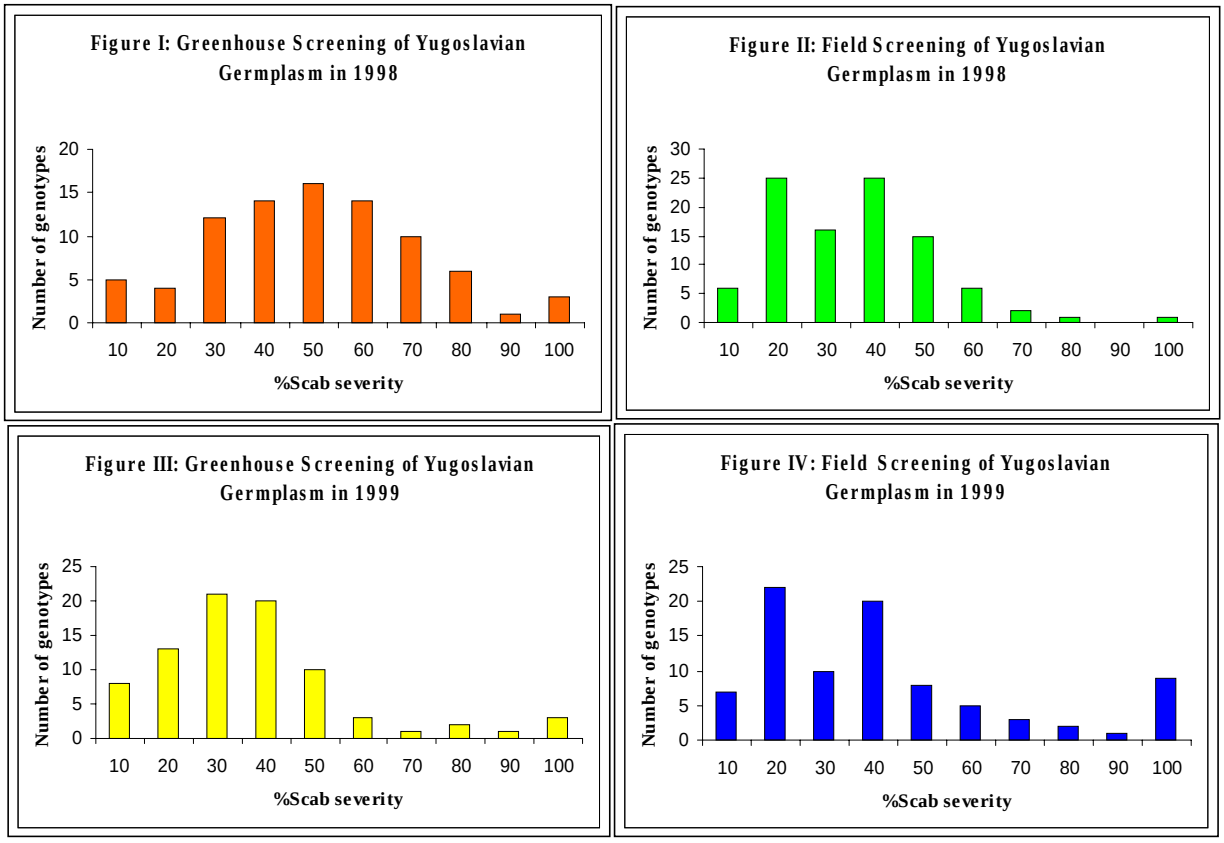

Figure I-IV: Normal Distribution Curve for mean FHB severity for Yugolsavian Germplasm for different years at different locations.

[1]VKR=Visual Kernel Rating
[2]TKR=Total Kernel Weight

DISCUSSION

No data transformation was required because all traits showed normal distribution. One way ANOVA and least significant difference (LSD) values indicated that there is a continuous level of disease severity from high to low instead of distinctly defined groups.

Highly significant correlation was observed between the two field years (r =0.62). There was moderate correlation between the two greenhouse experiments for disease severity (r =0.37).

Significant correlations were observed for percent scabby seed and severity for both the field years in both the reps. This indicated that these two traits may be the most effective scale for assessing scab resistance in the field. For individual environments, the correlation between incidence and severity was significant only in 1999 field year and the correlation between visual kernel scale and severity was significant only in 1998.

CONCLUSION

Few lines were found to be significantly more resistant than the resistant checks in all the four tests over two years. Lines were considered to be significantly different than the susceptible check ‘P2545’, only if:

1. Line was present in all the four experiments,
2. Data for both reps were present in the field both years,
3. More than ten plants per genotype were inoculated in the greenhouse both years.

Two genotypes were found to be significantly resistant to FHB at the level of 0.005 and six genotypes were found to be significantly resistant to FHB at the level of 0.01 than the susceptible check. Accession numbers of these plants along with the disease ratings are listed in Table IV.

Note from the Authors:

· Pedigree analysis of the resistant Yugoslavian wheat accessions revealed that these lines belong to a completely new lineage of resistant parents.
· After looking at the Yugoslavian wheat accessions in the greenhouse and field, we believe that some of these lines have exceptional agronomic traits (e.g. 24 spikelets in a single head) which may be interesting to evaluate for agronomic and quality trait analysis.

FUTURE WORK (1999-2000):

We will make crosses between resistant and susceptible lines of Yugoslavian accessions and with other sources of resistance that are currently available in different breeding programs. Following the crossing of these lines this year, we will be able to distribute small quantities of seed to interested persons.

REFERENCE

Bai, G. and Shaner, G. 1994. Scab of Wheat : Prospects for Control. Plant Disease, 78 : 760-766.

Parry, D. W., Jenkinson, P., McLeod, 1995. Fusarium ear blight (scab) in small grain cereals-a review, Plant Pathology:44, 207-238.

Schroeder, H. W. and Christensen, J.J (1963). Factors affecting resistance of wheat to scab caused by *Gibberella zeae*, Phytopathology 53:831-838.

Snijders, C. H. A, 1990. The inheritance of resistance to head blight caused by *Fusarium culmorum* in winter wheat. Euphytica 50: 11-18.

Stack, R.W. and McMullen, M.P. A visual scale to estimate severity of Fusarium head blight in wheat. NDSU Extension Service, North Dakota State University of Agriculture and Applied Science and U.S. Department of Agriculture, North Dakota.

Table I: Pearson Correlation Coefficients / Prob > |R| under Ho: Rho=0/Number of observations for four experiments

Source	Mean Severity	GH98	Field98	GH99	Field99
GH98	48	1	0.14	0.37	**0.35**
Field98	40	0.14	1	0.17	**0.62**
GH99	37	0.37	0.17	1	0.06
Field99	57	**0.35**	**0.62**	0.06	1

Table II: Correlations among other traits for the field year 1998

Source	HDate	Incidence	Severity	Index	VKR	% Scabby seed
Heading date	1	-0.14	-0.04	-0.07	0.34	0.38
Incidence	-0.14	1	0.38	0.5	0.17	0.15
Severity	-0.04	0.38	1	0.98	**0.47**	**0.51**
Index	-0.07	0.5	**0.98**	1	**0.46**	**0.49**
[1]VKR	0.34	0.17	**0.47**	**0.46**	1	**0.7**
% Scabby seed	0.38	0.15	**0.51**	**0.49**	**0.7**	1

Table III: Correlations among other traits for the field year1999							
Source	**Hdate**	**Incidence**	**Severity**	**Index**	**VKR**	**TKR**	**% Scabby seed**
Heading date	1	-0.63	-0.77	-0.77	-0.4	0.39	-0.5
Incidence	-0.63	1	**0.64**	**0.67**	0.08	-0.01	0.27
Severity	-0.77	**0.64**	1	**0.99**	0.3	-0.31	**0.54**
Index	-0.77	**0.67**	**0.99**	1	0.29	-0.3	**0.54**
[1]VKR	-0.4	0.08	0.3	0.29	1	-0.46	0.3
[2]TKR	0.39	-0.01	-0.31	-0.3	-0.46	1	-0.76
% Scabby seed	-0.5	0.27	**0.54**	**0.54**	0.3	-0.76	1

Table IV: Fusarium Head Blight Resistant Yugoslavian Wheat Germplasm tested in two years and two locations.							
Genotype	**FHB Incidence (%)**	**FHB Severity(%)**	**FHB index**	**Visual Kernel (%)**	**DON (ppm)**	**Scabby seed(%)**	**Significance**
Freedom	89	22.7	15.2	70	14.63	16.7	
Ning7840	85	23	14.95	65	10.7	14.2	
PI 306504	67	13.5	10.13	33	5.67	15.9	***
PI 251544	63	17.3	5.35	10	4.41	7.7	***
PI 184252	78.7	16.95	12.56	32.5	4.07	3.651	**
PI 434672	71.9	13.46	11.25	70	-	17.8	**
PI 221360	81.25	10.95	8.61	60	-	19.63	**
PI 221388	81.25	27.6	12.05	50	4.08	2.5	*
PI 284665	91.25	16.07	15.4	63	5.39	14.25	*
PI 284666	69.35	16.5	9.275	5	12.43	7.5	*
PI 470103	87.5	25.85	18.7	5.5	19.89	14.51	*
PI 221386	90	19.75	8.85	98	4.08	7.113	*
PI 221387	80	20.3	9.12	-	4.06	16.26	*
PI 259882	82.5	19.95	13.2	100	-	36.47	*
PI 358334	78.5	10.675	8.5	55	-	23.7	*
P2545	93	82	76.2	100	12	39	

*** significant at the level of 0.005
**significant at the level of 0.01
*significant at the level of 0.05

IDENTIFYING RESISTANCE AND THE RELATIONSHIP BETWEEN SPIKELET SYMPTOMS AND KERNEL INFECTIONS IN *FUSARIUM GRAMINEARUM* INFECTED SOFT RED WINTER WHEAT

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OBJECTIVES

1) To screen soft red winter wheat for Type II resistance to *Fusarium graminearum*.

2) To investigate the relationship between visual spikelet infection and kernel infection.

3) To examine the spread of *Fusarium graminearum* through the spike.

INTRODUCTION

Fusarium head blight (FHB), also known as head scab, caused by *Fusarium spp.,* has been a historically devastating disease of wheat and barley all around the world. In Kentucky, the prevalent cropping system of no till or minimal till wheat production works to influence head scab levels by providing sufficient inoculum levels. Incorporating FHB resistance into soft red winter wheat is considered to be the most effective control strategy.

Mesterhazy et al. (1999) report five different modes of resistance to FHB. Type II (resistance to spread within in the spike) is commonly measured in greenhouse inoculation experiments. Type IV (resistance to kernel infection) is less understood but has been researched. Mesterhazy reported in 1997 that there are genotypes that have less kernel infection than anticipated, based on FHB values. This experiment was completed to better understand the interaction of spikelet infection and kernel infection due to FHB and thus provide information on the most effective breeding and selection methods.

MATERIALS AND METHODS

In the fall of 1999, 29 soft red winter wheat lines and 21 F_1's were evaluated in the greenhouse for Type II resistance to *Fusarium graminearum*. The fifty wheat genotypes were planted in the greenhouse on October 11, 1999 in a completely randomized design.

Type II Screening: Macroconidial suspensions were prepared in the lab from a mixture of eleven different *F. graminearum* isolates. At the time of anthesis a central floret of each spike was marked with a permanent non-toxic pen and inoculated by pipetting 3 ml of the spore suspension containing approximately 1,000 spores. After inoculation, plants were placed in a humidity chamber for three consecutive nights. Plants were moved out of the chamber on the fourth day and scored for disease development on the 21st day post-inoculation. The number of diseased spikelets and the total number of spikelets were recorded

for each inoculated spike. The spikelet infection rate was calculated as the percentage of diseased spikelets per total spikelets.

Kernel Assessment: Each inoculated spike was harvested and the kernels from each spike were plated onto acidified potato dextrose broth agar to quantify the presence of *F. graminearum* in the developed kernels. Seeds from each spike were plated onto the agar according to the spatial arrangement of the spikelets from which they came. The number and position of blank spikelets containing no seed were recorded. Plates were incubated for 7 days at 20°C. After incubation, those kernels that showed the presence of *F. graminearum* were recorded.

RESULTS AND DISCUSSION

Type II Resistance

The 50 genotypes differed in their response to FHB (Table 1). For brevity, only twelve of the 50 genotype means are shown (Table 2). The number of replicates per genotype varied due to low numbers of F_1 seed and the loss of some seedlings due to de-vernalization.

Table 1: ANOVA Tables for Kernel Infection and Spikelet Infection

ANOVA Kernel Infection

Source	df	Sum of Squares	Mean Square	F value	
Genotype	49	52033.47	1061.91	2.11	***
Error	312	156814.99	502.61		
Total	361	208848.45			

*** p<0.001

ANOVA Spikelet Infection

Source	df	Sum of Squares	Mean Square	F value	
Genotype	49	33490.98	683.49	1.76	**
Error	312	121350.11	388.94		
Total	361	154841.1			

** p<0.01

Relationship Between Spikelet Infection and Kernel Infection

From Table 2 we see that some genotypes did have a lower kernel infection rate than expected from their spikelet infection rate. These genotypes would posses type IV resistance based on Mesterhazy's explanation. For example, Glory, which had a spikelet infection rate of 45.6%, had a kernel infection rate of only 30.5% (Table 2).

Effect on Selection

If we set a hypothetical selection criteria of 10% and keep only those genotypes showing less than 10% spikelet infection, 28 genotypes would have been selected. Of those 28 genotypes, 7 actually were above the 10% infection level based on kernel infection data and 4 genotypes would not have been selected based on spikelet infection but should have been selected based on kernel infection data.

Is there a significant difference between kernel infection rate and spikelet infection rate?

A one tailed t test was completed to compare the two overall means. The result from this test revealed that the difference between overall kernel infection mean and overall spikelet infection mean was not significant at the 5 or 10% level. Although the overall means are not different, differences in spikelet infection and kernel infection are noted on an individual genotype mean basis. The correlation coefficient between these two variables was $r = 0.51 (p<0.01)$. The relationship between kernel infection and spikelet infection is moderate and agrees with other correlation coefficients found in the literature (Masterhazy, 1999).

Table 2: Comparison of kernel and spikelet infection by *Fusarium graminearum* in several soft red winter wheat genotypes and F_1's.

Pedigree	Kernel Infection	Spikelet Infection	n
Patton/Glory	0	6.6	5
Patton/Foster	0	3.5	2
Ernie	1.42	3.34	10
Foster/91C-117-32	2.21	5.75	4
Coker 9663/91C-117-32	3.19	11.63	8
Foster	3.72	11.71	7
Patton	4.31	5.3	10
Glory/91C-117-32	6.34	8.38	8
Coker 9663	20.32	15.41	6
91C 117-32	22.51	13.07	8
90C 054-6	23.29	39.2	5
Glory	30.47	45.57	7
Overall Mean	15.55	12.94	50

Injection Inconsistencies

Table 3 provides a breakdown of all observations made in this study. In the first scenario where neither plant symptoms or fungus presence was recorded, the necessary conditions for FHB development did not occur. This could be attributed to nonviable spores, improper environmental conditions, or ill-timed injections. Most likely these escapes are due to

injections made prior or past anthesis, the most infectious stage. The next scenario describing visual symptoms but no actual fungus present in the kernels could be accounted for by early senescence fooling the human eye. A white head symptom has also been described in the literature where the wheat head is not actually infected with the fungus but assimilate is shut off to the head thus causing the white head appearance (Snijders et al, 1992). The final situation that warrants some attention is most troubling. No visual symptoms were noted in the plants but indeed they were infected and the fungus present in the kernels. It is not uncommon to isolate *F. graminearum* from sound looking kernels, yet not only did these kernels look sound but the spikelets looked sound as well. This scenario did not occur prevalently and could possibly be attributed again to the improper human judgement of symptoms.

Table 3: Comparison of Spikelet Infection Levels to Kernel Infection Levels

	Plates showing NO fungus present	Plates showing fungus present	Total Observations
Spikelets showing NO symptoms	38 (10.5%)	22 (6.07%)	60 (16.57%)
Spikelets showing symptoms	126 (34.8%)	176 (48.62%)	302 (83.42%)
Total Observations	164 (45.30%)	198 (54.69%)	362

What should n be?

Based on the error mean square contained in this study, 14 replicates would reduce error variance sufficiently to detect a difference of 10% in spikelet infection. Fifty-six replicates are needed to increase the detection level to 5%. To detect a difference of 10% in kernel infection, 16 replicates are sufficient. Eighty-one replicates are sufficient at the 5% detection level. Noting that 56 and 81 replicates are economically non-feasible for most university breeding programs, we recommend that 15 replicates be used in greenhouse experiments with similar levels of experimental variation. Of course the inherent variation within an experiment greatly influences the number of replicates needed. As the variation decreases the number of necessary replicates also decreases.

Spread Through the Spike

Plating the kernels in order, according to their arrangement on the spike, allowed us to follow the spread of the fungus through the spike. Based on the results from the plating data we could reconstruct the presence of the fungus within each spike. From this enormous amount of data it appears that the fungus spirals both up and down the spike infecting florets.

REFERENCES

Mesterhazy, A. 1997. Breeding for Resistance to Fusarium Head Blight of Wheat. *In* Dubin, H.J., L. Gilchrist, J. Reeves, and A. McNab, eds. *Fusarium Head Scab: Global Status and Future Prospects.* Mexico, D.F.: CIMMYT.

Mesterhazy, A., T. Bartok, C.G. Mirocha, and R. Komoroczy. 1999. Nature of Wheat Resistance to Fusarium Head Blight and the Role of Deoxynivalenol for Breeding. Plant Breeding 118: 97-110.

Snijders, C.H.A. and C.F. Krechting. 1992. Inhibition of Deoxynivalenol Translocation and Fungal Colonization in Fusarium Head Blight Resistant Wheat. Canadian Journal of Botany. 70: 1570-1576.

PROGRESS OF CHINA/CIMMYT SHUTTLE BREEDING AND GERMPLASM EXCHANGE AIMED AT COMBINING HIGH YIELD POTENTIAL WITH SCAB RESISTANCE

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INTRODUCTION

Fusarium head blight or head scab of wheat is a major disease in China, particularly in the Autumn-sown Spring Wheat Zones in the Middle and Low Yangtze Valleys and Southwestern China located in the Yangtze region, and the Northeast Spring Wheat Zone. About 7 million ha of Chinese wheat area is affected by head scab, and developing scab-resistant cultivars has been the major breeding objective in these areas.

Chinese wheat confers good resistance to head scab, and Sumai 3 is used worldwide as a resistance source, but its yield potential and agronomic performance need further improvement. On the other hand, CIMMYT wheats have short stems and good lodging resistance, high yield potential, good rust resistance, and acceptable quality. However, they are poorly adapted to humid Chinese environments and susceptible to head scab and leaf wilting.

In the mid 1980s, CIMMYT and China initiated a shuttle breeding and germplasm exchange program focusing on incorporating the scab resistance of Chinese wheats into high yielding CIMMYT germplasm. A formal agreement was signed between CIMMYT and the Chinese Academy of Agricultural Sciences (CAAS) in 1988. The target area has expanded greatly since 1995, and most major provincial agricultural academies have started cooperating with CIMMYT. This paper describes the progress of this joint project in terms of germplasm exchange and development, information exchange, and training.

Germplasm exchange and development

At CIMMYT, progress in breeding for scab resistance has built largely on the use of Chinese germplasm. In the last 15 years, around 700 Chinese commercial varieties, advanced lines, and important scab resistant wheats (such as Sumai 3, Ning 7840, Shanghai 3, Shanghai 4, Shanghai 5, Suzhou 6, Wuhan 3, and Chuanmai 18) have been sent to CIMMYT. A set of resistant bread wheat lines with good agronomic performance have been developed at CIMMYT through the use of Chinese germplasm, as reported by Gilchrist et al. (1997).

In general, two types of crosses, Chinese/CIMMYT and Chinese/CIMMYT//CIMMYT, are used in Mexico when using Chinese germplasm to improve wheats for other countries and mega-environments. However, in CIMMYT crosses directed towards China, crosses are Chinese/CIMMYT//Chinese or sometimes CIMMYT/Chinese//Chinese. Currently, Chinese

wheat can be found in the pedigrees of more than 50% of CIMMYT germplasm for high rainfall environments (ME2).

In addition to scab resistance, Chinese spring wheat also shows good resistance to Karnal bunt, helminthosporium leaf blotch, tan spot, and septoria diseases. A large number of CIMMYT Chinese crosses are made each year, and many Chinese derivatives are included in CIMMYT's international nurseries distributed throughout the world. The most outstanding CIMMYT bread wheat crosses under different mega-environments (MEs) with Chinese germplasm in their pedigrees are presented below. They have shown good adaptation to locations outside Mexico.

1. GUAMUCHIL 92 (=CATBIRD=CHUM 18/BAU) (CM 91045-6Y-0M-OY-1M-8Y-0B-0MEX)
2. ARIVECHIL M92 (LUAN=WUH1/GLEN/4/INIA 66/AG.DI//INIA 66/3/GEN (CM100587-E-0M-0Y-030M-8Y-1Y-0M-0MEX)
3. SHA 3/CBRD (CMSS92Y00595S)
4. WEAVER/WL3926//SW89.3064 (CMSS92Y01054T)
5. NG8675/CBRD (CMSS92Y00639S)
6. SW89.3064/STAR (CMBW91Y01627S)
7. XIANG82.2661/2*KAUZ (CMBW91Y02917M)

ME2-High Rainfall /ME3-Acid Soils

1. SHA4/CHIL (CM 91099)
2. CHIL/CHUM18 (CM92687)
3. XIANG82.2661/2*KAUZ (CMBW91Y02917M)
4. MILAN/SHA 7 (CM97550)
5. CHUM18//JUP/BJY (CM91046)
6. CBRD//VEE#10/2*PVN (CMSS93B01081S)
7. SHA3//SERI// G.C.W.1/SERI (CMBW91Y01596S)
8. HXL8088/DUCULA (CMSS93Y02492S)
9. BR14*2/SUM3//TNMU(CMBW91M02048S)

ME4-Low Rainfall

1. HXL 7573/2*BAU (CMBW91Y03634M)
2. NANJIANG 8646/KAUZ//BCN (CMBW8900966T)
3. HXL8246/KAUZ (CMBW90M2205)

ME5-High Temperature

1. G.C.W 1/SERI (CM86992)
2. SABUF (= SHA3//BUC/FLK) (CM95073)
3. SW8905124*2/FASAN (CMBW91Y03050F)
4. XIANG82.2661/2*KAUZ (CMBW91Y02917M)

More than 10,000 CIMMYT lines have been distributed to 40 Chinese wheat breeding programs, either in the form of regular international nurseries and special nurseries such as F3YZ and F3CHENGDU, or materials selected by Chinese visiting scientists in Mexico. In China, three-way cross of Chinese/CIMMYT//Chinese is employed. The following varieties directly evolved from the shuttle breeding project have been released in China. Presently, 5-7 million ha are cultivated to varieties carrying CIMMYT germplasm in their pedigree. Ningchun 4 and Xinchun 6 are the leading varieties in the Northwestern Spring Wheat Region and Xinjiang, respectively. In addition to that, good quality wheats such as Longmai 26, Zhongyou 9507, Jinan 17, and Liaochun 10 have quality conferred by CIMMYT wheats.

1. Ningmai 7 (Ning8931 = Shanghai 4-23B-0Y), Jiangsu Province
2. Ningmai 10 (Ning9415 = SHA7//PRL/VEE #6 (CM 95117)), Jiangsu Province
3. Chuanmai 25 (=1414/Chuanyu 5//Genaro 80), Sichuan Province
4. Chuanmai 30 (=SW3243, Alondra cross), Sichuan Province
5. Longmai 26 (=Long94-40830), hard wheat, Heilongjiang Province
6. Kenghong 16 (=CM95434), soft wheat, Heilongjiang Province
7. Dongfeng 1 (selected from CIMMYT/ICARDA nursery), Beijing
8. Zhongyou 9507, hard wheat, Beijing
9. Jinan 17, hard wheat, Shandong Province

In the 1999-2000 season, CIMMYT's China Office has offered three regional nurseries in China. Each nursery will consist of advanced lines and newly released varieties as well as introductions from other countries. To fulfill the need for quality improvement, two quality wheat nurseries have also been distributed.

