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.

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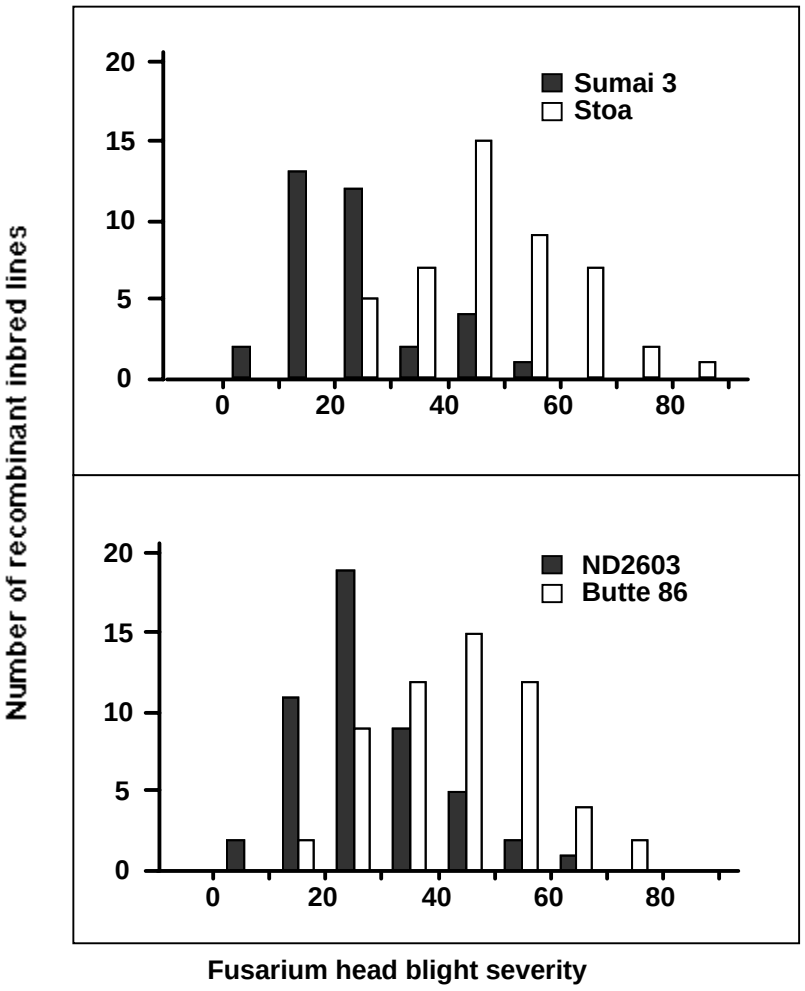

Figure 1. Histograms of Fusarium head blight severity for RI lines with resistant or susceptible parent alleles in the *Qfhs.ndsu-3BS* QTL region. Only those genotypes homozygous for this interval are included, bound by markers *Xgwm493* and *Xgwm533*.

