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PREFACE

Fusarium Head Blight emerged in the past decade as a widespread and powerful enemy of American agriculture. This disease, also known as 'Scab', inflicts yield and quality losses on farms in at least 18 states. Food industries throughout the U.S. incur losses from the cost of dealing with the toxin-contaminated grain that often accompanies scab infection. Combined losses to all steps in the food system are difficult to estimate, but the bill at the farm-gate alone is estimated to exceed 3.0 billion dollars since 1990.

The existing private, state, and federal research system of the U.S. handles most of the newly emergent pests and diseases. But some problems, like scab of wheat and barley, present unusual challenges that warrant new approaches to research. There are several reasons why scab falls into this category:

- Scab is an economic threat to growers, processors and consumers of wheat and barley throughout much of the U.S.;
- Research is needed in a wide array of disciplines, with possible solutions including:
  - resistant varieties (from conventional or biotechnology approaches),
  - alternative residue and rotation management schemes,
  - crop protection via chemical and biological controls, and
  - detoxification or alternative processing of contaminated grain;
- The interactions of the scab pathogen with local and regional farming practices are complex and poorly understood;
- Recent experience clearly signals that no single institution can bring to bear the critical mass of research this problem demands;
- Scab solutions are likely to be both site and system specific, which dictates involvement of local experts in all of the directly affected states;
- Opportunities for acquiring competitive funds for scab research from traditional federal sources are extremely scarce.

As a result of these special circumstances, scab of wheat and barley is one of several plant disease complexes accorded special status as "Emerging Diseases" in recent Federal budgets. National awareness of the serious nature of scab is a welcomed development. However, the real challenge comes in the design and implementation of a national, multi-disciplinary, and multi-institutional research system that can win the war on scab as quickly and efficiently as possible.

During the past three years, federal, state, and private sector scientists have worked closely with growers, input providers, millers, and food processors from across the country to design and fund just such a system. The result of these efforts is the U.S. Wheat and Barley Scab Initiative. In one sense, the Initiative is a self-organized "contact group" on scab. The goal of the U.S. Wheat and Barley Scab Initiative is to develop as quickly as possible effective control measures that minimize the threat of Fusarium head blight (scab) to the producers, processors, and consumers of wheat and barley. The Initiative is guided by a Steering Committee that includes growers, farm organizations, food processors (e.g., millers, bakers, pasta manufacturers, and brewers), scientists (from Land Grant universities, USDA, and private companies), and consumer groups. Eight members of the Steering Committee also serve on an Executive Committee. The Steering and Executive Committees are advised by a series of six research committees composed of recruited volunteers from the scientific leaders of the U.S. wheat and barley research communities. The composition

of all committees is designed to ensure balanced input from all commodities, regions, disciplines, and institutions engaged in the battle against scab. The Initiative's Networking and Facilitation Office, based at Michigan State University, facilitates the work of these committees. That office also promotes communication among the varied parties interested in scab, and represents the Initiative's only 'fixed' asset.

Each year, the Steering Committee submits to the USDA-ARS a comprehensive and optimized research plan designed to achieve the Initiative's goals. That plan is the Initiative's recommendation for how the USDA-ARS can most effectively employ the funds appropriated by the U.S. Congress for collaborative scab research. The relevance of the research plan is assured by the central role that industry (growers and processors) plays in the Initiative's work. Much of the work reported in this document were partially or completed funded with Initiative recommended ARS funding.

Congress has appropriated funds for this collaborative effort for three consecutive years (FY98: $500,000, FY99: $3.5M, and FY2000: $4.8M). The USDA also contributed funds to Initiative recommended funding with end-of-year funds in FY97 ($200,000), and discretionary funds in FY98 ($200,000). The $4.8M appropriated in the FY99 budget is part of ARS's base budget and does not require the yearly lobbying process associated with 'special grants' through the USDA- CSREES. In other words, there is every reason to expect re-appropriation of the $4.8 M on an annual basis for at least several years. Increases specifically associated with scab have also been made in the base budgets of specific ARS facilities in the past three years ($1.375M). Combined, these funds represent an unprecedented commitment to wheat and barley research at the Federal level.

The FY97 and FY98 funds were dispersed primarily as Cooperative Agreements through Dr. Bob Busch's offices (USDA-ARS, MN). The Scab Initiative's Research and Steering Committees undertook a process in 1998 that generated a comprehensive research plan for the funds appropriated in FY99 federal budget (October 1998 through September 2000). Every conceivable solution area was considered, and the resulting mix of research projects is truly comprehensive. The prioritization process was competitive by its very nature, since researchers had to demonstrate their ability to contribute to the overall solution. On the other hand, the final research agenda consists of a mix of projects identified via both directed and purely competitive processes. In almost all cases, funds proposed by the Initiative will be heavily leveraged by existing sources of investments in personnel, facilities, and supplies.

The resulting research plan for FY99 involved 66 principal investigators in 19 Land Grant Universities and the ARS. Collectively, 111 projects were funded for one year, most with starting dates (dates funds were available) in May or June of 1999. The details of the overall research plan were finalized at the 1998 Fusarium Head Blight Conference. Project details including end of fiscal year (September) progress reports can be viewed at the Initiative's website at WWW.SCABUSA.ORG . Much of the work reported in these proceedings was partially funded by Initiative recommended funding.

The unprecedented nature, magnitude, and scope of this collaboration required constant innovation by the administrative staffs of the Initiative, ARS, and the participating Land Grant Universities. We are happy to report that the ARS has been supremely cooperative and has exhibited degrees of flexibility and sensibility not commonly attributed to Federal agencies.

The Initiative recently solicited proposals for fiscal year 2000. For the first time, a formal call for proposals (actually pre-proposals) was issued. Proposals totaling $5.73M were received from a total of 84 scientists affiliated with 23 Land Grant Universities and the ARS. $1.55M of these funding requests is for new proposals, and 19 of the applicants are new to the Initiative. The six research area committees are finishing up a formal review process as these proceedings go to print. The Executive Committee will meet with the leaders of the Research Committees on the day before the formal start of the '99 FHB Forum. The Executive Committee will then prepare a proposal for a comprehensive research plan for consideration by the overall Steering Committee the following morning. Once the Steering Committee's input has been incorporated, the overall plan will be submitted to the ARS as the Initiative's formal recommendation for the use of the FY2000 funds. Investigators will need to make any necessary adjustments in their budgets and combine proposals on the basis of investigator to streamline the grant process for ARS. ARS then conducts an internal review process that we expect will endorse the great majority of the recommendations made by the Initiative.

Substantial research efforts were already underway in several states before anyone had notions of a national effort. However, the articles and poster abstracts included in this volume represent a veritable explosion of scab research. Promising new sources of host plant resistance genes are reported, along with substantial progress in molecular tagging of previously discovered genes. Conventional plant breeders are eliminating highly susceptible varieties and improving the resistance in new releases. Likewise, investments in biotech solutions have enabled progress in the arena of genetic engineering. Promising bio-control agents are reported here, and several reports confirm the efficacy of Folicur and possibly other fungicides. Good progress in unraveling the mysteries of the pathogen's life is evident. Some highly innovative methods are being employed in that effort, including employment of *Fusarium* that has a fluorescent gene from jellyfish, and radio controlled aircraft to document aerial spore movement. New knowledge is reported here regarding the fate and effect of DON in food products, as is new work on the toxicological properties of that mycotoxin.

The speed and magnitude of the success our industries have had in generating funds and associated research plans is an arguably unprecedented happening in U.S. plant agriculture. However, all we've really done is enabled ourselves to fully engage in the real challenge, which is the elimination of scab as a destructive force in the U.S. food system. We now have a substantial solution-discovery engine up and running. There are two fundamental challenges deserving of heightened focus during the 1999 FHB Forum and throughout the ensuing year. First, how do we increase the scientific productivity of the resources committed to scab research, and second, how do we minimize the time lag between discovery of a solution and its comprehensive employment in relevant at-risk systems? We believe that we've only begun to scratch the surface of the wealth of opportunities for synergistic collaboration on both of these issues. One means of addressing the first challenge (scientific productivity) is full employment of modern communication technologies, i.e., the Internet. The Internet has already played a significant role in the Initiative's history, both through email list servers, and through a central web-site. However, little if any progress has been made in moving towards the kinds of 'virtual communities' of scientists made possible by the real-time, or near real-time communication systems available through the web. The second challenge, the lab vs. real-world time lag, requires pro-active forward thinking by across-discipline alliances representing all aspects of the continuum from

the lab to the field or factory. In both cases, these challenges require continued investment and belief in research system components that transcend the individual research scientist.

In closing, we wish to commend the ongoing spirit of collaboration that enabled and sustains the U.S. Wheat and Barley Scab Initiative. We are honored to have the opportunity to work in the context of the Initiative and look forward to a productive 1999 National FHB Forum.

Tom Anderson and Rick Ward
Co-Chairs,U.S. Wheat & Barley Scab Initiative

SOME OF MY QUESTIONS, OPINIONS AND EXPECTATIONS ON BREEDING WHEAT FOR SCAB RESISTANCE

Dajun Liu

I feel greatly honored to be invited to attend this forum, which provides me with a rare chance to meet the many scientists involved in the US Wheat & Barley Scab Initiative. Dr. Rick Ward wanted me to speak at this forum, but I have been hesitating what to speak about since it seems that the Chinese scab studies have been frequently introduced and repeatedly documented long ago in this country. But, being a scientist engaging in wheat scab studies in China for more than 20 years, I hope to share with you some of my questions, opinions and expectations on breeding wheat for scab resistance. It would be my great pleasure, if my talk might be of help in your discussions.

I started to touch upon the wheat scab problem in the mid 1970s, when a nation-wide collaborative network of wheat breeding was just under reorganization, and the international exchange of Chinese wheat breeding was just recommenced. At that time, I was involved in the wheat breeding programs south of theYangtze River. In addition to yield and earliness, quite a strong emphasis was placed on scab resistance. Screening nurseries with artificially induced epidemics had been commonly used to determine the response of wheat breeding lines to scab disease. I will never forget my meeting with visitors from CIMMYT in late 1970s at Jiangsu Academy of Agricultural Sciences. Headed by Norman Borlaug, Haldore Hanson, the late Director General of CIMMYT and Glen Anderson, the late Director for Wheat Program of CIMMYT at the time were also in the group. Our guests were shocked by the severity of the scab epidemics in the nurseries. I suppose that the visit might have given them a deep impression about why CIMMYT wheat varieties could hardly be adapted to that part of China. In 1980, I was invited by Norman Borlaug to visit El Batan and Toluca, where I met Dr. Sajaya Rajaram for the first time. We had a vivid discussion about the necessity to also include breeding for scab resistance in the international wheat improvement program at CIMMYT. I was not surprised that CIMMYT initiated a program on scab resistance 2 years later. The cooperation between China and CIMMYT in breeding wheat for scab resistance has significantly strengthened.

Scab resistance always has been an important target of wheat breeding south of Yangtze River. This area consists of more than 10 provinces that grow wheat in China. However, we learned many lessons from the unsuccessful release of improved varieties developed in the early stages of Chinese wheat breeding that relies mainly on selections from landraces and exotic introductions. The breeders of the elder generation in China are well aware of stories related with such lessons. As a result of massive breeding efforts made since 1950s, a number of Chinese cultivars with scab tolerance or resistance were developed and popularized along the middle and lower reaches of Yangtze River. The earliest cultivars were Wan-Nian #2, selected from the improved variety ND 2419 (a cultivar selected from Strampelli's variety Mentana by late Professor Shanbao Jing, the late Directors of the Chinese Academy of Agricultural Sciences and Nanjing Agricultural College, the predecessor of Nanjing Agricultural University) and Wu-Mai #1, a selection from an introduced Italian variety Funo (Stampelli's too). Later, Er-Mai #6, an induced mutatant of ND2419, Su-Mai #2 & #3 and

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Xiang-Mai #1, produced from recombinations between Chinese and Italian genotypes, came out successively from the mid 1960s to mid 1970s. Interestingly, all the above-mentioned varieties with scab resistance or tolerance were developed without exception on the basis of Italian breeding products (such as Metana, Ardito, Giuliari and Funo etc), but a few Chinese landraces were involved. Of greater interest, the Brazilian variety Frontana, generally thought to have Type III resistance to scab disease also involves Italian variety Mentana in its pedigree. It is my belief that there should be some substantial reason explaining such a repeated use of Italian varieties in the international practice of breeding wheat for scab resistance. And it is also true that most of scab resistant or tolerant wheat cultivars or strains released after the 1970s in China (actually also in other countries) almost exclusively derived from the crosses involving Su-Mai #3 and its sister lines or derivatives. My question arose again: " Does it occur reasonably and inevitably?"

There is no doubt of the urgency to broaden the genetic resources for scab resistance used in wheat breeding not only because of restricted genetic basis, but also the inadequate level of resistance. In my view, the extensive and full use of genetic resources from landraces, related species and every possible origin should be strengthened. Of course, to reach the final goal through these approaches may take us a long time. But, a quotation from "Confucianism" might be of instructive importance: " No reach with haste. " It is my opinion that wheat landraces with precisely identified scab resistance from those areas where selection pressure has been existing for centuries, such as southern China and southern Japan, must be adequately evaluated , properly maintained and reasonably utilized. As for the genetic resources for scab resistance from related species and /or of even more remote origins, it seems that great potential will always accompany with increasingly appearing challenges. But, I do think the germplasms with incorporated alien genes shall be able to play unique and irreplaceable roles in breeding wheat for scab resistance in the near future.

In Addition to the germplasms related to breeding scab resistant wheat, I would still like to stress some other restraints that hinder a breakthrough in breeding advancement. To me, how to determine the breeding targets in terms of scab resistance appropriately, how to understand numerous basic aspects of the pathogenesis of the disease and the nature of host resistance better, how to refine the relevant methodology further still need to be studied in greater depth. To pave the way for a breeding breakthrough, continuous achievements in both practical and basic studies are indispensable. Being scientists from developing countries, our expectations of US scientists probably focus more on the basic research.

The reemergence of scab disease of wheat and barley in North America in the early 1990s is, indeed, a disaster for grain production as a whole. But it also brought something beneficial in the renaissance of scab studies on world wide scale. Thanks to the McKnight Foundation, my colleagues and I not only obtained more resources to strengthen our research programs, but also received valuable chance to learn from the recent achievements of the scab studies made in the US and Canada. We are very happy to see the establishment of the USWBSI and its active role. We greatly appreciate your efficient utilization of all the resources you have strived for in recent years. The achievements you made in the development and release of new varieties with improved scab resistance, genetic analysis and gene mapping, pathogen biology, epidemiology, mycotoxin studies have impressed us deeply. Indeed, all these achievements should be a great contribution to the international studies on scab. Concluding my talk I sincerely hope an international initiative to study scab in wheat and barley will soon emerge.

ISOLATION OF TRICHOTHECEN RESISTANT GENES FROM THE WHEAT CULTIVAR FRONTANA

N.J. Alexander[*1], S.L. Ziegenhorn[1,2], G.J. Muehlbauer[3], S.P. McCormick[1], and B. Kurtz[2]

ABSTRACT

Wheat head blight is a plant disease that is caused by the fungus *Fusarium graminearum*. This fungus produces a trichothecene toxin called deoxynivalenol (DON) that acts as a protein synthesis inhibitor and is thought to be correlated with the severity of the disease. It is reasoned that if plants could detoxify DON, then they may exhibit increased resistance to fungal invasion. The wheat cultivar Frontana exhibits limited resistance to infection by *Fusarium graminearum* and it is possible that this resistance is due to the expression of toxin resistant genes. Several cDNA libraries were made from both infected and non-infected wheat heads at differing time periods post-infection, a well as tissue cultures grown with or without DON for varying time periods. For screening, these cDNA libraries were put into a yeast vector and transformed into a yeast strain that is sensitive to the trichothecene toxins. Selection for resistant colonies was then done on toxin media. The resulting inserts were sequenced and compared by BLAST for homology with other sequences in GenBank. Only the fungal gene *TRI101* was isolated from the cDNA libraries made from *Fusarium*-infected wheat heads. This gene codes for an acetyltransferase that has been shown to detoxify a number of trichothecene toxins, including DON. Analyses are continuing on the remaining libraries.

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UPDATE ON DNA MARKERS FOR FUSARIUM HEAD BLIGHT RESISTANCE QTL IN TWO WHEAT POPULATIONS

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OBJECTIVES

Our objectives are to saturate the chromosome 3BS region, previously found to contain a *Fusarium* head blight resistance QTL, with additional DNA markers and develop markers for this region that may be used in a marker assisted selection scheme.

INTRODUCTION

We have identified quantitative trait loci (QTL) for *Fusarium* head blight (FHB) resistance in two wheat populations (Anderson et al., 1998a,b; Waldron et al., 1999). The most significant QTL for FHB was located on the short arm of chromosome 3B and designated *QFhs.ndsu-3B*. The best marker in this region, an AFLP from the primer pair *Eco*RI-agc/*Mse*I-cta explained 17.6% and 15.6% of the phenotypic variation in greenhouse-based evaluation of Type II (Mesterhazy, 1995) FHB resistance in Sumai 3/Stoa and ND2603/Butte 86 recombinant inbred populations, respectively.

MATERIALS AND METHODS

The populations used and methods of evaluation were described previously (Anderson et al., 1998b). Briefly, the populations are recombinant inbred lines from the cross of Sumai 3/Stoa (112 lines) and ND2603 (Sumai 3/Wheaton)/Butte 86 (139 lines). Each population was evaluated twice in the greenhouse for Type II (spread) resistance at North Dakota State University under the supervision of Dr. Robert Stack. The average of the two screenings were used in the analyses reported here.

Primers for the microsatellites (SSR) published by Röder et al. (1998) were synthesized and are being screened for polymorphism among the four parents of these populations as well as parents of other populations. Markers known to be located in putative QTL regions based on our previous research were given the highest priority. PCR amplification was as described by Röder et al. (1998) except 35 cycles of amplification were used instead of 45. Visualization of fragments was by electrophoresis in 5% polyacrilimide gels and silver staining according to the protocol of Bassam et al. (1991).

RESULTS AND DISCUSSION

Of the 142 microsatellite markers screened to date, 48 (34%) were polymorphic between Sumai 3 and Stoa and 79 (53%) between ND2603/Butte 86. The marker *Xgwm264* was mapped using all RIL from the Sumai 3/Stoa population. Unfortunately, this marker putatively mapped to 5BL instead of the expected location on 3BS (data not shown). The marker *Xgwm533* was mapped in the ND2603/Butte 86 population and explained 24.6% of the phenotypic variation in scab resistance in this population (Fig. 1). All lines have been screened with this marker two or three times. Due to discrepancies (2 cases) and apparent presence of heterogeneity in some lines (10 cases), 12 lines are considered missing data for this marker. This marker explains the highest proportion of FHB resistance of those that we have identified to date for a gene inherited from Sumai 3. Unfortunately, this microsatellite was not polymorphic between Sumai 3 and Stoa. Comparison of the interval maps for scab resis-

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tance in these two populations indicates that the scab QTL in these two populations is toward the proximal end of this chromosome with respect to the AFLP locus *XEagcMcta.1* that was mapped in both populations.

Other ongoing research in our lab is directed at 1) obtaining STS markers for the *XEagcMcta.1* AFLP locus; 2) verifying all markers in additional populations; and 3) implementing marker assisted selection in our wheat breeding program. We have cloned the critical fragment from the *XEagcMcta.1* AFLP locus, developed primers, and screened the parents, but have yet to reveal polymorphism.

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Figure 1. Frequency distribution of FHB severity for the ND2603 and Butte 86 alleles at the *Xgwm533* microsatellite locus among 127 ND2603/Butte 86 recombinant inbred lines.

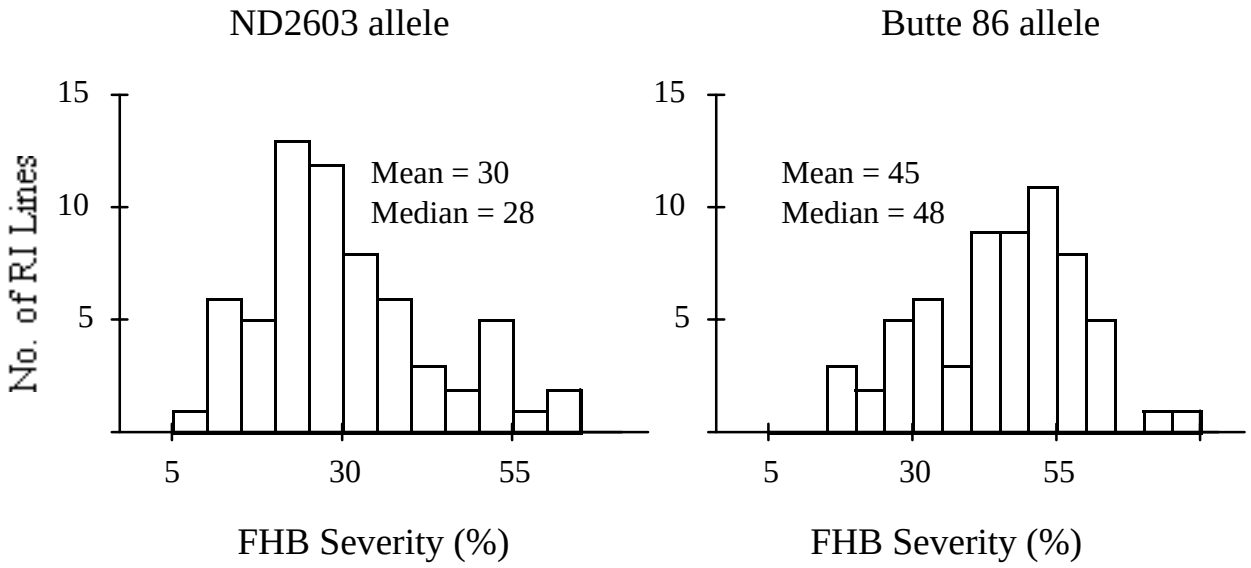
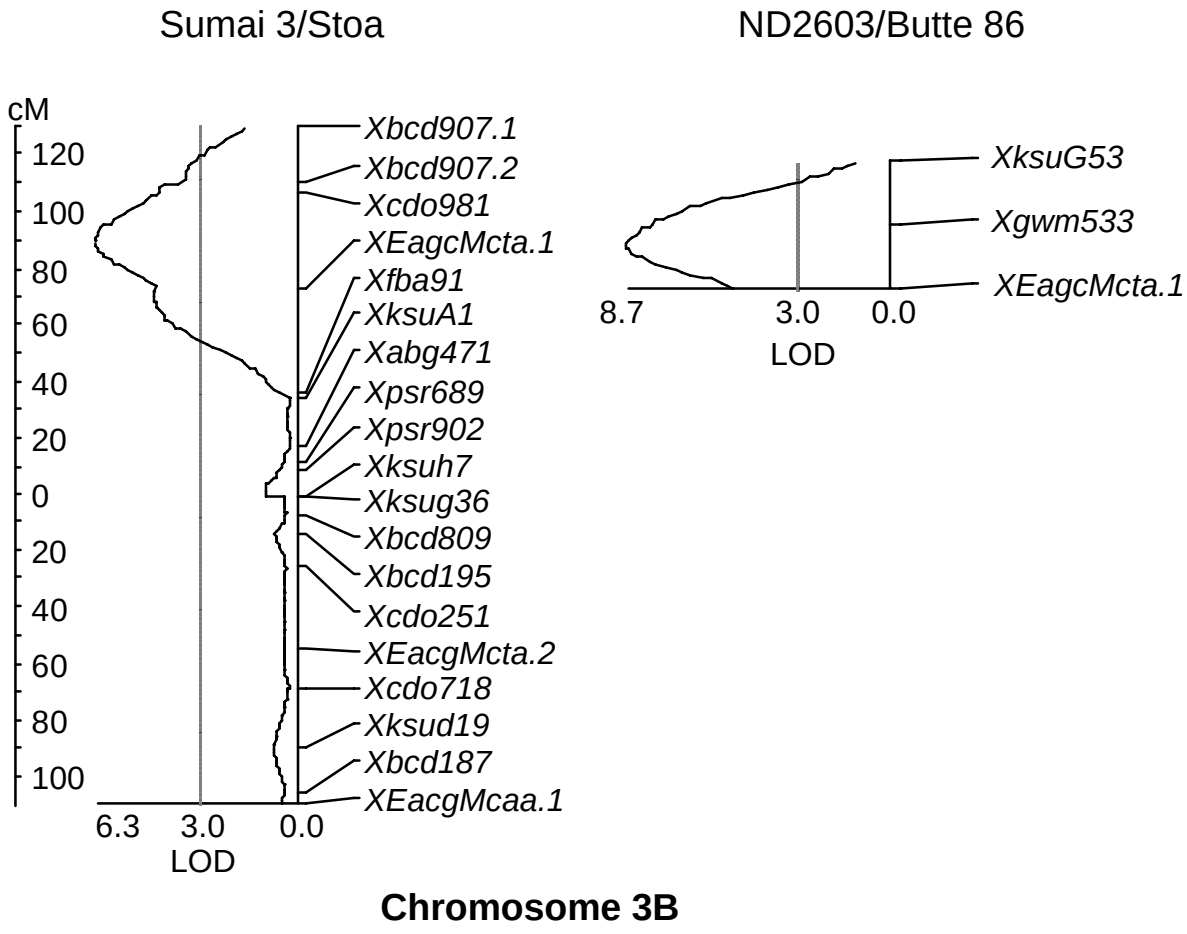


Figure 2. Interval analysis of chromosome 3B *Fusarium* head blight resistance in Sumai 3/Stoa and ND2603/Butte 86 recombinant inbred populations. Chromosomes are aligned at their common marker, *XEag.Mcta.1*. Zero on the cM scale indicates the position of the centromere.



USING AN AFLP MAP TO IDENTIFY SCAB RESISTANCE QTL IN WHEAT

Gui-Hua Bai[1,2], Frederic L. Kolb[3*], Gregory Shaner[4], and Leslie L. Domier[3,5]

ABSTRACT

Wheat scab, caused by *Fusarium graminearum*, is a destructive disease in the US. To identify quantitative trait loci (QTL) for scab resistance, we constructed a molecular map using amplified fragment length polymorphism (AFLP) markers with 133 F_9 recombinant inbred lines (RILs). The RILs were derived by single-seed descent from a cross between the resistant wheat cultivar Ning 7840 and the susceptible cultivar Clark. The F_7 to F_{10} RILs were evaluated in the greenhouse for resistance to scab by injecting conidia of *Fusarium graminearum* into a central floret. A genetic linkage map consisting of 27 linkage groups was constructed with 600 AFLP markers. Three QTL, one with a major effect, for scab resistance were identified by interval mapping and collectively explained up to 63% of the variation (R^2). Some of the AFLP markers will be converted into STS markers for marker-assisted selection in wheat breeding programs.

INTRODUCTION

Fusarium graminearum causes scab, an important wheat disease. Scab reduces wheat yield and grain quality. The mycotoxins produced by the fungus in infected grain are detrimental to humans and livestock. The use of resistant cultivars is one way to control the disease (Bai and Shaner, 1994). Scab resistance is a quantitative trait. Phenotypic evaluation of resistance is complicated, laborious, and confounded by environmental variation.