1. Winter and facultative wheat screening nursery: 24 institutes involved.
2. Autumn-sown spring wheat screening nursery: 11 institutes involved.
3. Spring-sown spring wheat screening nursery: 12 institutes involved.
4. Good quality wheat screening nursery for autumn-sown wheat region: 35 institutes involved.
5. CIMMYT good quality wheat screening nursery: 12 institutes are involved.

Human resources development

From 1985 to 2000, CIMMYT sponsored visits to Mexico by 95 Chinese scientists for a period from three weeks to one year. Fifty-six Chinese scientists have participated in the wheat improvement and cereal quality training courses, which last from two to eight months. CIMMYT has also supported the attendance at international conferences of more than 30 Chinese scientists. These opportunities have enhanced their scientific skills and improved their understanding of CIMMYT's wheat breeding methodology. Currently, most training

alumni and visiting scientists have been appointed to lead the wheat breeding program in their own institutes, and several have been promoted to directors of their institutes.

Information exchange

Chinese scientists receive many CIMMYT publications, and CIMMYT provides a channel for Chinese scientists to understand wheat breeding in other countries. The following CIMMYT publications have been published in Chinese. CIMMYT has also published three Wheat Special Reports on subjects related to Chinese wheat.

CIMMYT publications in Chinese:

- Zou Yuchun (translator), 1994. Collection of CIMMYT Wheat Breeding Papers, Sichuan Science and Technology Press.

- He Zhonghu (translator), 1995. CIMMYT Wheat Breeding Methodology, China Agrotech Press.

- Yang Yan (translator), 1999. Bunt and Smut Diseases of Wheat, Concepts and Methods of Disease Management, edited by R.D. Wilcoxson and E.E. Saari, China Agrotech Press.

- He Zhonghu (translator), 1999. Increasing Yield Potential in Wheat: Breaking the Barriers, edited by M.P. Reynolds, S. Rajaram, and A. McNab, China Science and Technology Press.

- Sun Jiazhu (translator), 2000. CIMMYT 1998-99 World Wheat Facts and Trends. Global Wheat Research in a Changing World: Challenges and Achievements, Beijing Academy of Agricultural Sciences.

CIMMYT publications regarding Chinese wheat:

- He Zhonghu and Chen Tianyou, 1991. Wheat and Wheat Breeding in China, CIMMYT Wheat Special Report No 2.

- Yang Zouping, 1994. Breeding for Resistance to Fusarium Head Blight of Wheat in the Mid- to Lower Yangtze River Valley of China, CIMMYT Wheat Special Report No 26.

- He Zhonghu and S. Rajaram, 1997. China/CIMMYT Collaboration on Wheat Breeding and Germplasm Exchange: Results of 10 years of Shuttle breeding (1984-94), Proceedings of a conference held in Beijing, China, July 3-5, 1995. CIMMYT Wheat Special Report No. 46

In addition to publications, CIMMYT and CAAS have also jointly organized several wheat breeding meetings and training courses, as presented below. They have greatly enhanced scientific exchange between CIMMYT and Chinese wheat breeding programs.

- CAAS-CIMMYT Wheat Breeding Meeting, Beijing, 1995
- China-CIMMYT Wheat Breeding Meeting, Henan, 1997
- China-CIMMYT Wheat Quality Training Course, 1998
- China-CIMMYT Spring Wheat Breeding Meeting, Inner Mongolia, 1999
- China-CIMMYT Wheat Quality Training Course, 1999
- National Wheat Breeding Meeting, Shandong, 2000
- CAAS-CIMMYT GXE Training Course, Beijing, 2000

REFERENCES

He, Z.H. and S. Rajaram, editors. 1997. China/CIMMYT Collaboration on Wheat Breeding and Germplasm Exchange: Results of 10 Years of Shuttle Breeding (1984-94). Wheat Special Report No 46. Mexico, D.F.: CIMMYT.

Dubin, H.J., L. Gilchrist, J. Reeves, and A. McNab, eds. 1997. Fusarium Head Scab: Global Status and Future Prospects. Mexico, D.F.: CIMMYT.

BREEDING FOR SCAB RESISTANCE IN SOFT WHITE WINTER WHEAT REPORT 1999-2000

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INTRODUCTION AND OBJECTIVES

Fusarium head blight or scab caused by *Fusarium graminearum* (*Gibberella zeae*) is a world-wide disease in wheat. During the last decade, it prevailed frequently in the North America. A consortium has been formed to tackle this problem in the USA and some progress has been made. In Michigan, scab has become one of the most serious obstacles to the Soft White Winter Wheat (SWWW) industry. Development and utilization of resistant varieties should be the most effective approach to control this disease. The main objectives of our study are:

To evaluate and screen various germplasm resources and breeding lines for resistance;

To develop resistant varieties adapted to Michigan and surrounding areas, and create elite germplasm with enhanced resistance and improved agronomic traits;

To study the inheritance and mechanisms of scab resistance, identify molecular markers associated with the resistance and apply them to the breeding program.

Here is a brief report on our experiments and advances during the crop season 1999-2000.

MATERIALS AND METHODS

In order to develop cultivars with scab resistance and adapted to Michigan and surrounding areas, single crossing, backcrossing and 3-way crossing were employed. Superior local varieties and Chinese resistant lines were used as the parents. At the same time, resistant to resistant crosses were made by single- and multiple-crossing to accumulate the resistance genes. SSD in the greenhouse and single plant selection in the field were practiced jointly.

Cultivars and advanced lines from different states and our breeding program were assessed for scab resistance. Single-floret inoculation and soil surface inoculation were adopted in the greenhouse and field, respectively. In order to classify the resistance of materials, Ning 7840 and Norm were used as the resistant and susceptible controls, respectively.

Before transplanting in the greenhouse, vernalization treatment for the materials (except for the spring type) was carried out at 0-4 °C for 9 weeks. During heading and flowering stages, single-floret inoculation was undertaken. 15 µl of *Fusarium graminearum* conidial spore suspension (5 x 104 spores/ml) was pipette injected into a basal floret in the central part of the spike. 10-20 spikes were inoculated per genotype. Mist-irrigation was given for three

days after inoculation and then the heads were watered three or four times a day to maintain high humidity and promote disease development. The number of scabby spikelets on inoculated heads was recorded three weeks after inoculation as follows (Jiang, 1998; Jiang et al, 1995):

0.5: only the inoculated floret showed the symptom;
1.0: only the inoculated spikelet showed the symptom;
1.8: inoculated spikelet and main rachis showed the symptom;
2.0 or more: number of the total scabby spikelets on the inoculated spike.

RESULTS AND DISCUSSION

Introduction of Exotic Resistance Germplasm

In order to meet the need for the development of scab-resistant varieties, 30 resistance resources were introduced into our breeding program. Most of these materials are new improved germplasm from China, such as highly-resistant W14, CJ 9306, CJ 9311; high yielding and scab resistant CJ 9403, CJ 9815, CJ 9807, CJ 8809, TFSL 037; white-grained and scab-resistant SH 19089, Shaan 85-2, CJ 9602; and so on (Jiang and Wu, 1996; Jiang, 1998; Griffey et al, 1999).

Establishment of Scab Screening System and Evaluation of the Germplasm Resources

A refined greenhouse screening system for scab resistance, based on the single-floret inoculation, has been established and fully implemented. 71 varieties and advance lines from different U.S. states and China were assessed this year. According to the mean number of scabby spikelets and the standard deviation in the controls Norm (10.39±0.60) and Ning 7840 (2.03±0.38), scab resistance was divided into the following 6 levels:

HR:	<1.3;	R:	1.3~2.8;	MR:	2.8~4.3;
MS:	4.3~7.5;	S:	7.5~13.3;	HS;	>13.3.

The results showed that there was a significant difference in the reaction to scab among genotypes and most were susceptible. Ten genotypes were resistant and 13 were moderately resistant (Table 1 and 2). W14 and its sister lines CJ 9306 and CJ 9311 were further proved to possess high resistance (Type II) (Jiang, 1997; Griffey et al, 1999). It seems that these 23 materials (R and MR) could be used in wheat breeding. However, further assessments are necessary.

The field screening was conducted at two sites. However, the infection did not take well and the data were not fit for publication. Insufficient humidity might be the reason for this, so further improvement of the mist-irrigation should be made.

Utilization of the Resistant Germplasm and Crossing

In order to transfer the resistance into the local cultivars, 25 combinations were made by crossing 9 new Chinese germplasms (W14, CJ 9807, CJ 9804, TFSL 037, CJ 9306, CJ 9815, CJ 9602, CJ 9403 and SH 19089) with 9 US winter wheat varieties (Caledonia, Goldfield, D 8006, D 6234, Ohio 552, VA96W-403WS, Freedom, Pioneer 25R26 and Ernie). 21 resistant by resistant combinations were made to accumulate the resistance genes using 12 strains (W14, CJ 9306, CJ 9311, TFSL037, CJ 8809, CJ 9807, CJ 9804, CJ 9815, CJ 9403, CJ 9602, SH 19089 and Yang 158). The hybrid seeds have been sown in the greenhouse. Backcrossing and multiple crossing will be conducted.

Inheritance Study and Establishment of RIL Populations

For a genetic study, 2 susceptible varieties (Norm and Veery) were crossed to each of 12 resistant genotypes. 24 F_1 hybrids have been planted in the greenhouse. In order to undertake scab resistance mapping and molecular marker assisted selection for the resistance, we initiated the establishment of RIL and DH populations. 6 $F_{3:4}$ populations (susceptible Veery was crossed to 6 resistant varieties) have been produced by SSD. This study is in progress.

REFERENCES

Griffey, CA, JL Chen, T Pridgen, M Chappell and J Shaw, 1999. Advances in researches on Fusarium head blight in the Virginia wheat breeding program. 1999 National Fusarium Head Blight Forum, 165-169.

Jiang, GL, 1998. Combining ability analysis of scab resistance for F_1 and F_2 in 4X5 factorial cross of common wheat. Wheat Information Service, 87: 31-38.

Jiang, GL, 1997. Breeding for resistance to Fusarium headblight in wheat. Cereal Research Communications, 25(3/2): 757-760.

Jiang, GL and ZS Wu, 1996. Development of scab-resistant gene pool and its application to wheat breeding. Journal of Nanjing Agricultural University, 19(1): 1-8.

Jiang, GL, ZS Wu, ZX Chen, SR Yu, and JM Wu, 1995. Effectiveness of population improvement through recurrent selection for scab resistance in wheat using dominant male-sterile gene ms2. Science in China (Series B), 38(11): 1361-1369.

Table 1. Frequency of different scab-resistance levels in wheat

Resistance level	HR	R	MR	MS	S	HS
Number of genotypes	3	7	13	24	23	1
Percentage of genotypes	4.23	9.86	18.31	33.8	32.39	1.41

Table 2. Number of scabby spikelets and the resistance levels in wheat cultivars

Variety name	Number of scabby spikelets	Resistance level	Variety name	Number of scabby spikletes	Resistance level
Hytest HTW9850	10.25	S	Hopewell	5	MS
Foster	10.11	S	Kaskaskia	2.27	R
Patton	5.76	MS	Lowell	5.3	MS
527W	9.63	S	Patterson	8.45	S
569W	7.67	S	Wakefield	9.33	S
Genesis 9939	3.47	MR	Frankenmuth	6.38	MS
Genesis 9953	6.4	MS	Pioneer Variety 2552	7.33	MS
Caledonia	6.38	MS	Pioneer Brand 25R57	8	S
NY86003-106	7.75	S	Pioneer Brand 25W60	5.13	MS
Superior	4.53	MS	Stine 455	9.67	S
Navigator	3.71	MR	Stine 488	12.73	S

GREENHOUSE AND FIELD EVALUATION OF RESISTANCE TO FUSARIUM HEAD BLIGHT IN SOFT RED WINTER WHEAT

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OBJECTIVES

1) To identify resistance to Fusarium head blight in the greenhouse and field screening trials.

2) To compare inoculum sources and methods.

Fusarium head blight (FHB) has caused significant losses in Kentucky's wheat crop in most years since 1991. The prevalent rotation in which growers are planting wheat after corn into minimally or no-tilled soil ensures abundant inoculum in most years. Therefore, breeding for FHB resistance is an essential component of the wheat breeding project at the University of Kentucky.

Entries in the 2000 Uniform North and Uniform South Winter Scab Screening Nurseries along with a number of advanced breeding lines were planted in the field in a randomized complete block design with four replications on 27 October 1999. Each plot consisted of four rows and measured 4ft by 4ft. The previous crop was corn (Zea mays L.) and the seedbed had been chisel plowed and disked. Entries in the greenhouse were planted in a completely randomized design with a variable number of replications.

The field inoculation protocol was as described by Van Sanford et al. (1999) with a few modifications. Approximately 100 g of sterilized water was added to the autoclaved corn in the Mason jars to provide adequate moisture for the pathogen to grow. On April 24, wheat plots were inoculated prior to heading by spreading 3.31g/ft^2 of the inoculated corn mixture. Plots were mist irrigated daily beginning on April 27. The irrigation system was set with an automatic timer programmed to mist irrigate the plots for 5 minutes with 15 minute intervals between the hours of 6 to 10 AM and 10 minutes with 20 minute intervals between the hours of 8 and 10 PM.

Disease evaluations were initiated on May 30 when scab symptoms were detected on several of the susceptible cultivars. Incidence was recorded as the number of infected heads per 50 heads sampled. Disease severity was assessed according to the Visual Scale for Estimating Head Blight in Wheat (Stack and McMullen, 1998). Fifty heads per plot were visually rated according to the Stack and McMullen scale.

The greenhouse injection procedure was as reported in Van Sanford et al. (1999).

'Clark' and 'Ernie' were grown in replicated plots in a randomized complete block design with four inoculum treatments: 1) *F. graminearum* colonized field corn (3.31g/ft^2) was spread

three weeks prior to anthesis, 2) a macroconidial suspension of 175 ml/plot at 50,000 sp/ml was sprayed, once at anthesis, and again one week post-anthesis, 3) a macroconidial spray was prepared from inoculum that had been frozen 4-6 months earlier, and 4) a non-inoculated control.

Data for the Uniform Scab Nurseries are presented in Tables 1 and 2.

The fresh inoculum treatment produced the greatest amount of disease. For cultivar Ernie, disease incidence, head severity and FHB index were significantly higher in the fresh-spray treatment when compared to the other treatments Table (3).

Disease symptoms were first noted in the fresh-spray treatment of the cultivar Clark one week after application. As the season progressed, it became evident that discerning infected spikelets on Clark would become increasingly more difficult due to the bronzing color of its glumes. Therefore, only disease incidence data was collected. No significant differences were observed among the treatments for disease incidence.

Several factors could influence why the fresh-spray treatment was more effective. By spraying macroconidial suspensions, you have the ability to control when to place the pathogen with the host at the most crucial time of susceptibility, at anthesis. Macroconidial germination rate was more than likely higher for the fresh inoculum suspensions when compared to frozen suspensions.

Table 3. Effect of inoculum treatment on cultivar Ernie.

Inoculum treatment	Incidence	Head Severity	FHB Index
1. Corn	44.44 b	12.41 b	5.53 b
2. Fresh Spray (50,000 sp/ml)	60.67 a	19.66 a	12.43 a
3. Frozen Spray (100,000	42.33 b	12.53 b	5.60 b
$LSD_{0.05}$	9.25	3.06	2.85
CV	28.13	30.8	54.2

REFERENCES

Bai, G.H. and Shaner, G. 1996. Variation in Fusarium graminearum and cultivar resistance towheat scab. Plant Dis. 80:975-979.

Stack, R.W. and M. P. McMullen. 1998. A Visual scale to estimate severity of Fusarium head blight in wheat. PP-1095. NDSU Extension Service.

Van Sanford, D.A., B. Kennedy, M. Hall, and C. Swanson. 1999. Greenhouse and field evaluation of resistance to Fusarium head blight in soft red winter wheat. 1999 National Fusarium Head Blight Forum Proceedings. 187-192.

Table 1: 2000 Uniform Southern Winter Wheat Screening Nursery, Lexington, Kentucky

		Field Data				Greenhouse Data			
Entry	Name	Height (inches)	Average Incidence*	Average Head Severity ^	Average Plot Severity#	21 dpi ⌘	Min	Max	Rank
South-1	Ernie	32.25	9.50	8.83	0.95	7.00	6.30	7.70	2
South-2	Futai 8944	37.25	8.50	12.59	0.89	9.70	4.50	30.00	7
South-3	SC 921285	31.50	18.00	10.07	1.67	68.00	5.90	100.00	20
South-4	SC 921299	30.50	16.50	7.44	1.23	71.70	17.60	100.00	22
South-5	SC 941292	36.50	25.50	9.38	2.34	38.20	5.00	100.00	15
South-6	Coker 9474	34.75	23.00	9.68	2.22	16.70	5.90	50.00	12
South-7	B950799	36.00	26.00	9.20	3.06	36.10	5.60	76.50	14
South-8	B930390	38.25	16.00	8.71	1.40	14.10	5.90	35.30	9
South-9	B961092	32.75	26.00	1.56	2.95	13.60	6.70	43.80	8
South-10	GA 89482-E7	35.75	21.00	14.22	2.32	62.20	6.30	100.00	19
South-11	GA 901146-E15	31.50	16.00	10.61	2.09	33.90	5.90	88.20	13
South-12	GA 90524-E35	30.00	26.00	8.65	2.40	14.90	5.60	50.00	11
South-13	GA 90552-AE33	36.00	27.50	8.77	2.42	48.30	5.00	100.00	17
South-14	Roane	33.50	29.00	8.21	2.54	8.00	4.80	26.30	4
South-15	VA96W-329	34.25	29.00	9.14	2.78	14.40	5.30	58.80	10
South-16	VA96W-326	36.00	22.50	8.20	2.00	68.80	6.30	100.00	21
South-17	VA96W-158	38.25	26.50	9.25	2.50	58.20	5.60	100.00	18
South-18	VA96W-348	35.50	9.50	7.44	0.77	39.80	5.30	100.00	16
South-19	NC96-13848	35.00	25.00	12.34	3.10	6.50	5.30	11.80	1
South-20	NC96-13965	34.75	17.50	10.18	2.16	8.50	6.30	17.60	6
South-21	NC96-13374	33.50	3.00	7.88	0.25	8.30	5.60	25.00	5
South-22	NC96-14629	32.50	10.50	10.94	1.16	7.90	5.90	18.80	3
	Average	34.38	19.64	9.24	1.96	29.76			
	CV	3.06	71.06	31.67	84.81	88.10			
	LSD (0.05)	1.48	0.20	4.31	2.35	26.10			

Footnotes apply to both Table 1 and Table 2.

* Incidence is reported as the percentage of scab infected heads per 50 heads.

^ Head Severity is reported as the percentage of scab infected spikelets per 50 heads excluding non-infected heads.

Plot Severity is reported as the percentage of scab infected spikelets per 50 heads including non-infected heads. This measurement is the same as the FHB Index.

⌘ Percentage of infected spikelets ((number of infected spikelets/total number of spikelets per head) x 100) 21 days post-inoculation.

● Data was not recorded due to late flowering.

Table 2. 2000 Uniform Northern Winter Wheat Screening Nursery, Lexington, Kentucky

		Field Data					Greenhouse Data			
Entry	Name	Height (inches)	Heading Date (May)	Average Incidence*	Average Head Severity ^	Average Plot Severity #	21 dpi ⌘	Min	Max	rank
North-1	Patterson	38.50	3.75	17.00	15.18	2.45	55.40	5.00	95.50	27
North-2	Freedom	38.75	4.50	35.50	9.94	3.44	6.10	4.50	14.30	3
North-3	Pioneer 2545	38.00	5.25	47.00	12.42	6.08	18.60	4.50	56.00	18
North-4	Ernie	33.00	2.00	15.50	7.73	1.26	6.50	5.90	7.10	6
North-5	NY87047W-6048	40.00	11.75	54.00	11.86	6.66	8.60	3.80	13.60	11
North-6	NY87047W-6041	40.00	14.00	35.50	9.43	3.32	11.80	4.20	23.10	13
North-7	NY87047W-7405	35.00	4.75	23.00	10.26	2.53	8.50	4.80	30.00	10
North-8	NY87048W-7387	43.00	13.75	25.50	8.24	2.10	6.00	4.50	10.50	2
North-9	NY87048W-7388	41.25	15.00	18.50	8.00	1.48	7.00	4.30	16.70	8
North-10	IL95-4162	38.00	3.25	20.00	7.84	1.65	6.20	5.60	6.70	4
North-11	IL96-7654	37.00	3.50	18.00	9.30	1.81	7.80	0.00	27.80	9
North-12	IL97-2945	40.75	3.25	16.50	7.00	1.16	16.00	4.30	41.20	16
North-13	IL96-3073	36.75	5.00	8.00	7.93	0.63	6.40	5.00	15.00	5
North-14	Roane	34.00	4.50	35.50	9.48	3.58	13.50	4.80	72.70	14
North-15	VA96W-329	33.25	5.00	33.00	11.91	3.93	54.30	5.90	100.00	26
North-16	VA96W-326	35.25	1.75	30.00	12.28	4.17	84.40	43.80	100.00	28
North-17	VA96W-250	33.25	2.00	39.00	11.24	4.33	21.20	5.00	35.00	20
North-18	VA96W-749	34.75	5.00	48.00	9.72	4.58	23.20	4.50	100.00	22
North-19	NE94654	38.00	10.00	30.50	10.12	2.98	22.60	4.30	40.90	21
North-20	MO 982030	32.00	1.00	35.50	8.26	2.94	20.80	6.30	88.20	19
North-21	MO 971022	32.25	1.25	21.50	9.16	1.93	26.30	5.60	100.00	23
North-22	MO 980725	35.75	5.00	30.50	9.97	3.02	16.60	4.30	100.00	17
North-23	MO 980525	39.50	10.50	22.50	12.10	2.66	●			
North-24	KY 90C-049-31	39.00	6.50	49.00	13.52	6.92	6.70	4.80	15.80	7
North-25	KY91C-117-33	37.75	5.00	21.50	10.08	2.37	9.40	4.80	20.00	12
North-26	OH645	38.00	4.75	27.50	9.66	2.52	52.30	5.90	100.00	25
North-27	OH650	39.50	4.00	32.50	9.65	3.09	26.90	5.60	56.30	24
North-28	OH661	37.50	4.00	31.50	11.88	3.71	5.60	4.80	6.30	1
North-29	OH688	41.70	9.75	28.50	8.33	2.38	14.10	5.30	35.30	15
	Average	37.29	5.85	29.33	10.09	3.09	20.10			
	CV	3.45	23.53	36.78	26.76	54.76	100.90			
	LSD (0.05)	1.81	1.93	0.15	3.78	2.39	20.60			

BREEDING FOR FUSARIUM HEAD BLIGHT RESISTANCE IN SOFT RED WINTER WHEAT

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OBJECTIVES

The long-term objective of this project is to develop soft red winter wheat genotypes with excellent resistance to scab combined with resistance to other diseases, high yield potential, and all of the other traits required in a successful variety. This is also one of the central goals of the U.S. Wheat and Barley Scab Initiative.

Short-term objectives for our project are:

- To combine genes for scab resistance from diverse sources,
- To identify breeding lines with better resistance to scab than any of the parents (transgressive segregrants), and
- To evaluate doubled haploid lines and identify scab resistant lines.

INTRODUCTION

Scab or Fusarium head blight (FHB), caused by *Fusarium graminearum* Schwabe and sometimes other Fusarium species, is a severe disease of wheat. Scab infection causes significant loss of grain yield, lower test weight, reduced grain quality, and reduced milling yield (McMullen et al. 1997). This results in substantial loss in value for both the producer and the wheat milling industry. Further, Fusarium species produce trichothecene mycotoxins that are detrimental to both humans and livestock. Damage due to Fusarium head blight was severe in Illinois in 1990, 1991, 1995 and 1996.

Introduction of scab resistant wheat varieties would contribute to improved food safety and reduce losses suffered by producers (Bai and Shaner, 1994). Different types of host plant resistance to scab have been described including: 1) resistance to initial infection, 2) resistance to invasion of plant tissue by the fungal hyphae, and 3) inhibition of translocation or degradation of deoxynivalenol, 4) resistance to accumulation of mycotoxins, and 5) yield tolerance (development of filled kernels in infected plants) (Stack, 2000). Resistance to scab is quantitative, and different mechanisms of resistance are controlled by different genes (Bai and Shaner, 1994). Development of varieties with a high level of scab resistance may require combining scab resistance from different sources (Singh and van Ginkel, 1997).