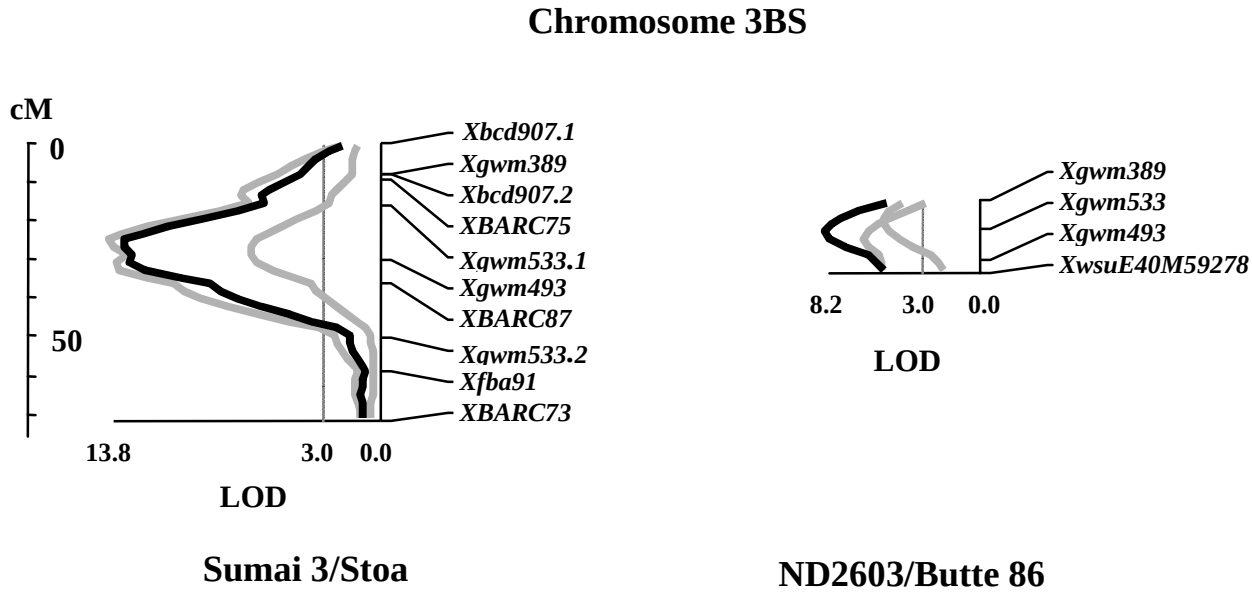

Figure 2. Interval analysis of data for chromosome 3B for Fusarium head blight resistance in the Sumai 3/Stoa and ND2603/Butte 86 recombinant inbred populations. The dark contour in each map represents the mean of the two experiments. The two lighter colored contours represent individual experiments. 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.

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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.

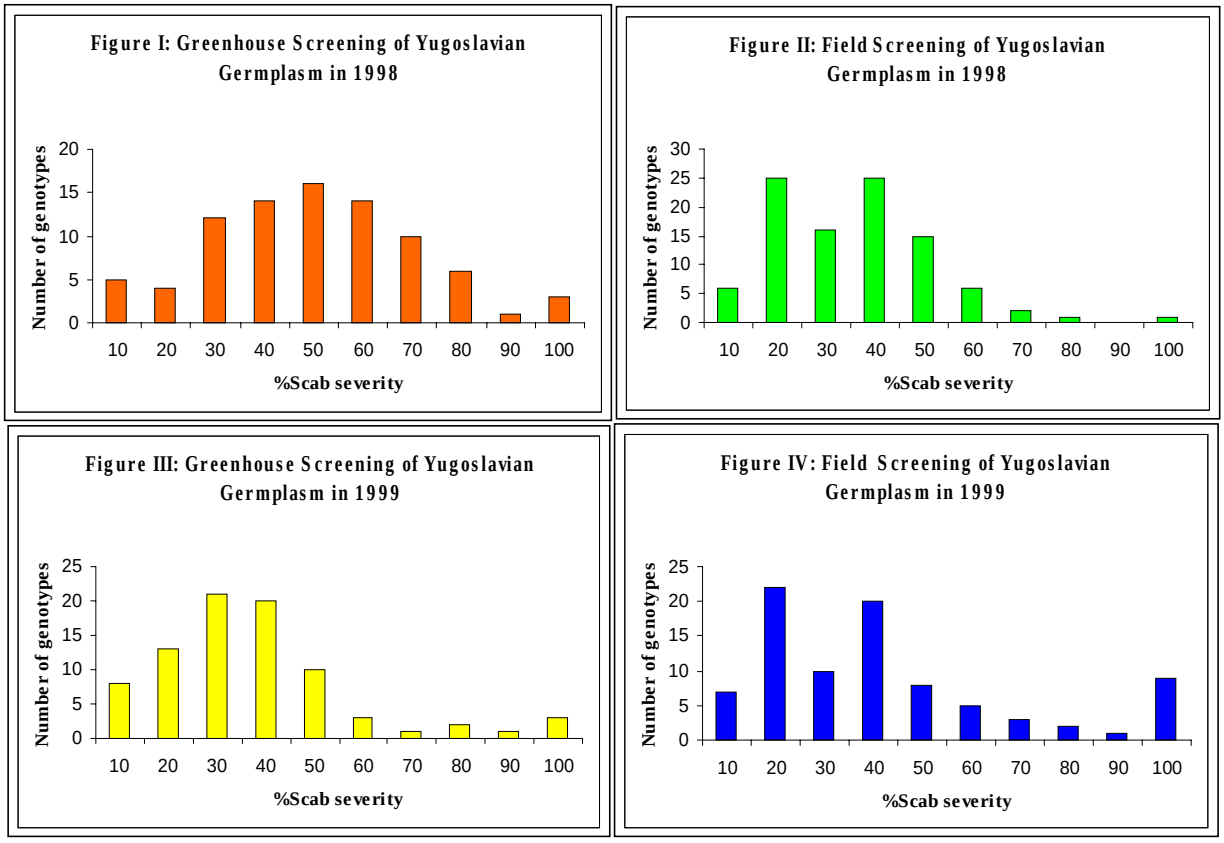

Figure I-IV: Normal Distribution Curve for mean FHB severity for Yugolsavian Germplasm for different years at different locations.

[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.