Use of molecular maps to identify quantitative trait loci (QTL) has been reported for important traits in many crops. Once a QTL is identified, molecular markers closely linked to it can be used as tags to selectively transfer the desired genes into different genetic backgrounds by marker-assisted selection. A restriction fragment length polymorphism (RFLP) map has been used to identify scab resistance QTL in 4 wheat chromosomes either from cultivar Sumai 3 or Stoa (Waldron et al., 1999). Recently, we used amplified fragment length polymorphism markers coupled with bulk segregant analysis to identify a major QTL for scab resistance that explained up to 53% of the phenotypic variation (Bai et al., 1999). Our objective in this study was to use an AFLP map to scan the wheat genome for additional QTL controlling scab resistance.

MATERIALS AND METHODS

Recombinant inbred lines (RILs) were derived by single-seed descent from a cross made between the cultivars Ning 7840 (resistant) and Clark (susceptible) in 1990 at Purdue University, Indiana. $F_5 - F_8$ RILs were evaluated for scab resistance in the greenhouse at Purdue University from 1994 to 1996 and the F_{10} RIL was evaluated at the University of Illinois in 1998. Nine to sixteen plants per family were evaluated by injecting 1000 conidia (a mixture of *Fusarium graminearum* isolates from Purdue University) into a central floret of a spike at early anthesis with a hypodermic syringe. To promote infection after inoculation, the plants were placed in a moist chamber for 3 days at 23°-25°C and 100% relative humidity. Spikelets showing symptoms were counted at 3, 9, 15 and 21 days after inoculation. Based on total spikelets per spike,

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counts of blighted spikelets at each of these days were converted to percentage of scabbed spikelets (severity). Final disease severity and area under the disease progress curves (AUDPC), calculated according to Shaner and Finney (1974), were used to characterize the reaction of each plant to infection. Plants from F_{10} families were individually harvested, and seeds were scored for percentage of scabbed kernels (PSK) and weighed to estimate hundred kernel weight (HKW).

DNA was isolated from Ning 7840, Clark, and the 133 F_9 RILs grown in a greenhouse at the University of Illinois using the CTAB procedure (Saghai-Maroof et al., 1984). For each sample, 500 ng genomic DNA was digested simultaneously with *Eco*RI and *Mse*I restriction enzymes. The genomic DNA fragments were ligated to *Eco*RI and *Mse*I adaptors to generate template DNAs for amplification. The *Eco*RI selective primers were labeled with ^{33}P-gATP. PCR was performed in two consecutive reactions in a thermocycler (MJ Research, Inc.). Primers for pre-amplification and selective amplification of genomic DNAs were designed according to Thomas, et al. (1995). Primer combinations showing high levels of AFLP polymorphism between two parents were used to evaluate all 133 RILs. Linkage analysis was performed with Mapmaker (V.2.0 for MacIntosh, Lander et al., 1987) with the Kosambi mapping function and using a maximum recombination fraction of 0.4 and a minimum LOD of 3. For QTL analysis, interval analysis was performed with Mapmaker/QTL (Lander et al., 1987).

RESULTS AND DISCUSSION

Of the 266 AFLP primer combinations tested, about 230 amplified clearly separated band patterns from parental DNA. Each primer combination amplified 70-140 bands, of which 1 to 18 bands were polymorphic. Sixty-three primers with relatively high levels of polymorphism between parents were selected to evaluate 133 RILs. In total, about 600 markers were mapped in 27 linkage groups covering a distance of 2251 cM. The number of markers within each linkage group ranged from 3 to 155. Clustering of AFLP was observed in several linkage groups, including linkage groups 1, 2, 3, 7 and 11. One major QTL for Type II resistance, as reported previously (Bai et al., 1999), was detected in all generations. R^2 for the QTL varied from 30 to 58%. Two other QTL were also detected and present in only some of the generations evaluated (Table 1). These two QTLs had minor effects on percentage of scabbed spikelets and marginally significant LOD values. They were more sensitive to environmental variation than was the QTL in LG12 since they were detected only in some generations.

F_{10} RILs were also evaluated for AUDPC, percentage of scabbed kernels and hundred kernel weight. The major QTL in linkage group 12 affects not only visual symptoms on the wheat spikes, but also seed infection. In the F_{10} the major QTL explained 53% to 58% of variation for the four scab measurements (Table 2). In breeding programs, this major QTL is an ideal candidate for marker-assisted selection. Four of the markers closely linked to the QTL were cloned and will be sequenced to develop STS markers. Interval mapping detected an additional QTL in linkage group 21 in the F_{10} RIL for AUDPC (Table. 2). This QTL was also associated with percentage of scabbed seeds and hundred seed weight, and therefore appears to have an effect on more than visual symptoms on spikes.

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Table 1. R^2 and LOD values of QTL for percentage of scabbed spikelets as detected by interval mapping for 5 RIL generations in the greenhouse

LG[a]	F_5		F_6		F_7		F_8		F_{10}	
	R^2	LOD	R^2	LOD	R^2	LOD	R^2	LOD	R^2	LOD
LG12	33.9	11.1	30.4	10.2	55.9	23.4	30.3	9.9	57.7	22.4
LG13	8.6	2.4	8.8	2.1	8.4	2.5	-	-	-	-
LG17	-	-	-	-	7.3	2.1	-	-	8.1	2.4
Total[b]	42.9	14.7	38.8	12.5	61.9	26.3	30.3	9.9	60.7	23.2

a. Linkage group number
b. Total variation explained by all the significant QTL detected by interval mapping and its LOD value.

Table 2. R^2 and LOD values of QTL for percentage of scabbed spikelets (PSS) at 21 days after inoculation, area under disease progress curve (AUDPC), percent scabbed kernels (PSK), and hundred kernel weight (HKW) as detected by interval mapping for 5 RIL generations in the greenhouse

LG[a]	PSS		AUDPC		PSK		HKW	
	R^2	LOD	R^2	LOD	R^2	LOD	R^2	LOD
LG12	57.7	22.4	55.4	21.5	57.7	21.7	52.8	19.0
LG17	8.1	2.4	7.5	2.2	-	-	-	-
LG21	-	-	12.3	2.4	12.5	2.4	17.0	3.0
Total[b]	60.7	23.2	58.1	22.2	63.2	23.1	59.2	22.0

a. Linkage group number
b. Total variation explained by all the significant QTL detected by interval mapping and its LOD value.

IDENTIFICATION OF GENES FOR RESISTANCE TO PATHOGENS IN NON-HOST PLANTS

Albert H. Ellingboe

ABSTRACT

Control of plant diseases via breeding disease resistant cultivars has been considered to be environmentally safe and cost effective. Control of disease by breeding is usually a never-ending endeavor because pathogens are living organisms capable of genetic changes that render the genes for resistance ineffective. A common observation is that there are not sufficient genes for resistance in the economic species so it has been necessary to introgress genes from related species into economic species. The proposal submitted to the USDA is to use a strategy devised for the specific purpose of identifying specific genes for resistance to a pathogen, *Fusarium moniliforme*, in a non-host species. The procedure uses a pathogen of the plant species to be mined as a vehicle to study the individual genes in the pathogen for which genes for resistance are sought. Once the specific genes in pathogen and host that control the interactions are identified, the procedures to clone the genes, and transfer the resistance genes to wheat and barley, will follow established procedures.

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AFLP LINKAGE MAP OF *GIBBERELLA ZEAE*

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ABSTRACT

A genetic map of *Gibberella zeae* could be useful in population genetics studies, map-based cloning of interesting genes, QTL analysis, and comparisons with related species. Complementary *nit* mutants of *G. zeae* strains R-5470 (from Japan) and Z-3639 (from Kansas) were crossed according to previously described methods. 99 *nit*+ progeny were selected and analyzed for polymorphisms using AFLP analysis. Analysis of thirty-three two-base selective primer pairs were analyzed revealing 1084 polymorphic loci of which 1039 unambiguously segregate into 9 linkage groups. The total map length of the genome from this analysis is estimated to be in excess of 2800 cM with an average interval of 2.7 map units per locus. Three linkage groups exhibit a high degree of segregation distortion. Selection of *nit*+ progeny may account for some but not all of the segregation distortion in the cross.

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ENHANCED SCAB RESISTANCE IN WINTER WHEAT GERMPLASM BY PLANT TRANSFORMATION

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ABSTRACT

Fusarium graminearum is an important and emerging pathogen of wheat not controlled effectively chemically or by genetic resistance in the world. Losses have occurred to the extent that wheat production in some parts of the US and Canada is severely threatened. Definition of the genetic and biochemical bases of the fungal pathogenesis may lead to the development of control strategies, but may take a significant period of time. Expression of known and novel antifungal genes in transgenic wheat, on the other hand, may provide quick, effective and durable scab control strategies. The ability to create transgenic wheat resistant to this fungus has significant impact to growers. We have identified two different types of genes, antifungal lactoferrin and antiapoptic genes, for resistance to the scab fungus. An in-vitro test indicated effectiveness of lactoferrin against the scab fungus. Similarly, transgenic tobacco plants expressing the antiapoptic genes were immune to a number of necrotrophic fungi. *Fusarium graminearum* is a necrotrophic fungus. In order to improve wheat transformation efficiency, a comparative study was conducted to ascertain co-expression, integration patterns and inheritance ratios of transgenes in wheat transformants derived from microprojectile bombardment and *Agrobacterium*-mediated transformation protocols. A total of 9 and 7 independent events were derived from the *Agrobacterium*-mediated and microprojectile systems, respectively. Wheat transformants were analyzed at either the R1 or R2 generation. Co-expression of the non-selected transgene (GUS) was observed in leaf tissue in 5 out of the 9 Agrobacterium-mediated events and 1 out the 7 microprojectile events. Co-expression in floral tissue was observed in 6 out of the 9 *Agrobacterium*-mediated events and 5 out of 7 microprojectile events. Although co-expression was observed at a higher frequency in the *Agrobacterium*-mediated transformants the 1 microprojectile event which GUS co-expression was observed in leaf, the expression level was significant higher than the 5 Agrobacterium-mediated events. Southern blot analysis on progeny revealed all 9 Agrobacterium-mediated events possessed 1 to 2 loci with 1 to 2 copies per locus, while 2 microprojectile events had fragmented copies with 1 extremely complex integration pattern.

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WHEAT SPIKE-*FUSARIUM GRAMINEARUM* MOLECULAR INTERACTIONS

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ABSTRACT

Fusarium Head Blight (FHB) of wheat is a crippling disease that causes severe economic losses in many of the wheat growing regions of the world. Our group is studying the interactions between wheat spikes and *F. graminearum* during infection. Microscopy of inoculated glumes revealed that the fungus appeared to penetrate through stomata, exhibited subcuticular growth along stomatal rows, colonized glume parenchyma cells and sporulated within 48-76 hours after infection (hai). No major differences in the timing of these events were found between Sumai 3 (resistant) and Wheaton (susceptible) genotypes. To study the host response to infection, we examined the expression of six defense response genes (peroxidase, PR-1, PR-2, PR-3, PR-4, and PR-5) in resistant (Sumai 3) and susceptible (Wheaton) genotypes during the initial 48 hai. In both genotypes, transcripts of the six defense response genes accumulated as early as 6-12 hai and peaked at 36-48 hai. Greater and earlier PR-4 and PR-5 transcript accumulation was observed in Sumai 3 as compared to Wheaton. These data indicated that wheat responds to infection by inducing a set of defense response genes. In a companion study, we examined whether infection induced a systemic response of the defense response genes. We found that infected plants of both resistant (Sumai 3) and susceptible (Wheaton) genotypes induced defense response genes in uninfected portions of the wheat spike, indicating that wheat mounts a systemic reponse to infection. Our results provide the first documentation of the infection pathways on wheat glumes and provide the first look at the host response at the molecular level.

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ENHANCED RESISTANCE TO SCAB BY GENETIC ENGINEERING WITH GENES FOR DEFENSE PROTEINS

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ABSTRACT

Pathogenesis-related proteins represent inducible defenses that help plants fight pathogen and pest infestations. This inducible defenses include biochemically diverse proteins fungal cell wall hydrolyzing enzymes (e.g. chitinases and beta -1,3-glucanases), inhibitors and membrane-permeabilizing proteins (e.g. thaumatin-like proteins, TLP). Genes/cDNA's for several of these proteins have been isolated from Rhizoctonia-infected rice and scab infected wheat in our laboratories. We have introduced some of these cDNA's/genes into calli derived from immature wheat embryos by biolistic procedures and regenerated transgenic plants from them. The regenerated plants were shown to possess the gene by Southern blotting and to express the transgenes by enzyme assays and western blotting using appropriate antisera for chitinase, -1,3-glucanases and TLP. One line of transgenic wheat plants expressing high levels of TLP at high level were tested for resistance to scab by the single floret inoculation method and were found to have significantly greater resistance to scab compared to control plants. Work is currently in progress to introduce combinations of chitinase, beta -1,3-glucanases and TLP to identify specific combinations of these pathogenesis-related proteins that would have the highest level of resistance to scab.

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

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OBJECTIVES

The aims of our project are to generate transgenic lines of hexaploid wheat (*T. aestivum*) expressing genes that encode candidate antifungal proteins, and to optimize the activities of the antifungal proteins in wheat for effectiveness against scab.

INTRODUCTION

Efforts are ongoing in laboratories throughout the world to obtain transgene-mediated resistance to *Fusarium* head blight in wheat, barley, and oat. Recently, Gill and coworkers reported that the expression of a rice thaumatin-like protein delayed the development of scab in transgenic wheat lines (Chen *et al*. 1999).

We transformed wheat with six genes encoding candidate anti-*Fusarium* proteins with different modes of antifungal action. The proteins are *Fusarium* DON acetyltransferase (from *FsTRI101*, McCormick *et al*., in press), *S. cerevisiae* multidrug transporter (from *PDR5*, Balzi *et al*. 1994), wheat thaumatin-like protein (from *tlp-1*, Rebmann *et al*. 1991), *Fusarium* glucanase (FvGlu), *Fusarium* endochitinase (FvChi1), and *Fusarium* exochitinase (FvChi2). The coding sequences of the genes are under constitutive regulation by the maize *Ubiquitin-1* promoter (Christensen and Quail 1996). A *Bgl*II restriction site in the original *Ubi-1* promoter was removed to facilitate the cloning of the coding sequences at the remaining *Bgl*II site in our monocot expression vector, pUBKBglII-. In this poster, we 1) present the pattern of GUS activity conferred by the modified *Ubi-1* promoter in wheat floret organs, 2) summarize the results of our transformation experiments, 3) discuss progress in quantifying transgene copy numbers and expression levels in wheat endosperm, and 4) discuss codon usage in candidate genes from fungal sources.

MATERIALS AND METHODS

Removal of the *Bgl*II restriction site in the *Ubi-1* promoter region of pUBK was carried out using the QuikChange™ Site-Directed Mutagenesis Kit and protocols (Stratagene, La Jolla, CA). Wheat transformation of cv. Bobwhite was carried out as described in Weeks *et al*. (1993), with modifications (Blechl and Anderson 1996). Floret organs of transformants expressing *uid*A under regulation of the modified *Ubi-1* promoter (lines B70-14b, AB7-203b, AB7-148) were assayed for GUS activity by histochemical staining (Gallagher 1992, Weeks *et al*. 1993). Leaf genomic DNA was obtained from a preparation of enriched nuclei (D'Ovidio *et al*. 1992). Total DNA was prepared from small leaf sections (Dellaporta *et al*. 1983). Total RNA was isolated from endosperm of homozygous transgenic lines at 15 to 25 days post-anthesis (Altenbach 1998). DNA and RNA blot analyses were done as described in Sambrook *et al*. (1989), using Zeta Probe nylon membrane (Bio-Rad, Hercules, CA). Hybridization probes consisted of coding sequence fragments that were radiolabeled with ^{32}P-a-dCTP. Codon usage data was generated using the Codon Usage Database at Kazusa (http://www.dna.affrc.go.jp/~nakamura/codon.html).

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RESULTS AND DISCUSSION

The modified *Ubi-1* promoter conferred high transient levels of GUS activity in triticale endosperm bombarded with *Ubi*::*uid*A (A. Blechl and G. Sharma, unpub.), and its activity was indistinguishable from that of the unmodified *Ubi-1* promoter in pACH15 (Christensen and Quail 1996). To characterize the modified *Ubi-1* promoter in wheat florets at anthesis, we examined GUS activity in several *Ubi*::*uid*A transformants. GUS activity was found primarily at the base and tips of the glume, lemma, and palea, and in pollen, brush and endosperm tissue, but not in the anther. Therefore, the modified *Ubi-1* promoter can confer gene expression in organs that are potential barriers to the spread of scab in the head.

Using transformation methods developed in the laboratory, we generated 31 transgenic lines of wheat carrying one of six candidate genes (Table I). To rapidly and accurately identify lines carrying the candidate transgenes, we used PCR to amplify transgene DNA from small amounts of leaf tissue. This approach was useful for identifying homozygotes and for following transgene inheritance in lines in which the bialaphos resistance marker gene was inactivated. To date, there are 2 to 11 lines representing each candidate gene, and a total of 16 lines homozygous for the candidate gene.

Table I. List of candidate genes and transgenic wheat lines.

Gene Name	Lines	Homozygotes	mRNA Detected
FsTRI101	4	3	in 1 of 3 tested
PDR5	11	6	in 0 of 4 tested
tlp-1	3	1	in 1 tested
FvChi1	8	4	in 1 of 3 tested
FvChi2	2	1	in 0 of 1 tested
FvGlu	3	1	in 1 tested

Gene Name	Mode of Action	Reference
FsTRI101	DON inactivator	McCormick, in press
PDR5	DON exporter	Balzi, 1994
tlp-1	Membrane permeabilizer	Rebmann, 1991
FvChi1	Chitin degradation	unpub.
FvChi2	Chitin degradation	unpub.
FvGlu	Glucan degradation	unpub.

Genomic DNA blots of four *FsTRI101* lines and three FvChi1 lines showed one to four integrated, intact copies of the candidate genes. In all but one of these lines, additional bands were present, indicating rearrangements. Expression of candidate mRNAs in the endosperm (~15-25 dpa) of available homozygous lines was determined using RNA blots. Our most highly-expressing line so far accumulated abundant levels of the wheat *tlp-1* mRNA in the endosperm. The remaining two *tlp-1* lines will be examined when homozygotes are identified. Expression of the fungal genes was generally lower. mRNAs of the expected sizes were detected in endosperm RNA from the homozygote carrying the FvGlu gene, from one of the four *FsTRI101* homozygotes, and from one of three FvChi1 homozygotes (Table I). None of the four *PDR5* lines tested to date accumulated detectable amounts of mRNA in the endosperm. Progress with RT-PCR, a more sensitive method for RNA detection, will be reported in the poster.

Genes from microbial and plant sources often differ in nucleotide composition, codon usage and other features that affect the expression of genes in a foreign host. Our initial computerized analyses indicated differences in codon usage of the candidate *Fusarium* genes as compared to a compilation of wheat genes (Table II). We speculate that the low levels of specific mRNAs in lines containing the fungal genes might be due to such differences. Codon usage analyses are under way for cereal genes expressed in endosperm, pollen and leaf, and for *Fusarium* genes. If necessary, strategies will be devised to modify the fungal genes for higher mRNA stability and translation (e.g., Iannacone *et al*. 1997; Lonsdale *et al*. 1998) in wheat floral tissues.

Table II. Third nucleotide frequencies in codons of *F. venenatum* candidate genes and wheat (*T. aestivum*) genes

A. FvGlu [a/] Amino acid	C,G	A,U	CG/AU Ratio [b/]	*T. aestivum* Ratio [c/]
F	15	0	>15	1.88
K	29	0	>29	4.36
N	14	1	14.0	2.37
Q	13	1	13.0	0.88
B. FvChi1 [d/] Amino acid	C,G	A,U	CG/AU Ratio [b/]	*T. aestivum* Ratio [c/]
G	11	28	0.4	1.70
K	14	1	14.0	4.36
N	20	4	5.0	2.37
R	4	11	0.4	2.33
C. FvChi2 [e/] Amino acid	C,G	A,U	CG/AU Ratio [b/]	*T. aestivum* Ratio [c/]
K	2	3	9.7	4.36

[a/] Contains a total of 302 codons; [b/] Ratio of codons NNC/G to NNA/U encoding the specified amino acid; [c/] Total of 119,403 codons from 353 wheat genes (www.kazusa.or.jp/codon/); [d/] Contains 400 codons; [e/] Contains 582 codons

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MOLECULAR MAPPING FOR *FUSARIUM* HEAD BLIGHT IN A RICL POPULATION OF TETRAPLOID WHEAT

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ABSTRACT

The extensive damage caused by *Fusarium* head blight (FHB) has made it necessary to develop resistant lines of durum and common wheat. Unlike hexaploid wheat, there are no useable sources of resistance in domesticated tetraploid cultivars. One species that shows promise as a source for FHB resistance is an accession of *Triticum turgidum* L. var. *dicoccoides* (Joppa, 1997). Langdon-dicoccoides disomic substitution lines [LDN(Dic)] were analyzed for Type II resistance. The Langdon durum line with a pair of chromosomes from an accession of *Triticum turgidum* L. var. *dicoccoides* [LDN(Dic-3A)] was shown to have reduced infection to FHB relative to the parents (Elias et al., 1996). A Recombinant Inbred Chromosome Line (RICL) population of 83 individuals derived from LDN(Dic-3A) has been analyzed over three seasons. These data, molecular marker (RFLP, AFLP, and microsatellite) mapping data and QTL analysis results futher delineating the location of FHB resistance loci on 3A are presented.

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TARGETING OF ANTIFUNGAL GENES TO INHIBIT GROWTH OF *FUSARIUM GRAMINEARUM* IN BARLEY

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OBJECTIVES

The objective of this research is to produce *Fusarium*-resistant barley through genetic transformation with specifically targeted native antifungal protein genes. Studies will examine the tissue preference and subcellular route(s) of Fusarium infection. This will guide strategies for future gene expression targeting. Tissue-specific promoters will be developed for lemma/palea and pericarp. Expression vectors will be developed to target antifungal genes (particularly hordothionin) to the intracellular space.

INTRODUCTION

Since 1993, a sustained *F. graminearum* epidemic has destroyed much of the U.S. barley and wheat crops. The production of mycotoxins (e.g., DON) by *Fusarium* makes the harvest unsuitable for food, feed or malting. At present, there are no known barley cultivars with biochemical resistance to *Fusarium*, although some have various levels of tolerance and avoidance. Thus, genetic transformation may be the only method for introducing biochemical resistance. This research seeks to redirect the expression of native genes encoding antifungal seed proteins so that they will be produced in the path of invading Fusarium hyphae.

Research is needed to determine the tissue preference and subcellular route of *F. graminearum* infection. Antifungal proteins must be expressed in the most appropriate tissue and subcellular compartment to avoid placing a metabolic burden on the plant and to minimize pressures which select for resistant pathogen strains. There is very little literature on microscopic studies of penetration by the *Fusarium* genera. In a review of *Fusarium* blight (Parry et al., 1995), the only histological study of *F. graminearum* (Pugh et al., 1933!) noted that hyphae can penetrate wheat kernel tissues both inter- and intracellularly. *F. oxysporum*, which causes root rot in flax, colonizes root cap cells through inter- and intracellular penetration (Kroes et al., 1998). Hyphae grow through the middle lamella and cause collapse of adjoining cells, prior to penetration. Early events in the infection of cotton root tip cells by *F. oxysporum* include the formation of a thin infection peg, without accompanying cell lysis (Rodriguezgalvez and Mendgen, 1995). The penetration of wheat stem cells by *F. culmorum* was shown to involve a stage in which hyphae penetrate the wall perpendicularly and then grow some distance through the intracellular space (Ebrahim-Nesbat et al., 1991). Our research, with a strain of *Fusarium* (gfp/Fusarium) transformed with the green fluorescent protein (gfp) gene of jellyfish, shows that when *Fusarium* colonization occurs on the lemma, hyphae can grow rapidly to the tip of the floret and colonize the extruded anthers or (later) stigmatic and ovary epithelial hairs. Hyphal growth rapidly proliferates downward and covers the pericarp. Hyphae can also penetrate the lemma and grow directly into the pericarp within two days from spore inoculation. It is therefore essential to express antifungal genes in these tissues.

Plants synthesize a variety of pathogenesis-related proteins (PRPs; Linthorst, 1991), often in

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response to pathogen invasion. While the mechanism of PR-1 proteins is unknown, PR-2 proteins include beta-(1,3)-glucanases, PR-3 proteins include chitinases, and PR-4 proteins bind chitin of hyphal cell walls (Hejgaard, 1992). PR-5 proteins are comprised of osmotin (Singh et al., 1987), PRHv-1 (Hahn et al., 1993), PWIR2 (Rebmann et al., 1991) and other thaumatin-like proteins (TLPs). Permatins are TLPs found in many cereals and are homologous with other PR-5 proteins. Permatin has been purified from maize and found to have antifungal properties (Roberts and Selitrennikoff, 1990). An additional group of PRPs is the highly basic low molecular weight thionin proteins.

Barley leaves and roots produce thionins related to seed hordothionin (HTH). These can be induced by fungi and have antifungal properties *in vitro* (Florack and Stickema, 1994). Barley leaf thionins are associated with the vacuole and cell wall (Reimann-Phillip et al., 1989). Both were shown to be toxic to the fungal pathogen of sugar cane *T. paradoxica* at 2.5 mg/ml. The Arabidopsis *Thi2.1* thionin gene is inducible by pathogenic fungi, and its constitutive over-expression leads to resistance to *F. oxysporum* (Epple et al., 1997). HTH has been expressed in tobacco and enhanced resistance to pathogenic fungi in some studies but not in others (Carmona et al, 1993; Epple, et al., 1997). Despite having these properties, the non-seed thionins apparently do not protect barley from *F. graminearum.* Low concentrations (ca. 5 mg/ml) of hordothionin completely suppress germination and growth of *F. graminearum* spores (unpublished data). However, HTH is found only in the starchy endosperm and is not available to prevent colonization by *Fusarium* on exposed floret tissues.

It is necessary to understand the mechanism of HTH targeting so that it can be rerouted to the intracellular space through the secretion pathway. Leaf thionins are synthesized as 17.4 kDa precursor proteins on membrane-bound polysomes (Ponz et al., 1983). The precursor protein contains an N-terminal signal peptide which facilitates transport into the ER lumen. A C-terminal acidic protein (AP) that constitutes about half of the precursor is processed away, leaving a mature protein of only 5 kDa. In leaves of tobacco transformed with barley HTH, the signal peptide of HTH was found to be essential for expression at the protein level, and the AP was required for high-level expression (Florack et al., 1994). HTH was not secreted into the intercellular spaces but was found mainly in the microsomal (vacuolar) and membrane fractions. The AP may not be involved in targeting in the leaf; the targeting signal might lie within the mature protein. Processing and targeting in the seed may be similar to that in the leaf. HTHs may be associated with protein bodies, since these are derived from the ER, as are vacuoles. Ponz et al. (1983) presented evidence that they are not associated with protein bodies but are instead extrinsically associated with the ER. However, earlier research determined that HTH is externally associated with protein bodies and is part of the protein-lipid matrix in which they are embedded (Carbonero et al., 1980).

MATERIALS AND METHODS

Route of Fusarium invasion

A strain of *F. graminearum* transformed with gfp has been produced by Tom Hohn (Novartis, NC (formerly at USDA, Peoria, IL)). The hyphae and spores are readily viewed by their green fluorescence with a Zeiss dissecting microscope equipped with a short wave blue light source. Several methods have been explored in this lab for visualizing the initial penetration events. Standard paraformaldehyde fixing and paraffin embedding techniques techniques failed because they destroyed gfp fluorescence. Cryostat sectioning preserved fluorescence, but spore attachments were apparently disrupted during

sectioning. A simpler technique has been devised whereby lemmas are infected for 6 h and then peeled into fine tissue strips. This preserves a high frequency of conidiospore attachments. These will be viewed by confocal microscopy (Keck Neural Imaging Center, Univ. of Wisconsin). Penetration by the fluorescent hyphae will be analyzed by processing of serial images at multiple depths of focus (Z-series).