MATERIALS AND METHODS

Specific crosses have been made to combine genes for scab resistance from different sources into genotypes that can be used in developing scab resistant varieties. Populations from single, three, and four - way crosses, as well as backcross populations, have been developed. Single crosses have been made involving different sources of resistance and breeding lines with other desirable traits. Three and four-way crosses were made to combine several sources of scab resistance and to combine scab resistance with other traits required in an adapted variety. Some populations have been produced from four-way crosses in which three of the parents are soft red winter wheat parents and the fourth parent is Ning 7840. Some populations are also being developed combining Type I resistance with Type II resistance.

Experimental breeding lines are evaluated for scab resistance and lines are selected using both a greenhouse inoculation method and an irrigated, inoculated field evaluation nursery. Procedures used in the field and greenhouse are being evaluated to improve techniques and increase efficiency.

Greenhouse evaluation of scab resistance: Needle inoculation of a single floret near the middle of a head is used for greenhouse evaluations (Bai and Shaner, 1994). A floret is inoculated with 2.0 µL of a balanced macroconidial suspension of 30 or more *F. graminearum* isolates. Following inoculation, plants are placed in a mist chamber for three days. Type II resistance is evaluated at 14 and 21, or 21 and 28 days after inoculation, depending upon the project.

Field evaluation of scab resistance: Experimental breeding lines for evaluation are planted in replicated one meter long rows. A mist irrigation system provides daily misting for two hours both morning and evening during flowering. Rows are inoculated by placing *F. graminearum* infected wheat seed on the ground between the rows. Data are collected on incidence and severity, and the FHB index is calculated. Incidence is assessed by visual estimation of the percentage of heads in a plot that show symptoms. Severity is determined as the average percentage of scabby spikelets over 7 to 10 random heads per plot. Seed is harvested, cleaned slightly, and rated to determine the percentage of shriveled and diseased kernels. A grain sample of the most resistant breeding lines is sent to Patrick Hart for deoxynivalenol (DON) analysis.

Scab resistant lines are evaluated for yield, agronomic traits, and resistance to other diseases. Scab resistance is only one trait required in an adapted variety. Combining scab resistance with many other traits is required for a successful, adapted variety.

We will evaluate doubled haploid lines produced from selected crosses. A wheat x maize hybridization method was used for production of doubled haploids (Chen, et al., 1999), with some modifications (S. Xu, personal communication). We selected a few three- and four-way crosses to work with, each involving more than one source of scab resistance and including adapted, high yield parents. Putative doubled haploid seed has been harvested from several plants, and seed will be planted in the greenhouse for increase.

RESULTS AND DISCUSSION - 1999-2000 PROGRESS

Four lines from the Illinois program were entered into the 2000 Cooperative Eastern Winter Wheat Fusarium Head Blight Screening Nursery. Based on the data available at this time, the lines from the University of Illinois program seem to be among the most scab resistant lines in the 2000 nursery. By entering these four breeding lines into the cooperative screening nursery these lines were made available to other breeders for use as parents.

About 300 single and three-way crosses were made with one or more scab resistant parents in each cross. In addition, 99 crosses were made with the objective of combining scab resistance genes from several sources. Many of the crosses in the second set involve parents with excellent scab resistance, but many of these parents are also unadapted or have low yield potential.

About 680 breeding lines were evaluated in replicated rows in the 2000 misted, inoculated scab evaluation field nursery. In addition, about 1500 entries from single plots and 1200 headrows were also evaluated in the field. Heads were selected from 35 F3 bulk populations grown in the field scab nursery, and 3040 headrows resulting from these selections were planted this fall (2000-01 season).

Plants from six segregating populations were screened in the greenhouse in 1999-2000. A total of 2220 plants were evaluated, and 844 plants (38%) were selected (most with Type II resistance equal to or better than Ernie).

Doubled haploid plants were produced using the wheat x maize hybridization technique. About 1770 embryos were rescued, 411 haploid seedlings were transplanted to the greenhouse, 327 plants survived the colchicine treatment, and an estimated 75% have produced seeds.

Scab resistant lines were evaluated in the field for many traits. Many of the lines with excellent scab resistance are unacceptable for other traits such as grain yield, milling and baking quality, standability, or resistance to other diseases. This problem is not resolved, but we are using backcrosses and three-way crosses to attempt to develop well-adapted scab resistant lines. We are also continuing to select and evaluate as many new experimental breeding lines as possible.

REFERENCES

Bai, G. and G. Shaner. 1994. Scab of wheat: Prospects for control. Plant Dis. 78:760-766.

Chin, J., C., C.A. Griffey, M. Chappell, J. Shaw, and T. Pridgen. 1999. Haploid production in twelve wheat F1 populations via the wheat x maize hybridization method. 1998 National Fusarium Head Blight Forum, Oct. 26-27, 1998. East Lansing, Michigan.

McMullen, M., R. Jones, and D. Gallenburg. 1997. Scab of wheat and barley: A re-emerging disease of devastating impact. Plant Dis. 81:1340-1348.

Singh, R. P., and M. van Ginkel. 1997. Breeding strategies for introgressing diverse scab

resistance into adapted wheats. In H. J. Dubin, L. Gilchrist, J. Reeves, and A. McNab, (eds.) Fusarium Head Scab: Status and Future Prospects. Mexico, D.F.: CIMMYT. p.86-92.

Stack, R.W. 2000. Return of an old problem: Fusarium head blight of small grains. Plant Health Progress - Plant Health Reviews - 22 June, 2000. http://www.planthealthprogress.org/current/reviews/fhb/article.htm

WINTER WHEAT BREEDING FOR SCAB RESISTANCE IN SOUTH DAKOTA

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ABSTRACT

Fusarium head blight (scab) causes losses of $200,000 to $800,000 in winter wheat in South Dakota per year. Losses of this magnitude have led to the development of programs devoted to finding sources of resistance to this disease. South Dakota State University (SDSU) has a program designed to test cultivars and elite and preliminary lines for their resistance to scab, identify winter wheat germplasm sources that show a high level of scab resistance, and develop populations segregating for scab resistance and desirable agronomic traits. The breeding plan consists of the F2 and F3 populations being grown as bulks under normal winter wheat production practices. Individual F3 plants will be evaluated for scab reaction by millet inoculation. F3:4 progeny rows are planted under normal winter wheat production practices and selected for agronomic performance. Individual head and the bulk will be harvested. In year4, F3:5 yield trials are grown and corresponding F4:5 progeny rows are grown in the scab nursery. Entries with good yield and scab reaction will be advanced by bulking superior progeny rows. This bulk will be planted in year 5 in F4:6 yield trials in the scab nursery and will also be screened in the greenhouse. Selections from year 5 will be advanced to multi-location yield trials in year 6. This program screened the following nurseries for scab resistance in 2000: Northern Regional Performance Nursery, Winter Wheat Regional Scab Nursery, South Dakota Crop Performance Trials (commercial varieties), SDSU Advanced Hard Red and Hard White Yield Trials, and SDSU Preliminary Hard Red and Hard White Yield Trials. Approximately 6000 plants were evaluated for scab resistance during the 2000 season. 1500 of the plants were kept and were planted into the field this fall. Scab resistance sources included in the selected populations included adapted spring wheats from the SDSU breeding program, Sumai 3 derived spring wheat lines, eastern European winter wheat lines, entries from the 1998 and 1999 regional winter wheat scab nursery, and adapted hard red and hard white breeding lines.

FUSARIUM HEAD BLIGHT RESISTANCE OF WHEAT LINE NING894037

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ABSTRACT

Fusarium head blight (FHB) of wheat (*Triticum aestivum* L.) caused by *Fusarium graminearum* Schwabe is a devastating disease worldwide. Characterization of new sources of FHB resistance is important to effectively develop improved wheat cultivars that have resistance to FHB. Wheat line Ning894037 was previously identified as inhibiting the spread of the disease after infection (type II resistance). In this study, 217 $F_{8:9}$ recombinant inbred lines were developed from a cross between Ning894037 and Alondra, a moderately susceptible cultivar developed in CIMMYT, and characterized for resistance to FHB in a field nursery at Lafayette, IN. At flowering, one floret of the 3rd or 4th spikelet from the tip of spikes was inoculated with a spore solution of *F. graminearum* and a plastic bag was immediately placed over inoculated spikes for 3 days and then removed. Ten primary spikes of the 217 lines and the two parent lines were inoculated. The number of infected spikelets and the total number of spikelets on inoculated spikes were recorded at 25 days after inoculation. On average, 18 % of spikelets of inoculated spikes of Ning894037 and 58 % of spikelets of Alondra became diseased. Percentage diseased spikelets for the 217 lines ranged continuously from 5% to 100%. The segregation pattern indicates that Ning894037 has at least one FHB resistant gene of significant effect and Alondra may have one resistance gene of small effect. There was no correlation between date of flowering and resistance to FHB.

FUSARIUM HEAD BLIGHT IN THE F-2 AND F-3 GENERATIONS OF A SPRING WHEAT RECOMBINANT POPULATION

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ABSTRACT

From the spring wheat cross ND2709/ND688, 107 individual F-2 plants and F2:3 families were screened for Fusarium head blight (FHB) reaction in greenhouse tests. ND2709 is a FHB-resistant line (Sumai3/Wheaton//Grandin) and ND688 is adapted but susceptible to FHB. On each individual F-2 plant 3-5 heads were inoculated at anthesis by spikelet injection with conidia of *F. graminearum*. Seed was saved from non-inoculated heads of the F-2 plants. Fifteen F2:3 seeds were planted in three replicates. Wheat heads were inoculated at anthesis as before. In both experiments, individual heads were scored for FHB symptoms on a 0-100% scale at 3.5 weeks postinoculation. Both the F-2 and F-3 generations showed highly significant differences in FHB, but the correlation between generations was poor (r = 0.37). Selecting the “best” and “worst” of the F-2 plants did result in population shifts in the F-2:3, but F-2 selection would have neither retained the most resistant F-3 lines nor discarded the most susceptible ones. Selection for FHB resistance in spring wheat should be deferred until at least the F-3 generation. (This poster was presented at the American Phytopathological Society North Central Division Meeting, Columbus, Ohio, June nnn 2000. The abstract will be published in Phytopathology 91(6) (Supplement in 2001).

MAINTAINING FUSARIUM HEAD BLIGHT RESISTANCE IN SPRING WHEAT THROUGH SUCCESSIVE BREEDING CYCLES

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ABSTRACT

Resistance in wheat to Fusarium Head Blight (FHB) is a character of highly complex inheritance. Introducing such a trait into commercial wheat and maintaining it through successive cycles of crossing to adapted but susceptible parents is a difficult task, requiring reliable disease testing procedures. For this purpose we have combined FHB screening in an inoculated, irrigated field nursery and greenhouse testing of elite materials. The resistant FHB response was retained in lines representing progeny from first, second, third, and fourth breeding cycles of several different spring wheat crosses. While some first and second cycle progeny showed good FHB resistance, none combined that resistance with the agronomic traits needed for commercial release. A few third cycle and several fourth cycle derived lines combined those traits and some are candidates for release as commercial cultivars. In spring of 2000, North Dakota State University released 'Alsen' wheat, a third cycle derivative combining moderate resistance to FHB from Sumai3 with agronomic and quality traits making it suitable for commercial production throughout North Dakota and adjacent regions. (This poster was presented at the National Association of Wheat Growers (NAWG) - 3rd Annual Wheat Industry Research Forum in Las Vegas, NV, Feb 10 - 11, 2000. The paper is published in the Proceedings at http://www.wheatworld.org/Proceedings2000/index.htm.)

SELECTING FOR FHB RESISTANCE IN EARLY GENERATIONS OF WINTER WHEAT POPULATIONS

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OBJECTIVES

The objective of this study was to inoculate the F_2 generation of known Resistant and Susceptible crosses of winter wheat with *Fusarium graminearum* to determine if it is possible to identify the more susceptible segregants and discard them as soon as possible in a breeding program. The moderately resistant and highly resistant segregants should be retained for further evaluation in later generations as they approach homozygousity. The second objective was to determine the correlation between visual symptoms and DON content in that F_2 population and then determine if visual selection was effective in isolating segregants with lower DON content.

INTRODUCTION

In wheat the two main types of resistance for Fusarium head blight (FHB) are generally recognized: Type 1 is resistance to initial infection, and Type 2 is the resistance to spread of symptoms within the head (Schroeder and Christensen, 1963). The genetic control of FHB resistance is very complex as different varieties carry resistance genes on different chromosomes (Weizhong, 1999). Two populations of winter wheat were used in this study. F_2 progeny derived from a cross of resistant (Frontana) and susceptible (Ruby) parents, with Type 1 resistance, and F_2 progeny derived from cross of resistant (WEKO60DH4 - a Sumai 3 derivative) and susceptible (Pioneer 2737W) parents, with Type 2 resistance. Sumai 3, and Frontana are among the most frequently used sources for FHB resistance around the world. An F_2 generation, as the first segregating generation, with a higher frequency of heterozygotes and highest frequency of plants with desirable alleles has been used in this study. It known that higher levels of heterozygosity reduces heritability, but if it were possible to discard all susceptible segregants as early as possible, smaller numbers of plants will be carried to the next generation, and significant economies in time and resources will be realized.

MATERIAL AND METHODS

Two populations of winter wheat were used in the study. For vernalization, the seeds were surface sterilized in 3 % sodium hypochlorite solution, soaked for six hours at 20 ^{0}C, patted dry and left to germinate for eighteen hours at 20^{0}C in plastic petri dishes. After eighteen hours, the moisture level was adjusted to 50 % of seed dry weight and the sealed petri dishes with germination paper disc, placed in a drawer in the cold storage room, at 4 ^{0}C. The dishes were weighed each week to keep the moisture level at 50 %. After eight weeks the plants were transferred to a soil mixture (three parts soil, two parts turface, and one part

peat moss), in the growth room which was maintained at 20/14^{0}C, with a 16-hour light cycle. When 50 % flowering stage was reached, each plant was inoculated with a suspension of macroconidia of *F. graminearum* (isolate DAOM178148, 50,000 spores/mL). The screening was done in the growth room from above. A spray inoculation method was used for the population with Type 1 resistance (Ruby/Frontana), where 2 mL of suspension of macroconidia of *F. graminearum* was sprayed onto individual heads. Point inoculation was used for population with Type 2 resistance (WEKO60DH4/2737W), where 10 Fl were injected into single florets. The plants were exposed to 100 % relative humidity for 48 hr, to initiate infection started. Infected spikelets were counted 7, 14, 21 days after inoculation, and area under disease progress curve (AUDPC) was calculated according to Shaner, and Finney (1977). Pearson correlation coefficients between AUDPC and 7, 14, 21 days after inoculation were calculated for each population. Deoxynivalenol (DON) content was estimated from groups of plants out of the Ruby/Frontana cross which representing most resistant, moderate resistant, and most susceptible progeny plants, compared with parents, using the competitive ELISA test (Sinha and Savard, 1996). Pearson correlation coefficients between AUDPC and DON were calculated.

RESULTS AND DISCUSSIONS

It is generally agreed that FHB resistance is controlled by multiple genes. In our study the segregation patterns in both populations confirmed more than one gene controlled resistance. The frequency distributions for AUDPC after inoculation with *F. graminearum* are shown in Figure 1 and 2. Transgressive segregants were obtained from both populations. The population with Type 2 resistance (WEKO60DH4/2737W) showed a higher frequency of transgressive segregants than the population with Type 1 resistance (Ruby/Frontana), suggested that genetic control of Type 1 and Type 2 resistance could be different. These results are consistent with those of Fedak et al. (1998).

Pearson correlation coefficients for AUDPC and 7, 14, 21 d after inoculation were significant for both populations. For the population with Type 2 resistance (WEKO60DH4/2737W) they were 0.72, 0.88, and 0.87, respectively, and for population with Type 1 resistance (Ruby/Frontana),

0.70, 0.93, and 0.89, respectively. According to our results from both populations, 14 days after inoculation is the best time for scoring FHB visually. Also, correlation coefficients between AUDPC and 14 days after inoculation were slightly stronger in population with Type 1 resistance, than in population with Type 2 resistance (0.93, and 0.88, respectively). Pearson correlation coefficient between AUDPC and DON, for the population with Type 1 resistance (Ruby/Frontana), was r = 0.70 and significant.

Since the majority of the plants in the F_2 generation are heterozygous they will continue to segregate. However based on the single trials that were conducted in this study it should be possible to eliminate the susceptible segregants at this stage and thus carry fewer and potentially more resistant lines into subsequent generations.

Future studies will involve growing selected progeny in field plots to determine relationship between growth chamber and field, relationship between F_2 plants and F_3 row performance

data, determine if spray inoculation of field plots is an effective tool to augment breeding programs. The lines showing the best FHB resistance in above trials will be used as parents to cross to commercial Ontario winter wheat cultivars in order to raise their FHB resistance levels.

REFERENCES

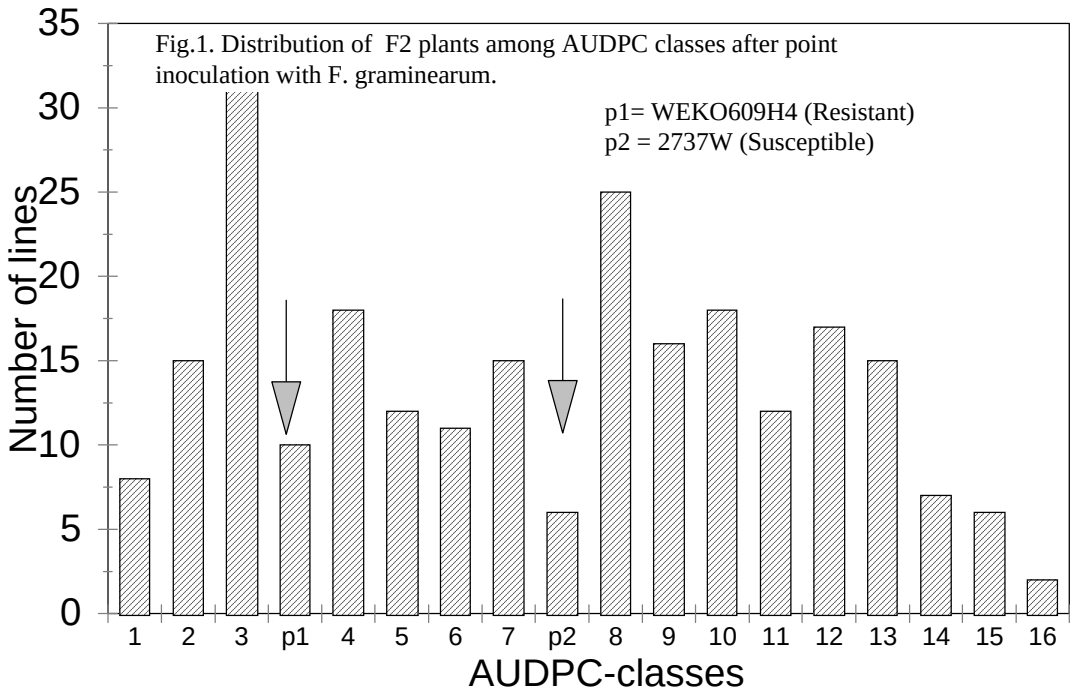
Fedak, G., J. Gilbert, Armstrong, K., Procunier, D., Tuiker, N., Bulter, G., Pandeya, R. 1998. Studies of Inheritance of Fusarium Head Blight Resistance in wheat. The 1998 National Fusarium Head Blight Forum. East Lansing, Michigan, USA. October 26-27, 1998. 25-28.

Schroeder, H. W., and Christensen, J. J. 1963. Factor affecting resistance of wheat to scab caused by *Gibberella zeae*. Phytopathology. 53: 831-838.

Sinha, R.C., and Savard, M.E. 1996. Comparison of immunoassay and gas chromatography methods for the detection of the mycotoxin deoxynivalenol in grain samples. Can J Plant Path 18:233-236.

Shaner, G., Finney, R. 1977. The effect of Nitrogen Fertilization on the Expression of Slow-Mildewing Resistance in Knox Wheat. Phytopathology. 1051-1056.

Weizhong, L. 1999. Wheat Scab in China: Breeding, Research, and Development. Canadian Workshop on Fusarium Head Blight. Winnipeg, Manitoba, November 28-30, 1999. 18-25.



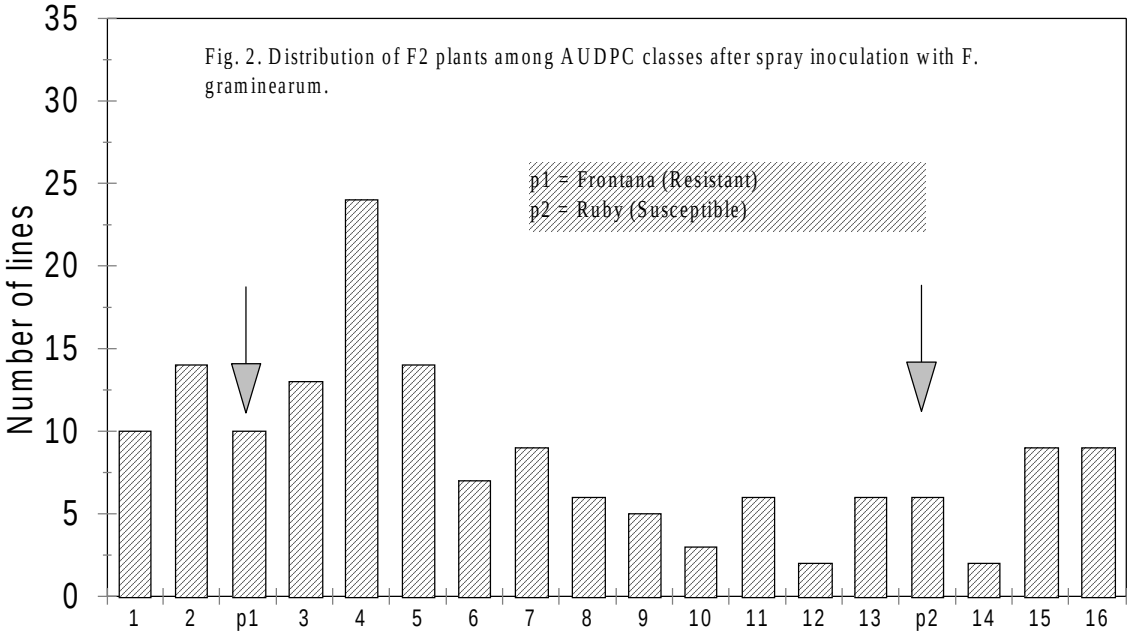


Fig. 2. Distribution of F2 plants among AUDPC classes after spray inoculation with F. graminearum.
p1 = Frontana (Resistant)
p2 = Ruby (Susceptible)
Number of lines
0
5
10
15
20
25
30
35
1
2
p1
3
4
5
6
7
8
9
10
11
12
13
p2
14
15
16

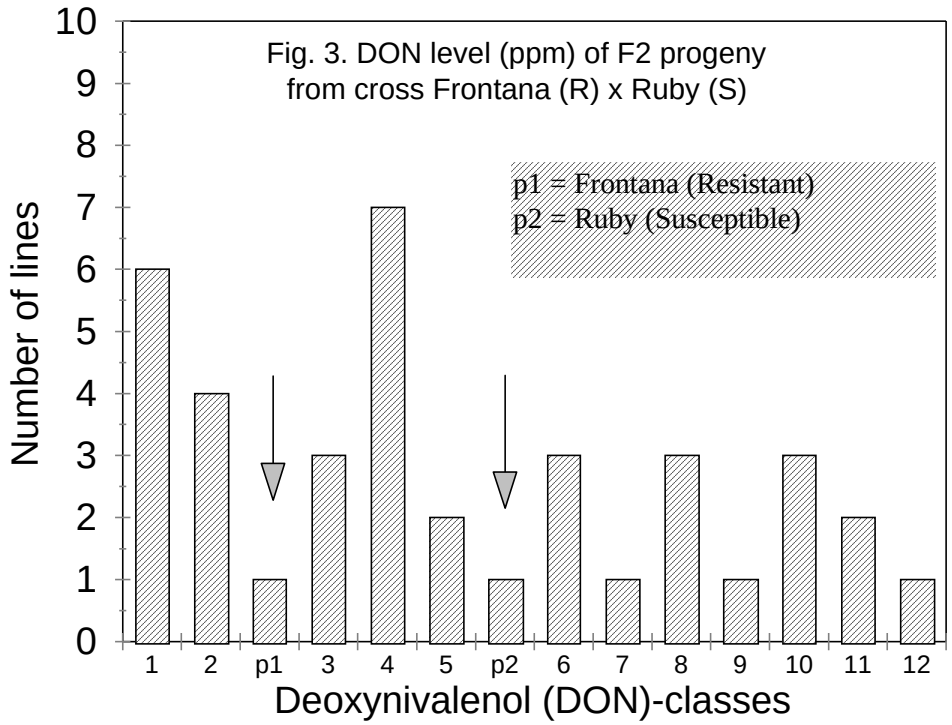


Fig. 3. DON level (ppm) of F2 progeny from cross Frontana (R) x Ruby (S)
p1 = Frontana (Resistant)
p2 = Ruby (Susceptible)
Number of lines
0
1
2
3
4
5
6
7
8
9
10
1
2
p1
3
4
5
p2
6
7
8
9
10
11
12
Deoxynivalenol (DON)-classes

MOVEMENT OF *FUSARIUM GRAMINEARUM* IN WHEAT SPIKES FOLLOWING GREENHOUSE INOCULATION

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OBJECTIVE

To relate visual spikelet infection following greenhouse inoculation to *F. graminearum* infection in individual spikelet components.