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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.

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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:

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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

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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.

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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

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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.

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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.

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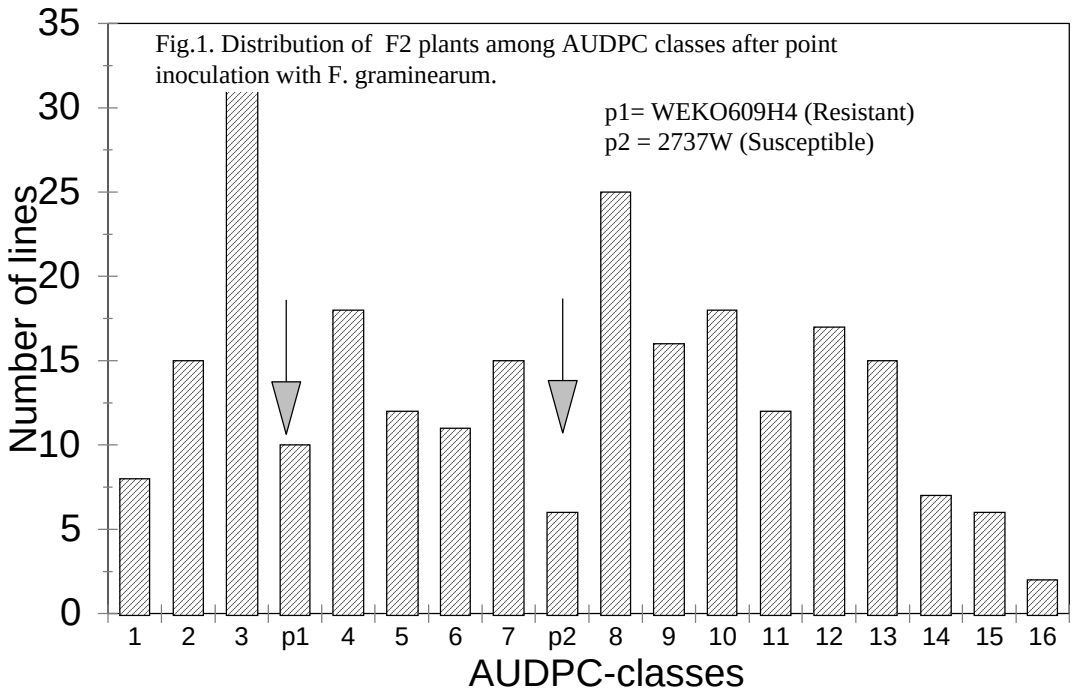

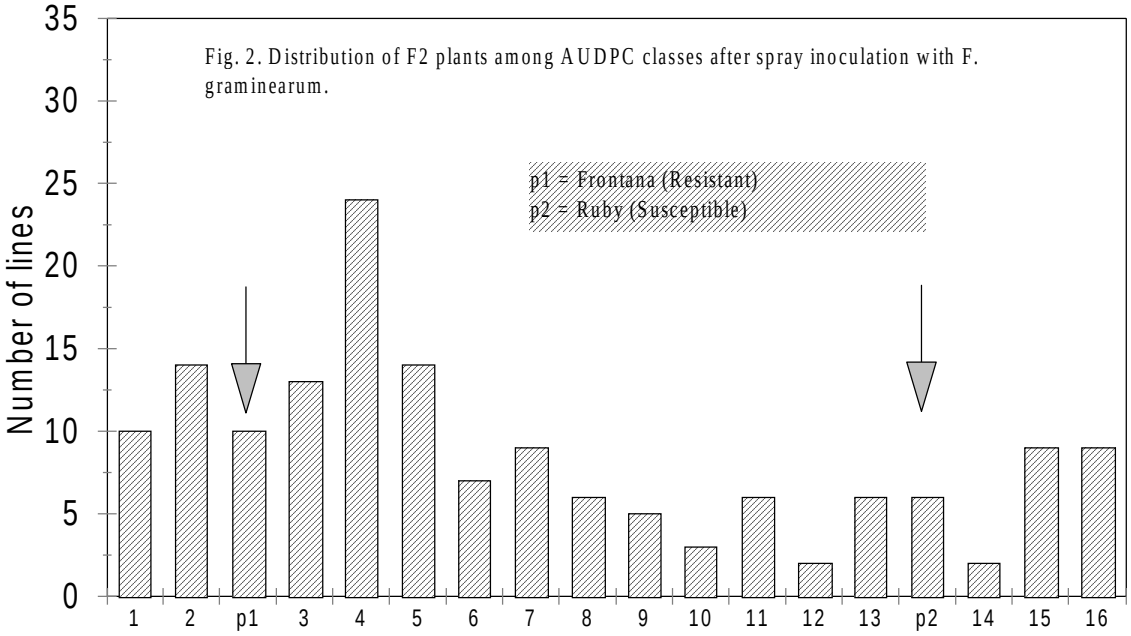
Fig. 2. Distribution of F2 plants among AUDPC classes after spray inoculation with F. graminearum.
p1 = Frontana (Resistant)
p2 = Ruby (Susceptible)
Number of lines
0
5
10
15
20
25
30
35
1
2
p1
3
4
5
6
7
8
9
10
11
12
13
p2
14
15
16