Screening for tissue-specific genes and production of promoters

The differential display technique (Liang and Pardee, 1992) is being used to detect genes expressed only in lemma and palea tissues or in the pericarp, using flag leaves as a control. RT-PCR reactions are conducted with 5'-end RAPD primers and ETVN 3'-end primers. ^{35}S-labeled products are separated by PAGE. Bands which appear in the spike lanes and not in leaf lanes are excised, reamplified, labeled with ^{32}P and used to probe RNA (northern) blots of lemma/palea, pericarp and leaf RNAs. Tissue-specific gene candidates will be cloned and sequenced, and the corresponding nuclear genes will be purified from a Morex genomic BAC library. This service is being generously provided by Andy Kleinhofs (Washington State U.). The deduced promoter regions will be subcloned and ligated in front of the gfp gene (provided by Jen Sheen, Harvard) to test promoter activity and tissue-specificity.

Vector construction and expression analysis

Transient expression studies of promoter activity will be conducted with the pAHC17 vector, which contains the Ubi promoter (and first intron), but has no selectable marker. Gfp has been inserted behind the Ubi promoter, so that it is expressed in barley and in stably transformed oat (Kaeppler et al., 1999). Vectors for stable expression of gfp and antifungal genes were prepared from the Ubi/GUS+Ubi/BAR vector pAHC25 by replacement of the GUS gene. Barley promoters will replace the Ubi promoter. The modified vectors will be attached to gold particles and used to transform barley through the biolistic (gene gun) approach (Wan and Lemaux,1994). Screening of putative transformants will be conducted using PCR on leaf DNA extracted by the CTAB procedure. Particle bombardments of isolated lemmas, pericarps and leaves will be conducted with candidate tissue-specific promoters, linked upstream from gfp. Studies will also be conducted to determine the optimal developmental stages for transient promoter/gfp reporter expression in each tissue, and bombardment conditions will be optimized for each to ensure valid tissue comparisons. If a promoter proves to be tissue-specific, promoter/HTH fusions will be constructed. Resulting transformants will be tested for *Fusarium* resistance.

Subcellular targeting of HTH

In order to target HTH to the intracellular space and/or to the secretion pathway, three constructs will be synthesized. These will be inserted into the pAHC25 expression vector in place of the GUS gene. Construct 1 will attempt to divert targeting from the vacuole by inserting a KDEL ER retention signal (Gomord and Faye, 1996) between the N-terminal signal peptide (SP) and GUS. It is predicted that the mature HTH-GUS fusion will be directed to the intracellular space by bulk flow. Construct 2 will contain the SP and mature protein, followed by GUS. Without the AP, the HTH-GUS fusion may be directed to the intracellular space. Construct 3 (control) will contain the SP, followed only by the GUS sequence. Construct 4 will contain the barley high-pI a-amylase signal sequence, preceding the HTH and GUS. This signal has been shown to direct gfp secretion in barley leaves (Nielson et al., 1999). GUS will be used as a reporter gene, instead of gfp, since much of gfp tends to localize at the nucleus. HTH components for these

constructs were subcloned from our full-length a-hordothionin clone, Thio12 (identical to barley genomic Hth-1,GenBank accession M23080), after removing introns. GUS was subcloned from the GUS gene in pAHC25. An oligonucleotide primer primer encoding KDEL has been synthesized. An oligonucleotide encoding the barley high-pI a-amylase signal sequence will be synthesized from the corresponding sequence in cDNA clone pM/C (Khursheed and Rogers, 1988). Constructs will be inserted into pAHC25 behind the Ubi promoter and intron. The vectors will be delivered into lemmas by particle bombardment. Subsequent compartmentalization will be assessed after 48 h by staining for GUS activity. Paraffin-embedded sectioned tissues will be viewed under oil immersion. If HTH-GUS is successfully targeted to the intracellular space, the targeting signal will be incorporated behind the our tissue-specific promoters.

RESULTS AND DISCUSSION

A previously unknown gene, D5, has been cloned and found to be expressed only in the lemma/palea tissues of the spike (Sathish et al.,1999). The promoter was subcloned from a BAC clone, provided by Andy Kleinhoffs. This was shown to drive the expression of a gfp reporter gene in a D5/gfp construct. In transient assays, expression was found in lemmas and not in leaves. We have successfully transformed Golden Promise with our cloned full-length HTH cDNA clone, thio12, and have so far found five transformants that produce HTH mRNA in seedling leaves. We have also produced transformants carrying the gene for another antifungal permatin protein, BARPERM1 (Nuutila et al., 1998; Skadsen et al., 1999). We are currently studying the expression of the *Barperm1* gene in developing seeds of untransformed barley. These studies showed that the permatin encoded by the Barperm1 gene is localized in the aleurone and ventral furrow of developing seeds (Sathish and Skadsen, ms. submitted).

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EXPLORING THE MOLECULAR MECHANISM OF FUSARIUM HEAD BLIGHT RESISTANCE AND DEVELOPING BREEDER-FRIENDLY DNA MARKERS TO FHB FOR WHEAT IMPROVEMENT

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OBJECTIVES

The goal of this project is to develop a molecular understanding of FHB development and to reveal the molecular mechanism of FHB resistance in wheat, while identifying breeder-friendly, PCR-based DNA markers for indirect selection of the FHB resistance in our breeding program. Our specific objectives in the past year were: 1) optimizing protocols for mRNA differential display and for AFLP; 2) assaying some newly introduced Chinese wheat lines for FHB resistance; 3) screening AFLP polymorphism among the parent lines; and 4) constructing mapping populations. Our objectives in the coming year will be: 1) identifying genes whose transcription is related to FHB development and/or to FHB resistance; 2) cloning, sequencing and analyzing the ESTs of the identified genes of interest; 3) continuing development of mapping population; and 4) screening the mapping population for AFLP markers for FHB resistance.

INTRODUCTION

An efficient way to battle FHB is to control it genetically. However, a major limiting factor is the lack of information about FHB resistant genes and resistance mechanism, especially at the molecular level. Adding to the difficulty associated with the utility of linkage-based DNA markers is the fact that FHB resistance in wheat is basically additive and is quantitatively inherited. In this project, we will first try to identify differentially expressed wheat and fusarium genes related to FHB development and/or FHB resistance in wheat, and then to clone, sequence and characterize the genes of interest or their expressed sequence tags (ESTs). Finally, we will investigate the physiological and biochemical pathway(s) that are involved as well as the physical locations where the genes are expressed. We expect to discover at the molecular level how wheat interacts with the scab pathogen and how Type II resistance inhibits FHB development.

Although the National Wheat and Barley Scab Initiative did not fund this project in FY1999, preliminary work has been initiated. We evaluated some newly introduced Chinese spring wheats for FHB resistance in the greenhouse. We have started investigating differentially expressed wheat and *Fusarium* genes between the FHB-susceptible and -resistant parents during FHB development (Figure 1). Searching for AFLP markers to FHB resistance has been initiated.

METHOD AND MATERIALS

Newly introduced Chinese wheat lines Yiyuan 2, Chuanyu 12, 10A, 88-1643 and 87-429 were evaluated for FHB resistance. Sumai 3 and Wheaton were used as controls. FHB evaluation was done in the greenhouse by spike spraying and floral inoculation. FHB severity, kernel yield/spike, and kernel quality were examined. Sumai 3 and Yiyuan 2 were also screened for AFLP polymorphism against Wheaton. Sumai 3, Wheaton, SS5 (a hybrid-derivative from a wheat intermediate wheatgrass cross) and LMPG-6 were used in the study of the differential display.

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High-quality, non-degraded total RNA was isolated from the samples with the guanidine hydrochloride extraction method (Logemann et al. 1987). The SuperScript RT-PCR kit (Life Technologies, Gaithersburg, MD, USA) was used to synthesize the first-stand cDNA. For the mRNA differential display (Liang and Pardee,1992; Welsh et al. 1992), the RNAimagetm kit from GenHunter (Nashville, TN, USA) was used and the protocol provided by the manufacturer was followed with necessary modifications. PCR products were separated on 6% denatured polyacrylamide sequencing gels and visualized by silver staining (Lohmann et al. 1995).

Genomic DNA was isolated with DNAzol ES (Molecular Research Center, Cincinnati, OH). A silver-staining-based AFLP protocol was developed on the basis of AFLP Analysis System (Life Technologies, Grand Island, NY) and DNA Silver Staining System (Promega, Madison, WI), and used to screen polymorphic loci among parental lines (Xing et al. 1999).

RESULTS AND DISCUSSION

Our preliminary FHB assay showed that Yiyuan 2, Chuanyu 12 and 10A showed some degrees of Type II resistance with an average FHB severity of 2.67, 1.38 and 1.86, respectively (Yen and Xing 1999). Our preliminary data also indicated that Yiyuan 2 may also have usable Type III and Type V resistances. Yiyuan 2 was crossed with susceptible line Wheaton and the hybrid populations have been advanced to F2.

Figure 1 shows an example of our mRNA differential display between FHB-resistant and FHB-susceptible wheat lines. We are now optimizing the protocol and will use it to discover differentially expressed ESTs between the FHB-susceptible and the FHB-resistant parents this winter. We plan to identify, clone, sequence and analyze the ESTs of interest. Sequence information obtained will be used to clone the full-length cDNAs and then the genes of both wheat and *F. graminearum* that are related to FHB development or resistance. Analyses of the cloned cDNAs or genes may greatly increase our knowledge on molecular pathogenesis of FHB and molecular mechanism of FHB resistance as well as on the molecular interaction between the pathogen and wheat, hence enhancing our ability to control FHB epidemics. The cloned ESTs will also be converted to PCR-based markers for indirect selection of FHB resistance.

Our preliminary AFLP assay with 51 PCR primer sets revealed a total of 587 and 686 polymorphic loci for the Yiyuan 2-Wheaton and the Sumai 3 - Wheaton pairs with an average of 11.5 and 13.5 polymorphic loci per primer set, respectively. Figure 2 shows an example of the AFLP polymorphism between Yiyuan 2 and Wheaton. We will start to screen the RILs for FHB-linked AFLP loci in the coming year. Identified AFLP loci will then be converted to PCR based markers for indirect selection for FHB resistance in our breeding program.

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Figure 1. (Right) An example of the mRNA differential display. The mRNA samples from FHB-free young seedlings were compared with differential display between FHB-resistant wheat SS5 (left) and FHB-susceptible wheat LMPG-6 (right). The mRNA differential display was done with the RNAimagetm kit from GenHunter (Nashville, TN) with the primer set of H T11C/H-AP2. The PCR products were separated on 6% denatured polyacrylamide sequencing gel and visualized with silver staining.

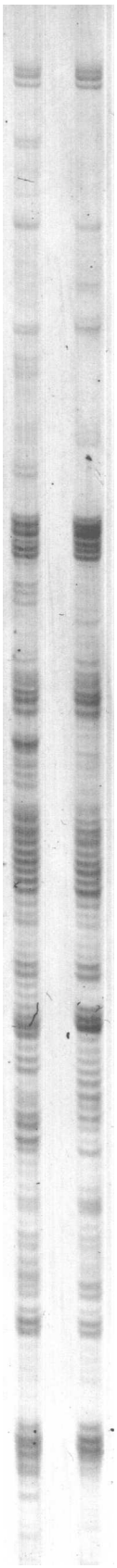

Figure 2. (Next page) AFLP polymorphism between FHB-resistant wheat Yiyuan 2 and FHB-susceptible wheat Wheaton, revealed by silver staining. Y: Yiyuan 2; W: Wheaton. Primer sets (from right to left): E ACA/ M-CTG, E-ACT/M-CAA, E-ACT/M-CAT, E-ACT/M-CAC, E-ACG/M-CAT, E-ACG/M-CTA, E-ACG/M-CAC, E-ACG/M-CTC, E-ACC/M-CAA, E-ACC/M-CAT, E-ACC/M-CTA, E-ACC/M-CTT, E-AGC/M-CAC, E-AGC/M-CAG, E-AGG/M-CAA, E-AGG/M-CAT, E-AGG/M-CAC, E-AGG/M-CAG, E-AGG/M-CTC, E-AGG/M-CTG; The numbers in the middle are the DNA standards (bp).

Figure 2.


WY
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WY
700
300
200
100

BTH-INDUCED GENE EXPRESSION IN WHEAT SPIKES DOES NOT PROVIDE RESISTANCE TO SCAB

G-Y. Yu and G. J. Muehlbauer*

ABSTRACT

To identify control measures for wheat scab, we investigated the potential of using benzo(1,2,3)thiadiazole-7-carbothioic acid S-methyl ester (BTH). BTH induces a set of genes referred to as wheat chemical induction (WCI) genes and provides resistance to pathogens in several crops. Systemic acquired resistance (SAR) has been generally associated with BTH application. We sprayed a 1 mM BTH formulation on wheat plants several days prior to anthesis and examined gene expression and resistance to scab. We examined wheat spikes and flag leaves of BTH-treated wheat for expression of pathogenesis-related (PR) genes that are commonly associated with SAR, and the five WCI genes that are specifically induced by BTH in wheat leaves. We found that all five WCI genes were induced by BTH in both flag leaves and in spikes, however, none of the six PR genes tested were induced. In our disease evaluations of BTH-treated plants, we found that this treatment did not provide significant resistance to point or spray inoculations of *Fusarium graminearum*. These data indicate that BTH application and the induction of WCI gene expression does not provide resistance to scab.

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USDA-ARS, OHIO STATE UNIVERSITY COOPERATIVE RESEARCH ON BIOLOGICALLY CONTROLLING FUSARIUM HEAD BLIGHT: 3. FIELD TESTING OF ANTAGONISTS

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OBJECTIVE

Determine if microbial strains effective in reducing Fusarium head blight severity in greenhouse bioassays are effective in reducing FHB in field trials on soft red winter wheat cultivars which vary in susceptibility to FHB.

INTRODUCTION

Over the last two years, research conducted at the NCAUR in Peoria, IL, in conjunction with The Ohio State University (Khan et al, 1998a; Khan et al., 1998b; Khan et al., 1999a; Khan et al., 1999b; Schisler et al., 1999a; Schisler et al., 1999b), has culminated in the isolation, screening and identification of several putative biological control agents for suppression of FHB. This update provides a summary of the results obtained in our 1999 field evaluation trials conducted on soft red winter wheat cultivars in Ohio and Illinois. Results of the 1998 field tests on the soft red winter wheat cultivar 'Pioneer 2545' were presented previously (Khan et al., 1998b; Schisler et al., 1999a).

MATERIALS AND METHODS

Isolation, screening and selection of microbial antagonists

As described previously (Khan et al., 1998a; Khan et al., 1998b; Schisler et al., 1999a Schisler et al., 1999b), approximately 740 strains of microorganisms were isolated from wheat anthers collected from fields in OH and IL and screened for their ability to either use choline as a sole carbon source or for *in vitro* inhibition of *Fusarium graminearum*. Strains capable of using choline or inhibiting *F. graminearum* were subsequently screened for their ability to suppress FHB in a series of greenhouse assays (Khan et al., 1998b). Strains capable of significantly reducing the severity or incidence of FHB were further evaluated for their ability to suppress various isolates of *F. graminearum* (Khan et al., 1999a; Khan et al., 1999b). Seven strains, four yeasts and three *Bacillus* sp., were selected for field testing on either or both durum and soft red winter wheat. The ability of several of these isolates at suppressing FHB on durum wheat is presented in another report at this symposium (Schisler et al., 1999b).

1999 Peoria, IL, and Wooster, OH, field trials of FHB antagonists on soft red winter wheat cultivars 'Pioneer 2545' and 'Freedom'

Three *Bacillus* sp. and three yeast isolates (Table 1) were screened on soft red winter wheat cultivars "Pioneer 2545" and "Freedom." These cultivars were selected because of their widespread use throughout the Midwest and in an attempt to acquire preliminary data regarding the integration of biocontrol and genetic resistance for managing FHB. Antagonists were produced in liquid culture in Fernbach flasks as described previously (Schisler et al., 1999b) and applied at the beginning of flowering in aqueous suspensions. Bacterial suspensions contained either 10% or 50% liquid culture or ~$2x10^8$ or $1x10^9$ cfu/ml, respectively. Yeast suspensions contained either 10% or 50% liquid culture or

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~$1x10^7$ or $5x10^7$ cfu/ml, respectively. Pathogen inoculum consisted of ascospores released from *F. graminearum* Z3639 colonized corn kernels scattered throughout the plots (~25-40 kernels/ m^2) 2 wk prior to wheat flowering. Non antagonist/buffer treated plants served as controls. Mist irrigation was applied regularly to promote FHB disease development. Plots were scored for disease severity and incidence and harvested to determine 100 kernel weights. Randomized complete block designs were used in both trials (*n*=4 in Peoria; *n*=6 in Wooster).

RESULTS AND DISCUSSION

In the field trial at Peoria, IL, five of the six antagonists tested reduced FHB disease on wheat cultivar 'Pioneer 2545' at one or both of the concentrations assayed (Table 2) despite poor environmental conditions for disease development across the Midwest. On cultivar 'Freedom', all six antagonists reduced FHB disease at one or both of the concentrations tested. At the Wooster, OH, field site, five of the six antagonists reduced FHB disease at one or both of the concentrations assayed (Table 3) on cultivar 'Pioneer 2545'. Yeast antagonist OH 71.4 reduced disease severity by nearly 56%. Antagonists OH 131.1, OH 181.1 and OH 182.9 reduced disease severity and disease incidence at both of the antagonist cell concentrations tested (Table 3). No appreciable amount of disease was observed on the 'Freedom' plots in Wooster.

The potential for these microbial antagonists to contribute to the management of FHB was demonstrated in these replicated field trials. Future research focusing on the: 1) development of liquid culture fermentation methodologies aimed at enhancing biocontrol agent efficacy; 2) determination of the genes, regulatory mechanisms or other cellular processes responsible for biocontrol agent efficacy; and, 3) continued evaluation of biocontrol agent efficacy under field conditions at various sites, on various hosts, and in tandem with other management practices, may greatly enhance our ability to manage FHB.

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Table 1. Microbial antagonists effective in reducing the severity of Fusarium head blight in greenhouse bioassays and selected for evaluation in the 1999 field tests.

Antagonist Strain	Accession Number	Type of Organism
AS 43.3	NRRL B-30210	*Bacillus* sp.
AS 43.4	NRRL B-30211	*Bacillus* sp.
OH 71.4	NRRL Y-30213	Yeast
OH 131.1	NRRL B-30212	*Bacillus* sp.
OH 181.1	NRRL Y-30215	Yeast
OH 182.9	NRRL Y-30216	Yeast

Table 3. Influence of two antagonist cell concentrations on Fusarium head blight development on soft red winter wheat cultivar Pioneer 2545" in a 1999 field trial at Wooster, Ohio[a].

Cultivar Pioneer 2545"				
	10% Antagonist		50% Antagonist	
Treatment	Disease[b] Severity (%)	Disease Incidence (%)	Disease Severity (%)	Disease Incidence (%)
F. graminearum	11.0	34.4	11.0	34.4
AS 43.3	9.8	31.4	10.3	33.9
AS 43.4	8.5	33.6	10.7	38.1
OH 71.4	4.8*	23.1*	9.6	30.6
OH 131.1	6.5*	25.3*	5.1*	22.0*
OH 181.1	6.7*	27.8*	6.3*	25.3*
OH 182.9	4.6*	23.1*	7.1*	27.0*

[a] Wheat heads were sprayed to run-off with an antagonist cell suspension. Naturally occurring inoculum of *F. graminearum* was supplemented with ascospores released from *F. graminearum* Z3639 colonized corn kernels that had been spread across the test plot (≈20 colonized kernels/m^2).

[b] Within a column means followed by an asterisk are significantly different from the *F. graminearum* control ($P \leq 0.05$).

Table 2. Influence of two antagonist cell concentrations on Fusarium head blight development on soft red winter wheat cultivars Pioneer 2545 and Freedom in a 1999 field trial at Peoria, Illinois[a].

	Cultivar Pioneer 2545"						Cultivar Freedom					
	10% Antagonist			50% Antagonist			10% Antagonist			50% Antagonist		
Treatment	Disease[b] Severity (%)	Disease Incidence (%)	100 kernel wt. (g)	Disease Severity (%)	Disease Incidence (%)	100 kernel wt. (g)	Disease Severity (%)	Disease Incidence (%)	100 kernel wt. (g)	Disease Severity (%)	Disease Incidence (%)	100 kernel wt. (g)
F. graminearum	2.0	11.2	3.3	2.0	11.2	3.3	1.0	8.3	3.1	1.0	8.3	3.1
AS 43.3	1.0	6.2*	3.3	2.6	16.7	3.2*	0.4	3.8*	3.2*	0.2*	3.3*	3.1
AS 43.4	0.4*	5.4*	3.3	2..2	12.5	3.3	0.4	4.6	3.1	0.3*	4.2*	3.2
OH 71.4	1.1	8.8	3.4	0.6*	6.2*	3.2*	0.7	5.4	3.3*	0.2*	2.9*	3.2
OH 131.1	1.9	10.4	3.2*	0.7*	4.6*	3.3	0.7	6.7	3.2	1.6	6.2	3.3*
OH 181.1	1.6	9.2	3.4	2.4	9.2	3.1*	0.2*	2.9*	3.2	0.5	3.8*	3.3*
OH 182.9	0.9*	5.4*	3.4	1.6	6.7*	3.4	0.3*	3.3*	3.2*	0.6	3.8*	3.0

[a] Wheat heads were sprayed to run-off with an antagonistic cell suspension. Naturally occurring inoculum of *F. graminearum* was supplemented with ascospores released from *F. graminearum* Z3639 colonized corn kernels that had been spread across the test plot (~20 colonized kernels/m^2).

[b] Within a column, means followed by an asterisk are significantly different from the *F. graminearum* control ($P \leq 0.05$).

OH 131.1	1.9	10.4	3.2*	0.7*	4.6*	3.3	0.7	6.7	3.2	1.6	6.2	3.3*
OH 181.1	1.6	9.2	3.4	2.4	9.2	3.1*	0.2*	2.9*	3.2	0.5	3.8*	3.3*
OH 182.9	0.9*	5.4*	3.4	1.6	6.7*	3.4	0.3*	3.3*	3.2*	0.6	3.8*	3.0

A NOVEL METHOD TO IDENTIFY *FUSARIUM* SPECIES THAT ATTACH TO ROOTS OF WHEAT AND BARLEY SEEDLINGS

J. Gilbert*, U. Kromer and B. McCallum

OBJECTIVES

To examine roots of seedlings grown from FHB-contaminated seed for infection by *Fusarium* spp.

INTRODUCTION

Seed dressings effectively control *Fusarium* species permitting infested seeds to emerge and thrive. However, it is not known if *Fusarium* added to the soil on infested seed is able to infect root systems of the emerging plants, or if the fungicide contains the fungus at the seed surface. Roots were examined to determine *Fusarium* species and levels of infection among 3 varieties each of spring wheat and barley treated with 2 seed dressings.

MATERIALS AND METHODS

Germination and level of infection were determined on 2 replicates of 100 seeds of cv.s Glenlea (extra strong), AC Morse (durum) and AC Vista (Canada Prairie Spring) wheats, fungicide-treated, or left as an untreated control. The three barley varieties were Stander, Robust and Foster. Experimental design was a 4 replicate split plot with variety as the main plot and treatments as the subplots. Plots were 0.9 X 3 m consisting of 4 rows each 30 cm apart. At GS 15-20 roots were washed thoroughly in water and frozen for later examination. Each root was surface sterilized in 0.1% NaOCl for 1 min and allowed to dry under a laminar flow hood before being placed on PDA agar plates and covered with a thin layer of Komada's medium (specific for *Fusarium* species). Plates were examined for colonies of *Fusarium* sp. and transferred to fresh PDA plates for identification.

RESULTS

Germination of pre-treated seed ranged from 73% to 91%, and for infection with *Fusarium* spp. 16% to 47% (Table 1). Foster barley germinated at 91%, despite high (31%) *Fusarium* infection. However, there were no significant treatment or variety differences in *Fusarium* spp. and number of isolations from roots. Nine *Fusarium* species were identified from the roots (Table 2). The predominant species isolated were *F. equiseti* (43.8% of isolations), *F. graminearum* (17.9%), and *F. sambucinum* (13.3%).

Table 1. Mean percent germination and level of infection in *Fusarium*-infected spring wheat and barley cultivars.

	Germination (%)	Infection with *Fusarium* sp. (%)
Glenlea	76	31
AC Morse	76	32
AC Vista	73	47
Stander	87	20
Foster	91	31
Robust	87	16

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Table 2. Percent isolations of *Fusarium* species from roots of spring wheat seedlings.

Species	Isolations (%)
F. equiseti	43.8
F. graminearum	17.9
F. sambucinum	13.3
F. sporotrichioides	8.0
F. solani	5.7
F. poae	3.4
F. culmorum	3.0
F. oxysporum	3.0
F. avenaceum	1.9

CONCLUSIONS

Seed treatment did not reduce *Fusarium* levels on roots compared to untreated controls. A successful method for isolating *Fusarium* species from whole roots was developed.

ACKNOWLEDGEMENTS

The technical assistance of R. Kaethler, K. Morgan and M. Idris is gratefully acknowledged.

SPRAYER MODIFICATIONS FOR ENHANCED CONTROL OF FUSARIUM HEAD BLIGHT WITH FUNGICIDES

Scott Halley[1], Jeremy Pederson[1], Marcia McMullen[1*], and John Lukach[2]

INTRODUCTION AND OBJECTIVES

Fusarium head blight (FHB) has been difficult to control with fungicides; due in part, to the difficult target a grain spike presents. Deposition and retention of fungicide on the grain spike is hindered by the vertical orientation of the spike, along with the interference of awns, and the waxy, glabrous surface of the spike. Conventional application methods intended primarily for foliar targets have shown to be ineffective in delivering fungicide to the grain spike. This study was initiated to identify spray application parameters of currently available equipment that are most effective in improving fungicide deposition on the spike. The primary objective of the study was to correlate the amount of fungicide delivered to the spike with FHB control. The control of fungal leaf spot on the flag leaf was also measured.

METHODS

Two types of spraying systems (CO_2 pressurized and air assist), several nozzle orientations, and two water volumes were evaluated for delivery of fungicide to the grain spike. Applications were made to Grandin hard red spring wheat (*Triticum aestivum*) at the early flowering period. A three percent v/v solution of orange Day Glo dye was included in the spray for detection of spray captured by the spike. Selected grain spikes were detached and examined under ultra violet light for fluorescence, and a digital camera system measured the percent of grain spike covered by the dye. FHB incidence, FHB head severity, FHB field severity, and flag leaf disease were evaluated at Zadok's 85. A grain-spawn inoculated with *Fusarium graminearum* was evenly spread over the plot area two weeks prior to flowering to enhance FHB infection. Plots were harvested with a plot combine, and yield and test weights were determined. Treatments were randomized in a complete block design, and data were analyzed using GLM, and Pearson's correlation coefficient.