INTRODUCTION

Resistance to FHB in wheat is quantitative and complete resistance has not been observed for any genotype (Snijders and Krechting, 1992). This has led to extensive evaluation of cultivars and germplasm in the greenhouse and field to determine the inheritance of resistance. Components of physiological resistance to infection have been identified and include resistance to the establishment of initial infection (Type I) and resistance to spread of the fungus within the spike (Type II). Wheat is generally most susceptible to FHB at flowering as the fungus first infects the extruded anthers and then moves throughout the developing caryopsis. Under favorable conditions spread of the disease within a spike may have two distinct stages: spread of the fungal symptoms into the rachis, and subsequent spread of symptoms through the rachis and into other spikelets (Bai and Shaner, 1996). Histological studies of pathogen spread in the spikelet provide evidence that the path of infection follows the vascular tissue, with most rapid growth in the longitudinal direction with slower transverse growth (Schroeder and Christensen, 1963). Ribichich et al. (2000) identified two paths of fungal invasion in the wheat spike. In the horizontal path the anthers and bracts of contiguous florets in the first spikelet were colonized, followed by movement through the rachis and rachilla to the contiguous spikelet. In the vertical path movement occurred in the spike through vascular bundles and parenchyma to spikelets above and below the original site of infection, followed by chlorosis, necrosis, and occlusion of the vascular bundles.

MATERIALS AND METHODS

Plants of 22 wheat varieties and breeding lines (Uniform Southern Scab Screening Nursery) with various levels of Type II resistance to FHB were established in pots in the greenhouse. At flowering, macroconidia spores were injected into a single floret (between lemma and palea) of a spikelet in the middle of eight spikes of each variety. Injections were made from a composite of 12 different isolates of *Fusarium graminearum.* After misting the inoculated spikes for three nights in a high humidity chamber to encourage fungal growth, the pots were moved to greenhouse conditions. Spikes and individual spikelets were visually rated for disease incidence and severity at 7, 14, 21 and 28 days post inoculation (dpi). At maturity the eight spikes from all genotypes were removed from the plants and held at laboratory temperatures.

The spikes from five genotypes (Ernie, SC 921299, GA 89482-E7, Roane and VA96W-326) which had high (100%) and low (7%) greenhouse infection were dissected into each component [rachis (section immediately below spikelet), glume, lemma, palea, seed] of the lowest floret of all spikelets on the spike. Each component was plated on a modified PCNB agar, grown in light at 25 ℃ for 14 d and examined for *F. graminearum* infection. The results of plating individual components were related to the visual greenhouse ratings of FHB spikelet infection. The remaining 18 genotypes were harvested, the seeds were hand threshed and will be evaluated as a composite sample (across 8 spikes) for *Fusarium spp.* seed infection and seed germination (Testing in Progress).

RESULTS

The percentage of infected spikelets [(number of infected spikelets/total number of spikelets per head) x 100] at 28 dpi in the greenhouse ranged from 7 (Ernie) to 100% (SC 921299). When individual components of the inoculated floret from each spikelet were evaluated, all five components for all genotypes were always infected with *F. graminearum.* There was usually little seed development in the inoculated floret. The mean infection of individual components of each floret for each genotype was determined by averaging the number of infected components across the total number of spikelets in eight spikes for that genotype.

F. graminearum infection for Ernie (average across 14 spikelets) for the rachis and seed components was 19 and 15%, respectively, which was more than double the 7% spikelet infection estimated in the greenhouse (data not ahown). Low levels of rachis infection moved to five spikelets below the point of inoculation (PI, floret inoculated), but only to two spikelets above the PI.

Although estimates of spikelet infection for Roane were at low levels (9%) in the greenhouse, much higher average levels of *F. graminearum* (across 19 spikelets) occurred for all components when plated in the laboratory (Fig. 1). Average rachis and seed infection was 40 and 29%, respectively, while the infection of glumes (26%), lemma (18%) and palea (17%) was lower but still double the visual estimates of spikelet severity. Rachis infection in Roane was 50 to 63% for all spikelet locations below the PI, but at low levels above this point. The other four components of the spikelet had similar trends of higher infection below the PI than above.

The three experimental breeding lines (VA 96W-326, SC-921299, GA-89482-E7) had much higher levels of spikelet infection in the greenhouse (72,100 and 80%, respectively). When spikelet components were evaluated, the average infection of the VA and SC breeding lines were lower than greenhouse estimates and the seed infection for GA line was 82% which was nearly identical with greenhouse spikelet infection (Fig. 1). Similar to Roane the infection levels of the rachis were always highest followed by infection in the seeds, glumes, lemma and palea. Seed infection for the VA and SC breeding lines was 18 and 42 percentage points lower than visual estimates of spikelet severity in the greenhouse. As for Roane the infection in all components for the VA and SC genotypes was consistantly high in spikelets below the PI, but dropped off sharply above this point. In contrast to the four other genotypes the levels of infection for all components of spikelets for the GA genotype remained high below and above the PI.

DISCUSSION

There was considerable difference between greenhouse estimates of *Fusarium* infection in spikelets and actual infection by *F. graminearum* in seeds and other spikelet components. The greenhouse estimates were much greater than laboratory infection for the VA and SC breeding lines, lower than the laboratory for Roane and about equal for Ernie (low levels) and GA breeding line (high levels). Does this mean that the inoculation system used by many plant breeders for screening and selection may be giving misleading results? Additional studies are planned to determine if the greenhouse estimates can be improved by more careful evaluation? Another concern may be that the levels in the laboratory are too high and misleading, however there was no doubt that *F. graminearum* infection occurred where indicated.

The movement of *F. graminearum* infection in the spike following inoculation was somewhat variable depending on genotype. There was a general trend, however for little infection above the inoculated spikelet in four of the five genotypes, while the spikelets below were infected. A review of the literature regarding the vascular system of the peduncle, spike, rachis and spikelets, shows that there are vascular bundles from transfer cells at the base of the spikelet that connect to the glumes, lemma, palea and developing ovule (Zee and O'Brien, 1971). Thus, if the *F. graminearum* moves via the phloem as proposed by Ribichich et al. (2000) from the inoculated floret, we see little reason why it shouldn't move to other components via the rachis both up and down the spike. The higher levels in the rachis than in other components would tend to support such movement. This is also supported by the research by Wang (1982) in which a grading system was developed based on rachis infection.

It has been proposed that spikelets above the point of infection are unable to obtain the nutrients and water needed for full development (Schroeder and Christensen,1963; Snijders and Krechting, 1992) and the tissue is killed to cause the white head symptoms. If this occurs there should be little or no seed development above the point of inoculation, however well developed seeds were found at the top of the spike in these studies, some of which had germinated in the PCNB media during the 14 day evaluation period.

The results of this preliminary investigation have shown that greenhouse visual ratings of inoculated spikelets may be poorly associated with the *F. graminearum* occurring in infected components the same individual spikelets. Our data has also shown that fungal movement in the spike occurs in three ways; localization around the point of inoculation (PI), movement both up and down the spike from the PI and movement primarily downward from the PI. Additional greenhouse and field studies are proposed to evaluate the movement of fungal hyphae into the various components of the spike following inoculation at various locations on the spike. These experiments will allow us to closely evaluate the method of single floret inoculation and possibly improve the accuracy of the visual rating system for spikelet infection.

REFERENCES

Bai, Guihua and Gregory Shaner. 1994. Scab of wheat: prospects for control. Plant Disease 78: 760-766.

Ribichich, K. F., S. Lopez, A. C. Vegetti. 2000. Histopathological spikelet changes produced by Fusarium grminearum in susceptible and resistant wheat cultivars. Plant Disease 84: 794-802.

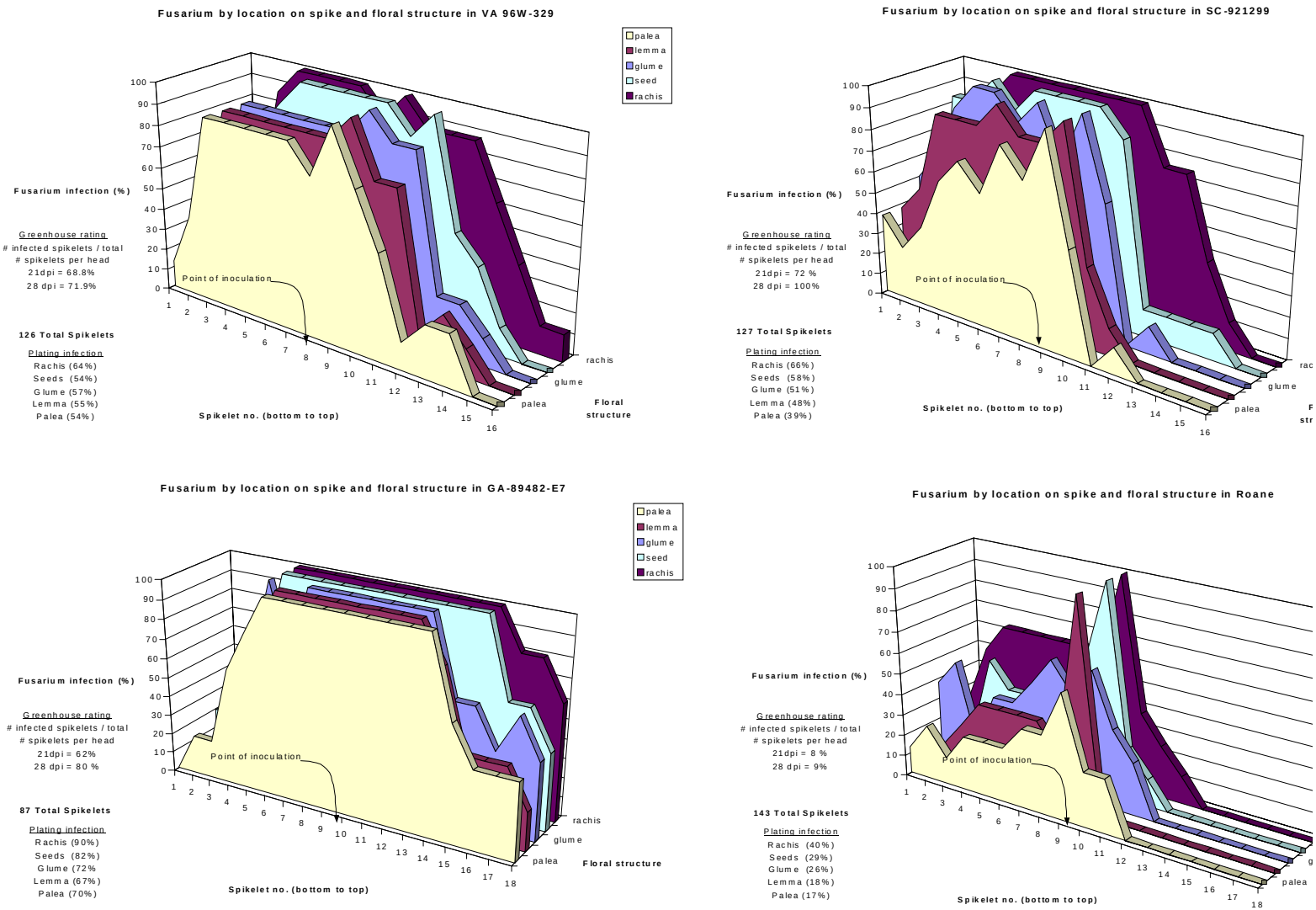
Schroeder, H. W. and J. J. Christensen. 1963. Factors affecting resistance of wheat to scab caused by Gibberella Zeae. Phytopathology 53:831-838.

Snijders, C. H. A. and C. F. Krechting. 1992. Inhibition of deoxynivalenol translocation and fungal colonization in *Fusarium* head blight resistant wheat. Canadian Journal of Botany 70: 1570-1576.

Wang, Y.-Z., X.-N. Yong and Q.-P. Xiao. 1982. The improvement of identification technique of scab resistance of wheat and the development of resistant sources. Sci. Agric.Sin. 5: 67-77.

Zee, S.-Y. and T. P. O'Brien. 1971. Vascular transfer cells in the wheat spikelet. Australian Journal of Biological Science 24:35-49.

Figure 1. Preliminary results of *Fusarium graminearum* infection in the components of wheat spikes for eight spikes and four g



FUSARIUM HEAD BLIGHT RESISTANCE IN SPRING WHEAT AND BARLEY: EFFECTIVE SCREENING NURSERIES

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OBJECTIVES

Our objective is to establish an effective screening nursery that (i) provides a range of environments with moderate to severe FHB disease pressure;(ii) identifies appropriate measures of FHB resistance; and (iii) includes advanced genetic materials from regional breeding programs.

INTRODUCTION

Disease resistant cultivars probably represent the best method of controlling FHB in spring wheat and barley. However, differences among cultivars/lines are pronounced only when environments favor disease development and traits directly related to FHB are measured. Our initial (1995-1996) approach was to compare several methods of inoculation (spore suspensions, crop residues, inoculated grain) and several measures of FHB (density segregation of infected seed, visual estimates of plant infection, and test weight, among others) using spring wheat and barley cultivars with fairly well known FHB susceptibility/tolerance. Later (1997-1998), our approach was to use different rates (0-150 lbs/a) of inoculated corn seed to establish varying levels of disease and to assess grain yield, kernel infection, deoxynivalenol concentration, kernel weight, and test weight as primary measures of FHB resistance. We have also (1999) used 10 fold differences in rates of inoculated corn seed to establish logarithmic increases in disease potential and then assessed appropriate disease-related traits. Every year since 1997, we have requested advanced lines and cultivars to include in the nursery from spring wheat and barley breeding programs in Manitoba, North and South Dakota, and Minnesota. This report describes research done in 2000 using differences in the length of time FHB was allowed to progress as a method of providing environments with moderate to severe FHB disease pressure. This approach was attempted because differing rates of inoculum did not provide the desired range of disease levels.

MATERIALS AND METHODS

Separate, misted nurseries were established for spring wheat and barley, each using a split-plot arrangement of a randomized complete block design with four replications. Six infection periods were whole plots and 20 cultivars/lines were subplots. Misting periods provided 0, 5, 10, 15, 20, and 25 days after heading (approximate mean of 20 entries) for FHB infection and progression. After allowing the appropriate time for disease progression, misting was discontinued and FHB was controlled by spraying designated whole plots with fungicide. Once initiated, spraying (6 oz./acre Folicur and 2 lb/acre Benlate) was repeated every five days. Cultivars/lines included susceptible and tolerant checks and advanced

lines from spring wheat and barley breeding programs in Manitoba, North and South Dakota, and Minnesota. In addition to standard measures of agronomic performance, estimates of FHB included visual scores of disease incidence and severity, percent kernel infection following plating on acidified potato dextrose agar (APDA) and dexynivalenol (DON) concentration. Data were analyzed using standard procedures for an analysis of variance of a split-plot arrangement of a randomized complete block design. Separate analyses were conducted for wheat and barley, considering infection periods and cultivars/lines as fixed effects. Also, for each crop, regression procedures described by Eberhart and Russell (1966) were used to determine cultivar/line responses to environments (infection periods) with varying amounts of disease pressure. Results (b values) from the regression analyses for DON concentration were used to separate cultivars/lines into three groups: susceptible, less susceptible and least susceptible.

RESULTS AND DISCUSSION

The percentage of infected spikes and spikelets increased with progressively longer infection period for both spring wheat and barley (Fig. 1 and 2)

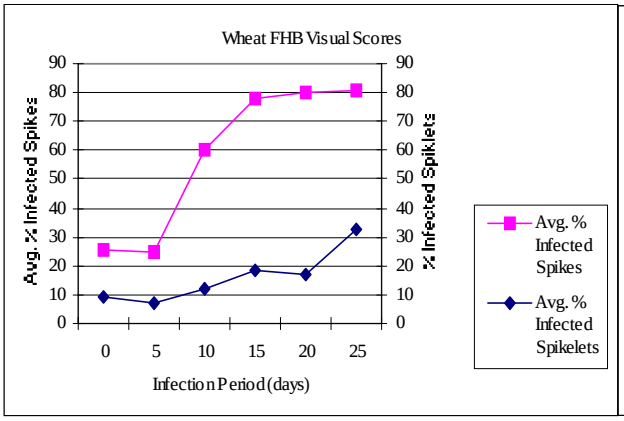

Figure 1

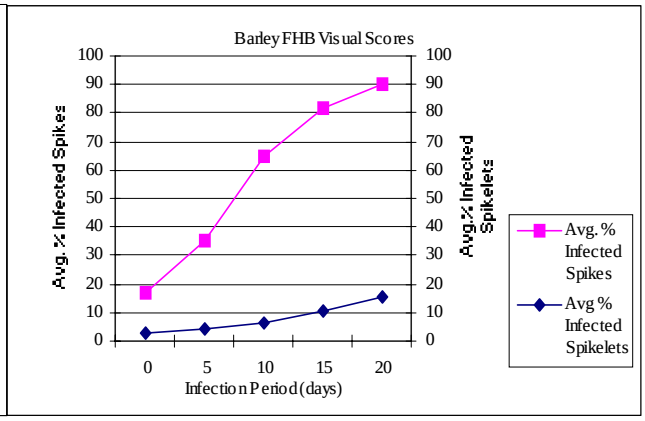

Figure 2

Kernel infection also increased when additional days were allowed for disease progression for both spring wheat and barley (Fig. 3 and 4). Furthermore, when the entries were separated into susceptibility classes on the basis of regression slopes, each class retained the same ranking as percent kernel infection increased with extended infection period (Fig. 3 and 4).

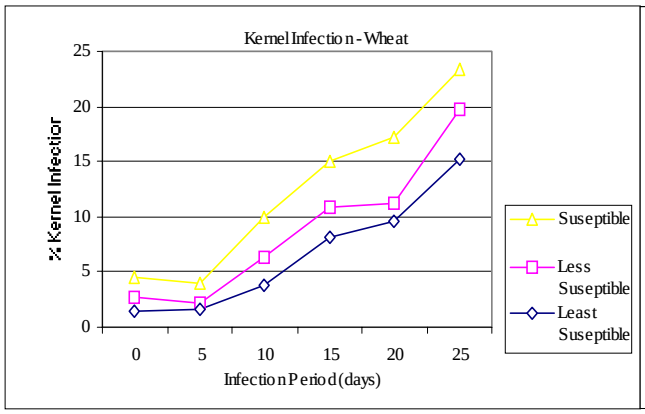

Figure 3

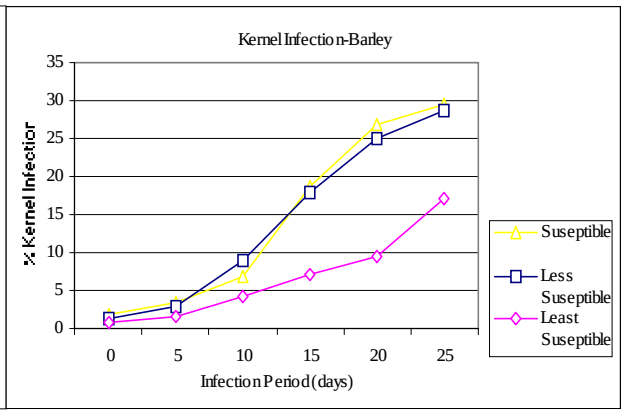

Figure 4

DON concentration followed a similar pattern of higher accumulation with time for both spring wheat and barley. Again, susceptibility classes retained their separation over most infection periods (Fig. 5 and 6).

Spring wheat, in contrast to barley, appears to have an initial lag period before visual symptoms, kernel infection, and DON concentration increase rapidly. This lag period may be related to the longer time period from heading to anthesis for spring wheat compared to spring barley. Field observation of the lines included in these experiments indicated that, in general, spring wheat

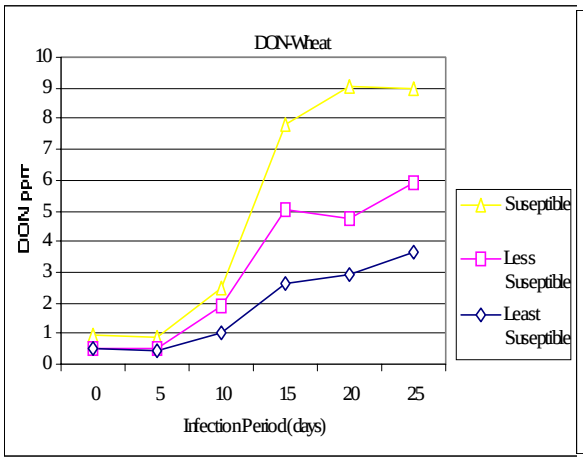

Figure 5

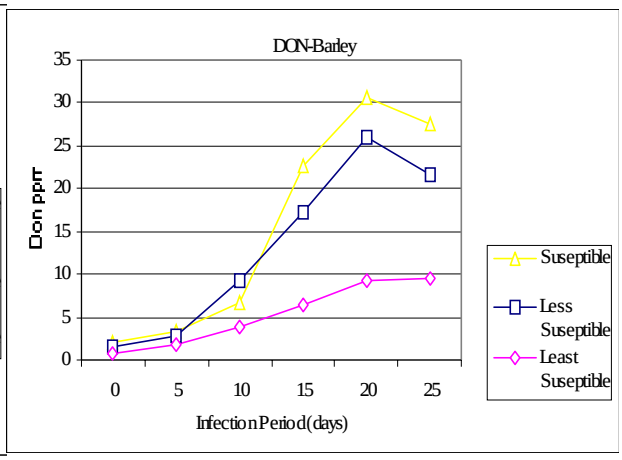

Figure 6

lines flowered within three to five days after heading. However, the spring barley lines normally flowered before or at heading. Increases in kernel infection and DON concentration in spring wheat and barley likely were affected by new infections that occurred throughout the entire 20 or 25 day infection period. New infections are indicated by the increase in

percentage of spikes infected over the entire infection period (Fig. 1 and 2). Therefore, misting duration from anthesis to at least 20 days postanthesis is especially important in determining final disease severity. However, additional environmental factors may also influence FHB disease levels.