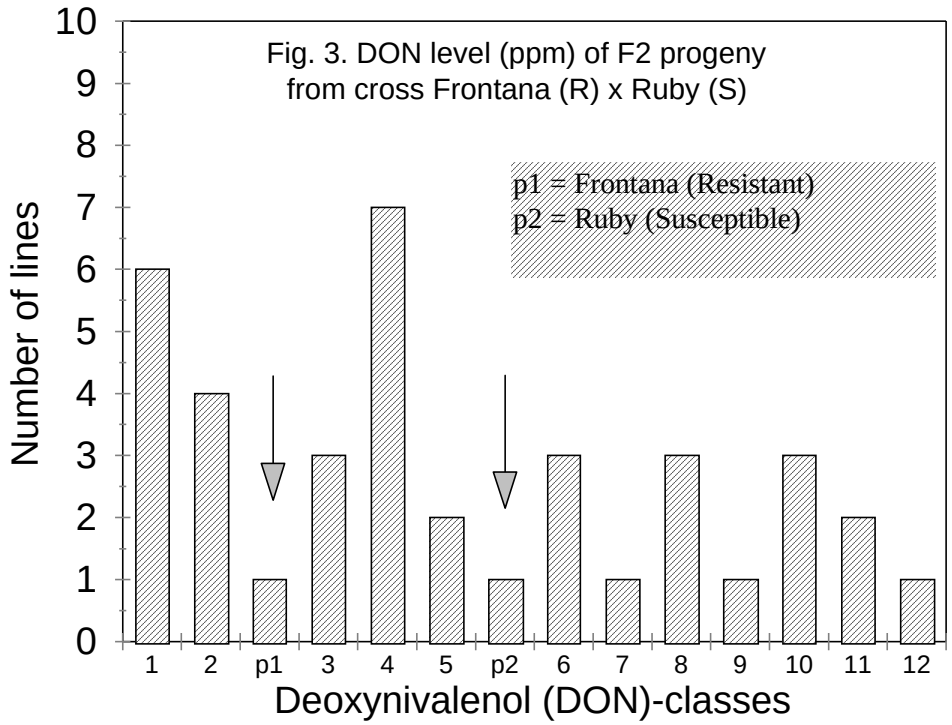
Fig. 3. DON level (ppm) of F2 progeny from cross Frontana (R) x Ruby (S)
p1 = Frontana (Resistant)
p2 = Ruby (Susceptible)
Number of lines
0
1
2
3
4
5
6
7
8
9
10
1
2
p1
3
4
5
p2
6
7
8
9
10
11
12
Deoxynivalenol (DON)-classes

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.