RESULTS

All applications of Folicur significantly reduced flag leaf necrosis and field severity over the check (Table1). Spike coverage increased by changing the nozzle orientation of Spray Air from vertical to 45º forward, and by changing the vertical orientation of the Standard sprayer nozzles to two XR11001 nozzles per opening, one angled forward, and one backward 30 degrees from horizontal (F+B). The XR11001 nozzles orientated F+B significantly improved control of field severity over straight down nozzle orientation treatments at 9 gallons per acre water volume. The XR11001 orientated F+B provided the lowest FHB incidence, FHB head severity, FHB field severity, yield, and test weight of all treatments. FHB parameters negatively correlated to amount of dye deposited on head (Table 2).

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Table 1. Comparison of Sprayers and Spray Nozzle Configurations for FHB Control

					FHB			Flag Leaf Necrosis				
Sprayer	Nozzle	Nozzle Orient.	gpa	Press-ure	Incid-ence	Head Severity	Field Severity	Leaf Rust	Leaf Spot[a]	Yield	Twt	Head Cover-age
				psi	%	%	%	%	%	bu/A	lbs/bu	%
Check	NA	NA	0	0	53.4	10.8	5.6	4.6	29.7	56.9	58.6	0.2
Spray Air	0.0424 Orifice	St Down	9	10	37.5	7.5	3.1	0	2.2	63.3	57.6	4.2
Spray Air	0.0424 Orifice	45" F	9	10	27.5	6.7	1.9	0	2.1	65.1	58.7	27.0
Standard	XR110 01	St Down	9	40	40.0	8.4	3.4	0.6	4.4	64.9	58.3	1.5
Standard	XR110 01	F+B	9	40	25.0	4.1	1.1	0	2.0	65.9	58.7	29.5
Spray Air	0.0424 Orifice	45" F	3	10	25.0	6.9	1.9	<0.1	2.8	64.5	57.7	10.6
LSD p>0.05					17.9	4.1	1.9	1.4	10.7	NS	NS	11.4
C.V. %					35	39	48	108	99	12	2	66

a. Leaf spots are a mix of tan spot and *Septoria nodorum* and *Septoria tritici*

Table 2. Pearson Correlation Coefficients between Coverage, Disease and Yield Parameters

	FHB Incidence	FBH Head Sev.	FHB Field Severity	Leaf Spot	Leaf Rust	Coverage	Yield	Test Weight
Incidence	1.0	0.49	0.87	0.78	0.61	-0.49	-0.69	-0.25
Severity	0.49	1.0	0.83	0.44	0.46	-0.28	-0.46	-0.08
Field Severity	0.87	0.83	1.0	0.80	0.68	-0.42	-0.63	-0.14
Leaf Spot	0.78	0.44	0.80	1.0	0.80	-0.35	-0.54	-0.04
Leaf Rust	0.61	0.46	0.68	0.80	1.0	-0.33	-0.42	<0.01
Coverage	-0.49	-0.28	-0.42	-0.35	-0.33	1.0	0.26	0.18
Yield	-0.69	-0.46	-0.63	-0.54	-0.42	0.26	1.0	0.54
Test Weight	-0.25	-0.08	-0.14	-0.04	<0.01	0.18	0.54	1.0

FUNGICIDE EFFICACY TRIALS AT MICHIGAN STATE UNIVERSITY

Patrick Hart[1*], Jared Froedtert[1], Richard Ward[2], Lee Siler[2], Gary Van Ee[3] and Richard Ledebuhr[3]

Fungicides were evaluated for their potential to reduce the incidence and severity of Fusarium Head Blight (FHB) in winter wheat, and concomitantly for a reduction in levels of deoxynivalenol (DON). Three projects associated with the use of fungicides in disease management were conducted: 1) evaluate efficacy of different fungicides as part of a regional Uniform Fungicide Trial; 2) the effect of different application methods on FHB; 3) the effect of fungicide application timing on FHB. Winter wheat varieties used in these experiments were planted in October 5th, 1998. Fertilizer and herbicides were applied as per Michigan State University recommendations. Corn inoculum infested with *Gibberella zeae* was spread over the fields on May 4th and again on May 14th. Low volume overhead irrigation was turned on May 13th. Irrigation was on for 15 minutes and off for ninety minutes, 24 hours/day from May 13th to June 14th. The irrigation was turned off for 24 hrs the days fungicides were applied. In projects 1 and 2 the variety Harus was used, and in project 3 the varieties Frankenmuth, Pioneer 2510, and Freedom were used. Perithecia were first observed on the corn inoculum on May 17th, and mature ascospores were first observed on May 28th. Fungicides in project 1 and 2 were applied to Harus at Feekes growth stage 10.5 on June 3rd. For the timing experiment, Project 3, Folicur (4 oz/acre + 0.06% induce) was applied on Pioneer 2510 and Freedom on 28 May (GS 10.1) and 1 June (GS 10.5). Frankenmuth was treated only at GS 10.5 on June 1st. Fungicides were applied with a CO_2 backpack sprayer and flat fan nozzles in projects 1 and 3. For project 2, Folicur was applied at GS 10.5 on the variety Harus using either a Proptech sprayer, or boom sprayer with flat fan nozzles directed at sixty degree angle above the horizontal. Folicur was applied at two rates, 2.5 or 4.0 oz per acre + 0.06% induce. All treatments in each project were replicated three times. Each plot was rated three times for disease incidence and severity using the scoring system developed by Stack and McMullen. Mature grain was harvested, milled and analyzed for DON by ELISA (Hart et al., 1998).

RESULTS

Project 1: evaluate efficacy of different fungicides as part of a regional Uniform Fungicide Trial.

Differences in yield, FHB incidence and severity, and DON were not significantly different between treatments including the untreated controls (Table 1). The Folicur treatment had the lowest amount of disease and DON, and highest yield. The previous two years levels of DON in Quadris treatments were often significantly higher than the untreated controls but not in 1999. Maintaining irrigation for two weeks after flowering may have provided an environment highly favorable for FHB to develop, more so than under natural conditions, and may have minimized differences that would normally be larger.

Project 2: the effect of different application methods on FHB.

All treatments had significantly lower levels of

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DON (P=0.05) than the untreated controls, but were not signicantly different from each other.(Table 2). Adjusting the angle of the flat fan nozzles appears to be an effective method of application (as is recommended by North Dakota extension). The apparent efficacy of lower rates is interesting, but should be validated over years and environments prior to being made a recommendation. Project two used the same variety and conditions as project one, but fungicides were applied more like a commercial operation because the plot size was 21 x 84 feet compared to 10 x 15 feet for project one. The significant differences in DON levels could have resulted from a more efficient application of fungicides compared to CO_2 back pack sprayer applications used in project one .

Project 3: the effect of fungicide application timing on FHB.

Concentrations of DON were significantly lower across all three varieties sprayed with Folicur at GS 10.5 (Table 3). DON concentrations were significantly lower in Freedom sprayed at GS 10.1, but not in Pioneer 2510. Frankenmuth was not sprayed at GS 10.1. These results are in line with the work of others also showing overall better efficacy in spring wheat and barley when fungicides were applied at GS.10.5 compared to GS. 10.1.

SUMMARY

Fungicides may be an effective management tool for FHB in winter wheat. Timing of application, and possibly the application method could influence the efficacy of fungicide treatments. Although differences between fungicides were not significant, the trend for Folicur to reduce FHB and DON below all other fungicide treatments is in line with previous years (Hart et al., 1998; McMullen, 1998).

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Table 1: Results from 1999 FHB Uniform Fungicide Trial

		Yield	DON	Disease Rating 15-June	
Treatment	**rate**	**(bu/a)**	**(ppm)**	**Incidence**	**Severity**
BASF 500 00F	15.3 fl oz/a	52.7	9.3	48%	3.5
Benlate / Manzate	0.5 lb/a , 1 lb/a	48.7	9.6	63%	4.8
Folicur	4 fl oz/a	55.2	6.0	58%	3.0
Penncozeb	1 lb/a	51.2	10.9	62%	4.7
Quadris (9.6 oz/a)	9.6 fl oz/a	50.0	7.7	42%	3.7
Quadris (12.3 oz/a)	12.3 fl oz/a	50.0	7.8	42%	3.8
Stratego (10oz/a)	10 fl oz/a	54.8	8.2	42%	3.7
Stratego (14oz/a)	14 fl oz/a	53.2	9.7	47%	3.5
Untreated	-	50.6	8.7	73%	4.8
		NS	**NS**	**NS**	**NS**

Table 2: Results from 1999 Sprayer Comparison Fungicide Trial

		DON	Disease Rating 29-June	
sprayer	**rate (oz/a)**	**(ppm)**	**Incidence**	**Severity**
Proptech	4.0	2.4*****	43.5	4.8
Proptech	2.5	6.5*****	40.4	5.1
Boom	4.0	5.9*****	35.2	4.6
Boom	2.5	3.4*****	42.9	5.6
Untreated	-	13.4	45.4	5.8

***** significantly different from the untreated control (p=0.05)

Table 3: Results from 1999 FHB Folicur Application Timing Fungicide Trial

	application	Yield	DON	Disease Rating 15-June	
variety	**stage**	**(bu/a)**	**(ppm)**	**Incidence**	**Severity**
Freedom	10.1	41.8	9.8*****	50%	3.3
Freedom	10.5	45.4	9.2*****	40%	2.3
Freedom	untreated	38.0	13.3	82%	3.8
Pioneer 2510	10.1	37.3	16.9	90%	4.0
Pioneer 2510	10.5	40.7	11.9*****	67%	4.0
Pioneer 2510	untreated	31.2	18.6	88%	4.5
Frankenmuth	10.5	37.3	7.8*****	22%	3.7
Frankenmuth	untreated	23.1	11.8	26%	5.0

***** significantly different from the untreated control (p=0.05)

USDA-ARS, OHIO STATE UNIVERSITY COOPERATIVE RESEARCH ON BIOLOGICALLY CONTROLLING FUSARIUM HEAD BLIGHT: 2. INFLUENCE OF PATHOGEN STRAIN, INOCULUM SPRAY SEQUENCE AND INOCULUM SPRAY TIME

N.I. Khan[1*], D.A. Schisler[2], and M.J. Boehm[1].

OBJECTIVES

To test the ability of microbial antagonists to reduce the severity of scab of wheat when coinoculated with any of three different isolates of *Gibberella zeae* and when sprayed at different times before or after the arrival of pathogen inoculum on wheat heads.

INTRODUCTION

Fusarium head blight (FHB), also known as wheat head scab, causes extensive damage to wheat throughout the world. In North America, the primary causal agent of FHB is *Gibberella zeae* (anamorph= *Fusarium graminearum*) which can produce potent toxins during colonization of grain including the estrogenic toxin zearalenone (F-2) (Vesonder and Hesseltine, 1980) and the trichothecene deoxynivalenol (vomitoxin) (Proctor et al., 1995) which can inhibit amino acid incorporation and protein production in plant tissues (Casale and Hart, 1988). Because wheat cultivars with a high degree of resistance are not currently available and the use of chemical control measures is complicated by concerns regarding potential residues and cost, biological control has been suggested as a promising method for reducing FHB (Khan et al., 1998).

In a separate report (Schisler et al., 1999), a screening method for selecting microbial antagonists that suppress FHB has been described as have biocontrol studies on durum wheat. In this study we explore the efficacy of our antagonists in reducing FHB incited by several isolates of *G. zeae* obtained from different geographical locations. We also studied how the sequence and timing of antagonist and pathogen inoculum arrival on the infection court influences the level of biological control observed. The field testing of these antagonists is reported elsewhere in this proceedings (Boehm et al., 1999).

MATERIALS AND METHODS

Greenhouse plant bioassay of biocontrol: point inoculation

Hard red spring wheat cultivar Norm was used in these experiments. Ten µl of a suspension containing conidia (10^5 conidia/ml), biocontrol agent (yeast at approximately $2x10^7$ cfu/ml; bacteria at approximately $5x10^8$ cfu/ml) and 0.04% Tween 80 in weak PO_4 buffer was used to inoculate the middle floret of a centrally located spikelet on a head. Heads inoculated only with conidia of *G. zeae* in the Tween 80-buffer suspension were the control. Three isolates of *G. zeae* (Z3639, DOAM, and Fg-9-96) were tested in a completely randomized experimental design with 16 heads per treatment. After inoculation, plants were kept in a plastic humidity chamber for 72 h and then transferred to greenhouse benches. Disease severity was visually estimated (Stack and McMullen, 1995) 10 days (data not shown) and 16 days after inoculation. One-hundred kernel weight and the percentage of healthy kernels (data not shown) were also assessed. The experiment was

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repeated at least once. The data on disease severity was normalized using arcsine transformation before statistical analysis using PC SAS (version 6.12).

Greenhouse plant bioassay of biocontrol: spray inoculation

A spray inoculation method was used to mimic the arrival of inoculum at the infection court in the field. Inoculum of *G. zeae* isolate Z3639 was sprayed on wheat heads immediately before or after and 4 h before or after spraying heads with microbial antagonists (formulation of pathogen and antagonist inoculum as described above). Heads sprayed only with conidia of *G. zeae* were the control. All other procedures were as described above.

RESULTS AND DISCUSSION

In the assays against *G. zeae* isolate Z3639, six antagonists, including four choline-utilizing strains, reduced disease as indicated by increased 100 kernel weights of microbially treated wheat heads ($P<0.05$, Table 1). Three antagonists decreased disease severity. Bacterial strain AS 43.3, AS 43.4, and yeast strain OH 182.9 reduced disease severity by >77%, 93%, and 56%, respectively. Treatments with antagonist strains AS 43.3, AS 43.4, and OH 182.9 increased the 100 kernel weight by >140%, 144 %, and 100 %, respectively ($P<0.05$, Table 1). In bioassays against isolate DOAM of *G. zeae***,** only bacterial strains AS 43.3 and AS 43.4 reduced disease as measured by reduction in disease severity, and disease incidence. Five antagonists increased 100 kernel weight (Table 1)**.** Conversely in bioassays using isolate Fg-9-96 of *G. zeae*, all antagonists except OH 72.4 increased 100 kernel weights and five of seven antagonists reduced disease severity ($P<0.05$, Table 1). Overall, bacterial strains AS 43.3 and AS 43.4 consistently reduced FHB disease regardless of the isolate of *G. zeae* used in the bioassays (Table 1). The other antagonist strains tended to be effective against one or two but not all three isolates of *G. zeae*. Further tests using a broader array of *G. zeae* isolates will provide an indication of how widely efficacious individual antagonist strains have the potential to be.

In spray inoculation experiments, all antagonists significantly reduced disease severity, regardless of the sequence, timing, and concentration of inoculum application ($P<0.05$, Table 2), though some antagonists did not increase 100 kernel weight when applied 4 h after inoculum of *G. zeae* (Table 3). These results suggest that biological control of FHB in the field may become less effective if cells of antagonists are significantly delayed in arriving at the infection court compared to inoculum of *G. zeae*.

Further studies on formulation technologies including identifying compounds that support growth of antagonist strains without enhancing the growth of *G. zeae* may offer a method of improving both the breadth and level of efficacy of FHB antagonists. Studies on optimizing liquid media to maximize biomass production and efficacy while utilizing cost effective nutrient sources, and field testing of antagonists showing promise in the greenhouse assays at different geographical locations are planned.

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Table 1. Influence of microbial antagonists on FHB incited by three isolates of *G. zeae* on the hard red spring wheat cultivar Norm [a]

	G. zeae Isolate								
	Z 3639			DOAM			Fg-9-96		
Treatment	Disease Severity (%)	Disease Incidence (%)	100 Kernel Weight (g)	Disease Severity (%)	Disease Incidence (%)	100 Kernel Weight (g)	Disease Severity (%)	Disease Incidence (%)	100 Kernel Weight (g)
G. zeae	90	95	1.5	76	91	1.8	54	66	3.2
AS 43.3	20 *[b]	63 *	3.6 *	17 *	41 *	3.7 *	3 *	3 *	4.0 *
AS 43.4	6 *	46 *	3.9 *	14 *	31 *	3.6 *	11 *	12 *	3.8 *
OH 71.4	78	82	1.9 *	75	87	2.0 *	3 *	12 *	4.0 *
OH 72.4	82	89	1.8	73	84	2.0 *	51	56	2.8 *
OH 131.1	79	89	2.1 *	75	87	1.9	26 *	34 *	3.8 *
OH 181.1	82	89	1.9 *	88	91	1.7 *	44 *	50	4.0 *
OH 182.9	39 *	72 *	3.0 *	69	84	2.0 *	51	65	3.5 *

[a]The middle floret of a central spikelet of a wheat head was co-inoculated with 10 l off a 25% suspension of antagonist liquid culture (10^7-10^8 cfu/ml) and *G. zeae* conidia (1 x 10^5 conidia/ml).
[b]Within a column, means followed by an * are significantly different from *G. zeae* control (P=0.05).

Table 2. Percent FHB disease severity when varying the time and sequence of pathogen and antagonist inoculum application to wheat heads

	% Disease Severity			
	Pathogen Inoculum Applied:			
Treatment	4 h Before Antagonist	Immediately Before Antagonist	Immediately After Antagonist	4 h After Antagonist
G. zeae Z3639	59	86	81	85
AS 43.3 (10%)[a]	13 *[b]	2 *	3 *	21 *
AS 43.3 (50%)	5 *	1 *	0 *	15 *
AS 43.4 (10%)	42 *	3 *	3 *	30 *
AS 43.4 (50%)	19 *	33 *	0 *	18 *
OH 71.4 (10%)	26 *	24 *	18 *	49 *
OH 71.4 (50%)	28 *	51 *	37 *	63 *
OH 182.9 (10%)	43 *	64 *	60 *	45 *
OH 182.9 (50%)	43 *	49 *	60 *	58 *

[a]Antagonists were applied at concentrations of 10% or 50% of a fully colonized complete liquid medium.
[b]Within a column, means followed by an * are significantly different from *G. zeae* control. Means were separated by Fisher protected LSD at P=0.05.

Table 3. One hundred kernel weights when varying the time and sequence of pathogen and antagonist inoculum application to wheat heads

	100 Kernel Weight (g)			
	Pathogen Inoculum Applied:			
Treatment	4 h Before Antagonist	Immediately Before Antagonist	Immediately After Antagonist	4 h After Antagonist
G. zeae Z3639	1.8	1.6	1.7	1.4
AS 43.3 (10%)[a]	2.4 *[b]	3.4 *	3.4 *	2.6 *
AS 43.3 (50%)	2.6 *	3.6 *	3.1 *	2.7 *
AS 43.4 (10%)	2.5 *	3.3 *	3.2 *	2.2 *
AS 43.4 (50%)	1.7	3.0 *	3.1 *	2.6 *
OH 71.4 (10%)	1.9	2.3 *	2.8 *	2.4 *
OH 71.4 (50%)	2.4 *	2.6 *	2.5 *	2.0 *
OH 182.9 (10%)	1.9	2.3 *	2.3 *	2.7 *
OH 182.9 (50%)	1.8	2.2 *	2.3 *	2.0 *

[a]Antagonists were applied at concentrations of 10% or 50% of a fully colonized complete liquid medium.
[b]Within a column, means followed by an * are significantly different from *G. zeae* control. Means were separated by Fisher protected LSD at P=0.05.

BIOLOGICAL CONTROL OF FUSARIUM HEAD BLIGHT (FHB) OF WHEAT BY *BACILLUS* STRAINS

Yongmei Luo and Bruce Bleakley*

ABSTRACT

Fusarium Head Blight (FHB) or scab, caused by some pathovars of *Fusarium graminearum* (teleomorph of sexual stage *Gibberella zeae*), is a fungal disease of wheat which can cause serious crop-yield reduction. Biocontrol agents that antagonize the pathogen could be useful in management strategies for controlling FHB. Four *Bacillus* stains designated 1BA, 1BC, 1BE and 1D3 were isolated from South Dakota wheat foliage as potential biocontrol agents of scab. Both whole cells and cell-free, concentrated ethyl-acetate extracts of the broth supernatants of each bacterial stain inhibited growth of the pathogen in plate assays. The compounds found in cell-free ethyl acetate extracts of bacterial culture supernatants were separated by thin layer chromatography (TLC) in a solvent system of 1-butanol:acetic acid:H_2O (3:1:1) and detected by UV light. Each strain had 11-12 individual spots that were separated on TLC plates. Extracts of 1BE and 1BC were used in disease nursery research in the summer of 1998. The results of MIXED t test statistical analysis showed significant reduction of scab symptoms on heads of spring wheat that received cell-free extracts of the *Bacillus* strains. Application of strain 1BE extract resulted in 25.88% disease reduction and application of strain 1BC extract resulted in 20.95% of disease reduction. The results showed that cell-free extracts of these *Bacillus* strains helped protect wheat against scab in a disease nursery, and that some biocontrol strains can be differentiated from one another based on TLC analysis of cell-free culture supernatant extracts of *Bacillus* strains. One or more of these *Bacillus* strains may prove useful in management schemes for controlling Fusarium Head Blight.

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CHEMICAL & BIOLOGICAL CONTROL OF FUSARIUM HEAD BLIGHT 1999 PROJECTS AND PROGRESS

Marcia McMullen[1*] and Gary Bergstrom[2]

OBJECTIVES

The control of Fusarium head blight (FHB = scab) has been difficult, because of the complex nature of the host/pathogen/environment interaction. While host resistance is the most promising and effective long-term management solution, wheat and barley farmers have needed some immediate solutions for keeping this disease from causing severe economic loss. In addition, optional tools for management even in the presence of host resistance may be necessary under severe epidemics or for crops or regions that don't have high levels of resistance available. The goals of the Chemical and Biological Control area of the National Wheat and Barley Scab Initiative have been: to evaluate fungicides and biological antagonists that will be effective, safe, easy to apply, and economic; to devise and test application methods that will enhance efficacy and economics; and to test the consistency of performance of fungicides, other antagonists, and methods across multiple environments. This discussion will highlight the 1999 research supported in this area through the U.S. Wheat and Barley Scab Initiative.

UNIFORM FUNGICIDE TRIAL

A set of core fungicide treatments was evaluated for efficacy against FHB across seven states (IN, MI, MN, MO, ND, OH, SD) in 1998 (McMullen, 1998). The data on efficacy of five products or product mixes in reducing FHB when applied at heading indicated an average of about 50% reduction in FHB severity occurred with some of the products. In 1999, additional fungicide treatments and locations were included in the Uniform Fungicide Trial. Nine fungicide treatments were compared to the untreated check in 14 states (AR, IL, IN, KY, MD, MI, MN, MO, NY, NC, ND, OH, SD, and VA). A summary report, with all collaborators and treatments listed, is provided elsewhere in these Proceedings - ***1999 Uniform Fungicide Trials to Identify Products Effective Against Fusarium Head Blight in Wheat,*** by M. McMullen, G. Milus, and L. Prom. Collaborators in these 14 states provided fungicide testing on three classes of wheat across very different environments, some of which had low to high levels of FHB in 1999, while others had drought conditions. The summary report indicates that, averaged over the environments that had FHB, eight of the nine fungicide treatments, applied once at flowering, significantly reduced FHB. DON levels were reduced on the average by 45% with the best treatment (Folicur at 6 fl oz/acre applied at flowering). Folicur fungicide had a Section 18 emergency exemption for use in many states in 1999, while two other promising treatments in these trials, BAS 500 and Stratego, are not yet registered for wheat. Greg Shaner, Purdue University, will provide some additional information on ***Scab and Fungicide testing in the Corn Belt***, during the 1999 Forum's session on the review of the Chemical and Biological Control Area.

Individual cooperators have also provided information on their fungicide results in several formats: manuscripts in these Scab Forum Proceedings; in Progress Reports provided on-

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line at http://www.scabusa.org/; in the *Fungicide and Nematicide Tests* publication; or at professional meetings. An example included in the 1999 Scab Initiative's Forum Proceedings is a report titled ***Fungicide Efficacy Trials at Michigan State University*** by P. Hart, J. Froedtert, R. Ward, L. Siler, G. VanEE, and R. Ledebuhr, all of Michigan State University. A. Grybauskas provided his 1999 results from Maryland in a poster titled ***Timing of Fungicide Applications for Fusarium Head Blight Management of Winter Wheat***, published in *Phytopathology* 89:S30. Among reports submitted to *Fungicide and Nematicide Tests* volume 55 are ***Efficacy of Fungicides for Control of Fusarium Head Blight on Wheat in Arkansas***, *1999* by L.K. Prom, E.A. Milus, and C. Weight, and ***Evaluation of Fungicides for Control of Fusarium Head Blight and Leaf Diseases on Winter Wheat at Columbia, MO., 1999*** by L.E. Sweets. The core set of fungicides also was tested against FHB on barley in Minnesota and North Dakota. The results of these tests are provided elsewhere in these Scab Forum Proceedings, under the title ***1999 Uniform Fungicide Trials for FHB Control in Barley***, by M. McMullen, R. Jones, J. Pederson, S. Halley, and J.Lukach.

BIOCONTROL STUDIES

Two sets of studies for evaluating biocontrol agents were supported by the National Scab Initiative in 1999. A cooperative project among G. Bergstrom and C. Stockwell, Cornell University, Ithaca, NY, and W. da Luz, EMBRAPA-TRIGO, Passo Fundo, Brazil, is described in a separate report provided in these Proceedings, titled ***Selection of Microbial Antagonists for Biological Control of Fusarium Head Blight of Wheat***. This team has isolated, preserved and characterized approximately 120 candidate biocontrol organisms from 70 different sources, and have developed bioassays to evaluate these isolates as to the most promising for efficacy against *Fusarium graminearum* and for tolerance to environmental stresses. This project is emphasizing organisms that are likely to be robust under harsh field conditions. This research project is evaluating the top candidate biocontrol organisms as protectant sprays during anthesis, as treatment of scabby wheat seed, and as treatment of infested maize debris.

Another group working on biocontrol includes M. Boehm and N. Khan at Ohio State University, Columbus, OH, and D. Schisler, USDA-ARS, Peoria, IL. They have provided results of their 1999 research in several poster presentations here at the Forum, titled ***Optimization and Field Testing of Biocontrol Agents Active Against Fusarium Head Blight***, and the team also will provide an oral summary during the presentation on the Chemical and Biological Control Area. This group has developed selective screening methods for identifying microbial strains with an enhanced likelihood of reducing the severity of FHB. They screened wheat anther colonists for their ability to metabolize choline and betaine, two *Fusarium graminearum* growth stimulating compounds found in anthers. They tested six strains of selected microorganisms in greenhouse bioassays and field tests in Illinois and Ohio, and plan to continue field testing in other environments. They also are looking at methods to enhance bioactivity of these microorganisms.

APPLICATION TECHNOLOGIES

Two groups in the United States have devoted considerable time and resources to evaluating methods of application of fungicides to improve FHB control. The grain spike is the site of infection, but the grain spike is a difficult target. Deposition and retention of fungicide on the grain spike is hindered by the vertical orientation of the spike, along with the interference of awns, and the waxy or glabrous surface of the glumes. Conventional application methods intended

primarily for foliar targets have shown to be ineffective in delivering fungicide to the grain spike. Generally, only a small percentage of the spray volume remains on the grain spike. Studies at North Dakota State University have been established to identify spray application parameters that are most effective in improving fungicide deposition on the spike. Angled, rather than vertically oriented, sprays have shown great improvement in deposition and disease control, and some air assist spray systems have also shown great promise. A report on some of these results in North Dakota is given within these Proceedings and as a poster titled ***Sprayer Modifications for Enhanced Control of Fusarium Head Blight with Fungicides***, by S. Halley, J., Pederson, M. McMullen, and J. Lukach.