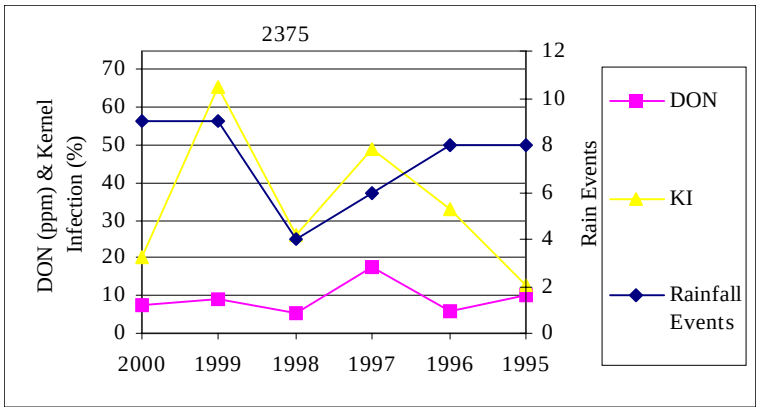

Figure 7

Evaluation of specific varieties grown in misted nurseries at Crookston, MN over the past six years indicate that rain events seem to override the effect of misting alone (Fig. 7). Using the variety 2375 as an example, over the years 1997-1999 rain frequency and kernel infection appear related. During 2000 the occurrence of rain was high but the duration was relatively short, possibly reducing the number of infections. During 1995 and 1996 rain occurred well after anthesis, thereby allowing a much shorter infection period. The frequency, duration, and timing of rain in relation to anthesis may result in disease levels that are in excess of those which could be attributable to misting alone.

Disease levels within an FHB screening nursery are affected by misting and environmental factors for at least twenty days following anthesis. Although rainfall can have an over riding influence, FHB levels can be modified by controlling the length of time the disease is allowed to progress and then stopping the disease by using repeated fungicide applications. The next step will be to determine a reliable indicator of disease level as the infection period progresses. Such an indicator would allow fungicide application to begin and misting to be terminated when the desired disease severity was attained.

REFERENCES

Eberhart, S.A. and W.A. Russell. Stability parameters for comparing varieties. 1996. Crop Sci. 6:36-40. 1996.

NEW RESISTANCES IN CIMMYT BREAD WHEAT GERMPLASM

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In the CIMMYT bread wheat breeding effort we generate materials intended for higher rainfall areas in the developing world, among other mega-environments. In high rainfall environments, the major wheat production constraints are diseases plus certain abiotic stresses, such as waterlogging, sprouting-prone conditions and, sometimes, nutrient imbalances (both deficiency and toxicity). However, the most observable stresses are the biotic ones. Of these, yellow or stripe rust (*Puccinia striiformis*) and leaf or brown rust (*P. recondita*) are often obvious, in particular the first, plus such foliar blights as *Septoria tritici*, tan spot (*Pyrenophora tritici-repentis*), and very occasionally *Septoria nodorum* and Fusarium *nivale* on the leaves. The main virus disease is BYDV.

Among diseases affecting the spike, Fusarium head scab (FHS), induced by various *Fusarium* species, is the number one problem, and seems to be expanding. The recent increase in this disease globally is probably due to the expansion of what are ironically called (from a disease standpoint) more sustainable production methods, such as zero, minimum, or reduced tillage, plus the intensification of rotations, in particular those including corn (maize), an alternate host of *Fusarium* spp.

As FHS spreads and causes damage by reducing the amount of harvested seed and contaminating the grain with toxins, joint efforts to combat this scourge have increased. This meeting is witness to such efforts. Key among approaches to control the disease is the incorporation of genetic resistance.

The CIMMYT program requests, receives, and specifically develops genetically diverse germplasm with resistance to FHS. Various reports documenting these sources are available (Gilchrist *et al*., 1997a, 1997b; 1999). Also, genetic studies aimed at determining modes of inheritance have been carried out and published (Singh *et al*., 1995; Van Ginkel *et al*., 1996). In recent years efforts by the pathology group have concentrated on differentiating germplasm in regard to the four types of resistance commonly applied in FHS (I, II, III, and IV). Our breeding strategy has focused on combining different resistances in adapted backgrounds (Singh and van Ginkel, 1997).

Two areas of recent research on FHS are reported here.

Three crosses were made among three resistance sources considered likely to be different based on their genealogy. We chose two parents (1 and 2, below) whose pedigrees contain no Chinese germplasm. The three parents were:

Gov/Az//Mus/3/Dodo/4/Bow	Bau/Milan	Catbird

Though the study continues, data from the first cycle of artificial inoculation with *Fusarium graminearum* isolates from Mexico have shown the following. It has proven very easy to select F5 lines that have levels of resistance twice that of either parent in all three possible intercrosses. See Figures 1, 2 and 3.

Although two of the parents were not derived from Chinese germplasm, progress could easily be made. This indicates that different genes with accumulative effects (additive or multiplicative) are available in “common” germplasm. In fact, all parents have a very desirable agronomic type, combine readily, and in many respects are rather good parents to use in a breeding program, apart from their FHS resistance.

Finally, we report that we have recently confirmed a group of relatively new CIMMYT bread wheat lines to have high levels of resistance to FHS, and that till now have not yet been commonly used around the world in breeding programs targeting scab. These entries are listed in the Table.

REFERENCES

Gilchrist, L., S. Rajaram, M. van Ginkel, A. Mujeeb-Kazi, and J. Franco. 1997a. Characterizing *Fusarium graminearum* resistance of CIMMYT bread wheat germplasm. *Cereal Research Communications* 25 (3-2): 655-657.

Gilchrist, L., S. Rajaram, A. Mujeeb-Kazi, M. van Ginkel, H. Vivar, and W. Pfeiffer. 1997b. Fusarium scab screening program at CIMMYT. Pp. 7-12 In: *Fusarium Head Scab: Global Status and Future Prospects.* H.J. Dubin, L. Gilchrist, J. Reeves, and A. McNab (eds.). Mexico, D.F.: CIMMYT.

Gilchrist, L.; Rajaram, S.; Ginkel, M. van; Velázquez, C.; Crossa, J. 1999. Transferring fusarium head blight resistance from bread wheat sources to high yielding advanced lines. In: National Fusarium Head Blight Forum; Ramkota Inn Sioux Falls, South Dakota; 5-7 Dec 1999. p. 162-163.

Singh, R.P., Hong Ma, and S. Rajaram. 1995. Genetic analysis of resistance to scab in spring wheat cultivar Frontana. Plant Dis. 79:238-240.

Singh, R.P., M. van Ginkel. 1997. Breeding strategies for introgressing diverse scab resistances into adapted wheats. Pp. 86-92 In: *Fusarium Head Scab: Global Status and Future Prospects.* H.J. Dubin, L. Gilchrist, J. Reeves, and A. McNab (eds.). Mexico, D.F.: CIMMYT.

Van Ginkel, M., W. van der Schaar, Y. Zhuping, and S. Rajaram. 1996. Inheritance of resistance to scab in two wheat cultivars from Brazil and China. Plant Dis. 80:863-867.

Table1. Newly confirmed CIMMYT bread wheat lines carrying Type II resistance to FHS with infection values of less than 6%. The first five entries are comparative checks.

Cross	Selection History	Resistance Type II (%)
MAYOOR	Check: Moderately Resistant	7.91
SUMAI#3	Check: Moderately Resistant	9.2
SERI/CEP80120	Check: Moderately Susceptible	14.84
FLYCATCHER	Check: Moderately Susceptible	21.04
BCN//DOY1/AE.SQUARROSA (447)	Check: Susceptible	32.93
SHA3/CBRD	CMSS92Y00595S-1SCM-0CHN-015Y-3SCM	2.5
NG8675/CBRD	CMSS92Y00639S-1-5SCM-2M-6Y-010SCM-0Y-0SCM	2.52
HXL8088/DUCULA	CMSS93Y02492S-2Y-010M-010Y-010M-10Y-1M-0Y-3SJ-0Y	2.59
CROC_1/AE.SQUARROSA (205)//BORL95	CIGM90.250-4Y-3B-4Y-0B-2M-24M-0Y-010SCM-0Y-0Y-0Y	3.41
GUAM92//PSN/BOW	CMSS92M01860S-015M-0Y-050M-0Y-11M-0Y	3.64
TNMU/3/JUP/BJY//SARA	CMBW91M02016S-0M-040Y-1AL-2AL-7Y-0M-3SJ-0Y	3.7
R37/GHL121//KAL/BB/3/JUP/MUS/4/2*YMI #6/5/CBRD	CMBW91Y01575S-4Y-010M-010Y-015M-2Y-0M-1SCM-010Y-010SCM-1PZ-0Y	4.31
MILAN/DUCULA	CMSS93B01075S-74Y-010M-010Y-010M-8Y-0M-2SJ-0Y	4.72
THB//MAYA/NAC/3/RABE/4/MILAN	CMSS92Y02157T-50Y-015M-010Y-010Y-9M-0Y	4.84
NG8319//SHA4/LIRA	CMBW90M2302-6M-010M-010Y-015M-6Y-0M-0ECU-0Y	4.84
SHA3/SERI//SHA4/LIRA	CMBW90M2468-12M-010M-010Y-015M-9Y-0M-0URY	4.85
R37/GHL121//KAL/BB/3/JUP/MUS/4/2*YMI #6/5/CBRD	CMBW91Y01575S-4Y-010M-010Y-015M-5Y-0M	4.92
NG8319//SHA4/LIRA	CMBW90M2302-6M-010M-010Y-015M-8Y-0M-5SJ-0Y	4.92
SHA3/SERI//SHA4/LIRA	CMBW90M2468-12M-010M-010Y-015M-6Y-0M-3SJ-0Y	5
KAUZ/TNMU	CMSS93B01069S-54Y-010M-010Y-010M-8Y-0M-3PZ-0Y	5
MAYOOR//TK SN1081/AE.SQUARROSA (222)	CASS94Y00009S-18PR-2M-0M-1Y-0M	5
SHA3/SERI//G.C.W 1/SERI	CMBW91Y01596S-2Y-010M-010Y-015M-6Y-0M-1SJ-0Y-010SCM-2PZ-0Y	5.26
HXL8088/DUCULA	CMSS93Y02492S-2Y-010M-010Y-010M-10Y-1M-0Y-2PZ-0Y	5.26
SHA3/CBRD	CMSS92Y00595S-4GH-0M-0SCM-0Y	5.26
TNMU/TUI	CMBW89M3847-64M-0AL-5AL-2B-0Y	5.3
ALUCAN/DUCULA	CMBW89M3764-36M-0AL-2AL-2B-0Y-5PZ-0Y	5.36
IAS64/ALDAN//URES/3/TNMU/4/TNMU	CMBW90M4487-0TOPY-14M-11AL-0AL-07Y-1M-0Y-1SJ-0Y	5.36
SABUF/5/BCN/4/RABI//GS/CRA/3/AE.SQUARROSA (190)	CASS94Y00042S-9PR-1M-0M-1Y-0M	5.51
793.3402//BUC/PVN/3/KAUZ/4/NJ8611	CMSS92Y02234T-7Y-015M-015Y-010M-2Y-0M-1SCM-010Y-010SCM-0Y	5.56
SHA3/SERI//SHA4/LIRA	CMBW90M2468-12M-010M-010Y-015M-9Y-0M-2SCM-010Y-010SCM-0Y-0SCM	5.61
SHA3/SERI//SHA4/LIRA	CMBW90M2468-12M-010M-010Y-015M-10Y-0M	5.65
TNMU/MUNIA	CMSS93B01052S-18Y-010M-010Y-010M-6Y-1M-0Y	5.66
NING8745/3/2*CHUM18//JUP/BJY	CMBW91Y02939M-030TOPM-9Y-010Y-015M-1Y-0M-0E-0ECU	5.74
R37/GHL121//KAL/BB/3/JUP/MUS/4/2*YMI #6/5/CBRD	CMBW91Y01575S-4Y-010M-010Y-015M-2Y-0M-1SCM-010Y-010SCM-3SJ-0Y	5.74
NG8675/CBRD	CMSS92Y00639S-1-5SCM-2M-6Y-010SCM-0Y	5.74
THB/CEP7780//SHA4/LIRA	CMBW90M2456-9M-010M-010Y-015M-10Y-0M	5.77
SHA3/CBRD	CMSS92Y00595S-5GH-0M-0Y-0SCM-0Y	5.85
NL456/VEE#5//PASA/3/BOW/GEN//KAUZ	CMSS93Y03376T-44Y-010Y-010M-010Y-8M-0Y	5.88
TUI/MILAN	CMSS92Y00540S-030Y-015M-0Y-0Y-18M-0Y	5.88
ISD-75-3-1/MO88//PRL/VEE#6	CMBW90M4731-0TOPY-42M-3Y-010M-3Y-9M-2KBY-05KBY-0B-0KEN	5.93

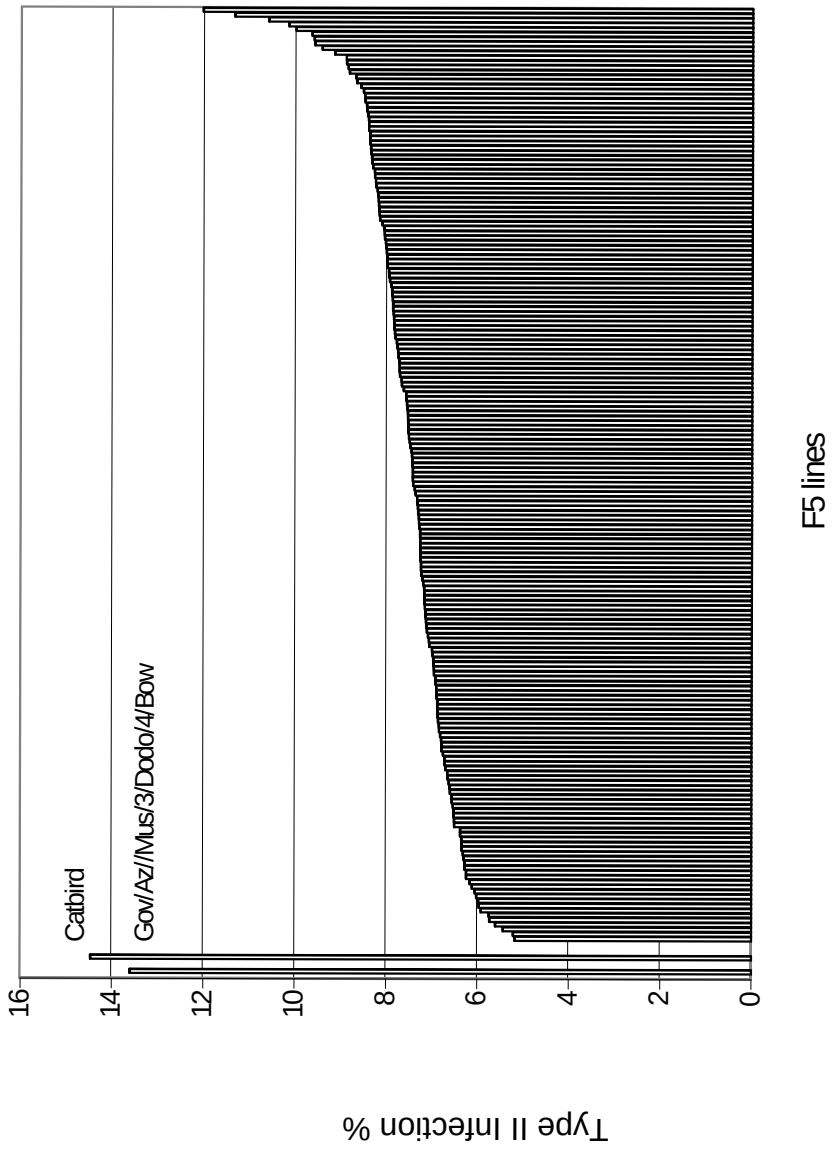

Fig. 1. Cross of Gov/Az//Mus/3/Dodo/4/Bow with Catbird. Both parents and 197 derived F5 lines are depicted against their response to infection to *Fusarium graminearum*, measured as Type II resistance.

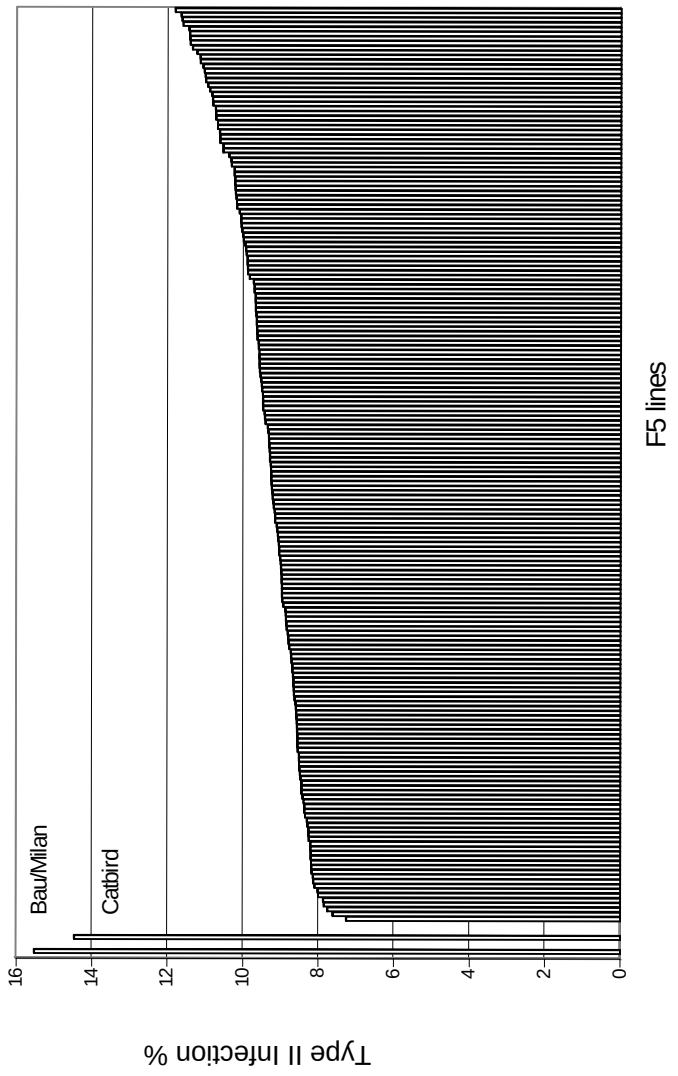

Fig. 2. Cross of Bau/Milan with Catbird. Both parents and195 derived F5 lines are depicted against their response to infection to *Fusarium graminearum*, measured as Type II resistance.

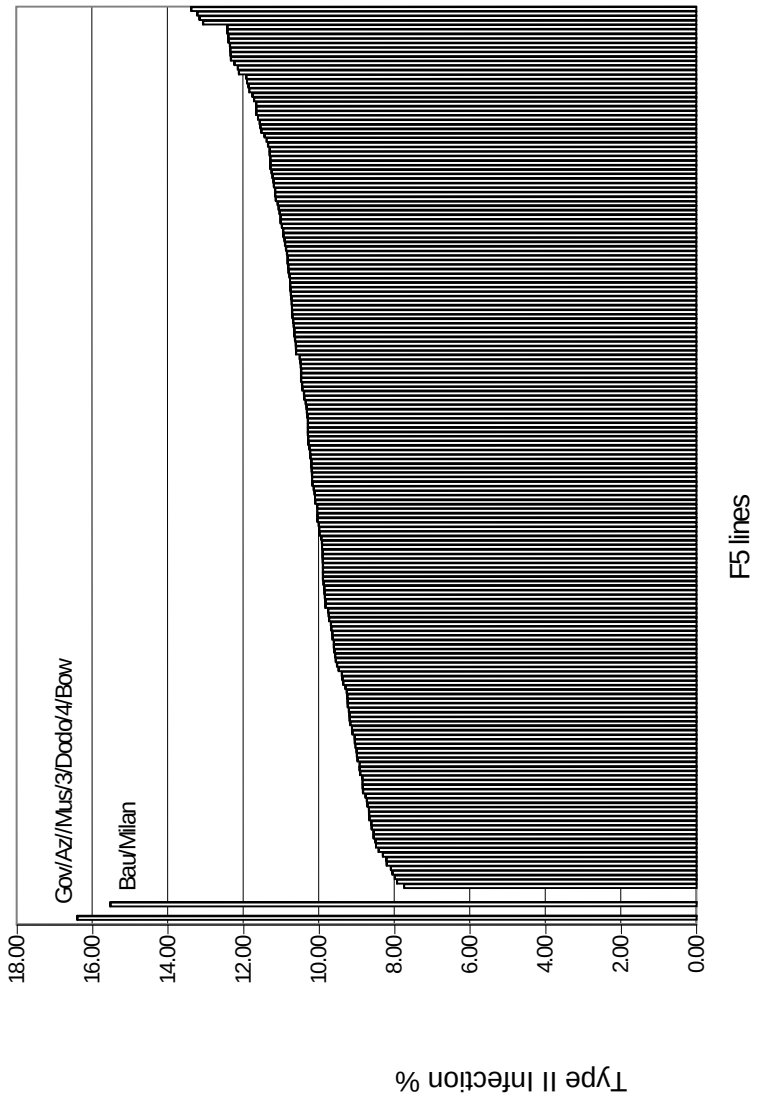

Fig. 3. Cross of Gov/Az//Mus/3/Dodo/4/Bow with Bau/Milan. Both parents and 195 derived F5 lines are depicted against their response to infection to *Fusarium graminearum*, measured as Type II resistance.

THE EFFECT OF DROUGHT STRESS ON SCAB DEVELOPMENT OF SPRING WHEAT

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ABSTRACT

Scab is one of the most important diseases affecting spring wheat production in the Northern Great Plain Area. We have observed that a wheat crop subjected to early season heat stress or drought stress seems to develop less scab than expected, even when conditions favor disease development during anthesis and grain fill. This research focuses on the effect of early season drought stress on scab development of spring wheat. In our preliminary experiment, 4 cultivars, ND2710, RUSS, WHEATON, and 2375 were treated with different water levels at both jointing and booting stage. The plants were inoculated with scab isolate Fg4 in the greenhouse. The percentage of affected spikelets at 21 days, percentages of tombstone kernels and shriveled seeds, seed weight per head, and kernel weight were measured and calculated. The results show that the drought-stressed plants within inoculated plant materials have significantly lower number of affected spikelets and tombstone kernels, compared to fully watered plants. The drought stressed plants tended to have a higher grain yield and kernel weight, but these differences were not significant. The severe level of disease generated in this study, likely overwhelmed any small difference induced by different water treatments.

INTRODUCTION

Fusarium head blight, also called scab, is a destructive disease in the humid and semihumid wheat growing areas of the world. So far, there are no wheat lines or closely related species found to be immune to scab (Hanson et al. 1950, Wan, et al. 1997). Breeders have been working on identifying and combining resistant gene from different sources to increase the level of resistance. Since the infection and development of scab in wheat highly depends on environmental conditions, the expression of resistance may not be stable across years and environments and this instability increases with higher susceptibility (Mesterhazy 1995).

The climate in South Dakota is often variable and unpredictable during the growing season. Periods of drought and high temperature stress during early plant development can be followed by wet periods conducive to scab development. Some studies have indicated that high temperature/drought stress right before inoculation can increase susceptibility because of weakness of plants (Schoeneweisis 1975, Beddis 1991). However, it has also been reported that stress can sometimes condition plants to resist pathogen attack (Joseph, 1995).

We have observed that a wheat crop subjected to early season heat stress or drought stress seems to develop less scab than expected, even when conditions favor disease development during anthesis and grain fill. What is the cause of this phenomenon? Does high temperature/water stress help wheat plants to prevent scab infection? This experiment investigates the interaction of pre-anthesis water stress and scab development.

PLANT MATERIALS AND METHODS

Four spring wheat lines, ND2710, RUSS, 2375, and WHEATON were used in this experiment. Drought treatment was conducted by withholding water up to 30% field capacity one time before rewatering at both jointing and booting stage while the other plants were kept at near field capacity. 10 ml of the scab suspension (40000 spore /ml) was injected into the spikelet near the middle part of the spike 1-2 days before flowering. 3-5 heads per replication were inoculated and the plants were incubated in a mist chamber for 24 hours with 100% humidity at 20ºC. The affected spikelet numbers (the total of infected and prematurely killed spikelets) at 21 days, tombstone kernels, shriveled kernels, seed number, grain weight per head, and kernel weight were recorded and calculated. The experiment was designed as a randomized complete block with 4 replications.

RESULTS AND DISCUSSION

Table 1. Effect of water stress on scab development and yield components

Scab	Water	% Affected spikelets	%Tombstone kernels	% Shriveled seeds	Seed No./Head	Yield (mg/Head)	Kernel Weight (mg)
Point	Fully	89.2	60.4	27.4	15.4	140	6.7
	Drought	78.9	41.8	38.9	10.9	150	9.7
	Fully vs Drought	*	**		*		
No	Fully	5.9	3.23	37.7	29.3	620	20.1
	Drought	17.4	13.7	27	15.6	360	20.6
	Fully vs Drought				**	**	
Scab vs No		**	**		**	**	**

* 5% significance
** 1% significance

The results showed significant differences in all the traits except percentage of shriveled kernels between inoculated and non-inoculated wheat plants (Table 1). The damage caused by scab was very severe. Up to 80% of the spikelets were affected by scab within inoculated plants. Drought stress severely reduced seed number and grain yield within non-inoculated plants.