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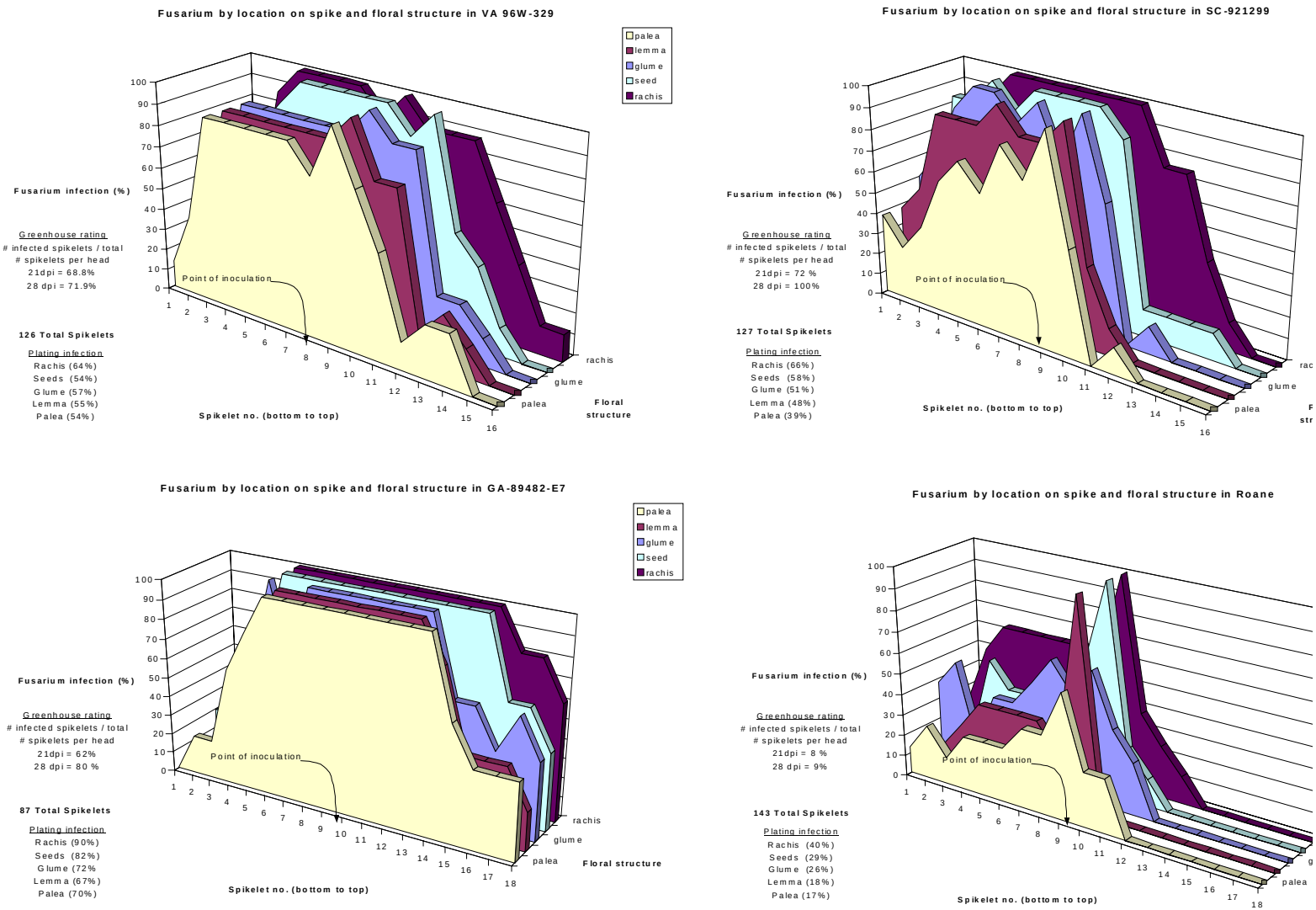
Figure 1. Preliminary results of *Fusarium graminearum* infection in the components of wheat spikes for eight spikes and four g



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)

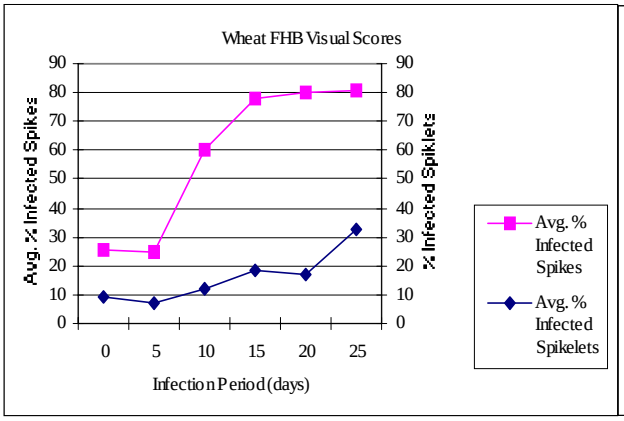

Figure 1

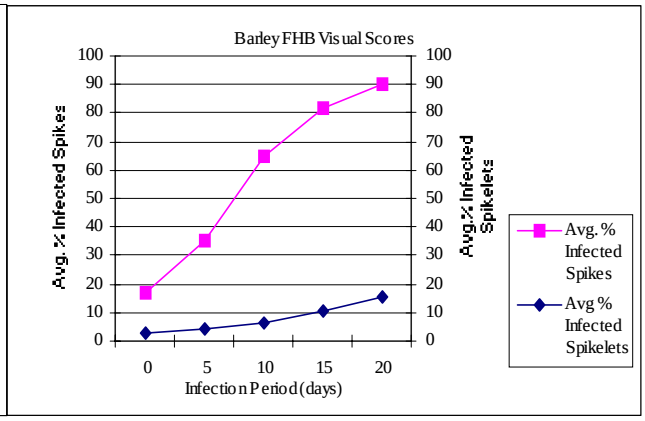

Figure 2

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).