At Michigan State University, Gary Van Ee and coworkers also have looked at spray application techniques, and compared the deposition characteristics of four different types of application equipment using a fluorescent dye tracer on the heads of wheat. They tested flat fan nozzles set at an angle from the horizontal and tested a modified version of the Proptec sprayer, an air assist type sprayer. Both methods provided average vomitoxin levels below 5 ppm while the untreated control exceeded 13 ppm. A summary of their results is available in the Initiative's Progress Reports and as part of a report included in this year's Forum Proceedings, titled ***Fungicide Efficacy Trials at Michigan State University***, by P. Hart, J, Froedtert, R. Ward, L. Siler, G. Van Ee, and R. Ledebuhr.

Overall, substantial progress has been made in the past year with identifying fungicides that are most efficacious in reducing FHB and vomitoxin, identifying biological agents that have good activity against *F. graminearum*, and in identifying application techniques that improve performance of these products. Additional research is needed in evaluating newer fungicide chemistries and rates of use across environments and grain classes, in evaluating biological agents in the field and in increasing their survivability and establishment, and in transferring application techniques to available, affordable, and practical equipment for producers.

1999 UNIFORM FUNGICIDE TRIALS TO IDENTIFY PRODUCTS EFFECTIVE AGAINST FUSARIUM HEAD BLIGHT IN WHEAT

Marcia McMullen[1*], Gene Milus[2] and Louis Prom[2]

INTRODUCTION

Wheat growers are very interested in finding effective fungicides that will substantially control Fusarium head blight (FHB) and be safe and economical to use. The severity of the FHB epidemics in the US in 1993, 1996, and 1997 led to interest in a cooperative project to evaluate a core set of fungicide treatments across wheat classes and environments. During these epidemics, few fungicides had federal registration for heading application to wheat. In 1998, a uniform fungicide trial was conducted across seven states (ND, MN, SD, OH, IN, KY, MO), with five fungicides or fungicide mixes (Benlate + mancozeb, Folicur, Tilt, Quadris, Quadris + Benlate) evaluated for reducing FHB when applied to wheat at the flowering stage. In 1998, only Benlate and various mancozebs had full registration; Folicur received Special Emergency Exemptions (Section 18) for use in many states; Tilt had 24C state labels for heading application in other states; and Quadris became registered after the growing season. In 1998, only three of the seven states had substantial levels of FHB in which to evaluate efficacy against FHB; across these three states (ND, SD, MN), FHB was reduced by an average of about 50% with the best treatments (McMullen et al., 1997). More information was needed on these and additional products potentially close to registration, and additional sites with potential for FHB were needed for effective evaluation of product efficacy across environments.

METHODS

Plant Pathologists in 14 states participated in the 1999 uniform fungicide trial. These states represented hard red spring wheat, hard red winter wheat, soft red winter wheat, and soft white winter wheat production areas. The states and the principal cooperators were:

Arkansas	*Gene Milus, Univ. of Arkansas, Fayetteville*
Illinois	*Wayne Pedersen, Univ. of Illinois, Urbana*
Indiana	*Greg Shaner, Purdue Univ., West Lafayette*
Kentucky	*Don Hershman, Univ. of Kentucky, Princeton*
Maryland	*Arvydas Grybauskas, Univ. of Maryland, College Park*
Michigan	*Pat Hart, Michigan State Univ., East Lansing*
Minnesota	*Roger Jones, Univ. of Minnesota, St. Paul*
Missouri	*Laura Sweets, Univ. of Missouri, Columbia*
New York	*Gary Bergstrom, Cornell Univ., Ithaca*
North Carolina	*Steven Leath, North Carolina State Univ., Raleigh*
North Dakota	*Marcia McMullen, North Dakota State Univ., Fargo*
Ohio	*Pat Lipps, Ohio State Univ., Wooster*
South Dakota	*Marty Draper, South Dakota State Univ., Brookings*
Virginia	*Erik Stromberg, Virginia Tech, Blacksburg*

The treatment list in 1999 included a nontreated check and nine fungicide treatments, including several new experimental products, various timings of application of two products, and two rates of one product (Table 1). Two of the products tested were strobilurin chemistries (BAS 500, Quadris) one was a triazole fungicide (Folicur), two treatments tested Stratego [a combination product of a triazole (Tilt) and an experimental strobilurin], and two treatments contained the protectant mancozeb fungicide (Penncozeb, Benlate + mancozeb). At some, but not all locations, artificial inoculum of *Fusarium graminearum* was applied as infected corn or corn and barley grain, distributed evenly over the plot area 10 to 14 days before flowering. At some sites, water was added to the plot sites prior to flowering and during flowering via a

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misting system or irrigation system. Fungicides were applied at the flowering stage of development, unless otherwise indicated. Fungicides were generally applied with a CO_2 pressurized hand held sprayer, delivering from 18-25 gpa. Nozzle configurations on sprayers varied among states, as did plot size. Disease parameters measured at soft dough stage of development included FHB incidence (% of heads showing symptoms), FHB head severity (% of head area infected), FHB index (= field severity = incidence x head severity), deoxynivalenol (DON) content in ppm, Fusarium damaged kernels (FDK), and % flag leaf disease, as well.

Table 1. Uniform fungicide treatments evaluated in 1999 for control of Fusarium head blight

Treatment	Products Manufacturer	Rate of product/acre	Adjuvant, if applied	Timing of application [a]
Control	-----	-----	-----	-----
Folicur	Bayer	6 fl oz	0.06 % v/v Induce	Feekes 10.51
Benlate + Manzate	DuPont + Griffin	0.5 lb + 1 lb	0.25% v/v CS7	Feekes 10.51
Penncozeb	AtoChem	1 lb + 1 lb		Feekes 10.3 + 10.51
BAS 500	BASF	15.3 fl oz	1 % v/v Agridex	Feekes 10.3
BAS 500	BASF	15.3 fl oz	1 % v/v Agridex	Feekes 10.51
Stratego	Novartis	10 fl oz		Feekes 10.51
Stratego	Novartis	14 fl oz		Feekes 10.51
Quadris	Zeneca	12.3 fl oz		Feekes 10.51
Quadris	Zeneca	9.2 fl oz		Feekes 10.51

[a] Feekes growth stage 10.3 = 50% heading; Feekes growth stage 10.51 = early flowering

RESULTS

Several of the 14 states participating in the 1999 trial had severe to moderate drought during the growing season, and little or no FHB developed. These states included New York, North Carolina, Ohio, Virginia, and Indiana. The fungicide trial in Arkansas had the greatest level of FHB, a test site on spring wheat in Watertown, SD had moderate levels of FHB, while other states or sites had FHB index levels below five when averaged across all treatments. Some states, such as Michigan, Illinois, Minnesota, Missouri, and Maryland had FHB in 1999, but the index level or field severity in untreated plots averaged below five percent. To examine how treatments performed across locations, and determine variability across locations, Gene Milus and Louis Prom did the following analyses for FHB index (field severity), Fusarium damaged kernels (FDK), and DON levels. Statistical analyses were done using mean FHB data from various locations that reported on all ten treatments of the uniform list or had FDK and DON data. Yields were not included in these analyses because they may reflect leaf disease control as well as FHB control. Individual reports from each state are provided in the state's progress reports provided to the U.S. Wheat and Barley Scab Initiative and listed on the Internet at http://www.scabusa.org/.

FHB Index (Field Severity)

The FHB index values were significantly different among locations and treatments (Table 2). The highest severities were at Fayetteville, AR, and the lowest average FHB index in this analysis was at Princeton, KY. The Fayetteville, AR location had an FHB index that was about the same as the sum of the other nine locations. When data was re-analyzed without Fayetteville data, treatments and locations were still significantly different. Variation in FHB index across sites indicates the problems associated with achieving enough infection and a uniformity of infection across locations. Factors affecting FHB infection include amount of inoculum available or applied, variety tested, amount of water applied, and natural environmental conditions.

All treatments significantly reduced the FHB index over the untreated control, when averaged across all 10 locations (Table 2), but few treatments varied significantly from each other. Penncozeb and Quadris appeared to be the least effective in reducing FHB, while Stratego at 14 fl oz, BAS 500 applied at flowering, and Folicur were the most effective.

FDK levels

Five trials in these studies had useable data for Fusarium damaged kernels (FDK) and locations were significantly different, but treatments were not (Table 3). Of the five trials, the Fayetteville, AR study had significantly more FDK than the other sites, while the Watertown, SD study on winter wheat had the least. Data were analyzed with (5 sites) and without (4 sites) the Fayetteville location.

DON levels

At the time of submitting this report, only eight locations in the uniform trials had useable or available data for DON; there were significant differences among the locations, but not among the treatments (Table 4). When Fayetteville data were excluded (7 sites), treatments were significantly different at $P = 0.08$. The nontreated control was intermediate among the treatments, and no treatment had significant reduction in DON over the control. Treatments of Penncozeb, Quadris, BAS 500 at heading or Stratego at the low rate averaged above or equal to the control in DON levels.

CONCLUSIONS

FHB levels varied considerably across testing sites in 1999. Additional sites with the presence of FHB at moderate levels and with all treatments included would have added to the data and provided more information about product efficacy. The best treatments in these composite analyses were the Folicur treatment and the BAS 500 applied at flowering, reducing the FHB index by about 50%, DON levels by about 22%. Additional improvements are needed to achieve substantial control of FHB with fungicides. Possibilities for improved control include application technologies that deposit more fungicide on the heads and untested experimental fungicides.

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Table 2. FHB Index (incidence x head severity) across locations and across treatments

Location[a]	Mean FHB Index across treatments	t grouping LSD (P=0.05)	Treatment	Mean FHB Index across locations	t grouping LSD (P=0.05)
Fayetteville, AR	25.1	A	Control	7.9	A
Watertown, SD	7.2	B	Penncozeb	6.2	B
Carrington, ND	4.2	C	Quadris 9.2 fl oz	5.9	CB
Langdon, ND	3.6	DC	Quadris 12.3 fl oz	5.3	CBD
Groton, SD	2.8	DCE	Stratego 10 fl oz	4.8	CBD
Fargo, ND	2.5	DFE	BAS 500 at 50% heading	4.8	CBD
Watertown, SD	2.4	DFE	Benlate + mancozeb	4.8	CBD
Keysburg, KY	1.4	FE	Stratego 14 fl oz	4.5	CD
Columbia, MO	1.2	F	BAS 500 at flowering	4.2	D
Princeton, KY	1.1	F	Folicur	4.0	D
LSD (P=0.05)	1.4		LSD (P=0.05)	1.4	

[a] Treatments applied to soft red winter wheat, hard red winter wheat, or hard red spring wheat

Table 3. Fusarium damaged kernel (FDK) levels across locations and treatments.

Location	Mean FDK 5 sites	t grouping 5 sites	Mean FDK 4 sites	t grouping 4 sites
Fayetteville, AR	61.6	A		
Langdon, ND	8.0	B	8.0	A
Watertown, SD	7.0	CB	7.0	A
Groton, SD	4.1	CD	4.1	B
Watertown, SD[a]	1.6	D	1.6	C
LSD (P=0.05)	3.3		1.1	

[a] hard red winter wheat

Treatments	Mean FDK 5 sites	Mean FDK 4 sites
Quadris 9.2 fl oz	23.0	7.3
Quadris 12.3 fl oz	19.6	6.3
BAS 500 at heading	18.5	5.6
Stratego 10 fl oz	16.9	4.9
Control	16.8	6.0
Stratego 14 fl oz	16.4	4.9
BAS 500 at flowering	15.3	3.9
Penncozeb	14.2	5.4
Folicur	14.1	4.6
Benlate + Mancozeb	14.0	4.1
LSD	NS	NS

Table 4. DON levels across eight locations and across fungicide treatments.

Location	Mean DON (ppm)	t grouping
Fayetteville, AR	14.8	A
Langdon, ND	10.8	B
E. Lansing, MI	8.6	C
Groton, SD	7.9	C
Columbia, MO	0.7	D
Princeton, KY	0.6	D
Urbana, IL	0.5	D
Keysburg, KY	0.5	D
LSD (P=0.05)	1.2	

Treatment	Mean DON (ppm)
Penncozeb	7.8
Quadris 9.2 fl oz	6.2
Quadris 12.3 fl oz	6.1
Stratego 10 fl oz	5.6
Control	5.6
BAS 500 at heading	5.6
Stratego 14 fl oz	5.1
Benlate + Mancozeb	5.0
BAS 500 at flowering	4.9
Folicur	4.3
LSD	NS

1999 UNIFORM FUNGICIDE TRIALS FOR FHB CONTROL IN BARLEY

Marcia McMullen[1*], Roger Jones[2], Jeremy Pedersen[1], Scott Halley[1], and John Lukach[3]

INTRODUCTION AND OBJECTIVES

Barley growers are very interested in finding effective fungicides that will substantially control Fusarium head blight (FHB) and reduce the deoxynivalenol (DON) content in the harvested grain. The severity of recent FHB epidemics in the US led to a cooperative project to evaluate a core set of fungicide treatments on barley.

METHODS

In 1999, fungicide tests on barley were done at two locations in North Dakota and one in Minnesota. A set of 10 treatments plus an untreated check were evaluated at Fargo and at Langdon, ND on Stander barley (Table 1), while six of these treatments were evaluated at Crookston, MN on MnBrite barley. MnBrite is a newly released variety from the Univ. of Minnesota that has more tolerance to FHB than Stander. Treatments were applied with CO2 backpack sprayers at 18-20 gpa at 40 psi during heading. Grain spawn inoculated with *Fusarium graminearum* was distributed evenly among plots at the Langdon and Fargo sites approximately 10 days before heading. The Crookston site was not inoculated. Plots at each site were in a randomized complete block design with four replicates per treatment. Disease ratings were taken at soft dough stage of kernel development. Disease parameters included FHB incidence (% of heads showing symptoms), FHB head severity (% head area infected), FHB index (= field severity = incidence x head severity), deoxynivalenol (DON) content in ppm, and % flag leaf disease.

RESULTS

FHB Index (Field Severity)

FHB levels were generally low across all three sites in 1999, with the untreated control having an FHB index of 1.3-1.5 at Fargo and Crookston, and 6.4 at Langdon. The mean FHB index values ranged from 0.8-3.2 when averaged across locations (Table 1). At Fargo, all treatments gave significantly lower FHB values than the untreated check, but treatments were not significantly different from each other. At Crookston, the differences among all treatments were non-significant. At the Langdon location, all treatments resulted in a significantly lower FHB index value than the untreated, and the BAS 500 treatment at heading, the Stratego at 14 fl oz, and Quadris at the 12.5 fl oz rate gave significantly lower FHB severity ratings than some of the other treatments. The mean values across locations (Table 1) indicate that the Benlate + mancozeb treatment, the Penncozeb treatment, and the BAS 500 treatment applied prior to full head emergence resulted in the highest FHB field severity ratings.

Deoxynivalenol (DON Levels)

At the time of submitting this report, only the Crookston location had DON analysis completed. At Crookston, all treatments significantly reduced DON levels over the untreated control, but all levels of DON were low, under 1 ppm. Only the Benlate + mancozeb treatment significantly reduced the DON levels over the other fungicide treatments.

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Leaf Disease

Leaf disease levels also were low across the sites, with flag leaf necrosis due to net blotch infection in untreated checks at 1.3% at Fargo, 1.8% at Crookston, and 38% at Langdon.
At Langdon, where net blotch was most severe, all treatments significantly reduced this disease over the untreated check, but the Benlate + mancozeb treatment and the Penncozeb treatment resulted in greater net blotch than other treatments.

Table 1. Fungicide treatments evaluated in 1999 on barley for control of Fusarium head blight (FHB) and effect on FHB field severity

Treatment	Product s Manufacturer	Rate of product/acre	Adjuvant, if applied	Timing of application [a]	Mean FHB Field Severity across locations
Control	-----	-----	-----	-----	3.1
Folicur	Bayer	6 fl oz	0.06 % v/v Induce	Feekes 10.51	1.6
Benlate + Manzate	DuPont + Griffin	0.5 lb + 1 lb	0.25% v/v CS7	Feekes 10.51	3.2
Penncozeb	AtoChem	1 lb + 1 lb		Feekes 10.3 + 10.51	2.2
BAS 500	BASF	15.3 fl oz	1 % v/v Agridex	Feekes 10.3	2.1
BAS 500	BASF	15.3 fl oz	1 % v/v Agridex	Feekes 10.51	0.8
Stratego	Novartis	10 fl oz		Feekes 10.51	1.8
Stratego	Novartis	14 fl oz		Feekes 10.51	1.0
Quadris	Zeneca	12.3 fl oz		Feekes 10.51	1.0
Quadris	Zeneca	9.2 fl oz		Feekes 10.51	1.1
Folicur	Bayer	4 fl oz	0.06% v/v Induce	Feekes 10.51	1.0

[a] Feekes growth stage 10.3 = 50% heading; Feekes growth stage 10.51 = head completely emerged

"SCES": AN OBJECTIVE FUNGICIDE COVERAGE EVALUATION SYSTEM FOR CONTROL OF FUSARIUM HEAD BLIGHT

S. Panigrahi[1*], H. Gu[1], V. Hofman[1], M. McMullen[2], and S. Halley[2]

INTRODUCTION

Effective chemical spray coverage on wheat or barley heads is critical for scab disease control and high crop yield. Effective chemical application is also always desirable for protection of the environment, and it will reduce input costs and increase wheat production profits. Furthermore, by thoroughly understanding spray coverage on wheat heads, we can learn how chemicals work on wheat heads. Variations produced by different combinations of spray parameters can be understood and thus the best spray configuration can be recommended.

In order to process spray images acquired, appropriate software needed to be developed for processing and analyzing images. Image processing techniques have been implemented in some spray coverage analysis applications. The most important step in analyzing these spray images is to segment the images correctly. That is to correctly separate the spray droplets from background in the images. One of the techniques is to use histogram-based thresholding techniques. Many algorithms have been potential to accomplish this task, for example, the modified Otsu's algorithm (Panigrahi et al., 1995), the edge-based algorithm (Kohler, 1979), multi-modal algorithm (Beveridge et al., 1989), moment-preserving algorithm (Tsai, 1985), entropy method (Kapur, 1985) and Shapiro's automatic thresholding algorithm (Shapiro, 1992). All these algorithms were implemented in our study.

OBJECTIVES

The objectives of this paper were to evaluate and compare the performances of different thresholding algorithms for quantifying spray coverage on wheat/barley head.

MATERIALS AND METHODS

Image Acquisition Methods

Wheat heads were sprayed using a mixture of chemicals and Dayglow Blaze Orange (EPX-15 from Day-Glo Color Inc.) at 1.0 % volumetric concentration. Surfactant Latron CS-7 (.05%) was also used along with the Dayglow Blaze Orange dye tracer to provide better-contrasted images. The sprayed wheat heads were put in a light chamber under Ultra Violet light (long wavelength). Dye-covered images of the wheat heads were taken with a high-resolution low light CCD camera (VI470, Optronics) with high integration time (two seconds). Two optical filters were used with cut off and cut on at 700 nm and 520 nm respectively. These filters filtered out unwanted backgrounds while provide best spectral ranges for the dye tracer to pass through. The images taken with the CCD camera were digitized and captured with a Coreco (TCI-SE) frame grabber. The same wheat head images were also acquired under visible lighting. The spray coverage was calculated by dividing the image area of sprayed chemical by image area of wheat head.

Algorithms Used

Figure 1 shows the flow chart of the image processing/analyzing software used for processing the sprayed images of wheat heads. These procedures were applied to both the regular as well as the dye-covered wheat head images.

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Algorithm 1: Modified Otsu's Algorithm (Panigrahi et al., 1995)

At first, Otsu's algorithm (Otsu, 1979) was used to calculate the threshold 'th'. The Otsu's method is based on discriminate analysis. The following criterion was used to determine the threshold,

(1) th = max $\{\sigma_B^2(th)\}$, for 0 <= th <= L and
$\sigma_B^2 = [\mu_T w(th) - \mu(th)]^2 / \{w(th)[1-w(th)]\}$

where w(th) and μ(th) are the zeroth and the first order cumulative moments of the histogram up to 'th' level and is defined by:

(2) $$w(th) = \sum_{i=0}^{th} p_i \ , \ \mu(th) = \sum_{i=0}^{th} ip_i$$

μ_T, the total mean level of the image, is given by:

(3) $$\mu_T = \sum_{i=0}^{L-1} ip_i$$

where

(4) $p_i = n_i/N$, $p_i > 0$ and $\sum_{i=0}^{L-1} p_i = 1$

and L represents the total number of gray level (256), n_i represents the number of pixels at any gray level 'i' and N represents the total number of pixels in the image. After 'th' was found, statistical procedures were implemented to to find a more accurate threshold

Algorithm 2: Shapiro's Automatic Threshold Algorithm (Shapiro, 1992)

Shapiro (1992) developed an adaptive method to find an optimum threshold. This method utilizes local thresholding techniques with subimages. The method is independent of object/background area ratio and insensitive to noises. Let us define a subimage R with size *m* x *m* pixels.

1) For each R, calculate g_{min}, g_{max}, g_{mid} where g_{min} is the minimum pixel value in subimage R, g_{max} is the maximum pixel value in the subimage R and g_{mid} is the average of the above two,
2) For each R, find g_1 and g_2 where g_1 is the histogram mean in the range of g_{min} to g_{mid} while g_2 is the histogram mean in the range of g_{mid}+1 to g_{max} , and find standard deviation, s of histogram of the whole subimage.
3) For each threshold candidate 'th' from 0 to 255, calculate weight function for each subimage with following formula,

(5) $$W(R,th) = \begin{cases} \exp\{-\dfrac{4.5(th - g_{mid}^{\ 2})}{(g_{mid} - g_1)^2}\}, \\ \quad if\ th \in [g_1, g_{mid}]; \\ \exp\{-\dfrac{4.5(th - g_{mid}^{\ 2})}{(g_{mid} + 1 - g_2)^2}\}, \\ \quad if\ th \in [g_{mid} + 1, g_2]; \\ 0, \textit{elsewhere} \end{cases}$$

4) for each threshold candidate 'th', find 'dt' where 'dt' is calculated using

(6) $$dt = \sum_{for\ all\ R} \sigma(R) \times W(R,th),$$

and find 'jt' with jt = dt / n where n is the number of subimages with non zero weight functions.
5) For all threshold candidates 'th', finds the one that maximizes 'jt' and that becomes the optimum threshold.

Algorithm 3: Edge-based algorithm (Kohler, 1979)

Kohler (1979) has proposed an algorithm that will select a threshold automatically so as to maximize the global average contrast of edges. Such method performs multiple passes over the image, creating histograms of contrast per threshold-value, from which the maximum is chosen. For each pass of the algorithm, two stages are established. In the first stage, each edge in the image is examined and

two histograms are build, one for total-contrast per threshold-value and the other edge-count per threshold-value. In the second stage, a third histogram of average-contrast per threshold-value is computed by dividing the total-contrast histogram by the edge-count histogram. Threshold is chosen based on an user defined minimum contrast criterion.

Algorithm 4: Entropy Method (Kapur, 1985)

Kapur (1985) has introduced a method that will find optimal threshold value that will maximize the information content of both the object and background. For each threshold value, the object and background distributions are derived from the original gray level distribution of the image and the two respective entropy values are calculated. Their sum is then used to represent the information content of both the object and background mentioned above. The optimal threshold is chosen as the gray level at which the sum of the two entropy values is maximum.

Algorithm 5: Moment Method (Tsai, 1985)

In this method, the threshold values are computed deterministically in such a way that the moments of an image to be thresholded are preserved in the output (binary) image. The i-th moment is calculated as

(7) $$m_i = \frac{1}{n}\sum_{g=0}^{l-1} g^i h(g), \quad i = 1,2,3$$

where n is the total number of pixels in the image. The threshold value t is obtained from the gray level histogram of the image by choosing t as the p_0-tile, where p_0 is given by

(8) $$p_0 = \frac{z - m_1}{(c_1^2 - 4c_0)^{1/2}}$$

and

(9) $$c_0 = \frac{m_1 m_3 - m_2^2}{m_2 - m_1^2}$$

(10) $$c_1 = \frac{m_1 m_2 - m_3}{m_2 - m_1^2}$$

(11) $$z = \tfrac{1}{2}\{(c_1^2 - 4c_0)^{1/2} - c_1\}$$

Algorithm 6: Multi-Modal Method (Beveridge et al., 1989)

Beveridge et al. (1989) has developed a region segmentation algorithm called multi-modal method developed from the Localized Histogram Segmentation algorithm. There are two phases to the histogram segmentation process.

In the first phase, the image is partitioned into a set of rectangular local subimages called sectors. The histogram (frequency distribution) of pixel values from the input image is then computed for each of these sectors. Within the histogram of each sector "significant" clusters are identified by a means of a peak-valley analysis.

The second phase of the process involves the peak addition in which "ambiguous" peaks in the histogram can be verified according to their presence or absence in adjacent sectors. The intensity value of each of the selected peaks is then used as the output label for all pixels in the input sector that map to the corresponding peak. The domain of this mapping includes all intensity values lying between the valleys on either side of the peak.

RESULTS AND DISCUSSIONS

All these algorithms were implemented using a image processing software called Aphelion. Several methods were available in the software package except the modified Otsu's method and Shapiro's Automatic Method. These two methods were then developed using Microsoft C/C++ and introduced in Aphelion environment as user defined operators.

Wheat heads with high, medium and low spray coverage were evaluated as well as their images

under regular lighting. Ten images of each category were chosen randomly to evaluate the performances of these algorithms. The images were binarized according to thresholds selected by different methods. The same images were also binarized according to a threshold selected by manual thresholding. The images thresholded manually served as a standard for other methods to be compared against.

The histograms of all segmented images were calculated. The object pixels in the manually segmented image were compared with those in the images segmented by each algorithm. Table 1-4 lists the results of the comparisons.

For high, medium and low coverage spray images of wheat heads, the average pixel difference is the least for the edge-based method. They are at 312, 165 and 73 pixels respectively. Since these spray images are sizes of 640 by 480, which contain 307200 total number of pixels, therefore, differences in the magnitude of hundreds or even lower portion of thousands are very small. The edge-based method was the best for these images followed by entropy method, modified Otsu's method, moment method, multi-modal method and Shapiro's method respectively. However, Shapiro's method seems to work well on low coverage wheat heads with average pixel difference of only 335 pixels, which ranked right behind edge-based method.

For wheat head images under visible lighting, the modified Otsu's method performs the best with an average pixel difference of 2394 pixels. The edge-based method failed miserably in this case. The other methods did not perform any better with average pixel differences of tens of thousands of pixels.

Figures 2(a), (b) show sprayed images of wheat head and its corresponding segmented image. Similarly, figures 3(a), (b) show an image of wheat head under visible lighting and its corresponding segmented image.

SUMMARY AND CONCLUSIONS

Image processing technique were developed and evaluated to process and analyze images of sprayed wheat head. The evaluation of six automatic thresholding methods worked reasonably well for most of the images with the edge method performs the best for sprayed images and the modified Otsu's method performs the best for images under regular lighting. Some of these methods were not exactly right for several low quality images. But our findings are satisfactory. Currently, we are extending the same study to 1000 images to obtain consistency and accuracy of such findings. Thought it might not be possible to find a thresholding method that would provide exact 100% segmentation accuracy for all types of images, we postulate that based on our current research, an optimum technique will be developed.