The main purpose of this research was to find out if the pre-anthesis drought stress affected the level of scab. The results indicate (Table 1) that the plant materials subjected to drought stress had less affected spikelets 21 days after inoculation, fewer tombstone kernels, and fewer kernels per head. The inoculated drought stressed plants tended to have a higher

grain yield and kernel weight compared with inoculated well-watered plants, but these differences were not significant.

Although the reduced percent of tombstone kernels and higher kernel weight could have contributed to the slightly higher grain yield, the reduction in seed number resulted in no gain in grain yield. Also the high level of scab in this experiment could have overwhelmed any small differences induced by different water treatments.

To further investigate the interaction of pre-anthesis stress and scab development, the experiment is currently being conducted in growth chambers under different water and temperature regimes. The environment provided by the growth chambers should be more consistent than the greenhouse environment. We will also attempt to generate less disease pressure to better distinguish differences due to the imposed stress treatments.

REFERENCE

Beddis, A.L. & L.W. Burgess. 1992. The influence of plant water stress on infection and colonization of wheat seedlings by *Fusarium graminearum* Group 1. Phytopathology 82:78-83.

Hanson, E.W., E.R. Ausemus, and E.C. Stakman. 1950. Varietal resistance of spring wheats to fusarial head blights. Phytopathology 40:902-914.

Mesterhazy A. 1995. Types and components of resistance to Fusarium head blight of wheat. Plant Breeding 114:377-386.

Joseph, K.U. 1995. Phytoalexins, stress metabolism, and disease resistance in plants. Annu. Rev. Phytopathology 33:275-97.

Wan, Yong-fang, Chi Yen, Jun-liang Yang and Deng-Cai Liu. 1997. The diversity of resources resistant to scab in *Triticeae* (*Poaceae*). Wheat Information Services 84:7-12.

Schoeneweisis, D.F. 1995. Predisposition, stress, and plant disease. Annu. Rev. Phytopathology 13:193-211.

NCR-184 2000 ARKANSAS STATE REPORT

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WHEAT PRODUCTION

Arkansas growers harvested about 1,000,000 acres of soft red winter wheat with a record state average of 56 bu / acre. Growing conditions were excellent throughout the season. The major constraint to production was a stripe rust epidemic that was much more severe than any stripe rust epidemic in collective memories. About 300,000 acres were sprayed with Tilt fungicide to control stripe rust. Many more acres would have benefited from a Tilt application. Average yield likely would have exceeded 60 bu / acre if stripe rust did not occur.

FHB SITUATION

FHB was found in trace amounts in several fields and experimental plots in east-central Arkansas. The effects of FHB on yield, test weight, and DON appeared to be negligible.

CURRENT FHB PROJECTS

Robert Bacon's breeding program has several advanced lines that appear to have FHB resistance from early CIMMYT spring wheat selections and Chinese lines, and these will be screened more rigorously during the coming year. He has also made additional crosses with adapted Arkansas breeding lines and resistant soft red winter wheats and eastern European winter wheats.

Gene Milus has advanced lines from populations derived primarily from recent CIMMYT spring wheat cultivars and lines. These lines are in the process of being screened more rigorously for FHB resistance in the greenhouse and at several field locations. Steve Harrison, wheat breeder at Louisiana State University, is collaborating with this project by screening lines at two locations in Louisiana. Selections made in Louisiana and Arkansas have been exchanged each year. Additional sources of "durable" leaf rust, stripe rust, and leaf blotch resistances have been incorporated into the same two agronomic parents used for FHB resistance, and a recurrent selection program is planned to combine resistances.

Milus has planted the uniform winter wheat, southern winter wheat, and Bacon's scab nurseries in field screening nurseries and in the greenhouse for type 2 evaluations.

Scab occurs occasionally at low levels on rice in Arkansas. Former postdoc, Louis Prom, collected, identified, and stored many Fusarium isolates from rice in Arkansas. This project was discontinued after his departure, but Milus would be willing to provide cultures of these isolates to interested scientists.

NCR-184 MANAGEMENT OF HEAD SCAB IN SMALL GRAINS ILLINOIS REPORT - NOVEMBER, 2000

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ILLINOIS WHEAT PRODUCTION

The estimated wheat yield in Illinois in 2000 was 57 bushels per acre. This was three bushels per acre below last year's average of 60 bushels per acre, and four bushels per acre below the record state average of 61 bushels per acre set in 1997. Acreage harvested was about 920 thousand acres, down about 10 % from 1999, and the first time in many years that the harvested wheat acreage has dropped below one million acres. Wheat production in Illinois in 2000 was about 52.4 million bushels. This was a 13 % decrease from the 1999 production of 60.6 million bushels. In general, the winter was very mild in Illinois, and the crop developed rapidly in the spring. Wheat was harvested earlier than average in some of the southern regions, but rainy weather delayed harvest in some areas. One of the biggest problems for Illinois wheat producers in 2000 was getting the wheat harvested. Several widespread storms delayed harvest for many farmers and severely reduced test weights. Scab damage was spotty in 2000 with significant losses in some localized areas, but little damage due to scab overall. In spite of good yields for many farmers in 2000, the number of wheat acres planted for 2001 is projected to be significantly reduced.

UNIVERSITY OF ILLINOIS RESEARCH

Breeding for Scab Resistance in Soft Red Winter Wheat: Development of scab resistant germplasm and varieties is a major research emphasis in the wheat breeding program. The long-term objective is the development of soft red winter wheat genotypes with excellent resistance to scab combined with resistance to other diseases, high yield potential, and acceptable winter hardiness and milling and baking quality. Our short-term objectives are: 1) to combine genes for resistance to scab from diverse sources; 2) to evaluate the genotypes produced from crosses and identify those with resistance to scab; 3) to identify molecular markers associated with genes for resistance to scab; and 4) to work toward using molecular markers to assist in breeding for scab resistance.

Four Illinois breeding lines in the 2000 Cooperative Eastern Winter Wheat Fusarium Head Blight Screening Nursery were among the most scab resistant lines in the nursery. These lines have potential as parents, represent sources of resistance that are different from the Chinese sources of resistance, and are in soft red winter wheat backgrounds. These lines were made available to other breeders by entering them into the Cooperative Eastern Winter Wheat Fusarium Head Blight Screening Nursery.

About 680 breeding lines were evaluated in the misted, inoculated field nursery in 2000. Material evaluated included germplasm reported to be tolerant / resistant, current varieties, and experimental breeding lines. Individual heads were selected from 35 segregating

populations grown in the field nursery. About 3000 headrows resulting from these selections have been planted this season (2000-01). About 2220 individual plants from six segregating populations were evaluated in the greenhouse scab screening, and about 844 plants with Type II scab resistance equal to or better than Ernie were selected.

We are continuing to select lines from segregating populations, evaluate lines, and increase the number of lines selected from crosses with potential scab resistance using both greenhouse and field procedures with misting systems and inoculation. In summary, new lines with scab resistance were identified, and the agronomic performance of previously identified lines was evaluated.

Research on Molecular Markers: Using a population of lines from a cross of resistant and susceptible cultivars, we continued to conduct research on identification of molecular markers linked to scab resistance. Three microsatellite markers on the short arm of chromosome 3B were linked integrated into an AFLP linkage group containing a major QTL for scab resistance. The order of the three microsatellite markers from telomere to centromere is Xgwm389-Xgwm533-Xgwm493, and the genetic distances between Xgwm389 and Xgwm533, and between Xgwm533 and Xgwm493 are 5.3 cM and 4.8 cM, respectively. Based on single factor analysis of scab resistance data from evaluation of four generations, Xgwm533 is the microsatellite marker most closely associated with the major scab resistance QTL. Based on F_{10} scab resistance data, Xgwm389, Xgwm533, and Xgwm493 explained 36%, 44%, and 34% of the phenotypic variation for scab resistance, respectively. Combined with AFLP mapping data, an integrated linkage map with AFLP and microsatellite markers was constructed. Interval analysis based on the integrated map of AFLP and microsatellite markers showed that Xgwm389 and Xgwm493 flank the major scab resistance QTL for scab resistance. Mapping of the three microsatellite markers on eight 3BS deletion lines showed that Xgwm389 is located distally to breakpoint 3BS-3, and Xgwm533 and Xgwm493 are located between two breakpoints, 3BS-3 and 3BS-8. Thus, the chromosome region containing the major QTL is located distally to the breakpoint 3BS-8. PCR products amplified by the three microsatellite markers can be separated and detected clearly on standard agarose gel. They should be applicable in marker-assisted selection for scab resistance. This research is in cooperation with Guihua Bai, Oklahoma State University; Greg Shaner, Purdue University; and Les Domier, USDA-ARS at Urbana, Illinois.

PUBLICATIONS

Kolb, F. L., and N. J. Smith. 2000. Registration of 'Kaskaskia' wheat. Crop Sci. 40:1494-1495.

Kolb, F. L., G-H. Bai, G. J. Muehlbauer, J. A. Anderson, K. P. Smith, and G. Fedak. 2001. Host plant resistance genes for Fusarium head blight: Mapping and manipulation with molecular markers. Crop Sci. 40: (accepted).

MANAGEMENT OF SCAB OF SMALL GRAINS NCR-184 2000 INDIANA STATE REPORT

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Scab was sporadic and generally not severe in Indiana in 2000, although some rain fell during flowering. Where scab was found, incidence was generally low. Greatest intensity of disease appeared to be in the northern part of the state.

CURRENT RESEARCH PROGRAMS

As part of a collaborative study on epidemiology of scab of wheat, a study was conducted at the Purdue Agronomy Research Center in Tippecanoe County. Spores were recovered from the air and from wheat heads consistently during the time wheat was flowering. A report can be found in the Forum proceedings. (Shaner and Buechley)

Work was also formally initiated on some “new” sources of resistance to Fusarium head blight. Several accessions were previously screened and reselected lines for resistance. During the spring of 2000, crosses were made between these lines and susceptible cultivars, or between these lines and previously studied resistant cultivars (Sumai 3 and Ning 7840). Additional crosses are being made during the fall of 2000. Once F3 families and backcross F2 families have been developed, analysis of resistance will begin. (Shaner and Buechley).

In the breeding program, cultivars and advanced breeding lines with partial resistance to head blight have been crossed with other lines that show Type II resistance. Field selection is also continuing for lines with a low incidence of head blight. Selection is based on performance at multiple field sites. The most promising lines have been submitted to the Cooperative FHB Wheat Nursery for 2001. A mist irrigation system was established in a nursery at the Purdue Agronomy Research Center for head blight resistance screening. (Ohm, Shen, Drake, Sharma)

Several recombinant inbred populations, derived from crosses between wheat accessions with resistance to *F. graminearum* and susceptible cultivars, are under development. Analysis of these will begin in the spring of 2001. Once families have been reliably classified, by repeated testing, a search for molecular markers will begin. (Ohm)

A recombinant inbred population from a cross between ‘Goldfield’ and ‘Patterson’ is being developed to study the inheritance of low incidence resistance. In the field, Goldfield typically shows only one-fourth to one-fifth the head blight incidence that is observed in Patterson. (Ohm)

Several fungicides were evaluated for efficacy against head blight at two locations in Indiana. Although head blight was not severe, a few treatments reduced incidence of head blight and scabby kernels, and level of DON in grain, compared to the untreated controls. At the southern Indiana site, we observed that some strobilurin treatments increased DON levels compared to the control. A more complete report is provided in the Forum proceedings. (Shaner and Buechley)

Three cDNA libraries were constructed with RNAs isolated from different *Gibberella zeae* PH-1 cultures. Around 4000 random clones from these libraries have been sequenced as Expressed Sequence Tags (EST). Numerous genes that are potentially important for plant infection have been identified in these ESTs (www.genomics.purdue.edu/~jxu/Fgr). A 7x-coverage cosmid library was also constructed with the vector pMocosX, which contains the hygromycin resistance marker suitable for *G. zeae* transformation. In addition, we have accumulated 9000 BAC clones with an average insert size of 60 kb and are in the process of improving the quality of BAC clones. (Xu)

Cereal classes and acreage in Indiana

Indiana produces soft red winter wheat. In 2000, Indiana farmers harvested 510,000 acres, at an average yield of 69 bu/A, for a total production of 35.2 million bushels.

ANNUAL REPORT FOR 2000 NCR-184 – IOWA

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Wheat production and head blight in Iowa in 1999. Winter wheat acreage harvested for grain was very low in 2000, estimated at 18 thousand acres, down 13 thousand acres (42 percent) from 1999. The yield of 47 bushels per acre is up four bushels per acre from last year. Production at 846 thousand bushels is down 37 percent from one year ago. Weather in 2000 was characterized by extremely dry conditions during the flowering period. The result was that Fusarium head blight had a negligible effect on the wheat crop in Iowa this year. Early summer rains promoted scab development in spring wheat and barley, but Iowa's acreage of these crops is extremely low.

Fusarium head blight research. In 1999 we participated in the uniform scab nursery for spring wheat. Plots were planted in soybean stubble and no irrigation or inoculum was provided. Head blight severity reached about 30% in susceptible checks and was 2-3% in the best experimental lines. Deoxynivalenol concentrations ranged from 2.5 to 25.6 ppm, and there was a significant correlation between disease severity and DON. We also participated in the uniform fungicide trials with a winter wheat variety, but scab did not occur in the plots and there were no treatment effects. This fall we planted the uniform scab nursery for winter wheat. The Iowa State University Department of Agronomy has hired a new small grain breeder, Dr. Jean-Luc Jannink, but his emphasis is likely to be on oats.

NCR-184 STATE REPORT KANSAS 2000

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FHB SITUATION IN 2000 IN KANSAS

Kansas wheat generally had average yields and average disease levels in 2000. Barley yellow dwarf, leaf rust, and strawbreaker foot rot were major diseases. Stripe rust was the worst in many years. Fusarium head blight was not reported anywhere in the state.

Programs and personnel involved in FHB research

Breeding program and scab resistance screening: For the third year, a field screening nursery was operated at Manhattan by Bill Bockus, Mark Davis, and Bob Bowden. Plots were inoculated with corn kernels infested with one aggressive isolate of *Gibberella zeae*. Plots were irrigated with fine impact sprinklers for 3 minutes per hour each night starting at heading.

The Uniform Winter Wheat Fusarium Head Blight Nursery was rated at four dates. NY87048-7387, NY87048-7388, MO980525, IL97-2945, OH688, IL96-3073, and VA96W-329 looked the best in our plot this year.

In the Kansas cultivar screening test, Agripro Hondo, Clark's Cream, Heyne, and AP7510 had the best resistance. The newest KSU release, Trego, was identified as highly susceptible to scab. Fortunately, this white wheat variety is targeted to western Kansas where conditions are seldom conducive to scab. The new white noodle wheat, Lakin, had very good resistance to scab.

The Kansas Intrastate Nursery was screened and several highly susceptible lines were identified and will be discarded. Several new breeding lines had resistance comparable to our best cultivars.

We have been breeding for low polyphenol oxidase (ppo) in our white noodle wheat program to reduce noodle browning. Since ppo is thought to be a disease defense gene, André Rosa, a student in plant breeding, attempted to correlated scab resistance and ppo in several populations. Preliminary results suggest that there is no correlation.

Transgenic resistance: S. (Krishnan) Muthukrishnan (Biochemistry), Harold Trick (Plant Pathology), Bikram Gill (Plant Pathology), and George Liang (Agronomy) are cooperating on transgenic resistance. Transgenic plants had an increased level of resistance to scab. Spring wheat, 'Bobwhite', a scab-susceptible cultivar was transformed with pAHC20 vectors carrying the *bar* gene and the gene of interest under the control of maize ubiquitin promoter. Several transgenic lines containing single or pyramids of different combinations of PR-

proteins have been identified and are being propagated and tested for resistance to scab in the greenhouse.

Pathogen genetics and variability: Jim Jurgenson, Bob Bowden, and John Leslie finished work on a genetic map of a cross between a strain of *Gibberella zeae* from Kansas and a strain from Japan using AFLP markers. There were 441 unique AFLP loci arranged on nine linkage groups. One linkage group appears to have an intercalary inversion. This could be significant for introgression of genes between Asian and North American populations. Ron Plattner and Nancy Alexander (USDA mycotoxin unit at Peoria) provided data that toxin type (DON vs. NIV) and amount are segregating in this cross. These loci were independent. The toxin type co-segregated with a polymorphism for the *Tri5* gene. Therefore the DON/NIV switch is in the trichothecene cluster.

Kurt Zeller is doing a study comparing populations of *G. zeae* from the Corn Belt using AFLPs in cooperation with Bob Bowden and John Leslie. Populations from Illinois, Kansas, Minnesota, New York, North Dakota, Ohio, and Virginia had high genotypic diversity. However, all populations had very similar allele frequencies. Therefore it appears the population in the region is panmictic with little or no divergence with increasing distance. Kurt is also working with Dr. Yin-won Lee from Seoul National University to look at diversity in South Korean populations of *G. zeae.*

Ivette Vargas, an M.S. student with Bob Bowden, is initiating studies of South American populations of *G. zeae* and comparing them to North American populations. Populations from Uruguay and Brazil are in hand. Further populations are being sought from Paraguay and Argentina.

NCR-184 2000 KENTUCKY STATE REPORT

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FUSARIUM HEAD BLIGHT STATUS DURING 2000

Fusarium head blight (FHB) levels were very low throughout most of Kentucky during spring 2000. An occasional field was significantly affected because of timely rains, but these incidences were anomalies.

CURRENT RESEARCH PROJECTS

Field and Greenhouse Screening - Marla Hall, Brenda Kennedy, Liu Hua and David VanSanford

Numerous soft red wheat cultivars, breeding lines, entries in the Uniform Northern and Southern Scab Nurseries, and approximately 1400 exotic accessions were evaluated under mist irrigation in a field near Lexington, Kentucky. Autoclaved corn seed, artificially infested with *Fusarium graminearum,* was used to inoculate the nursery. Most of these lines were evaluated in the greenhouse for Type II resistance. 2000 data showed considerable variation; some breeding lines showed good Type II resistance as well as an apparent combining ability for this trait.

Inheritance Studies - Marla Hall, Liu Hua, and David VanSanford

A number of populations were synthesized from wheat parents with reportedly different sources of resistance to FHB. S_1 lines will be evaluated under mist irrigation in an inoculated nursery to elucidate inheritance of resistance. Two diallel series of crosses were made for Type II resistance and DON accumulation. These F_1's will be evaluated in the field and greenhouse.

Breeding Program - David VanSanford

Numerous crosses have been made to various sources of resistance, within and outside the soft red wheat market class.

Uniform FHB Fungicide Test - Donald Hershman and Scott VanSickle

Tests were performed at two locations in west Kentucky during 2000. One test was in south central Kentucky (Logan County) and one was in far west Kentucky (Fulton County). Both tests relied on natural inocula and neither test was irrigated. Six fungicides and a non-treated check were evaluated at both locations. An additional fungicidal treatment was evaluated at the Fulton County test. Due to very low disease pressure at both test sites, no differences were detected between any of the treatments in regard to disease control or

yield effects. Plans for 2001 are to evaluate treatments at single test site which is both artificially inoculated with *F. graminearum* and mist-irrigated.

FHB Field Survey - Donald Hershman, Scott VanSickle and Philip Needham

2000 marked the third year of a state FHB survey. During the three years of the survey, 261 grower fields in Kentucy and several adjacent counties in Indiana, Illinois, Missouri and Tennessee were evaluated for FHB incidence and severity. These disease ratings were regressed against levels of corn residue left on the soil surface after planting the previous fall. In all three years, there was very poor and highly variable association detected between corn residue on the soil surface (measured in the fall) and FHB ratings. Data suggests that factors other than corn residue, such as weather, drive FHB epidemics. Widespread incidence of inocula of the FHB fungi probably exists in Kentucky due to the large number of widely-scattered corn fields which exist throughout wheat producing areas of the state. We hypothesize that this reality negates the in-field influence of corn residue on FHB inocula when environmental conditions favor FHB. In contrast, inocula sources are irrelevant in years when overall weather conditons do not favor FHB. The effect of in-field inocula on FHB epidemics may be greater in areas where FHB fungi are not widely distributed because of limited crop acreage of susceptible crops or do to certain crop rotations or cropping systems used.

Movement of *Fusarium graminearum* in Wheat Spikes Following Greenhouse Inoculation - Dennis TeKrony, David VanSanford, Jason Argyris, and Brenda Kennedy

The single floret inoculation system is commonly used to monitor visible infection of spikelets to determine the level of Type II resistance which exists in wheat. A preliminary investigation was conducted to relate visual spike and spikelet infection to actual presence of *F. graminearum* within components (rachis, glume, lemma, palea, and seed) of spikes and spikelets. Five genotypes were evaluated in the greenhouse following single floret inoculation with an isolate of *F. graminearum*. Results suggest that visual rating of inoculated spikes may be a poor indicator of actual levels of F. graminearum which exist in infected spikes and spikelets. Data also show that fungal movement in spikes occurs in three ways: 1) localization around the point of inoculation (PI); 2) movement up and down the spike from the PI; and 3) movement primarily downward from the PI. This research indicates that a potential weakness may exist in breeding programs which use visual FHB ratings to determine the level of Type II resistance following single floret inoculation. By providing for a better understanding of actual movement of *F. graminearum* within infected spikes and spikelets, it is hoped that a more precise method of measuring Type II resistance under greenhouse conditions might be developed. This, in turn, would improve the efficiency of breeding for FHB resistance.

2000 NCR-184 STATE REPORT MANAGEMENT OF HEAD SCAB OF SMALL GRAINS

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State situation: FHB occurred in Michigan in 2000. The incidence varied between less than 1% to greater than 40%. The occurrence was state wide, but with the higher incidence and severity in the western half of the state. DON was a significant problem with many end users of wheat who limited the DON content of the unfinished wheat to 1 ppm.

Research Reports

Variability of deoxynivalenol in individual fields of wheat. In a 1996 and 1998 statistical study on winter and spring wheats, a grain probe sampling protocol was developed to predict levels of deoxynivalenol (DON) in FHB infected grain. The variability was greater between probes in the 1998 study compared with the 1996 study. In the 1996 study, the DON average from four probe samples was within 1 ppm of the upper limit of the estimated truck average (95% confidence), or within 0.5 ppm on either side of the estimated truck average (95% confidence). Four probes from the 1998 study predicted the average within 3 ppm of the upper limit (95% confidence), or within 1.5 ppm on either side of the average (95% confidence), thus reflecting the increased variability of DON distribution in the trucks. Two of the five trucks from 1998 had DON means below 10 ppm (5.9 and 9.2 ppm), and four probes predicted the mean (95% confidence) within 2 ppm and 1 ppm, for the upper limit and for either side of the mean respectively.

An in field evaulation of the above sampling recommendations was conducted throughout Michigan in 2000. The DON analysis of the sampled grain has not been completed. In addition, a new study was implemented to estimate in field variability of DON in order to develop a simple but statistically reliable sampling recommendation for individual fields.

In planta expression of a peptide that mimics the binding of DON to DON specific antibody. The objective of this research project is to investigate the affect of DONPEP on resistance characteristics in Arabidopsis to the effects of deoxynivalenol, a virulence factor produced by *Gibberell zeae* (*Fusarium graminearum*) which is responsible for the disease in wheat know as Fusarium head blight (FHB). Our longer term goal is to develop transgenic wheat plants that exhibit resistance to G. zeae. This project is one part of a larger project that is investigating a broad range of alternatives to reduce the severity of this disease, including variety selection, planting multiple varieties, fungicides, tillage and biological control. It appears, however, that resistance is the only acceptable long-term approach.