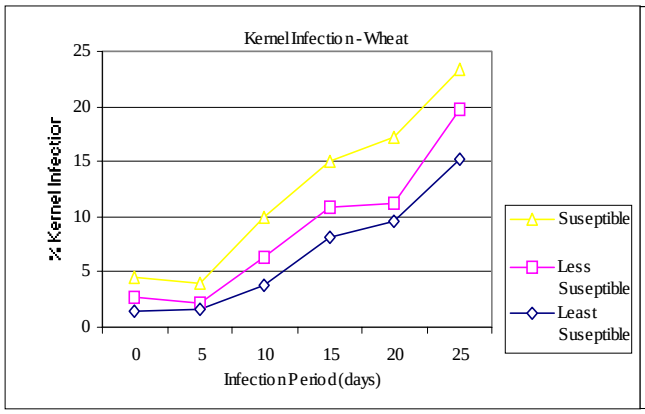

Figure 3

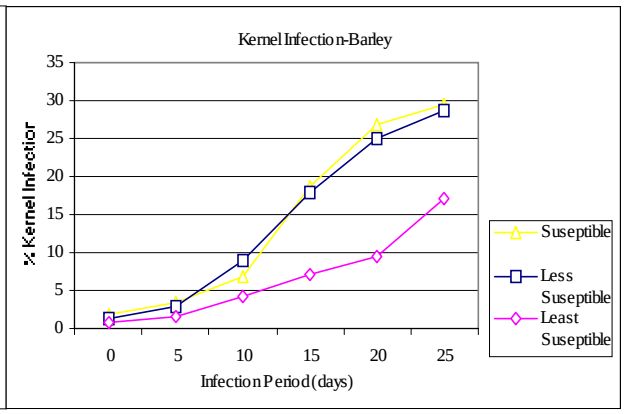

Figure 4

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

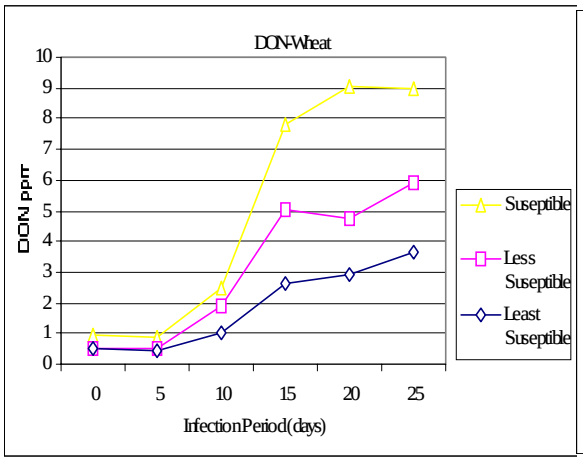

Figure 5

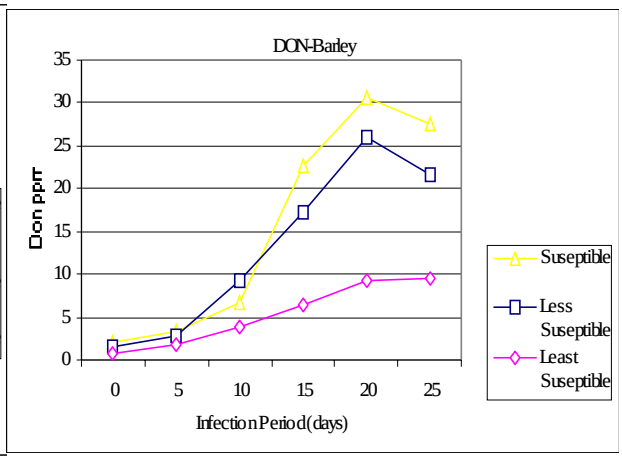

Figure 6

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.

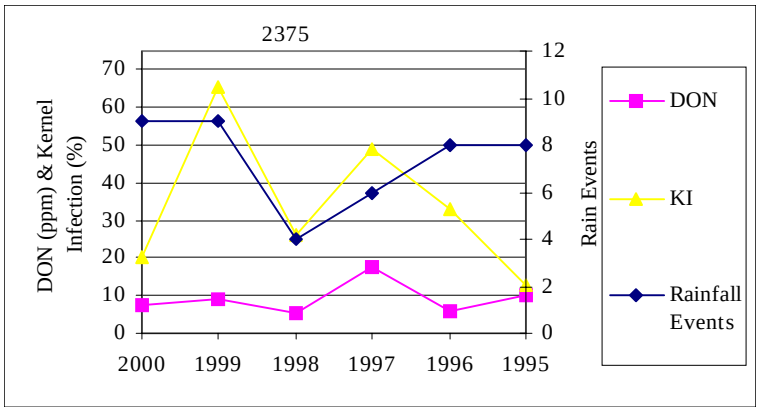

Figure 7

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.