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Figure 1. Flow Chart Image Processing Software

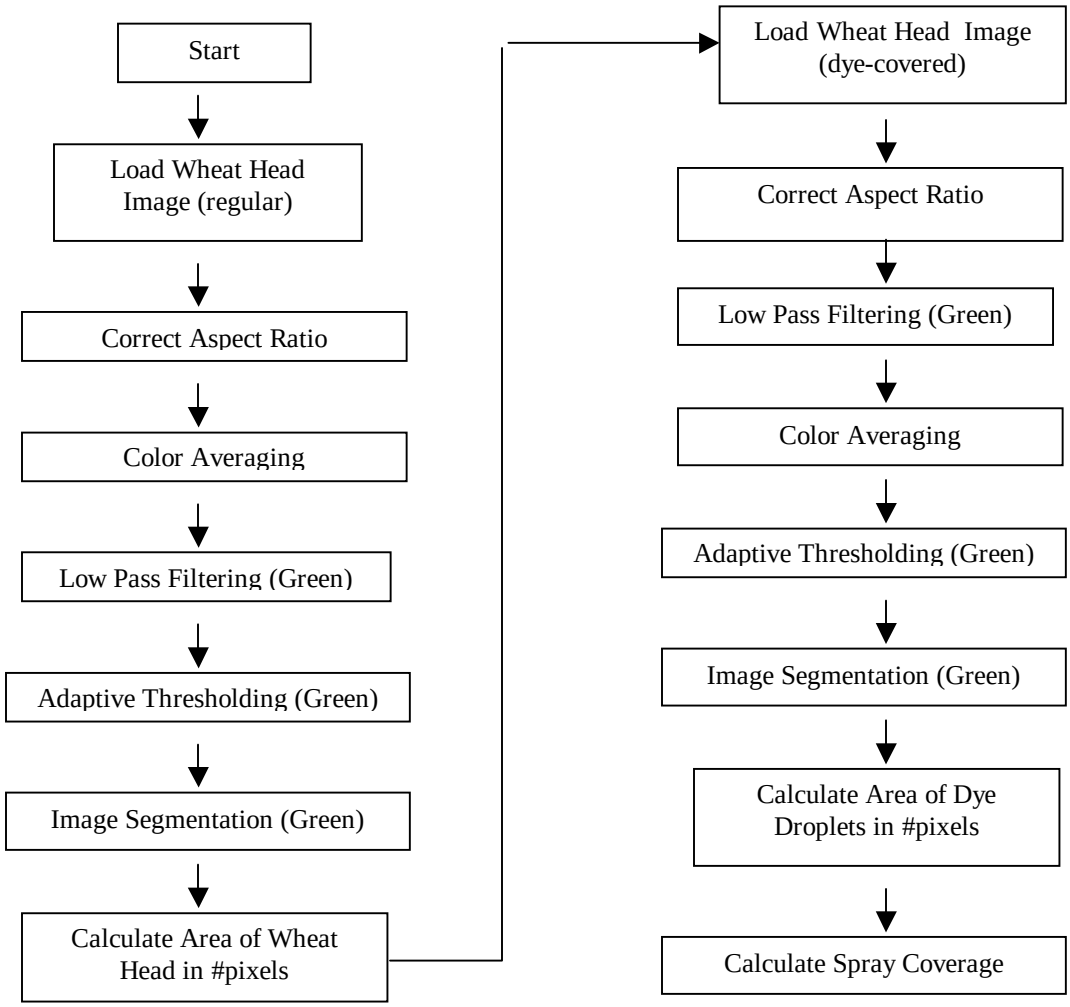

Table 1. Threshold Comparisons (Object Pixel Differences) of High Spray Coverage Wheat Heads

Image No.	Edge-Based Method	Moment Method	Entropy Method	Multi-modal Method	Modified Otsu's Method	Shapiro's Method
1	373	9866	4452	44739	4807	277582
2	0	20198	5244	477	6921	272988
3	336	9527	4359	42770	3677	273108
4	571	14064	7146	3011	8100	2196
5	0	18439	7450	1148	8479	2561
6	692	10322	4796	437	7180	2073
7	270	11089	7986	2337	6420	1685
8	706	9088	4984	97072	7990	2310
9	0	53263	11290	523	5944	1538
10	173	7233	4803	87562	9109	1660
Avg.	**312.1**	**16308.9**	**6251**	**28007.6**	**6862.7**	**83770.1**

Table 2. Threshold Comparisons (Object Pixel Differences) of Medium Spray Coverage Wheat Heads

Image No.	Edge-Based Method	Moment Method	Entropy Method	Multi-modal Method	Modified Otsu's Method	
1	76	9628	4760	49200	Not Available	
2	0	6240	2222	47907	Not Available	
3	170	9644	2330	41441	Not Available	
4	0	6138	2375	42084	Not Available	
5	245	15277	2893	435	Not Available	
6	99	12510	3128	2122	3351	
7	242	7973	2273	46764	3265	
8	219	10074	3099	46627	4396	
9	438	8984	4742	575	4635	
Avg.	**165.44444**	**9607.556**	**3091.333**	**30795**	**3911.75**	**2**

Table 3. Threshold Comparisons (Object Pixel Differences) of Low Spray Coverage Wheat Heads

Image No.	Edge-Based Method	Moment Method	Entropy Method	Multi-modal Method	Modified Otsu's Method	Shapiro's Method
1	0	11570	2886	2949	3462	704
2	166	10452	1926	101572	2374	355
3	92	10416	2189	89022	2659	434
4	0	17101	3898	87446	3539	688
5	106	463	896	101838	1557	84
6	19	7734	1462	86665	1865	283
7	116	7302	1521	87021	1825	300
8	50	5535	1197	102201	1782	274
9	146	690	848	76929	1636	166
10	36	318	420	549	1855	64
Avg.	**73.1**	**7158.1**	**1724.3**	**73619.2**	**2255.4**	**335.2**

Table 4. Threshold Comparisons (Object Pixel Differences) of Wheat Heads Under Visible Lighting

Image No.	Edge-Based Method	Moment Method	Entropy Method	Multi-modal Method	Modified Otsu's Method	
1	4733	18546	5876	3059	604	
2	15571	38990	1438	42265	9551	
3	648	14662	213638	910	167	
4	3364	15789	207814	63963	1099	
5	2359	17727	4951	3027	387	
6	253885	17370	4999	2073	750	
7	243293	14362	209125	1167	801	
8	1779	14605	217450	509	35	
9	15540	36048	1238	15540	8156	
Avg.	**60130.222**	**20899.89**	**96281**	**14723.6667**	**2394.444444**	**2**

Figure 2(a) -- A typical sprayed image of wheat head

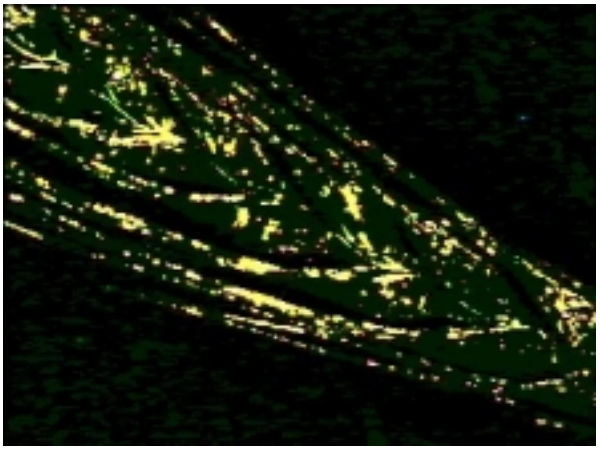

Figure 2(b) -- Segmented sprayed image using edge-based method

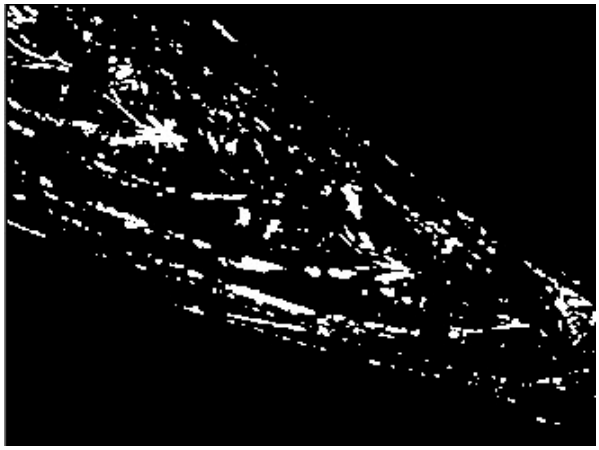

Figure 3(a) -- A typical image of wheat head under visible lighting

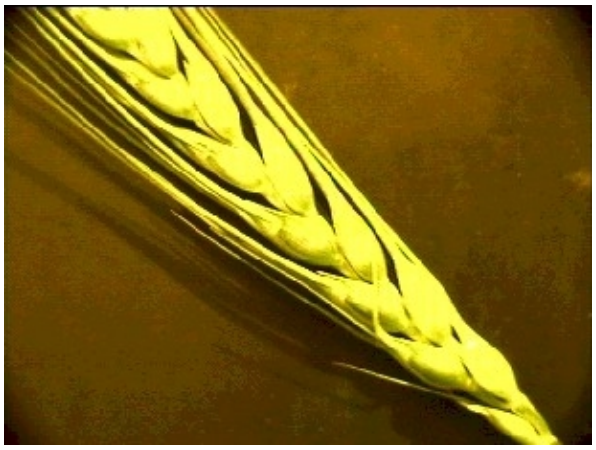

Figure 3(b) Segmented wheat image using modified Otsu s method

USDA-ARS, OHIO STATE UNIVERSITY COOPERATIVE RESEARCH ON BIOLOGICALLY CONTROLLING FUSARIUM HEAD BLIGHT: 1. ANTAGONIST SELECTION AND TESTING ON DURUM WHEAT

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OBJECTIVE

Determine if microbial strains that are effective in reducing Fusarium head blight disease severity on hard red spring and soft red winter wheats are effective in reducing the disease in greenhouse and field tests on durum wheats.

INTRODUCTION

Fusarium head blight (FHB), also known as wheat scab, is a devastating disease of wheat and barley that is primarily incited by the fungus *Gibberella zeae* (anamorph= *Fusarium graminearum*). FHB epidemics cause extensive damage to wheat and barley in humid and semihumid wheat growing areas of the world (McMullen et al., 1997). In durum wheats, the pathogen-produced toxin deoxynivalenol (DON) is retained in semolina at approximately 50%, and *G. zeae* has a strong adverse effect on pasta color when *G. zeae* damaged kernels make up as little as 2% of a lot (Dexter et al. 1997).

High levels of *G. zeae* inoculum are generally found on susceptible wheat heads in fields that experience FHB epidemics (Francl et al., 1999). The use of biotic agents to reduce the severity of FHB holds considerable promise since their application to wheat heads can be timed to be coincident with the most susceptible stages in wheat development: anthesis to early milk (Fernando et al., 1997). Mechanisms of biological control activity that have been identified in a variety of pathosystems include preemptive colonization of infection sites, induced systemic disease resistance, mycoparasitism, nutrient competition and antibiotic production. A traditional method for selecting putative biological control agents is an *in vitro* Petri plate bioassay designed to identify those strains capable of producing antibiotics that inhibit mycelial growth of a pathogen. Unfortunately, the results of Petri plate antagonism assays regularly do not correlate with the biocontrol efficacy of the same strains tested against the pathogen on plants (Reddy et al., 1993).

In research conducted at the NCAUR in Peoria, IL, in cooperation with The Ohio State University (Boehm et al., 1999; Khan et al, 1998; Khan et al., 1999) a selective microbial screening method (Schisler and Slininger, 1997) that does not rely on the traditional Petri plate antagonism assay was developed. *Gibberella zeae* primarily infects the heads of wheat plants from the time of flowering until the soft dough stage of head development (Fernando et al., 1997). Strange and Smith (1978) observed that choline and betaine, compounds present in anthers and wheat heads, stimulated germ tube elongation of conidia of *G. zeae*. We postulated that some of the microorganisms present on wheat anthers may be effective in biologically controlling FHB. Furthermore, since choline and betaine provide a growth stimulus to the pathogen, we surmised that screening anther colonists for their ability to metabolize these growth stimulating compounds (choline and betaine) could provide a method for significantly narrowing the search for antagonists of *G. zeae*.

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Over 700 strains of microorganisms were isolated from anthers collected from wheat plants across Illinois and Ohio. Approximately 55 of more than 700 strains of microorganisms assayed utilized choline as a sole carbon source. Of the seven microbial strains that significantly reduced FHB disease in repeated greenhouse trials, five of the strains were able to utilize choline as a sole carbon source while the remaining two were not. Selecting from a collection of microbial strains those that utilize choline, therefore, was an effective method for rapidly narrowing the search for microbial strains that reduce FHB in wheat. Four effective biocontrol strains (Table 1) were chosen for use in multiple greenhouse tests and a field test of the efficacy of these strains to reduce FHB on durum wheat.

MATERIALS AND METHODS

Greenhouse testing of FHB antagonists on durum cultivars "Renville" and "Ben."

Seedlings were grown in pasteurized potting mix in a growth chamber for 8 weeks prior to transfer to greenhouse benches. Inoculum of the four microbial antagonists (Table 1) used in experiments was grown in a complete liquid medium in Erlenmeyer flasks. Conidia of *G. zeae* isolate Z 3639 were produced on CV-8 agar. At the onset of wheat head flowering, a centrally located spikelet of a wheat head was inoculated with an aqueous suspension containing antagonist cells and broth, 1 x 10^5 conidia/ml of *G. zeae*, 0.04% Tween 80, and a weak phosphate buffer. Antagonists' colony forming units were approximately 2 x10^7 cfu/ml for yeast antagonists and 5 x 10^8 cfu/ml for bacterial antagonists. Controls consisted of "*G. zeae* only" and "tween-buffer only" treatments. Wheat plants were incubated at high relative humidity for 72 h and were scored for disease severity and incidence 16 days after inoculation. There were at least four heads per replication and four replications per treatment. Treatments were distributed in a completely randomized design, and data from experiments that were conducted at least twice were combined. Differences between treatments were determined using analysis of variance (ANOVA) and means separated from controls using Fisher's protected LSD test.

1999 Peoria, IL, field trial of FHB antagonists on durum cultivars "Renville" and "Ben."

Inoculum of antagonists generally was produced as described above except in larger volumes in Fernbach flasks. Due to abnormally hot field conditions from boot through crop maturation (high temperatures ranging from 30-36 C), inoculum of *F. graminearum* was provided as conidia produced as described earlier and as ascospores released from *F. graminearum* Z 3639 colonized corn kernels scattered throughout the plot (?approx 25 kernels/ m^2) 2 weeks prior to wheat flowering. Antagonists and conidia were applied at flowering in aqueous suspensions. Mist irrigation was applied regularly and heavily to promote FHB disease development. Plots were scored for disease severity and incidence.

RESULTS AND DISCUSSION

In greenhouse tests, all four antagonists significantly reduced disease severity on cultivar Renville compared to the positive control (Z3639), and three of the four reduced disease incidence (Table 2). Bacterial antagonist AS 43.3 decreased disease severity by >90% and disease incidence by >75%. Two of the four biocontrol treatments increased, and two of the four decreased the percentage of kernels that were scored as visually faultless though this subjective rating was more severe than the quantitative rating of 100 kernel weights where three of four biocontrol agents significantly increased this factor. Three of four antagonist treatments reduced FHB disease severity on cultivar Ben in greenhouse tests, and two of four reduced FHB disease incidence (Table 3). Because plants were harvested somewhat prematurely in one of the two experiments with Ben, the percent visually faultless kernels and 100 kernel weight data is difficult to interpret.

Though hot dry weather caused an extremely unfavorable season for growing durum wheat and for inciting scab disease, some significant treatment effects with field grown Renville were observed (Table 4). Bacterium AS 43.3 and yeast OH 182.9 significantly reduced disease severity. OH 182.9 also reduced disease incidence. For the Ben portion of the field trial, high levels of variability precluded statistically separating any of the means obtained (data not shown).

With these results, the considerable potential for applying these microbes to reduce the severity of FHB on durum wheat has now been demonstrated. Further studies on durum wheat, including planned cooperative field studies at locations where durum wheats are traditionally grown, should help clarify what role these biocontrol agents could play in the integrated management of FHB.

ACKNOWLEDGEMENTS

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prematurely in one of the two experiments with Ben, the percent visually faultless kernels and 100 kernel weight data is difficult to interpret.

Table 1. Microbial antagonists used to reduce the severity of FHB on durum wheat

Antagonist Strain	Accession Number	Type of Microorganism
AS 43.3	NRRL B-30210	Gram positive bacterium
AS 43.4	NRRL B-30211	Gram positive bacterium
OH 71.4	NRRL Y-30213	Yeast
OH 182.9	NRRL Y-30216	Yeast

Table 2. Influence of microbial antagonists on FHB in greenhouse tests on durum cultivar Renville

Treatment	Percent Disease Severity[a]	Percent Incidence	Percent Visually Faultless Kernels[b]	100 Kernel Weight (g)
G. zeae Z3639	50	96	25	1.9
Buffer	0 *	0 *	90 *	2.8 *
AS 43.3	3 *	21 *	46 *	2.3 *
AS 43.4	17 *	62 *	53 *	2.4 *
OH 71.4	36 *	83	11 *	1.9
OH 182.9	27 *	79 *	18 *	2.1 *

[a]Means in a column followed by an * are significantly different from the *G. zeae* Z3639 control (P=0.05). Means were separated using Fisher s protected LSD.
[b]Perfectly formed kernels with no discoloration or sunken areas were scored as faultless.

Table 3. Influence of microbial antagonists on FHB in greenhouse tests on durum cultivar Ben

Treatment	Percent Disease Severity[a]	Percent Incidence	Percent Visually Faultless Kernels[b]	100 Kernel Weight (g)
G. zeae Z3639	46	97	37	3.2
Buffer	0 *	0 *	69 *	3.6 *
AS 43.3	10 *	41 *	68 *	3.4 *
AS 43.4	9 *	44 *	69 *	3.2
OH 71.4	26 *	87	54 *	3.0 *
OH 182.9	43	87	30 *	2.7 *

[a]Means in a column followed by an * are significantly different from the *G. zeae* Z3639 control (P=0.05). Means were separated using Fisher s protected LSD.
[b]Perfectly formed kernels with no discoloration or sunken areas were scored as faultless.

Table 4. 1999 Peoria field results: microbial antagonists against FHB on durum wheat cultivar Renville.

Treatment	Percent Disease Severity[a]	Percent Incidence
G. zeae Z3639	1.9	18.8
AS 43.3	1.3 *	14.8
AS 43.4	1.9	18.4
OH 71.4	1.9	16.7
OH 182.9	1.3 *	11.8 *

[a]Means in a column with an * are significantly different from the *G. zeae* Z3639 control (P=0.05). Means were separated using Fisher s protected LSD.

SELECTION OF MICROBIAL ANTAGONISTS FOR BIOLOGICAL CONTROL OF FUSARIUM HEAD BLIGHT OF WHEAT

C.A. Stockwell[1*], G.C. Bergstrom[1] and W.C. da Luz[2]

OBJECTIVES

To select microbial antagonists effective in controlling *Fusarium graminearum* when applied to cereal spikes, seed, or crop residue, and to evaluate bio-compatible residue treatments for their ability to interfere with perithecial development or ascospore release.

INTRODUCTION

US wheat and barley producers, grain elevators, brewing, malting, and milling industries have suffered severe economic losses in recent years due to Fusarium head blight (FHB) caused by *Fusarium graminearum* (teleomorph=*Gibberella zeae*) (McMullen et al., 1997). Biological control is looked to as an additional strategy in an integrative approach to FHB management in cereals. FHB may be amenable to biocontrol for two reasons: 1) the disease cycle is monocyclic and 2) the length of time during which the plant can be infected is quite short, from flowering to kernel soft dough stage.

Screening microoganisms to control wheat scab was initiated in Brazil a decade ago (Luz, 1988). Treatment with individual bioprotectants significantly diminished the severity of the disease under field conditions, raising the yield of wheat between 7 and 31 % when compared to untreated plants (Perondi et al. 1996). In laboratory assays, *Paenibacillus macerans, Pseudomonas putida* and *Sporobolomyces roseus* reduced the *in vitro* growth of *F. graminearum* up to 95 - 100 % (Stockwell et al., 1997). In greenhouse trials, flowering spikes co-inoculated with *Paenibacilus macerans* and *F. graminearum* yielded seeds with one tenth the mycotoxin DON (=vomitoxin) concentration (ppm by HPLC analysis) as that found in the seeds from plants inoculated with the pathogen alone (Stockwell, et al., unpublished).

A project was initiated in May 1999 to select microbial isolates with potential for biological control of FHB when applied to cereal spikes, seed or infected crop residues. We are using the following protocols for selection and evaluation of candidate microbial isolates.

MATERIALS AND METHODS

Initial selection and evaluation of isolates

Several bioassays have been adopted in order to efficiently evaluate the large number of isolates and choose the most promising organisms and bio-compatible treatments for inclusion in greenhouse and field trials.

The funnel-method test (Luz, 1990), which compares the effect of the diffusate of individual test organisms on the radial growth of *F. graminearum*, is used to evaluate the isolates for antibiosis. Bioassays which have been employed to identify organisms tolerant to environmental stresses include: NaCl-nutrient medium for osmotic stress tolerance, an assay for UV tolerance and heat tolerance for selection of spore-formers.

Evaluations as protectant sprays during anthesis

Candidate biocontrol organisms are evaluated for their efficacy as protectants by co-inoculating them

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with the pathogen onto the spikes of glasshouse-grown wheat plants during anthesis. The most promising of the newly acquired isolates will be evaluated in this way as well as re-confirming the results of previously-tested biocontrol agents. In the 2000 field season, *Paenibacillus macerans* and possibly several other outstanding biocontrol candidates will be included as treatments alongside chemical treatments in the Uniform Fungicide Trial at Aurora, NY. Sufficient primary inoculum is assured by the distribution of artificially-infested maize kernels throught the experimental plot. This material produces mature perithecia by the time the wheat reaches anthesis.

Evaluations as treatment of scabby wheat seed

The isolates will be evaluated on scabby seed for their ability to improve emergence and seedling vigor using both *in vitro* and glasshouse assays. The laboratory assay uses 24-well culture plates to evaluate the efficacy of candidate biocontrol agents, bio-compatible treatments and combinations of treatments. In the glasshouse assay, treated scabby seed are planted in sterile soil mix and rated for percent germination and seedling height.

Evaluations as treatment of infested maize debris

An *in vitro* ascospore discharge inhibition assay is used to identify organisms and other bio-compatible treatments of maize debris which will reduce perithecial formation and ascospore discharge. Several treatments are being evaluated during autumn/winter 1999-2000 for their ability to interrupt the production of primary inoculum on artificially-infested maize stalk pieces exposed to ambient environmental conditions.

RESULTS AND DISCUSSION

We have isolated, preserved and characterized approximately 120 candidate biocontrol organisms, from 70 different sources including wheat, maize, and over-wintered maize debris, wild grasses and sedges on non-agricultural land, and air-borne samples. In addition, one of the authors, Dr. Wilmar Luz of EMBRAPA-TRIGO, Brazil provided us with 14 promising microorganisms which, when used as seed treatments in field trials in Brazil, were shown to reduce losses due to several soil-borne fungal diseases of wheat and maize.

In the 1999 field season, the very promising candidate biocontrol agent, *Paenibacillus macerans*, was included as a treatment alongside chemical treatments in the Uniform Fungicide Trial at Aurora, NY. Unfortunately, the lack of precipitation at anthesis resulted in negligible infection of all treatments. This biocontrol treatment will be included again in the 2000 field trials.

One of the key issues in the development of efficient biocontrol agents is *adaptability*. Organisms which give good control in *in vitro* assays may be ineffective or unreliable under field conditions. For this reason the selection of organisms which are likely to be robust under harsh field conditions is emphasized in this project. Spore-forming bacteria are desirable as biocontrol agents because of their tolerance to environmental stresses and their stability in commercial formulations. Several candidate biocontrol agents already have been identified which exhibit both antibiosis and tolerance to several environmental stresses. We are optimistic that we will find among our collection one or more isolates that will significantly control *Fusarium graminearum*, alone or in combination with other treatments when applied to cereal spikes, seed or infected crop residues.

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USE OF A GFP STRAIN OF *FUSARIUM GRAMINEARUM* FOR HISTOLOGICAL INVESTIGATION OF INFECTED BARLEY

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ABSTRACT

We are investigating development of *Fusarium graminearum* on and in barley florets and other organs using a transformed strain of the fungus containing a constitutively expressed gene for green fluorescent protein (GFP). The GFP strain fluoresces green when viewed by fluorescence microscopy under blue or blue-violet incident light. The GFP strain is easier to see in inoculated living florets, leaf or coleoptile tissues (as viewed by epifluoresence microscopy) than are wild strains (viewed by conventional light microscopy). To remove surface mycelium which can obscure fungus development within underlying tissues, a 2 to 1 (v/v) solution of cellulose acetate in acetone is applied to the tissue surface, allowed to dry for a few minutes, then stripped away, leaving the tissue surface intact and free of mycelium. In initial experiments, inoculum in the form of mycelium growing on mung bean agar blocks (2x12x0.5 mm) was applied to cut ends of detached leaves, coleoptiles, the palea, and the lemma from Robust (susceptible) and Chevron (partially resistant) barley. After 24 hr, the block was removed. The fungus grew both into and on top of the inoculated tissues. By three days after inoculation, hyphae within tissues grew 2.9-3.1 mm in leaves, 1.2 - 1.5 mm in coleoptiles and paleas, but less than 0.2 mm in lemmas (as measured from the cut ends to the advancing hyphal front). In leaves, the distance was increased to 4.5 - 5.3 mm if the inoculum block was left in place 48 hr instead of 24 hr. Hyphae within tissues were subcuticular and intercellular. Plant cells in colonized leaves remained green 2-2.5 mm behind the advancing hyphae front. Tissues more than 2.5 mm from the front became chlorotic, losing the red fluorescence of chlorophyll. On the surface of the tissue, mycelium advanced about 0.3 mm ahead of the underlying subcuticular and intercellular hyphae. Hyphae appeared to grow into and out of leaf stomates. The results show that fungus development was limited in paleas, lemmas and coleoptiles compared to development in leaves, that exogenous nutrients can increase amount of colonization in leaves, and that development was the same in tissues of Robust and Chevron. Based on experience gained in these experiments, we will use the GFP strain to monitor pathways of infection from spot inoculations on exposed surfaces of the palea and lemma of intact barley heads, and to investigate the probable role of anthers in promoting head colonization. (This poster was presented at the 16th Annual Barley Researchers' Workshop, Idaho Falls, July 11-15, 1999. The abstract will be in the 1999 Barley Newsletter.)