To investigate the function of DONPEP.2 and its possible antagonistic interaction with DON in planta, we cloned DONPEP.2 into plant expression vectors by fusing with green fluorescent protein (GFP) using several strategies. First, DONPEP.2 was fused to the C-terminus of GFP under control of enhanced CaMV 35S promoter in the binary vector pCAMBIA. Sec-

ond, DONPEP.2 was fused to the N-terminus of GFP in the binary vector pEZT-NL. Third, two additional constructs were made to introduce PR1b signal peptide in front of DONPEP.2-GFP fusion in the binary vector pEZT-NL.

These constructs have been introduced into Arabidopsis (Columbia wild type) by Agrobacterium tumefaciens mediated transformation. These constructs will be transformed into wheat in collaboration with Dr. Patricia Okubara at the USDA-ARS, Western Regional Research Center, Albany, California. After transgenic plants are obtained, cell localization of DONPEP.2 will be observed, and its interaction with DON will also be investigated. Our rational for a two plant system approach is that regeneration of Arabidopsis is rapid compared to wheat, and should allow us to characterize the DON-DONPEPTIDE interaction, and come to a better understanding of how the DONPEP should be manipulated in planta to be most effective.

Fungicide trials on wheat to reduce FHB and DON levels.

Fungicide trials to evaluate effects on FHB development were conducted in 2000. Symptom development in the experimental plots was erratic and no conclusions concerning the effect of the fungicides in disease incidence, disease severity, or DON levels were obtained.

DON Diagnostic Services Laboratory

In 2000 over 3,000 samples have been analyzed by mid-November with approximately 1,000 samples remaining. These should be completed by the end of November. The number of sample submitted for analysis was about twice as many as in 1999, and the levels of DON appeared to be higher than in 1999. A newer Neogen ELISA was evaulated and used in place of the ELISA used in 1999. This test is called the 5/5 test, compared to the 10/10 test, and reduces both incubation times by five minutes.

USEFULNESS OF FINDINGS:

The year 2000 FHB epidemic was unusual in that the visual expression of symptoms, both incidenc and severity, would have predicted lower than detected levels of DON. A rudimentary FHB prediction system based on the amount of rain immediately preceding and during heading was fairly accurate at predicting where FHB would be the most severe. However, actual determination of FHB failed to predict the higher than expected DON levels. This suggested the importance of estimating DON in individual fields. This is important not only to growers and processors, but estimates of DON variability in research plots has not been reported or studied.

WORK PLANNED FOR NEXT YEAR:

The statistical study on sampling will be continued. Work will continue on the peptide mimic, including the development of transgenic plants, and a determination of the possible role the mimic may have in reducing the toxic effects of DON as a virulence factor in wheat and possibly barley. Work will also continue on the development of recombinant antibody to

DON, and identification of other peptide mimics that may be useful in elucidating the receptor ligands associated with DON toxicity.

PUBLICATIONS

Yuan, Q., W. Hu, J. J. Pestka, S. He, and L. P. Hart. 2000. Expression of a functional anti-zearalenone antibody in transgenic Arabidopsis plants. Appl. Environ. Microbiol. 66:3499-3505.

Hart, L. P., O. Schabenberger, and F. Kong. 1999. Validating sampling strategies for vomitoxin in the midwestern U. S. Proceedings 1999 National Fusarium Head Blight Forum. p. 111-114.

Hart, L. P., J. Froedtert, R. Ward, L. Siler, G. VanEE, and R. Lederbuhr. 1999. Fungicide efficacy trials at Michigan State University. Proceedings 1999 National Fusarium Head Blight Forum. p. 53-55.

2000 NCR-184 MANAGEMENT OF FUSARIUM HEAD BLIGHT OF SMALL GRAINS - MINNESOTA STATE REPORT

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In 2000 Minnesota spring wheat production was similar to 1999 with production estimated at 2 million acres. Durum wheat was estimated at 2,000 acres, down 3,000 acres from 1999. Winter wheat acres were estimated at 20,000 acres. Minnesota's barley acreage increased to an estimated 240,000 acres, up 70,000 from a record low in 1999. The 2000, season was generally good for small grains production with state yields of barley averaging 64 bu/A and spring, durum and winter wheat averaging 49, 51, and 46 bu/A respectively.

A dry winter and early spring resulted in much of the Minnesota cereal crop being planted well ahead of the 5-year average. The early part of the growing season was generally cool and dry and afforded crops an excellent start with little disease development. Continued dry conditions posed concern to crops in southwest and southern Minnesota toward the end of May. Rainfall, shortly before anthesis, relieved dry soil conditions but raised concerns over the development of foliar diseases, particularly leaf rust and Fusarium head blight. While Fusarium level were generally low and yield losses minor, however levels of deoxynivalenol in harvested barley prevented much of the Minnesota crop from being sold as malt quality.

Septoria, powdery mildew, tan spot, and Fusarium head blight were observed on wheat but infections were generally low and yield losses were light. Leaf rust of wheat was also observed, but disease development was later and slower than last year and the resulting disease was not as widespread or severe as in 1999. Common root rot and crown rot were widespread and likely contributed to significant yield losses in some crops, root rots were especially prevalent in those fields that became waterlogged following heavy rainfall.

Aphid populations were much lower than last year and aphid damage was minimal. Army-worms became a problem in some fields late in the season and insecticide applications were required in some locations to check large populations in wheat and barley fields.

Minnesota's Fusarium head blight program was established in 1993 and has continued to expand. A brief outline of the current researchers and their projects follow:

Wheat - variety development, germplasm introduction, and biotechnology

J.A. Anderson (Agron. & Plant Genetics), R.H. Busch (USDA-ARS, retired), R. Dill-Macky (Plant Path.), J.A. Kolmer (USDA-ARS), G. Nelson (AES-WCROC), G.J. Muehlbauer (Agron. & Plant Genetics), J.J. Wiersma (AES-NWROC), J.V. Wiersma (AES-NWROC), W. Xie (Plant Path.)

Breeding high yielding hard red spring wheat varieties with resistance to FHB and acceptable agronomic and end-use characteristics is a primary focus of the Minnesota wheat breeding program. Approximately 1,000 lines are tested for FHB resistance using point inoculation in the greenhouse annually. Field screening for FHB resistance of approximately 11,000 rows (preliminary and advanced lines) was conducted this year in replicated inoculated and mist irrigated nurseries at three locations in Minnesota. Sources of resistance being utilized by the wheat breeding program include: Sumai 3, Wang-shui-bai, Ning 7840, Ning 8306, Ning 8331, Fujian 5114, Fujian 5125, Fujian 60096, Yumai 7, Yan-shi 9, and Er-mai 9. Recent releases from the program include HJ98 (1998) and McVey (1999). Dr Robert H. Busch wheat geneticist and spring wheat breeder retired this year. Dr. Busch can be credited for the release of 11 wheat varieties.

Barley - variety development, germplasm introduction, and biotechnology

K. Smith (Agron. & Plant Genetics), D.C. Rasmusson (Agron. & Plant Genetics, retired), R. Dill-Macky (Plant Path.), G. Nelson (AES-WCROC), G.J. Muehlbauer (Agron. & Plant Genetics), J.J. Wiersma (AES-NWROC), J.V. Wiersma (AES-NWROC), W. Xie (Plant Path.)

Developing barley varieties with resistance to *Fusarium* and reduced levels of deoxynivalenol with acceptable agronomic and malting quality characteristics have been the primary objectives of the Minnesota barley breeding program over the past five years. In 2000, over 7,500 rows of breeding lines were evaluated for resistance to *Fusarium* in field experiments conducted at three locations in Minnesota and additional 250 lines were screened in the greenhouse. Sources of resistance utilized in the barley breeding program include; AC Sterling, AC Oxbow, Atahuapla, Chevron, and Zhedar. DON testing of field evaluated materials is an important aspect of this program. MNBrite, released in 1998, has partial resistance to FHB but is not considered a malting variety by the brewing industry. Lacey, released in 1999, ranked first in yield comparisons with Robust, Stander, Foster, and MNBrite. Lacey appears to have a sound quality profile although industry scale testing has yet to determine the variety's malting status. Lacey appears to be similar to Robust with respect to resistance to Fusarium head blight. Donald C. Rasmusson retired from the University of Minnesota last month. Varieties including Morex, Excel, Robust, and Stander released from Don Rasmusson's program have dominated the barley acreage in the Upper Midwest.

Biotechnology - molecular studies of host response, germplasm enhancement, and genetics of pathogenicity

W.R. Bushnell (USDA-ARS), H.C. Kistler (USDA-ARS), G.J. Muehlbauer (Agron. & Plant Genetics), R.J. Zeyen (Plant Path.)

This diverse group of projects includes studies aimed at the isolation of resistance genes, mapping of resistance genes with molecular markers and the development of resistant wheat and barley varieties through genetic engineering. Transformation systems for wheat and barley have been developed and mapping of resistance genes in barley and wheat populations have been undertaken. Methods to expedite the selection of antifungal proteins

best suited for utilization in transformation are ongoing. Studies to examine the pathways of floret infection of *Fusarium* with experiments utilizing a green fluorescent protein (GFP) labeled *Fusarium* isolate which facilitates observation of fungal establishment in host tissues are continuing. The diversity of *Fusarium graminearum* is also being examined by testing the relative aggressiveness of isolates collected throughout the US and representative strains from the world collection.

Chemical and cultural control of Fusarium head blight

R. Dill-Macky (Plant Path), R.K. Jones (Plant Path), A.L. Sims (AES-NWROC)

Evaluation of candidate fungicides for efficacy in suppression of FHB have been undertaken as part of a multistate cooperative effort. To successfully integrate fungicide treatments, a quantitative PCR method for estimating inoculum potential is being tested. Studies to examine the relationship between residue decomposition and *Fusarium* survival and inoculum potential are continuing and studies to examine the effect of burning on the survival of *Fusarium* in residue have been initiated.

NCR-184 COMMITTEE- MANAGEMENT OF HEAD SCAB IN SMALL GRAINS 2000 MISSOURI REPORT

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Winter Wheat Production in Missouri and the 2000 FHB Situation in Missouri

Most of the Missouri wheat acreage is soft red winter wheat with a minimal number of hard red winter wheat acres. Fall seedings for the 2000 winter wheat crop in Missouri totaled 1 million acres, up 2 percent from the 1999 crop seeded acreage. Of the 1 million acres planted, 950,000 acres were harvested. Missouri wheat production in 2000 totaled 49,400 million bushels, up from last year's production of 44,160 million bushels. Missouri yields averaged 52 bushels per acre, up 4 bushels from last year's average yield of 48 bushels per acre.

1999 was an "interesting" year for wheat production and a fairly poor year for Fusarium head blight in most of Missouri. The winter of 1999-2000 was the eighth warmest winter on record (records going back to 1895). Most of Missouri was in a state of drought entering the year 2000. On January 1, 2000, topsoil moisture supply was rated as 24 percent very short, 42 percent short and 33 percent adequate for the state. Precipitation remained well below average until mid June. The wheat crop headed unusually early with 34 percent of the crop reported as heading for the week ending April 30. This was 9 days ahead of normal and the most advanced for that date since 1981. These unusually dry conditions as most of the state's wheat crop was flowering resulted in low levels of scab. Localized rainfall lead to scab problems in those areas but the incidence and severity of scab was minimal in Missouri in 2000.

The most serious disease problems on wheat during the 2000 season were virus diseases. Wheat spindle streak mosaic, wheat soilborne mosaic, barley yellow dwarf and wheat streak mosaic were all widespread and, in many fields, severe. Most samples tested by ELISA were positive for more than one of the viruses with wheat spindle streak mosaic and wheat streak mosaic being the most commonly found combination. Leaf rust and Septoria leaf blotch came in late in the season and did not move up to the flag leaves until well past heading. Stripe rust developed in higher than normal levels in southeastern Missouri and was found in low levels throughout the state. Losses from foliage diseases were low for most of Missouri. Missouri did have a Special Local Need Registration (Section 24c Registration) for Tilt which extended the time of application to Feeke's Growth Stage 10.5. However, because of the low level of foliage diseases few growers took advantage of the Tilt label change or the new federal label for Quadris on wheat.

The rains came just as much of the state was moving into wheat harvest. By June 18, 2000, farmers had harvested 40 percent of the crop which was about 11 days ahead of normal. Winter wheat harvesting was virtually complete by July 16, 6 days ahead of normal. How-

ever, the rain delays resulted in lowered test weights, decline in quality and weed problems in fields. There were some reports of wheat sprouting in the field and many reports of wheat heads or plants turning black in the field.

Quality of wheat seed tested by the Missouri Seed Improvement Association has been extremely variable this season. Lots coming in for germination tests look fairly normal. However, germination rates have dropped dramatically from bin germs to final germs. Lots with bin germs in the 90's had final germs in mid 50's to 70's. In one extreme case the germ of a lot dropped from a bin germ of 94 to a final germ of 14. When viewed with magnification it was possible to see that many kernels had sprouted and the sprout had been broken off. Dead seeds in the final germ tests were covered with various storage molds. The drop in germination rates appears to have been from sprouting and weathering (moisture and heat) in the field as harvest was delayed by wet conditions.

There are no official estimates of the number of acres planted to wheat this fall. Fall harvest was early. Rains have caused some delays in wheat seeding but as of November 5, 89 percent of the wheat acres had been planted.

Current Scab Research at the University of Missouri

Uniform Scab Fungicide Trial: The University of Missouri did participate in the Uniform Scab Fungicide Trial coordinated by Dr. Marcia McMullen, NDSU. Eight fungicide treatments were evaluated on Madison and Roane. FHB occurred in extremely low levels throughout the plot. There were no statistically significant differences in yield, ppm of DON, % incidence of FHB, % FHB severity or % field severity between the untreated control and any of the nine fungicide treatments. Results of this trial are given in more detail in the report for this initiative project.

Breeding Program: The University of Missouri's Wheat Breeding Program has a major emphasis on accelerating the development of scab resistant soft red winter wheat that was initiated in 1993 and significantly enhanced in the last 3 years with funds from the National Wheat and Barley Scab Initiative. Routine screening of all advanced lines in the breeding program has enabled the identification of numerous pedigrees with good to acceptable levels of scab resistance. Resistance is for reduced spread and incidence coupled with kernel retention. Once verified, lines will be entered into the Uniform Winter Wheat Scab Nursery and selected pedigrees, which differ from Ernie by descent, will be used in crossing programs to study the genetics of their resistance.

Beyond screening, the incorporation of resistance genes identified through germplasm screening programs is essential to the continued improvement of Fusarium head blight resistance in winter wheat. We currently are incorporating genes from Sumai 3, Ning 7840, Frontana, and several CIMMYT sources. In addition, we routinely use soft red winter wheats sources including Ernie, Patton, Goldfield, Freedom and several of our own lines expressing good levels of resistance. Chinese and Yugoslavian accessions showing good levels of resistance have been added to our crossing block for 2000.

Germplasm Evaluation Center: Missouri was identified as a germplasm evaluation center for the National Wheat and Barley Scab Initiative with responsibility for identifying new sources of resistance in winter wheat. Screening of Asian accessions has been completed, results have been posted on the web, and seed will be available for distribution to interested scientists at the 2000 Scab Forum. Approximately 2000 accessions from the Balkans are being screened in 2000-2001. Resistances identified in the first group of 1006 are being verified during the fall of 2000 and data will be presented at the 2000 Scab Forum.

CIMMYT Germplasm Introduction Partnership: Approximately 75 wheat lines will be introduced into the US through the National Scab Initiative's partnership with CIMMYT. Lines will include 23 lines from China with diverse resistances, 7 lines from Romania and a number of lines from CIMMYT's bread wheat breeding and wide crossing programs. These lines will be quarantined in Missouri and then distributed to interested breeders in the spring of 2000.

Genetic Studies: Studies investigating the inheritance of resistance in Ernie are currently underway utilizing the Missouri breeding line MO 94-317, a widely adapted and highly inbred (F_{12}) line, as the susceptible parent. It has high yield and excellent milling and baking quality but is highly susceptible to scab with a FHBI of $\geq$ 0.9 and poor kernel quality under disease pressure.

Conventional Six Generation Means and Variance Analyses: A set of populations (F_1, reciprocal F_1, F_2, BC_1 and BC_2) from the cross Ernie x MO 94-317 is currently under development for conventional genetic analysis of the scab resistance in Ernie. Population development will be completed in 2000/2001 and genetic analyses will be conducted in 2001. Both Type II and Type III resistance data for each generation initially will be examined for goodness-of-fit (based on P^2 analysis) to simple Mendelian ratios. Where data collected fail to fit a simple dominant/recessive genetic model, generation means and variance analyses will be conducted.

Monosomic Analyses: Monosomic plants from each of the 21 Chinese Spring monosomics developed at the University of Missouri by Dr. E.R. Sears have been crossed with Ernie in an effort to identify critical chromosomes influencing scab resistance in Ernie. In addition, the results of this study will help focus molecular work aimed at identifying markers associated with genes for scab resistance in this cultivar.

Molecular Analysis: A set of F_3 derived F_9 recombinant inbred lines (RIL's) has been developed from the cross Ernie x MO 94-317 which will be used to map resistance genes in Ernie. Results from screening F_6 RIL's suggest that resistance in Ernie is heritable and relatively simply inherited. Mapping of gene(s) associated with resistance is expected to begin in January 2001 using RFLP, AFLP and SSR markers.

FUSARIUM HEAD BLIGHT IN 2000 NCR-184 NEBRASKA STATE REPORT

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Fusarium head blight incidence - John Watkins (Plant Pathology)

Fusarium head blight incidence in commercial wheat fields was very low in 2000 due to an extended drought throughout the growing season. Two thirds of Nebraska's winter wheat is grown in areas where drought is common i.e. the panhandle and the southwest, and Fusarium head blight is rare. There is some concern that irrigated wheat in these two areas could be affected by Fusarium blight if rains come at flowering because these fields are intensively managed and there is ample moisture to support the crop. As occurred in dry-land wheat, the incidence of Fusarium head blight in irrigated wheat was neglidgeable in 2000. Only one certified seed sample tested by the Nebraska Crop Improvement Association in 2000 tested positive for Fusarium head blight.

Enhance scab resistance in winter wheat germplasm by plant transformation - A.Mitra, Marty Dickman and Julie Schimelfenig (Plant Pathology), Tom Clemente and Shirley Sato (Biotechnology Center)

In this research the key goal was to increase the sources of resistance to Fusarium head blight (FHB) though the use of novel, highly biologically active genes from nontraditional sources by collecting a set of inhibitor of programmed cell death (PCD) or antiapoptotic genes (lead candidate genes: IAP and ced9) and antifungal genes (lead gene: lactoferrin). The objective was to transform Bobwhite with these genes using microprojectile or *Agrobacterium tumefaciens* mediated transformation, grow the progeny and screen them for resistance or tolerance to FHB. As these genes may not be effective, a second objective was to continue collecting genes that may be effective in reducing the devastating effects of FHB.

Using microprojectile and *Agrobacterium tumefaciens* mediated transformation, 24 events with IAP, 3 with ced9 and 24 with lactoferrin were made. The strategy in using antiapoptotic genes is to affect the infection process which requires testing at the whole plant level. The strategy in using lactoferrin is to express a known antifungal protein, which should affect FHB. Extracts from transgenic tobacco plants expressing lactoferrin (when compared to transgenic tobacco plants not expressing lactoferrin) were shown to inhibit FHB growth in petri dishes. The progeny from the IAP, ced9, and lactoferrin transgenic lines have been screened in the growth chamber. Preliminary results from our screen for FHB for IAP identified some families expressing a level of tolerance to FHB that merits further testing. The preliminary results for lactoferrin were also promising. Plants containing lactoferrin (57.6$\pm$ 8.8, mean $\pm$ standard error) had a lower level of FHB than plants, which did not contain lactoferrin (91.2$\pm$10.1). For both genes, transgenic families having the highest level of tolerance and appropriate controls (the best conventionally developed FHB susceptible and

resistance lines, and nontransgenic Bobwhite) are being retested to confirm these preliminary results. Additional genes (e.g. the antiapoptotic gene, Bcl-xl, and some derivatives) have been collected for and are being inserted into wheat.

To enhance variety development of scab resistant varieties - P. Stephen Baenziger (Agronomy) and Julie Schimelfenig (Plant Pathology)

The main objective was to develop germplasm that is tolerant to Fusarium head blight (FHB), which will be the future base for cultivar development for the high rainfall and irrigated acreage in the central Great Plains. To meet this goal, adapted and exotic germplasm was collected from throughout the world and crossed into adapted hard red and white winter wheat cultivars. Once the transgenic FHB tolerance is verified, those transgenes will be rapidly incorporated and pyramided into common wheat. Traditionally about one third of Nebraska=s wheat acreage is in the FHB risk area (between 600 to 700,000 acres) and the University of Nebraska wheat breeding program has developed cultivars grown on 80% of Nebraska, as well as being widely grown in the FHB risk areas in adjacent states in South Dakota and Kansas. In addition to collecting germplasm, a key need has been to develop effective screens to allow selection for FHB tolerance.

Elite germplasm has been collected from the northern Great Plains and eastern United States, as well as from China and evaluated for agronomic performance. The crossing continues as would be expected in any traditional breeding effort. An effective greenhouse screen has been developed, which will be used mainly for better parent identification. This screen has been used to evaluate transgenic materials. A field-screening nursery based on mist irrigation with appropriate controls to screen 1000 lines is being built and will be used in 2001.

The most significant accomplishments for 2000 were the continued incorporation and generation advance of FHB tolerant germplasm, the development of a FHB tolerance greenhouse screen, and the purchase of necessary equipment for a FHB tolerance field screen. NE94654, recommended for release in 2000-2001, appears to have low level of FHB tolerance. NE94654 is a line that seems well adapted to FHB risk areas of Nebraska.

NCR-184 STATE REPORT NEW YORK 2000

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FHB Situation in 2000 in New York

FHB was widespread across the soft winter wheat production area of New York in 2000, with disease incidience varying from field to field. Visual symptoms seemed to be delayed until about 3 weeks after flowering, possibly due to cool temperatures, especially at night, during grain formation. In general, the disease had only a modest impact on grain yields, but test weights were reduced. Vomitoxin contamination in the range of 1 to 4 ppm was common in commercial grain lots received at flour mills, thus many loads were rejected or discounted. Preharvest sprouting was also a severe problem in 2000 in New York wheat as was Stagonospora nodorum blotch. A cooperative survey between Cornell and the Star of the West Flour Mill in Churchville, NY is being conducted to assess associations among vomitoxin level, incidence of *Fusarium* infected grains, and geographic location of production fields in the 2000 crop.

Programs and Personnel Involved in FHB Research

Winter wheat cultivar evaluation

One site of the winter wheat cooperative scab nursery is located at Ithaca, NY. Conditions were conducive for scab development, which was promoted with irrigation and the provision of grain spawn inoculum in the plots. See the report by Lipps and Engle for a summary of results at all locations. Two New York lines, NY87047W-7387 and NY87047W-7388, were among the top five entries for reduced FHB severity and lowered DON content. In addition to the standard 29 cooperative lines, an additional 50 regionally-adapted varieties and lines are also being evaluated. Also, scab reaction of over 75 lines derived from crosses of New York-adapted winter wheat cultivars with Chinese sources of resistance is being assessed.

Personnel: Mark Sorrells and David Benscher (CU Plant Breeding); Gary Bergstrom and Stan Kawamoto (CU Plant Pathology)

Fungicide Evaluation

One site of the uniform fungicide trial is located at Aurora, NY. See the summary report by McMullen and Milus in this volume. Fungicides and biocontrols were applied by foliar spray utilizing the dual (forward-backward) flat fan nozzle system configured by North Dakota researchers. Grain spawn inoculum was spread in the border areas and the plots were irrigated following anthesis. Rainfall was also frequent from heading through grain formation, conditions conducive for scab. Leaf blights were not significant at this location. No treatment resulted in a significant increase in yield, though plots treated with Folicur had the highest yields (Table 1). Various treatments induced moderate reductions in scab incidence,

Fusarium damaged kernels, DON contamination, and improvement of test weight, but not to the extent required for economic feasibility. Overall, the most promising treatment for scab and DON reduction was the combined application of the bioprotectant Trigo Cor 1448 with 4 fl oz of Folicur. Personnel: Stanley Kawamoto, Christine Stockwell, Gary Bergstrom (CU Plant Pathology); William Cox and Dilwyn Otis (CU Crop and Soil Sciences)

Biological Control

Microbial antagonists of Fusarium graminearum are being isolated and characterized for potential application to wheat spikes, seed, and crop residue. See the report by Stockwell et al in this volume.