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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.

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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

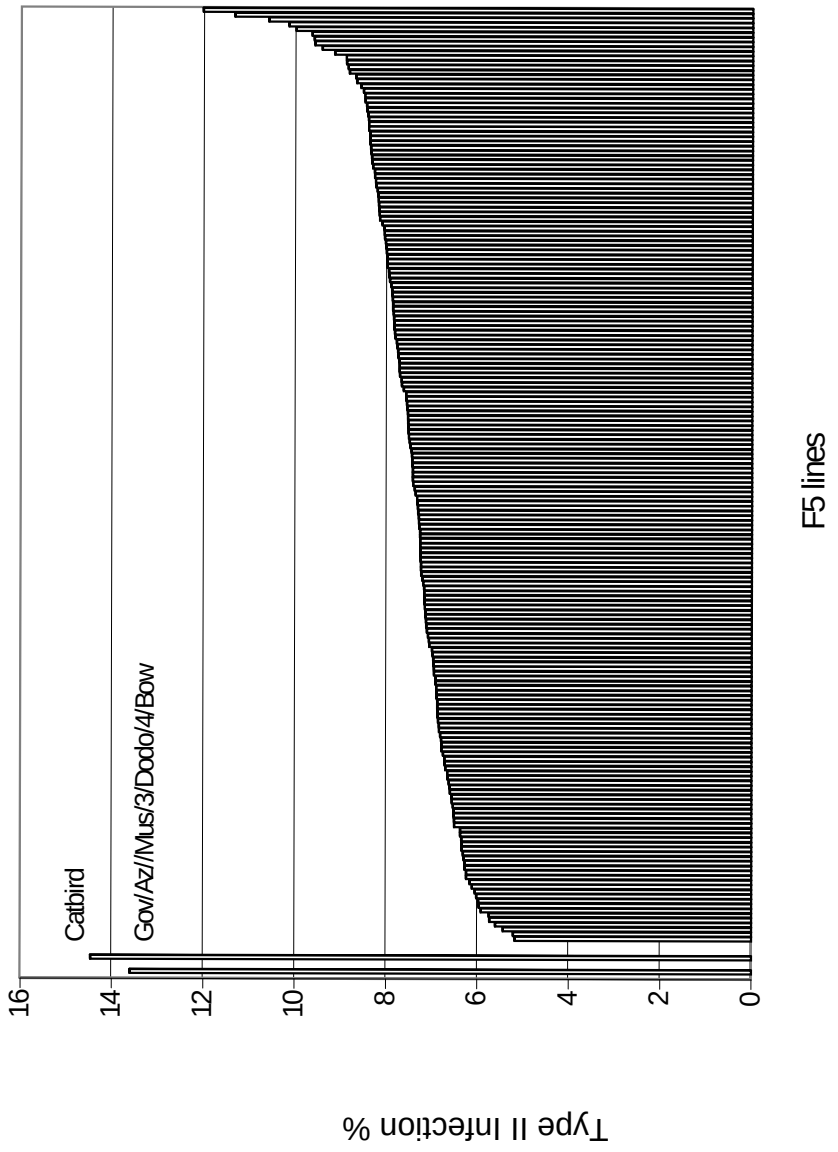

Fig. 1. Cross of Gov/Az//Mus/3/Dodo/4/Bow with Catbird. Both parents and 197 derived F5 lines are depicted against their response to infection to *Fusarium graminearum*, measured as Type II resistance.