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GENETIC ANALYSIS OF A *GIBBERELLA ZEAE* MUTANT WITH ALTERED MORPHOLOGY, REPRODUCTION, AND VIRULENCE

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ABSTRACT

Gibberella zeae (*Fusarium graminearum*) causes both wheat ear blight and maize ear rot, with yield reduction and mycotoxin contamination of the infected grain. During routine experiments to produce and regenerate protoplasts of *G. zeae* field strain GZ3639, we identified a mutant, designated *ear*$^-$ for ear rot, that was significantly reduced in ability to cause both wheat ear blight and maize ear rot. *Ear*$^-$ mutants produced normal macroconidia and were similar to the wild-type strain in growth and morphology on V8-juice agar medium, but were reduced in pigmentation on a range of other media. *Ear*$^-$ mutants were unable to produce perithecia, but were able to function as males in outcrosses with strain GZ3639. To conduct genetic analysis, strains were marked with different auxotrophic mutations, and heterozygous perithecia were identified by the presence of recombinant prototrophic progeny. Among random ascospores from heterozygous perithecia, 48 *ear*$^+$ and 26 *ear*$^-$ progeny were recovered, a slight deviation from the 1:1 segregation ratio expected for a single gene. Among these progeny, reduced pigmentation and female sterility showed perfect cosegregation with reduced virulence on wheat ears. These data suggest that a single genetic change may affect both morphogenesis and virulence of *G. zeae*. Future work will elucidate the genetic basis of this mutation and the function of the gene products in wheat ear blight and maize ear rot.

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ROLE OF ENVIRONMENT AND INOCULUM LEVEL IN WHEAT FUSARIUM HEAD BLIGHT DEVELOPMENT

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OBJECTIVES

Develop a forecasting system for wheat Fusarium head blight based on environment and pathogen propagule levels.

INTRODUCTION

Fusarium head blight (FHB) of wheat, caused by *Gibberella* zeae (Schw.) Petch., has proven to be a difficult disease to manage in many wheat producing regions of the world (Parry et al. 1995). In North America, severe epidemics of FHB have occurred in recent years resulting in large crop losses due to direct yield reduction and complications with mycotoxin contaminated grain (McMullen 1997). Management options for FHB include host resistance, crop rotation, tillage to destroy crop residues, and fungicide applications; however, research has shown that no one management option has been effective in controlling FHB (Parry et al. 1995, Bai and Shaner 1994). Successful disease management will require multiple strategies. The development of a reliable disease forecasting system would improve the ability of wheat producers to deal with the prognosis of disease and facilitate timely applications of chemical or biological control agents.

An early attempt to predict epidemics of FHB was based on observations of regional environmental variables correlated with severe epidemics (Atanasoff 1920). In 1965, Nakagawa et al. proposed a forecasting system for that utilized a regression equation for disease prediction based on environmental variables highly correlated with incidence of seed infection. Moschini and Fortugno (1996) proposed the use of linear regression equations developed from historical records of disease and environmental conditions in Argentina. More recently, Francl et al. (1999) reported on the importance of both inoculum level and environment as components of a FHB epidemic. Therefore, a thorough understanding of the factors contributing to the development of epidemics is needed to develop a disease forecasting system and identify potentially successful disease control options.

One approach to developing a disease forecasting system for FHB is to monitor and quantify the relationships between host, pathogen and environment. In order to define the role of these factors in the disease cycle of FHB and ascertain the effects of regional differences in environment and agricultural practices, a cooperative effort was initiated in 1999. Researchers in Ohio, North Dakota, Indiana, South Dakota, and Manitoba, Canada have completed replicated field trials in their respective locations. By presenting here the results from the Ohio and North Dakota locations, we demonstrate the type of information that such research can generate.

MATERIALS AND METHODS

Replicated plots of susceptible wheat cultivar, Hopewell, were planted near Wooster, Ohio, in the fall of 1998. Similar plots of the susceptible hard red spring wheat cultivar, Norm, were planted in the spring in Fargo, North Dakota. At

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both locations daily variation in inoculum level was monitored by arbitrarily selecting five wheat spikes each day from the replicated plots. Wheat spikes were placed into 50 ml of sterile deionized water, shaken to dislodge spores, and spikes then were discarded. A 0.5-ml aliquot was transferred to each of three plates of Komada's selective medium. Cultures were incubated for 10-14 days and Fusarium type colonies were transferred to potato dextrose agar and carrot agar to identify *Gibberella zeae*. Numbers of colony forming units per head were calculated as an indicator of viable inoculum. A Burkard volumetric spore sampler (sampling rate =16.5 L/min) located within the crop canopy (Ohio) or on an adjacent no-till area (ND) was used to provide supplemental information about the presence of airborne inoculum. Samples were collected every 24 h and observed microscopically for the presence of *G. zeae* ascospores and macroconidia. Disease severity and incidence of plants were evaluated by assessing 30 arbitrarily selected wheat spikes at the soft dough stage of growth (GS 83) (Zadoks et al. 1974).

Environmental variables, including ambient air temperature, relative humidity (RH), and precipitation, were measured in the wheat field with an automated datalogger (Campbell Scientific Inc.). The presence of moisture within the wheat canopy was measured with six replicate flat-plate resistance sensors. All wetness sensors were coated with an off-white latex paint and oriented south at the same height as the wheat spikes (80-90 cm).

RESULTS AND DISCUSSION

The environment in Wooster, Ohio, was favorable for wheat growth during the 1998-1999 growing season. Warm fall temperatures and a mild winter resulted in excellent winter survival of the crop. In the spring, favorable temperatures prevailed and precipitation events provided adequate moisture during the early stages of crop vegetative growth. As the crop progressed into the boot and heading growth stages (GS 45 to GS 59), dry conditions prevailed as precipitation events became less frequent (Figure 1). However, precipitation events and high RH were common during crop anthesis (GS 60 to GS 69). Four of the 7 days during crop anthesis corresponded with >11 h of wetness duration, >80% average RH, and measurable precipitation. Temperature during anthesis ranged from 10 to 20EC, and the average temperature was 15EC.

The daily average number of *G. zeae* colony forming units per wheat spike in Ohio ranged from 0 to 16 between heading emergence (GS 50) and early milk (GS 73) growth stages (Figure 1). The median number of colony forming units of *G. zeae* per day was 1 between head emergence and early milk stage of growth. During anthesis, the median daily number of colony forming units was 2. The Burkard sampler detected only low numbers of *G. zeae* spores; however, other fungi common to the air flora of Ohio were readily detected. The highest number of *G. zeae* spores collected was 1000 spores in a 24-h period (mean density 40 spores/m^3, which coincided to the watery ripe stage of growth (GS 71). Ascospores were the observed more frequently and in greater density than macroconidia. Final disease incidence and severity were less than 0.1% at Wooster, Ohio.

The environment in North Dakota was warm and frequent precipitation events provided adequate moisture for crop development. Three precipitation events occurred between boot (GS 45) and completion of heading (GS 60) stages of growth (Figure 2). Three of the 4 days during crop anthesis (GS 60 to GS 69) coincided with >9 h of wetness duration. RH during anthesis ranged from 75% to 87%, with an average of 80%. Ambient air temperature during crop anthesis ranged from 18 to 24EC, and the average temperature was 22EC.

Between the heading and early milk stages of growth, the daily average number of *G. zeae* colony forming units per wheat spike in North Dakota ranged from 0 to 44 (Figure 2). The median number of colony forming units of *G. zeae* per day was 4 during the same time period. While the crop was flowering, the median number of colony forming units per wheat spike was 7. The number and frequency of *G. zeae* spores collected in a Burkard spore sampler was greater than that collected in Wooster, Ohio. Although a low number of macroconidia were observed, ascospores were the major component of the *G. zeae* inoculum in Fargo. Final disease incidence at Fargo was 18%, and disease severity was 1.2%.

The median number of colony forming units per wheat spike observed at Fargo and Wooster were consistent with inoculum levels reported by Francl et al. (1999) for other non-epidemic years. Differences in disease level observed at the two locations may be the result of differences in inoculum level and timing of inoculum release events (Figures 1 & 2). At Wooster, dry conditions prior to anthesis may have resulted in delayed development of *G. zeae* perithecia, which would explain the low levels of inoculum detected during anthesis. Both the Ohio and North Dakota locations appeared to have wetness duration, RH and precipitation levels sufficient for infection by *G. zeae*. However, average ambient air temperature was 7EC lower at Wooster than in Fargo during crop anthesis, and may have also resulted in lower disease levels at Wooster. Based on this information we hypothesize that there are at least two critical periods of environmental conditions. The first critical environmental period occurs prior to head emergence and appears to be important for conidiogenesis, development of perithecia and ascospore maturation. The second critical period occurs while the crop is flowering and corresponds to inoculation and infection events. In the future, regional disease forecasts for FHB may be possible as more information from these and other wheat producing regions further defines the role of environment in the completion of FHB disease cycle events.

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Figure 1. The role of environment and *Gibberella zeae* inoculum level in the development of wheat Fusarium head blight was evaluated at Wooster, Ohio during the 1999 growing season. A bioassay was used to monitor the average daily number of colony forming units (CFU) per wheat spike in replicated field plots was used to monitor inoculum concentrations. Inoculum levels were combined with corresponding 24 h summaries of temperature, relative humidity, precipitation, and wetness duration.

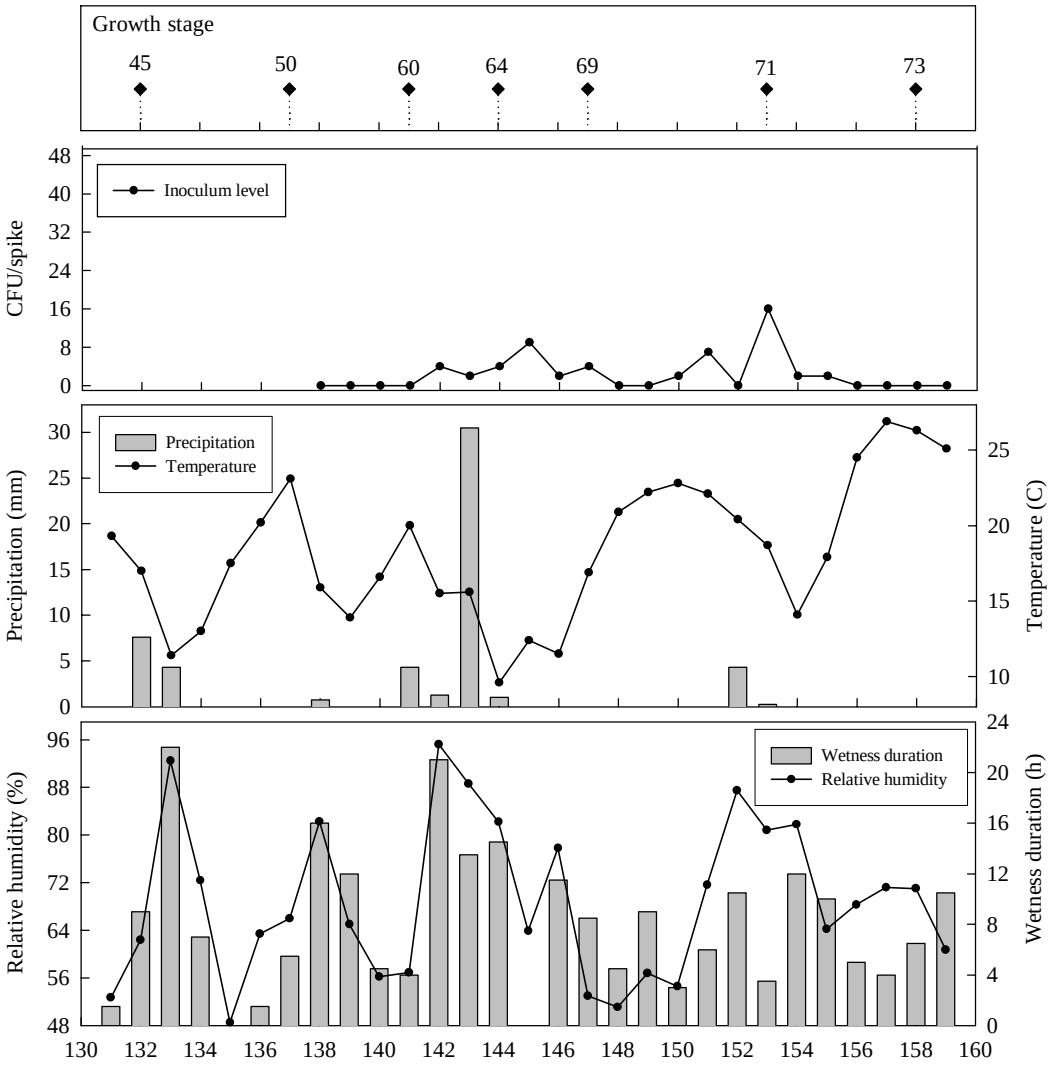
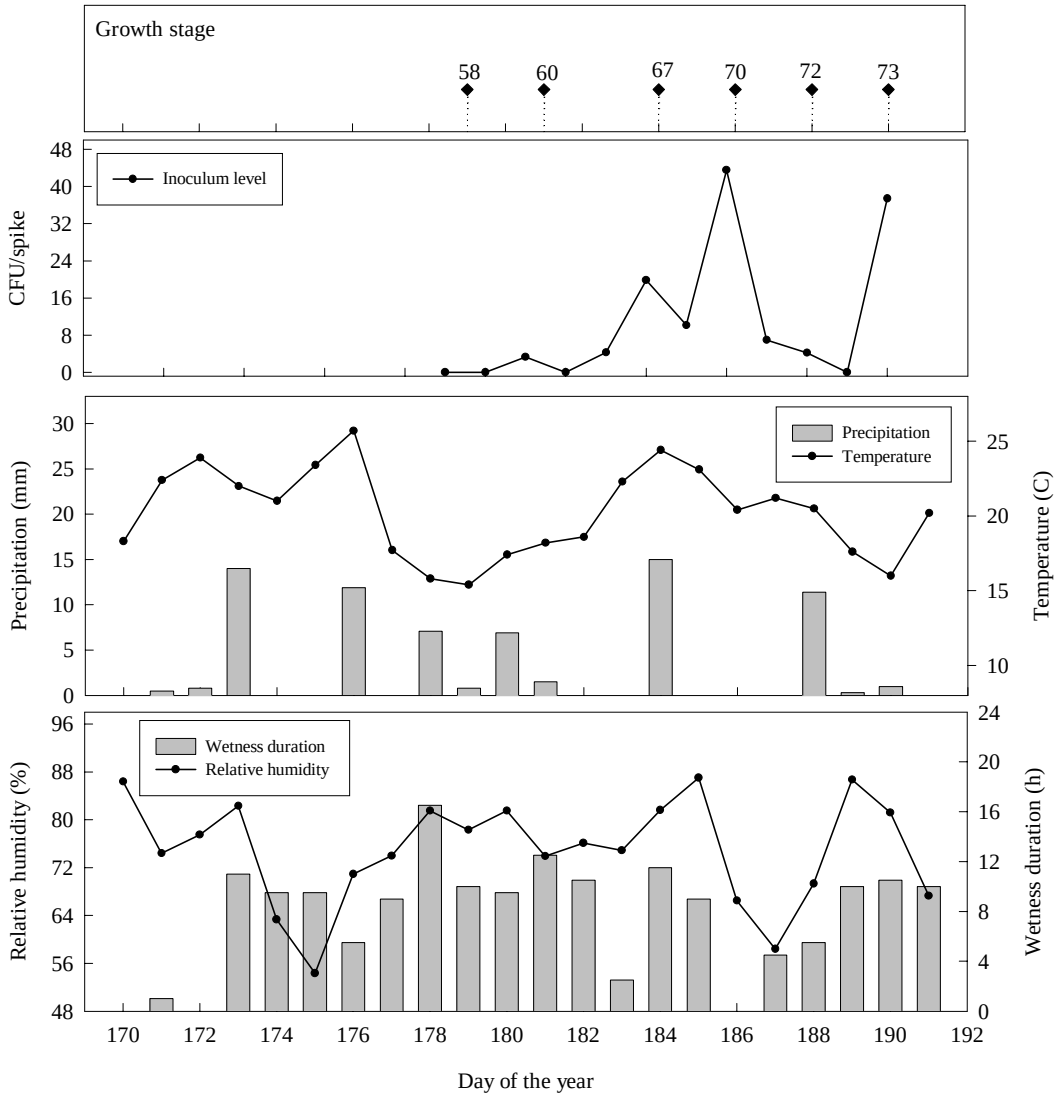


Figure 2. During the 1999 growing season in Fargo, North Dakota, the role of environmental conditions and *Gibberella zeae* inoculum levels in the development of wheat Fusarium head blight was evaluated. The daily average number of colony forming units (CFU) per wheat spike in replicated field plots was monitored with a bioassay. Inoculum levels were combined with 24 h summaries of temperature, relative humidity, precipitation, and wetness duration.



A WHEAT HEAD CULTURE SYSTEM TO SCREEN FOR PATHOGENICITY MUTANTS OF *GIBBERELLA ZEAE*

Iffa Gaffoor, Anita Davelos, and Frances Trail*

ABSTRACT

An *in vitro* assay was developed to screen mutants of *Gibberella zeae* PH-1 for loss of pathogenicity. Wheat heads at anthesis were excised and placed in culture medium prior to inoculation with a suspension of macroconidia. The wheat heads were then incubated for the development of symptoms. The success of this method was evaluated by comparing the rate of infection in excised wheat heads of Norm (a susceptible variety of wheat) to that in intact wheat heads and by assessing the stability of resistance of Ning (a resistant line of wheat) in excised heads.

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USE OF REMOTE PILOTED VEHICLES IN AEROBIOLOGICAL STUDIES OF *GIBBERELLA ZEAE*

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OBJECTIVE

To determine whether viable ascospores of *Gibberella zeae* are dispersed in the planetary boundary layer of the lower atmosphere, and thus, exhibit the potential for regional dissemination.

INTRODUCTION

Gibberella zeae (Schw.) Petch (anamorph *Fusarium graminearum* Schwabe) is the causal pathogen of head blight of wheat and barley. Ascospores are forcibly discharged from perithecia, becoming airborne and infecting wheat spikes during flowering (Paulitz, 1996). Knowledge of the vertical profile of fungal spores in the air is important for a better understanding of the pathogen's distribution. Since the transition zone between the surface boundary layer (SBL) and the planetary boundary layer (PBL) is considered to be 2-2.5 times the crop canopy height in agricultural settings (Huschke, 1989) sampling the atmosphere between 0-152m is critical for understanding the initiation of long-ranged transport of this pathogen. Knowledge of the aerobiology of *G. zeae* will contribute to a better understanding of the regional epidemiology of Fusarium head blight (FHB).

MATERIALS AND METHODS

Two 2.4-m wingspan remote piloted vehicles (RPV's) were used to collect ascospores of *G. zeae* in the lower atmosphere. RPV's sampling devices were vertically mounted Petri dishes containing Komada's agar 1.75% as a collection surface and were outfitted with the Petri dish top as a door which can be remotely open when sampling periods were initiated. Each sampling device has the capacity to sample an average of $8m^3$ of air per minute. A total of 60 collection flights were performed during June 7- June 30, 1999 at Cornell's Musgrave Agronomy Farm near Aurora, NY. The landscape features wooded areas and small agricultural fields of corn, soybean, oat, wheat and fallow fields. Collections were made at approximately 15m aboveground and most circular flight patterns overlapped a plot of winter wheat in which we scattered autoclaved corn kernels infested with *G. zeae* (Gz014NY98) on May 14. Due to dry conditions at anthesis in early June, no FHB developed in the inoculated winter wheat.

RESULTS AND DISCUSSION

The use of RPV's is a feasible method for collecting viable ascospores of *G. zeae* from the lower atmosphere. Viable ascospores were collected each night, under a range of atmospheric conditions, and even following 7 days without a local rainfall event (Fig. 1). Therefore, airborne inoculum of *G. zeae* was present prior to and through the anthesis period of local winter wheat. The fact that FHB did not develop can be attributed to a lack of moisture favoring infection of wheat florets, rather than to

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a lack of inoculum. Peak colony counts of *G. zeae* were obtained during evenings with heavy cloud cover (Table 1) which increases the vertical mixing of air currents in the lower atmosphere.

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Fig.1. Precipitation at Musgrave Agronomy Farm at Aurora (NY) during June, 1999.

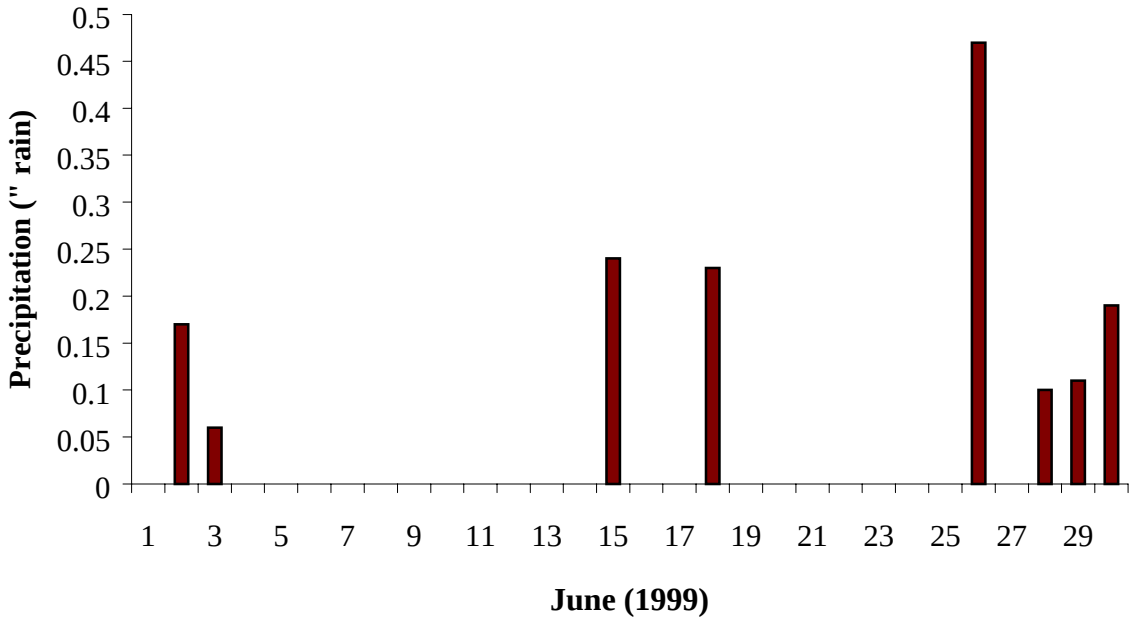

Table 1. Peak colony counts for *Gibberella zeae* collected using RPV s.

Date	Number of flights	Collection Time (PM)	Daily Temp (F)	Peak colony counts/15min	Flight conditions
June 7, 1999	3	6:30-8:15	93/67	10 @ 6:30	No cloud cover, no wind
June 8, 1999	6	6:30-9:30	95/66	27@ 9:30	No cloud cover, no wind
June 10, 1999	10	6:21-10:25	78/60	60 @ 9:15	Heavy cloud cover, very windy and cold
June 16, 1999	11	6:30-10:30	63/42	40 @ 8:40	Low cloud cover, no wind, cold
June 23, 1999	14	4:45-10:05	84/58	47 @ 4:45	Cloud cover, no wind, hot
June 25, 1999	5	4:40-5:45	90/67	23 @ 4:45	Hot, no wind, no cloud cover
June 30, 1999	11	4:50-9:45	78/49	87 @ 8:30*	Heavy cloud cover, no wind, cold

*flying 8m directly above inoculated wheat plot

SURVIVAL AND INOCULUM POTENTIAL OF FUSARIUM GRAMINEARUM IN WHEAT RESIDUES[1]

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OBJECTIVE

To determine the relationship between wheat residue decomposition and the survival and inoculum potential of *Fusarium graminearum*.

INTRODUCTION

Fusarium head blight (FHB) is economically important in wheat and barley worldwide (4,9). The disease has also become a major problem in the midwestern United States, producing devastating losses to growers in recent years (2,5). The principal pathogen associated with FHB in Minnesota is *Fusarium graminearum* Schwabe and its perfect stage *Gibberella zeae* (Schw.) Petch. (10).
F. graminearum survives saprophytically on residues of hosts like corn, wheat, barley and other cereals and some grasses (8). Management practices like crop rotation with non-host crops and plowing infected residues have been recommended to reduce primary inoculum of *F. graminearum* (1). The establishment of strategies for the management of *Fusarium* infected residue will require a thorough understanding of the viability and inoculum potential of *F.graminearum* in relation to residue decomposition.
A field experiment was established to examine the relationship between the decomposition of wheat residue and the survival and inoculum potential of *F. graminearum*. Data from this research may assist growers in establishing appropriate crop rotation periods and effective tillage systems in the management of *F.graminearum* infected residues.

MATERIALS AND METHODS

Stems of hard spring wheat residue (cv. Russ) in sections 24 cm long were placed in fiberglass mesh bags. Each bag contained 20 g of residue equivalent to 2857 kg/ha. Three hundred bags with inoculated residue (*F. graminearum* Group 2) were used for the determination of survival and inoculum potential of *F. graminearum.* Non-inoculated residue was used in a paired study of residue decomposition. Isolation from inoculated and non-inoculated nodes were made to determine the effect of the inoculation.

Field experiment

The residue was placed in the field in October 1997 at the Northwest Experiment Station, Crookston, MN.

Treatments:
1. Chisel plow, residue on soil surface
2. Chisel plow, residue buried at 7.5-10 cm depth
3. Chisel plow, residue buried at 15-20 cm depth
4. Moldboard plow, residue buried at 15-20 cm depth

Seventy-five samples in each treatment [15 residue bags (samples) in each strip, 5 replicates (strips)] were used to allow monthly samplings from April to October/November in 1998 and 1999. One set of samples from each strip was randomly collected during the sampling period. The residue was left undisturbed during the winter months when the ground was frozen.

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Determinations

The decomposition of the residue was determined by dry matter loss. Survival of *F. graminearum* was determined on stem pieces (30 per sample) 1.5 cm long including a single node. Pieces were surface disinfected, placed on pentachlorobenzene (PCNB) agar (6) and incubated at 20-22°C with 12 hr light per day for seven days. *'Fusarium'* colonies were counted and *F. graminearum* colonies were determined following transfer of 10 random colonies to carnation leaf agar (CLA) (3). CLA cultures were incubated at 20-22°C with 12 hr light per day for 10 days. Perithecia formation indicated *F. graminearum* Group 2 isolates. *Fusarium* colonies not forming perithecia were identified to species according to the descriptions of Nelson *et al*. (7).

Inoculum potential of *F. graminearum*: In April, May, July and September 1998, 24 stem/node pieces from each sample were surface disinfected and placed on sterile sand in culture boxes with vented lids. Nodes were incubated at 20-22°C with 12 hr light per day for 21 days and kept moistened. Ascospores were collected on silicone greased slides placed 2 cm above the nodes for 24 hours. The number of ascospores collected per slide and replicate were counted under the compound microscope. Due to reductions in the number of nodes recovered in November 1998 and subsequent samples, a weight of nodes was evaluated. Nodes with mature perithecia were placed in sterile distilled water (dilution 1:20) and a drop of Tween 20. Nodes were soaked for 10 hours to allow ascospore discharge and finally shaken for 10 minutes. Three aliquots (0.03 ml) were obtained from each treatment and used to determine the ascospore concentration.

Survival and inoculum potential data were subjected to ANOVA and treatments means were compared by Tukey test (P=0.05) using SAS (SAS Institute, 1985).

RESULTS AND DISCUSSION

F. graminearum was isolated from 96% and 97% of non-inoculated and inoculated nodes, respectively, suggesting that the inoculation had little impact on the colonization of wheat residue by *F. graminearum*.

Preliminary results (October 1997-May 1999) for the dry matter remaining and survival and inoculum potential *of F. graminearum* are presented in Tables 1, 2 and 3.

Buried residue decomposed substantially faster than residue at the soil surface (Table 1). No differences were evident in decomposition rate between the chisel plowed and moldboard plowed treatments buried at 15-20 cm. Approximately 20 and 50% of residue dry matter content remained in buried and surface residue treatments in May 1999, respectively.