Personnel: Christine Stockwell, Stanley Kawamoto, Gary Bergstrom (CU Plant Pathology); Wilmar da Luz (Embrapa Trigo, Passo Fundo, Brazil)

Aerobiology/Epidemiology

Research is continuing with the use of remote piloted aircraft to study the aerobiology of *Gibberella zeae* ascospores in the lower atmosphere in order to better understand the potential of regional dispersal of airborne inoculum. Efficient recovery of isolates aloft with the same AFLP pattern as a clonal isolate released on the ground in grain spawn strongly indicates that ground level inocula gain access to the planetary boundary layer. Also under investigation are the effects of environmental conditions on the discharge of mature ascospores from perithecia. Research is being conducted in laboratory chambers and under field conditions. Maldonado-Ramirez *et al* in this volume report on initial results in examining the temporal patterns of ascospore release from a corn stalk substrate under natural conditions. Three papers by Shah *et al* in this volume report on spatial aspects of FHB and epidemiological considerations associated with seed infection incidence.

Personnel: Sandra Maldonado-Ramirez, Gary Bergstrom (CU Plant Pathology); Elson Shields (CU Entomology); David Gadoury (CU Plant Pathology, Geneva campus); Don Aylor (Connecticut Ag Experiment Station); Robert Bowden, Kurt Zeller (Kansas State University)

Table 1. Effect of foliar treatment at anthesis on scab incidence, Fusarium-damaged kernels, yield, test weight, and DON contamination in Caledonia winter wheat in Aurora, NY in 2000.

Treatment and amount	Scab (spike) incidence on 26 Jun (%)	*Fusarium* damaged kernels (%)	Test weight @ 13.5% moisture (lb/bu)	Yield @13.5% moisture (bu/A)	DON ppm
Nontreated	14.1	8	56.1	74	10.7
AMS 21616	na	7.1	56.7	77.4	12.6
BAS 500F (12.3 fl oz/A) + Agridex COC (1% v/v)	11.7	6.7	56.7	73.5	14.3
BAS 500F (6.2 fl oz/A) + Agridex COC (1% v/v) + Folicur 3.6F (2 fl oz/A)	14	9.9	56.4	80.1	15.1
Folicur 3.6F (4 fl oz/A) + Induce (0.125% v/v)	11.1	6.7	57	82.4	9
Folicur 3.6F (6 fl oz) + Induce (0.125% v/v)	na	5.6	58	80.4	10.5
HI 2036 (5 lb/A)	23.5	9.1	54.5	76.7	13.7
Quadris 2.08SC (9.2 fl oz/A) + Benlate 50WP (0.25lb/A)	16.2	6.8	56	76.2	11.2
Stratego E (14 fl oz/A) + Induce (0.125% v/v)	11.6	8.8	56.6	74.1	12.9
Tilt (4 fl oz/A)	10.5	6.2	56.8	74.1	9.6
TrigoCor 1448 biocontrol	11.7	7.5	56.6	74.2	8.2
TrigoCor 1448 biocontrol + Folicur 3.6F (4 fl oz/A) + Induce (0.125% v/v)	8.7	5.2	58.3	80.1	8
TrigoCor 4712 biocontrol	11.9	7.1	56.6	77.3	9.4
TrigoCor 9790 biocontrol	17	8.2	56.5	77.2	14.4
LSD (*P* =0.05)	0.6	0.2	1.1	NS	3.8
CV (%)	14	12.4	0.1	12.1	23.5

NCR-184 REPORT 2000 - NORTH DAKOTA

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The FHB situation in North Dakota in 2000 and its impact on small grain crops. Results provided by Marcia McMullen, extension plant pathologist, who conducted a survey of 1200 grain crops across ND in 2000. Statewide, Fusarium Head Blight (FHB) was about twice as severe in 2000 than in 1999. Individual severely affected fields of spring wheat and barley could be found in parts of central ND and in fields of durum in north central, west central and northwest ND. There were many late planted durum crops which may have increased the problem. Several of the worst-affected counties were in more western locations which have not seen severe FHB in the past. Overall, wheat losses to FHB in 2000 were moderate, about 6% statewide, but averaging over 11% in the durum region. Yield loss in barley was less than 2% statewide but DON levels were often above 0.5 ppm in affected fields.

Overview of present research programs. The FHB research effort at NDSU continued to be a large one in 2000. Six NDSU departments, three NDSU Research & Extension Centers, and the USDA-ARS Northern Crop Sciences Laboratory located on the NDSU campus, all were involved in research on FHB. Many of the projects received funding from the scab initiative and reports from those investigators are included in the forum proceedings. Several of the projects are cooperative efforts between state and federal scientists.

While the principal research emphasis at North Dakota State Univ. continues to be on breeding for resistance to FHB, and classical and molecular genetics of resistance, there is active research in several other areas including epidemiology, soil microbial ecology, physiology and biochemistry, grain quality, food science, disease survey, and chemical control.

FHB resistance is being sought in breeding programs for spring wheat, spring wheat, durum wheat, and barley. Methods to obtain resistant varieties include both conventional and molecular plant breeding methods. These efforts utilize inoculated-irrigated field nurseries and greenhouse testing. Sources of resistance for spring wheat being used include lines from China, Japan, Hungary, and Brazil. Sources for durum include world collection materials and accessions from ICARDA. Similar diverse sources are being evaluated for barley.

The highlight of the year was the release of ‘Alsen’, a hard red spring wheat combining moderately high resistance to FHB with excellent grain quality and good agronomics. This release is fortuitous as certain other varieties with partial FHB resistance or yield tolerance to FHB are being lost because of race changes in wheat leaf rust.

Units involved in FHB Research.

NDSU:
Dept. of Plant Pathology
Dept. of Plant Sciences.
Dept. of Soil Science.
Dept. of Cereal and Food Sciences.
Dept. of Agricultural Engineering.
Dept. of Veterinary Science and Microbiology.
NDSU Extension Service
NDAES Research-Extension Centers at Langdon, ND, Carrington, ND, Minot, ND.

USDA
USDA-ARS Northern Crop Sciences Laboratory, Fargo

NCR-184 MANAGEMENT OF HEAD SCAB OF SMALL GRAINS: 2000 OHIO REPORT

Patrick E. Lipps[1]*, Erick D. Dewolf[1], Laurence V. Madden[1], Anju Gupta[2] and Jessica S. Engle[1]

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One million, five thousand acres of soft red winter wheat were planted in Ohio in the fall of 1999 for harvest in 2000. Highly favorable weather conditions for winter survival and spring growth lead to a record state average yield of 72 bu/A. There was very little damage from head scab, although most fields had trace levels of disease. Fields with the highest level of scab did not exceed 5-10% incidence. Yield losses probably range from 0 to 3%, but state wide the average yield loss was less than 0.1%. Cool temperatures during and after anthesis probably limited scab development because precipitation for the period was adequate to favor infection.

Research

Research efforts at OSU were focused on: disease forecasting, screening germ plasm, breeding for disease resistance, and evaluation of fungicide efficacy.

Disease forecasting: De Wolf, Madden, Lipps

A) We are participating in a cooperative program with North Dakota, South Dakota, Indiana, and Manitoba to monitor inoculum levels, environmental parameters and disease severity in replicated plots. Information from multiple sites is being used to develop a disease forcasting system. The cooperative effort is necessary to assess the effect of regional variation in cropping practices, tillage and climate on inoculum levels and subsequent disease level across the wheat producing regions. Volumetric air sampling and a wheat head bioassay are being used to monitor fluctuations in the levels of inoculum reaching heads. Automated environmental monitoring instrumentation is used to measure temperature, relative humidity, precipitaion, solar radiation, wind speed, and moisture status of the crop. This is the second year of our monitoring project.

B) Erick De Wolf and Larry Madden have developed a scab risk assessment model based on historical weather information and scab severities. The model was constructed from hourly temperature, relative humidity and precipitation data from 50 location years from Ohio, North Dakota, Kansas and Missouri. Stepwise regression identified two time periods in which three environmental parameters were critical to reasonably accurate prediction: 1) duration of precipitation and duration of temperature between 15 and 30C for 7 days prior to crop anthesis and 2) duration of temperature between 15 and 30C and corresponding relative humidity above 90% for the 10 days post anthesis. This model has had 84% prediction accuracy in classifying epidemics with greater than 10% scab severity.

C) Erick De Wolf is examining the effect of temperature and moisture content of crop residues on development of perithecia by *Gibberella zeae*. He has adapted a sensor to monitor the moisture content of corn residues over time. Experiments are in progress using enclosed moisture chambers in growth chambers to accurately control temperature and moisture content of the residues. Preliminary results indicate that temperature and moisture have a profound influence on perithecial development. Moisture sensors were used in corn residues in the field during the 2000 growing season to document fluctuations in moisture content in corn residues and examine perithecial development under a wheat crop canopy.

Breeding for scab resistance: Lipps, Gupta, Engle

A) The departments of Hort and Crop Science and Plant Pathology are cooperating to develop varieties with resistance to head scab. Four avenues of research are being followed; 1) evaluation of varieties and advanced lines for resistance, 2) evaluate and select lines with combined resistance to FHB and Stagonospora blotch, 3) incorporate resistance from sources identified within the breeding program into elite lines and 4) increase the level of resistance above current levels by incorporating new genes and gene combinations from diverse germplasm sources. During the year the following germ plasm were screened for resistance in field nurseries: 200 Advanced breeding lines, 128 resistant by susceptible crosses, 101 resistant by resistant crosses, 443 scab resistant by Stagonospora resistant crosses, and 1428 early generation head row selections. In addition the resistant by susceptible crosses were evaluated in the greenhouse.

B) Anju Gupta screened a population fo 189 Yugoslavian lines obtained from the National Plant Germpalsm System to identify new sources of resistance that could be incorporated into the breeding program. These lines were evaluated in the greenhouse and the field for two years. Results of the project are being presented the Fusarium Head Blight Forum (December 2000).

C) Anju Gupta has been identifying quantitative trait loci associated with resistance to FHB by screening two different populations using microsatellite markers. The populations being evaluated are a resistant by susceptible cross (Ning 7840 x OH 542) and a resistant by resistant cross (Ning 7840 x Freedom). This project will be complete in 2001.

Fungicide efficacy and dissemination of information: Lipps, De Wolf

We are participating in the cooperative effort of the Chemical and Biological Control section headed by M. McMullen and G. Bergstrom. Five fungicides and two biologicals were evaluated in 2000 using procedures and rates outlined by the project leaders. Measurable precipitation occurred 4 of the 7 days of anthesis, but average daily temperatures below 15 C limited disease development. Continued rain favored Stagonospora glume blotch. The biological agents (TrigoCor 1448 and 9790) did not limit glume blotch development. BAS 500 and Tilt reduced the level of disease to less than 50% of the untreated control. BAS 500 (56.7 bu/A) and Folicur (50.1 bu/A) treated plots had significantly higher yield (LSD 5 bu/A) than the untreated control (42.3 bu/A). Weather observations were used to predict the risk of head scab during the critical anthesis period. Weekly reports were provided to wheat growers through the Ohio State University Extension electronic newsletter Crop Observation and Recommendation Network (C.O.R.N.).

NCR-184, MANAGMENT OF HEAD SCAB OF SMALL GRAINS 2000 SOUTH DAKOTA STATE REPORT

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2000 SCAB DEVELOPMENT IN SOUTH DAKOTA

Minimal scab was observed across the state in South Dakota in 2000 although some fields in north and eastern part of the state had a higher incidence. Scab index (statewide average) was estimated at 2%. (M. Draper and Y. Jin).

CURRENT RESEARCH PROJECTS

Germplasm introduction and evaluation. The overall project goal is to identify new sources of scab resistance in spring wheat and to introgress the resistances into adapted materials. Spring wheat accessions from targeted regions of the world and relatives of wheat were evaluated in inoculated field nurseries and in the greenhouse. A scheme of three-nursery system was implemented in the germplasm evaluation process. Accessions were evaluated in the Preliminary Screening Nursery (PSN) initially. Selections from PSN were re-evaluated in the greenhouse to derive entries for Elite Germplasm Nursery (EGN). Most elite selections from EGN were entered into the Uniform Regional Scab Nursery for spring wheat for testing at multiple environments and for direct access by researchers. Elite selections were used for crossing to introgress the resistance into adapted germplasm. (Y. Jin)

Epidemiology. Ongoing research on several aspects of scab epidemiology includes effects of soil moisture/wetness on inoculum development, ascospore survival and accumulation on plant surface, and monitoring inoculum and environments. A sensor was developed to monitor moisture at the soil/air interface and integrated into an automated weather station. This sensor may help to understand the effects of soil surface wetness on the inoculum development. South Dakota continues to serve as one of the testing sites in a collaborative project, collecting data on environmental conditions, inoculum dynamics and disease development during the crop season in an attempt to develop a disease forecasting system. (Y. Jin)

Breeding for scab resistance in spring wheat. Greenhouse and field screening nurseries are used to evaluate early generation and advanced lines for scab resistance. All entries in the advanced yield trials are at least moderately resistant to scab. This is dramatically different from a few years ago when the spring wheat breeding program first began to evaluate for resistance to scab. We continue to see an increase in the number of lines that have good agronomic performance and good scab resistance. Eighteen lines in our 2000 advanced yield trials had scab resistance equal to Sumai 3. Ten of the eighteen lines had superior grain yield under heavy scab pressure compared to our best yielding commercial cultivars

(Russ and Oxen). Six of the eighteen lines had grain yield equal to Russ and Oxen in replicated yield trials grown under natural conditions. (J. Rudd)

Breeding for scab resistance in winter wheat. The first step in developing scab resistant hard winter wheat varieties is to assess the genetic variability for resistance in existing cultivars and advanced breeding lines. Scab resistance sources in the winter crossing block included adapted spring wheats from the SDSU breeding program, Sumai 3 derived spring wheat lines, eastern European winter wheat lines, entries from the 1998 regional winter wheat scab nursery, and adapted hard red and hard white breeding lines. Approximately 200 crosses with scab resistant sources were made and the segregating populations will be evaluated in 2000. Approximately 6000 plants were evaluated for scab resistance during the 1999 season and selections were planted into the field this fall. The following nurseries were screened for scab resistance in 2000: Northern Regional Performance Nursery; Winter Wheat Regional Scab Nursery; South Dakota Crop Performance Trials; SDSU Advanced Hard Red and Hard White Yield Trials; SDSU Preliminary Hard Red and Hard White Yield Trials. (A. Ibrahim)

Fungicide efficacy studies. South Dakota participated in the uniform fungicide trial for scab suppression. Two hard red spring wheat cultivars were planted at three locations each and treated at anthesis with the seven core treatments. The treatments were also applied to two hard red winter wheat cultivars planted at a single location. The winter wheat location was lost due to poor stand associated with root rotting diseases and cheat grass pressure. Plots were evaluated for protection of the flag leaf against diseases as well as for average incidence of scab infected heads, average head severity of scab, average plot severity of scab, Fusarium damaged kernels (FDK), deoxynivalenol (DON) content in the harvested grain, grain yield, protein and test weight of harvested grain. Under ambient conditions, scab was not severe. The greatest scab occurred at the South Shore, SD location. All treatments significantly reduced diseased leaf area ($P_{0.05}$) on spring wheat at the Groton location. Scab was not reduced significantly at any location. The most severe scab observed was at South Shore, SD with 3.9% scab index in the untreated plots. Scab was present at less that 1% disease index at the other two locations. Folicur (4 fl. oz./A), BAS 500 (12.3 fl. oz./A), and BAS 500 (6.2 fl. oz./A) tank mixed with Folicur (2 fl. oz./A) were the most effective treatments numerically. In other treatments, Folicur applied at the 6 fl. oz./A rate numerically outperformed the 4 fl. oz./A rate for scab index at South Shore. Metconazole and Folicur at 6 fl. oz./A were the most effective treatments tested. When scab or leaf diseases were reduced numerically by a fungicide, no significant increase in yield was realized. A mist irrigation system was completed late in the summer at the Brookings site that will be used to ensure moisture at anthesis in 2001 studies. (M. Draper)

Molecular biology and DNA markers for scab resistance. One of big obstacles in fighting scab epidemics is that little is known about the nature of scab resistance, particularly at molecular level. Our research aims at addressing this problem and getting insight into the molecular mechanism of *F. graminearum*-wheat interaction. Our current objectives were to identify, clone, sequence and analyze ESTs related to scab resistance by comparing the DDPCR revealed EST profiles of spring wheat cultivars Sumai 3 (resistant) and Wheaton (susceptible) before and after inoculation with *F. graminerum*. A total of 144 primer combinations were tested. Several gene expression patterns were observed: 1) constitutively ex-

pressed in Sumai 3; 2) constitutively expressed in Wheaton; 3) induced expression in inoculated Sumai 3 and Wheaton only; 4) induced expression in inoculated Wheaton only; and 5) induced expression in inoculated Sumai 3 only. ESTs of the last category are most likely related to scab resistance genes. Three such ESTs, *EST12G*, *EST15AU* and *EST15AD*, were cloned with PCR-Trap cloning kit (GenHunter Corporation, Nashville, TN) and sequenced using ABI automatic sequencer. A sequence similarity-search of GeneBank database revealed that *EST15AU* is 94% similar to part of a wheat mRNA for polypeptide elongation factor 1 beta'; *EST15AD* has three homologous regions (with 86% identity) with an EST sequence from a pathogen induced sorghum bicolor cDNA; *EST12G* is almost identical (with 99% identity) to a part of minus strand of a wheat gene for chloroplast ATP synthase CF-O subunit I and III. Confirmation of the accurate relationship of these three ESTs with scab resistance by genetic analysis is in progress. (Y. Yen)

Biological control studies. South Dakota began screening biological control agents in the field in 1999. During the summer of 2000 the project was expanded with six agents evaluated as whole cell treatments. A mist irrigation system was completed too late in the year to provide moisture during anthesis. As a result, scab index values were quite low in 2000. Nonetheless, the results appear promising. Two of the agents screened were from the SDSU collection that had been selected based on suppression of tan spot in greenhouse tests and *Fusarium graminearum* in culture plate tests appeared to have some activity in the field. The other four isolates had been selected elsewhere for antimicrobial activity. Suppression of scab at the very low levels of severity was similar to Folicur. None of the agents reduced leaf disease. At the low levels of disease present in the field an evaluation is not reliable, but the agents tested do appear to have promise for continued testing. (B. Bleakley and M. Draper)

PERSONNEL INVOLVED IN SCAB RESEARCH

Researchers/Project: Y. Jin/Small Grain Pathology; M. Draper/Extension Plant Pathology; J. Rudd/Spring Wheat Breeding; A. Ibriham/WinterWheat Breeding; B. Bleakley/Soil Microbiology; Y. Yen/Cytogenetics-Molecular Biology.

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NCR 184: VIRGINIA 2000 SSTATE REPORT ON *FUSARIUM* HEAD BLIGHT

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PRODUCTION AND SCAB DEVELOPMENT

In Virginia, 205,000 acres of soft red winter (SRW) wheat were harvested in 2000, with a state average yield of 60 Bu/A. The harvested acreage has declined by 40,000 acres since 1998, when scab epidemics devastated the crop. The incidence of *Fusarium* Head Blight (FHB) was sparse in the 1999-2000 season due to dry conditions prevailing throughout much of the flowering stage.

FUNGICIDE EFFICACY STUDIES

Research aimed at developing a means to control FHB in wheat with the application of a single fungicide, multiple fungicides, or a biological agent on wheat heads prior to or during anthesis was conducted this past year. Effectiveness of fungicide treatments on the control of FHB could not be assessed this past year due to the absence of FHB development, despite no-till planting of plots into chopped corn stubble.

ASSESSMENT OF SCAB RESISTANCE IN SRW WHEAT

One of the major objectives of our program has been to assess the variation among SRW wheat genotypes for resistance or susceptibility to FHB. Thirty SRW wheat genotypes, including many commercial cultivars, were evaluated in irrigated nurseries for reaction to FHB at two locations in inoculated and non-inoculated plots. At Blacksburg, inoculum was applied as a spore suspension to each genotype at flowering and again seven days post-anthesis. At Warsaw, colonized corn was spread in plots two weeks prior to anthesis.

Among the 30 genotypes evaluated in year 2000, FHB Index varied from 9 to 69 at Blacksburg and from 8 to 81 at Warsaw. Yield losses varied from 9 to 49% at Blacksburg and from 0 to 36% at Warsaw. Reductions in test weight varied from 4 to 23% at Blacksburg and from 0 to 14% at Warsaw. Percentage of scabby seeds varied from 24 to 90% at Blacksburg and from 10 to 85% at Warsaw. DON concentration varied from 0.5 to 15.9 PPM at Blacksburg and from 3.0 to 57.9 PPM at Warsaw. In tests conducted over the past 3 years, disease severity and yield loss often were significantly lower in cultivars Ernie, Freedom, Roane and INW9824 than others. Other genotypes exhibiting resistance included AgriPro Patton, AgriPro Foster, IL94-1909, IL94-1549, and NY87048W-7388.

BREEDING FOR SCAB RESISTANCE

Thirty-six scab resistant sources (21 Chinese, 2 French, 1 Japanese, 2 Canadian and 10 SRW wheat lines) have been used as parents in the breeding program to incorporate and/or combine Type II and other types of resistance. This year, 89 F_3, 101 F_2, 162 F_1, and 42 BC_2F_1 populations will be advanced. Scab resistance of 50 advanced lines and 532 doubled haploid and F_6 lines, selected from 2460 F_5 lines evaluated at Warsaw last year, will be evaluated for scab resistance in an inoculated nursery at Blacksburg and for other agronomic traits in trials at Warsaw in 2001. In addition, agronomic performance of 2960 F_5 lines, selected from inoculated tests of 53 F_4 populations, will be evaluated in a head-row nursery at Warsaw.

Type II resistance derived from nine different sources is being backcrossed into11 different SRW wheat backgrounds, of which three possess other types of scab resistance. Four BC_3F_1, 15 BC_2F_1 and 3 BC_1F_1 populations will be crossed with their respective recurrent parent this year. Subsequently, this will result in the development of near-isogenic lines with type II resistance incorporated into adapted SRW wheat backgrounds and facilitate pyramiding of different types of resistance.

Approximately 240 doubled haploid lines derived from 12 crosses were evaluated for scab resistance and other agronomic traits this past year, and nearly 450 additional haploid plants derived from nine crosses were produced. In the coming year, the maize x wheat hybridization method will be used to produce doubled-haploid progeny in ten multi-parent F_1 populations comprised of adapted resistance sources. Development of resistant lines using the comprehensive breeding techniques proposed above will provide the fundamental basis for pyramiding and improving scab resistance in SRW wheat varieties.

INHERITANCE AND MAPPING STUDIES

Three scab resistance sources, W14, Shaan 85 and Ernie, were crossed with the susceptible SRW wheat cultivar Madison and/or Pioneer 2684, and their F_2 progeny were evaluated for resistance using a single floret inoculation method in greenhouse tests. Two complementary genes with major effects were found to confer scab resistance in W14 and Shaan 85 based on similar segregation patterns of F_2 populations for type II, III and IV resistance characterized by scab severity, DON content and percentage of infected kernels, respectively. One to two genes were found to confer resistance in SRW wheat Ernie. The $F_{2:3}$ families of mapping populations Pioneer2684 x W14 and Madison x W14 will be characterized for severity, DON content and scabby seeds in greenhouse tests this coming year to confirm genetic segregation data obtained from F_2 populations last year.

To date, 62 SSR markers have been characterized in the parents and F_2 bulks of the two mapping populations; twenty-one (34%) of the 62 markers were polymorphic in both populations. Preliminary mapping data indicate that marker GMS533 located on chromosome 3BS (Anderson et al. 1998; 2001) likely is associated with resistance in W14. A second putative marker GMS410 identified in the susceptible parent Pioneer 2684 also was associated with resistance. These results will be verified in the coming year. Additional markers, SSR and other types, will be evaluated in the two mapping populations to search for additional puta-

tive QTLs associated with resistance, saturate chromosome regions associated with resistance, and develop a skeletal map. This research has the potential to identify new QTL associated with scab resistance, provide additional markers linked to previously reported QTLs, and to identify markers that are effective across genetic backgrounds; all of which are essential for successful exploitation of marker-assisted selection.