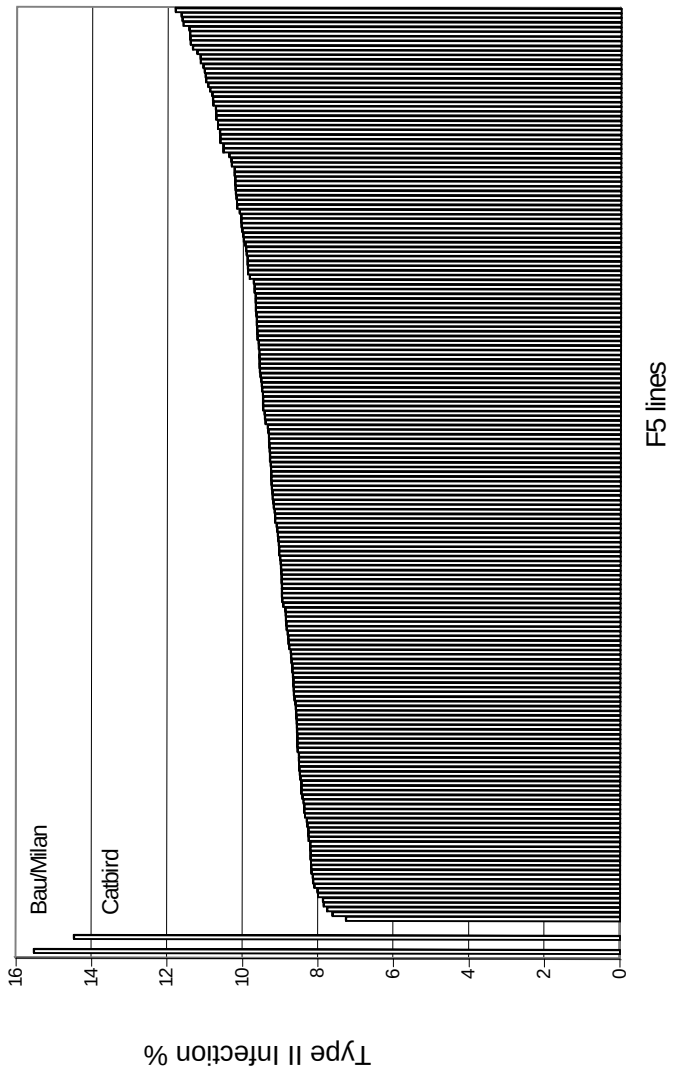

Fig. 2. Cross of Bau/Milan with Catbird. Both parents and195 derived F5 lines are depicted against their response to infection to *Fusarium graminearum*, measured as Type II resistance.

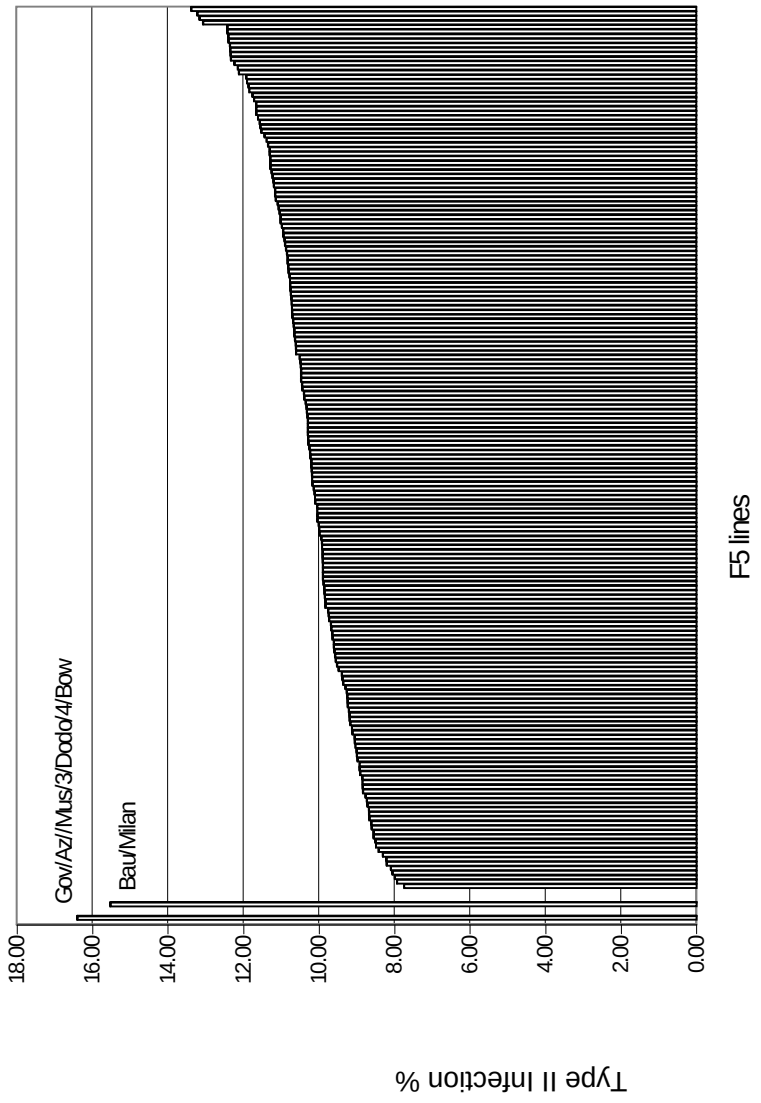

Fig. 3. Cross of Gov/Az//Mus/3/Dodo/4/Bow with Bau/Milan. Both parents and 195 derived F5 lines are depicted against their response to infection to *Fusarium graminearum*, measured as Type II resistance.

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.

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