Significant reductions in the colonization of residue by *F. graminearum* were observed once the residue had been in the field for at least eight months (Table 2) in those treatments where the residue had been buried. Even when a marked decreased in the survival of *F. graminearum* occurred in July 1998, the number of *Fusarium* spp. colonies isolated from residue samples tended to remain the same. This suggests that other species, probably with higher competitive ability, were colonizing the residue in preference to *F. graminearum*. Other *Fusarium* species recovered were: *F. sporotrichoides, F. equiseti, F. culmorum, F. semitectum, F. avenaceum, F. sambucinum, F. solani*.

Preliminary results of inoculum potential indicate that residue, from which *F. graminearum* can be isolated, support perithecial development and the production of mature ascospores (Table 3).

The reduction in the amount of residue colonization by *F. graminearum* is related to the decomposition of residues. Surface residues, which decomposed more slowly, seem to provide a host to *F. graminearum* for a longer period of time than buried residues. Residue from which *F. graminearum* can be isolated support perithecial development and ascospore production.

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Table 1. Percent wheat residue dry matter, relative to initial weight at placement (October 1997), at sampling times between April 1998 and May 1997 in four tillage/residue placement depth treatments.

	Percent dry weight of wheat residue remaining at sampling										
	1997	1998								1999	
Treatments	Oct.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Apr.	May
Chisel plow surface	100[p]	94	86	85	76	72	61	55	50	53	43
Chisel plow 7.5-10 cm	100	86	75	62	46	39	22	19	18	21	21
Chisel plow 15-20 cm	100	88	77	66	51	41	28	19	22	22	22
Moldboard plow 15-20 cm	100	87	79	68	55	41	32	28	21	23	22

[p] values given are the means of five replicates

Table 2. Percent colonization of the nodes of wheat residue by F. graminearum in four tillage/residue placement depth treatments at time of residue placement (October 1997) and at sampling times between April 1998 and May 1999.

	Colonization of wheat residues by *F. graminearum* (%)										
	1997	1998								1999	
Treatments	Oct.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Apr.	May
Chisel plow surface	96[q]	93	96	96a[r]	69a	76a	80a	78a	81a	63a	67
Chisel plow 7.5-10 cm	96	81	91	93ab	57ab	61ab	60ab	58b	77ab	61ab	63
Chisel plow 15-20 cm	96	97	83	80b	46b	40b	56ab	57b	69ab	61ab	54
Moldboard plow 15-20 cm	96	81	90	87ab	47b	40b	50b	52b	51b	45b	50

[q] values given are the means of five replicates

[r] values followed by different letters are significantly different at *P=0.05*

Table 3. Inoculum potential of *F. graminearum*.

Sampling month	Chisel plow surface	Chisel plow 7.5-10 cm	Chisel plow 15-20 cm	Moldboard plow 15-20 cm
April 1998	55[s]	97	48	56
May 1998	50	72	27	33
July 1998	39	48	20	23
September 1998	49	58	31	27
November 1998	10[t]	10	16	12
April 1999	34	72	30	7
May 1999	104a[u]	40ab	21b	105a

[s] Ascospores per cm^2

[t] Thousands of ascospores per g of residue

[u] Means followed by the different letters are significantly

EPIDEMIOLOGY OF FUSARIUM HEAD BLIGHT OF WHEAT IN INDIANA, 1999

David Thomas, George Buechley and Gregory Shaner*

Scab, or Fusarium head blight, has been a difficult disease to control throughout the world because of the lack of effective disease management practices. Traditional disease control options (disease resistant cultivars, crop rotations, tillage to destroy residues, and fungicides) have not been used because of lack of availability, excessive cost, or negative impacts on soil conservation (McMullen et al. 1997). Evaluation of these various management options has shown that no one approach is effective in controlling Fusarium head blight. Bringing Fusarium head blight under control will require multiple disease management strategies (Parry et al. 1995, Bai and Shaner 1994), coupled with greater understanding of the epidemiology of the disease.

MATERIALS AND METHODS

The experiment was conducted in a long-term tillage-rotation experiment at the Purdue Agricultural Research Center (PARC) in Tippecanoe County (west central Indiana). Wheat plots adjacent to plots that had corn residue from the 1998 crop year were the site of spore sampling and assessment of disease incidence and severity.

A Burkard Cyclone sampler was placed in the wheat plot when plants were at the boot stage of development (GS 45). A continuous corn plot that received minimum tillage was located just west of this plot.

The spore sampler was operated from 19 May to 13 June. The Burkard drew in air at a rate of 16.5 liters per minute. The Eppendorf collection tube was replaced every 24 hours, between 10:00 and 11:00 am each morning. The contents of Eppendorf tube were processed directly after sampling, or placed in a freezer for processing later.

Spores were recovered from the Eppendorf tube by adding 1.5 ml of sterile water, shaking on a Vortex mixer for 1 minute and spreading 1 ml of the suspension evenly over a petri plate containing Komada's medium. The plate was placed under 24-hr UV light at 25°C. A 5ml sample of the water remaining in the Eppendorf was examined with a hemacytometer.

On 18 May, approximately 500 wheat heads between growth stages 48 and 50 were selected along the west edge of the long-term tillage plot. They were covered with waxed paper bags to shield them from airborne *Gibberella zeae* spores. Beginning on 20 May, 5 heads were uncovered and tagged each morning between 10:00 and 11:00 am. The heads were collected at the same time the following morning and 5 more heads were uncovered and tagged. The collected heads were then covered with plastic and taken directly to the lab for processing. This process was repeated until 11 June.

The 5 heads were shaken in 50 ml of sterile water for 1 minute. Five 1-ml portions of the washing were transferred to each of five plates of Komada's medium, which were placed under 24 hr UV light at 25°C.

For all platings, when colonies were clearly visible on the plates (approx. 0.5 cm in

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diameter), all *F. graminearum* colonies were counted.

Blighted heads and total heads in 20 arbitrarily-selected 61-cm lengths of row were counted on 17 June. Scab incidence was calculated as the percent of scabby heads in the 20 samples. On 22 June, 50 scabbed heads were taken for estimation of severity of infection. The spikelets on each head were counted, as well as blighted spikelets. These heads were threshed by hand, and total as well as scabbed kernels were counted.

RESULTS

Daily average temperatures varied dramatically at the beginning of the flowering season, ranging from 21.7°C on day 142 to 10.3°C on day 145 (Fig. 1). Average daily temperatures rose from then, reaching 13.9°C by the end of flowering and 27.8°C by the end of the sampling period. Precipitation occurred on only 6 days during this period, for a total of 87 mm; 38 mm fell during flowering (days 141-146).

The Burkard sampler collected spores on most days from 23 May through 11 June, when sampling ceased (Fig. 2). Although the highest number of spores collected occurred on the day with maximum rainfall during the sampling period, there was no consistent relation between rainfall amount and number of spores collected. The number of spores collected during the time wheat was flowering was quite low. The spore counts rose after anthesis (growth stage 70, day 147).

In addition to direct enumeration with a hemacytometer of spores of *Gibberella zeae* collected by the Burkard sampler, washings from the Eppendorf tube were plated on Komada's medium. Estimates of the number of CFUs/ml by this method were considerably lower than estimates from the hemacytometer counts (Fig. 3). There was a low correlation between the hemacytometer counts and the counts from Komada's medium (R=0.46). The CFU counts from the Komada's platings showed less relationship to precipitation than did the hemacytometer counts.

Air spora was also estimated by exposing heads for 24-hr periods and then washing them and plating the washing on Komada's medium. As with the Burkard samples, this method detected propagules of G. zeae on most days (Fig. 4). However, propagules were detected on only 2 days during anthesis, and this was toward the end of anthesis.

The mean incidence of scab calculated from the 20 61-cm field samples was 6.6% (st. dev.= 3.7%). On those heads that were blighted, mean severity was 29.9% (st. dev.= 23%). Mean incidence of scabby kernels from blighted heads was 8.2% (st. dev.= 11%).

DISCUSSION

Declining temperatures and only two, nonconsecutive rain events during anthesis evidently prevented severe development of head blight. Three consecutive days with rain occurred during the late milk stage of development, in conjunction with moderate temperatures. A large spore flight was detected on the third day of rain, but this evidently did not result in heavy infection. As found in a previous study (Francl et al. 1999), there was no clear association between precipitation and number of spores detected.

The spore number estimates derived from direct examination of the Eppendorf tube washing with a hemacytometer were more than two orders of magnitude greater than the estimates obtained by plating the washing on Komada's medium. There was also a low correlation between the methods of estimation. This discrepancy

between the two methods may arise in part from crowding of colonies on the Komada's plates. On the days where the hemacytometer counts revealed large numbers of spores, competition among colonies on the Komada's medium would probably have prevented all potential colonies from developing. Several platings resulted in the development of nearly 200 colonies, which covered the entire surface of the Komada's medium. A concentration of 200 CFUs/ml is probably the maximum for a reliable count on Komada's medium without further dilution of the Eppendorf tube washing. Dilution problems were evidently not the only reason for discrepancy between the two types of data, however. Given that competition may have restricted the number of colonies on Komada's medium, we regard the hemacytometer counts as a more reliable representation of the ambient spore concentrations.

Although the mean severity of blight on symptomatic heads was 30%, the incidence of scabby kernels from these heads was much lower. The likely explanation for this difference is that many of the scabby spikelets contained no kernels when dissected.

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Fig.1: Temperature and precipitation data from the beginning of anthesis to the end of sampling Day 140 is May 20.

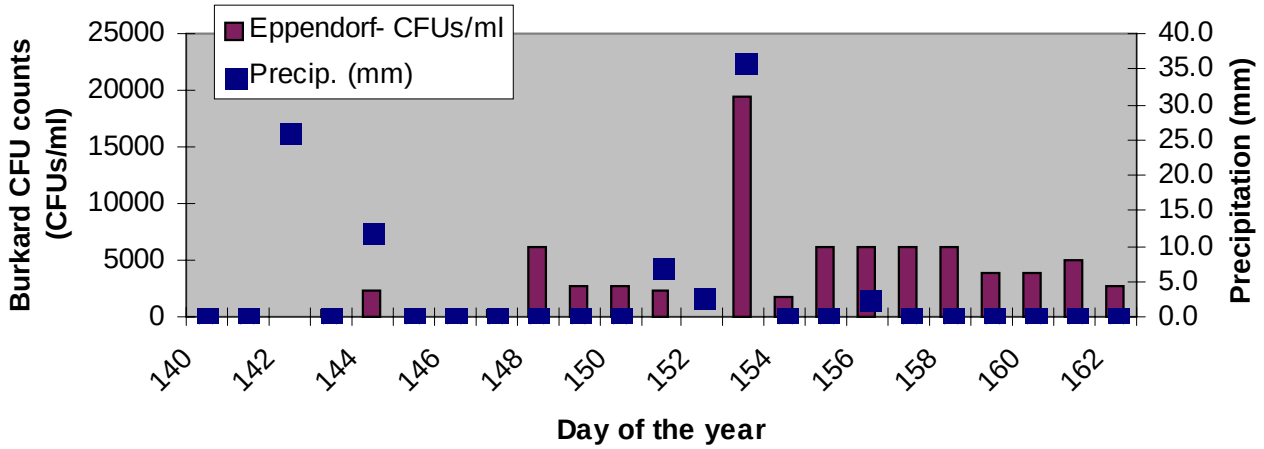


Fig.2: Hemacytometer counts of spores of *Gibberella zeae* from the Burkard sampler compared to precipitation data.

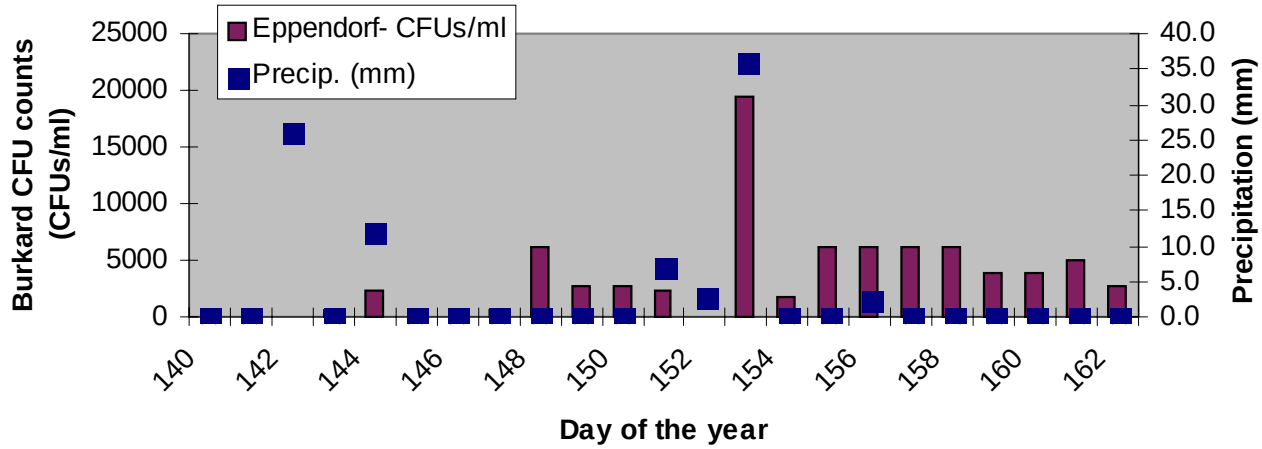


Fig.3: Concentrations of propagules of *Gibberella zeae* recovered from wheat heads exposed for 24 hours (calculated from Komada's plating) compared to precipitation.

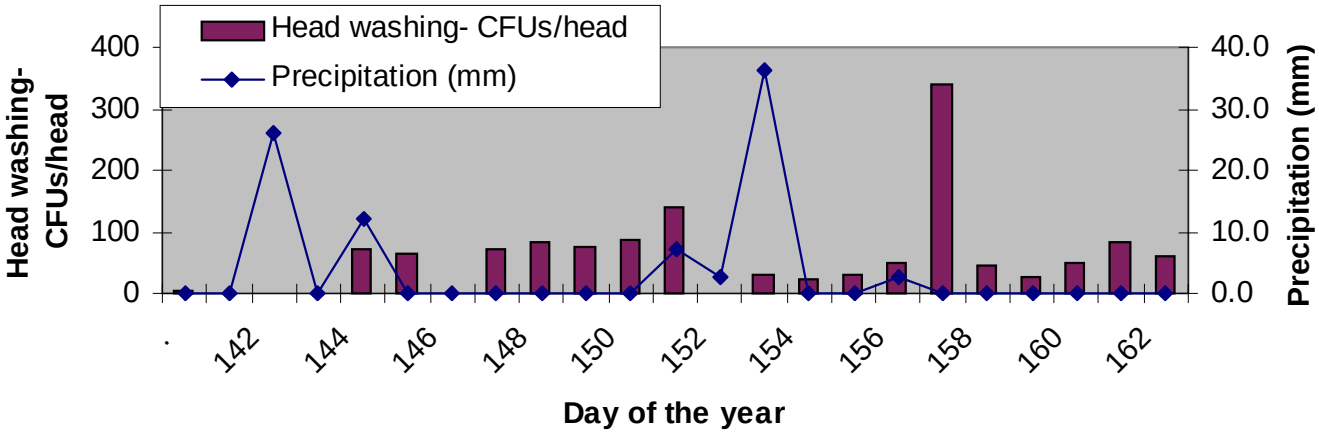
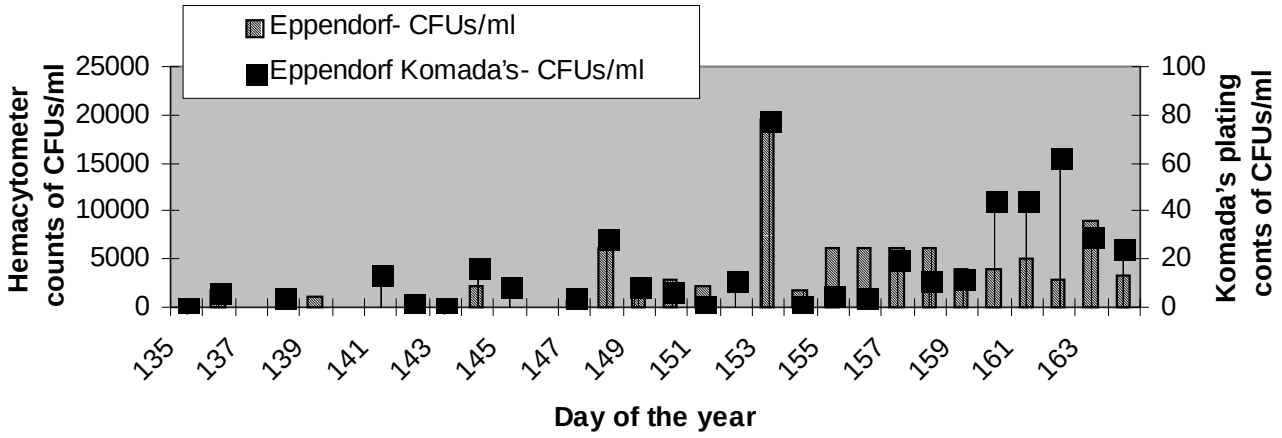


Fig.4: Comparison of counts of propagules of *Gibberella zeae* collected by a Burkard sampler using hemacytometer (bars) and by counting colonies on Komada's medium (squares).



PLANT RESIDUE IN THE CONTROL OF FUSARIUM HEAD BLIGHT

Robert L. Todd*, Robert Stack, Edward Deibert, and John Enz

ABSTRACT

Many approaches have been taken to control the disease Fusarium Head Blight, such as development of resistant-varieties, chemical control, crop rotation, and recently, attention to residue management. The fungus, *Fusarium graminearum*, causing Fusarium Head Blight persists and multiplies on infected crop residues of small grains and corn. It is the consensus of many investigators that *Fusarium* control via residue management may provide one means to control this disease. Treatments might include "plowing down" residue or use of nitrogen fertilization to enhance the decomposition process.

The research presented in this review is part of an ongoing investigation to establish the correlation between residue management and the survival of *Fusarium*. Residue decomposition and fusarium survival are quantified when wheat, barley and corn plant residues are placed on and below the soil surface. Cover crop and nitrogen (N) fertilizer treatments are included as well as monitoring parameters related to decomposition such as soil temperature and water, carbon to nitrogen ratio and lignin content of the residue. If *Fusarium graminearum* survival is related to residue decomposition, then residue management strategies which enhance displacement of *Fusarium* might be developed. Since residue decomposition is a microbial process, manipulation of the indigenous microorganisms might accelerate the loss of *Fusarium.*

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ASCOSPORE FORMATION AND DISCHARGE IN *GIBBERELLA ZEAE*

Frances Trail*, Corrie Andries and Haixin Xu

ABSTRACT

Gibberella zeae infects wheat and barley heads in flower by airborne ascospores that are forcibly ejected from perithecia produced on corn and wheat debris. We have been monitoring the timing of perithecium formation on crop debris in fields in southern Michigan since 1997. Since perithecia are ephemeral, the timing of formation may be critical to control of head scab. Results of our survey will be presented. We have completed a study of the development of perithecia in culture to coincide with our ongoing study of perithecium development on crop debris. The structure of the perithecium has implications on its ability to function effectively in ascospore dispersal. The cellular structure of the crop debris may also have important implications on perithecium formation.

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AFLP DIVERSITY OF *GIBBERELLA ZEAE*

K. A. Zeller, R. L. Bowden*, and J. F. Leslie

ABSTRACT

Fifty wheat heads with scab were sampled from a 0.25-m^2 quadrat in Kansas in 1993 and a similar quadrat was sampled in North Dakota in 1994. Isolations were made from the top, middle, and bottom of each head. For both quadrats, AFLP banding patterns showed that more than one AFLP haplotype colonized most heads. Several haplotypes appeared on more than one head, suggesting secondary colonization. AFLP haplotypes correlated very well with a previous vegetative compatibility group (VCG) diversity study on the same isolates. Allele frequencies in the two quadrats were similar. This suggests little genetic differentiation over large distances in the Great Plains region. Further studies with greater sample sizes are planned.

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DIAGNOSTIC VOMITOXIN (DON) SERVICES IN 1999-2000

Howard H. Casper

INTRODUCTION

Fusarium Head Blight (FHB) emerged in the 1990's as an important problem for American agriculture. Resolving the FHB problem involves cooperative efforts and a multidisciplinary approach, including analytical assays for vomitoxin in the new wheat and barley varieties. In 1999, the US Wheat and Barley Scab Initiative provided grants, for diagnostic vomitoxin (DON) services, to 4 laboratories in Michigan, Minnesota, and North Dakota. The following information provides an insight into the methods, quality assurance and number of samples processed by each of these laboratories.

MATERIALS AND METHODS

The following information provides names, addresses and analytical techniques for the 4 laboratories. The 3 laboratories in Minnesota and North Dakota all use the method of Tacke (1), followed by GC/EC or GC/MS quantitation.

1. MICHIGAN
Lab Director: L. Patrick Hart, Ph.D., Dept. of Botany & Plant Pathology, Michigan State University, East Lansing, MI 48824, Phone: 517-353-9428, Fax: 517-353-5598,
e-mail: hartl@pilot.msu.edu
Method: Water extraction and DON quantitation with the Neogen Veratox kit
Sample Types: Wheat and barley
Intralab Quality Assurance: Wheat Pool (2.2 ppm DON)

2. MINNESOTA
Lab Director: Weiping Xie, Ph.D., Dept. of Plant Pathology, University of Minnesota, 495 Borlaug Hall, 1991 Upper Buford Circle, St. Paul, MN 55108, Phone: 612-625-2751, Fax: 612-625-9728, e-mail: weipingx@puccini.crl.umn.edu
Method: Acetonitrile: water extraction, silylation and DON, 15 A-DON, 3 A-DON quantitation by GC/MS, plus a screen for 8 other trichothecenes.
Sample Types: Wheat and barley
Intralab Quality Assurance: Wheat Pool (12.4 ppm)

3. NORTH DAKOTA
Lab Director: Howard H. Casper, Ph.D., Dept. Vet. and Micro. Science, North Dakota State University, Fargo, ND 58105, Phone: 701-231-7529, Fax: 701-231-7514,
e-mail: hcasper@ndsuext.nodak.edu
Method: Acetonitrile: water extraction, silylation and DON, Nivalenol, 15 A-DON, quantitation by GC/EC. Full screens for 17 mycotoxins can also be done by GC/MS.
Sample Types: Wheat and barley
Intralab Quality Assurance: Wheat Pool (1.8 ppm DON), Malt Pool (1.5 ppm DON), Barley Pool (3.2 ppm DON).

4. NORTH DAKOTA
Lab Director: Paul B. Schwarz, Ph.D., Dept. Cereal Science, North Dakota State University, Fargo, ND 58105, Phone: 701-231-7732, Fax: 701-231-7723
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Method: Acetonitrile: water extraction, silylation and DON quantitation by GC/EC
Samples Types: Barley and malt products
Intralab Quality Assurance: Malt Pool (2.0 ppm DON)

5. PROFICIENCY CHECK SAMPLES

During the FHB meeting in Michigan in 1998, there was some discussion on the exchange of check samples between the 4 laboratories. In August of 1999, North Dakota State University (NDSU) collected wheat and barley samples from local sources and distributed these samples on a monthly basis. A wheat sample and a barley sample was sent on each occasion and the data was collected from each laboratory within one week. Each laboratory did the DON analyses in their normal fashion. These check samples allowed each laboratory to evaluate the accuracy and precision of their system and whether corrective actions were necessary.

RESULTS AND DISCUSSION

The information that was available from the 4 laboratories pertaining to quality assurance, number of samples analyzed and proficiency check samples is listed in Tables I, II and III.

The data in Table I shows that the intralab coefficient of variation for the 4 labs varies from 6 to 16% on the control pools that were analyzed with the test samples. This is a reasonable precision considering the objective of separating the high versus low producers of vomitoxin. The interlab proficiency check samples demonstrated that the 4 laboratories are getting similar results, and there were no major differences between the labs. Considering the intralab coefficient of variation, it is unlikely that there is a significant statistical difference between the different labs. The ELISA kit (2) provided a reasonable intralab coefficient of variation and the overall data was

Table I. Intralab Vomitoxin Quality Assurance (QA) Data for July November, 1999.

1.	MICH P. Hart	QA: Wheat Pool;	n = 24,	Ave = 2.2 ppm,	cv = 9%
2.	MINN W. Xie	QA: Wheat Pool;	n = 31,	Ave = 12.4 ppm,	cv = 6%
3.	ND P. Schwarz	QA: Malt Pool;	n = 98,	Ave = 2.0 ppm,	cv = 16%
4.	ND H. Casper	QA: Wheat Pool;	n = 42,	Ave = 1.8 ppm,	cv = 9%
		: Malt Pool;	n = 42,	Ave = 1.5 ppm,	cv = 10%
		: Barley Pool;	n = 42,	Ave = 3.2 ppm,	cv = 9%

Table II. Estimated Vomitoxin Assays for 1999 2000.

	Method	PIs	States	Samples	CV
MICH P. Hart	ELISA	~9	~8	~2,200	~9%
MINN W. Xie	GC/MS	~13	~1	~3,400	~6%
ND H. Casper	GC/ECD	~8	~4	~3,800	~9%
ND P. Schwarz	GC/ECD	~7	~2	~3,500	~16%

PIs = Research scientists supported by DON assays
CV = Coefficient of variation for control pools

Table III. Interlab Vomitoxin Proficiency Check Samples.

			ppm DON				
Lab	**Method**	**Grain**	**Aug. 99**	**Sep. 99**	**Oct. 99**	**Nov. 99**	**Ave.**
MICH P. Hart	Elisa	Wheat	3.7	1.6	4.8	12.5	5.6
MINN W. Xie	GC/MS	Wheat	2.6	1.0	4.0	14.4	5.5
ND H. Casper	GC/EC	Wheat	3.0	0.9	3.2	12.2	4.8
ND P. Schwarz	GC/EC	Wheat	3.0	0.8	2.0	9.6	3.9
Ave.			3.1	1.1	3.5	12.2	
MICH P. Hart	Elisa	Barley	1.1	7.4	6.4	2.4	4.3
MINN W. Xie	GC/MS	Barley	1.0	6.8	6.5	3.3	4.4
ND H. Casper	GC/EC	Barley	1.0	6.2	5.9	3.0	4.0
ND P. Schwarz	GC/EC	Barley	0.9	5.0	5.2	2.2	3.3
Ave.			1.0	6.4	6.0	2.8	

not significantly different from the chromatographic techniques. One NDSU lab (P. Schwarz) might be coming in slightly lower than the other 3 labs and this difference is being evaluated by Dr. Schwarz. Similarly, the other NDSU lab (H. Casper) experienced a higher coefficient of variation (~9%) for 1999 than he saw in 1998 (~6%) and will be evaluating this factor. The vomitoxin assay by chromatographic techniques does have certain steps (i.e., glassware) that may cause variation, but the cause and effect are not well defined. Information that is accumulated at each of the chromatographic laboratories, on improved precision, will be shared. In the research campaign for 1999-2000, we estimate that ~13,000 samples will be processed by the 4 laboratories for ~37 principal investigators in ~15 states. The 4 laboratories have met the criteria of the grants from the US Wheat and Barley Scab Initiative and probably processed more samples than originally planned.

The interlab proficiency check samples will probably be continued in the FHB research campaign for 2000-2001. Each laboratory will be evaluating means of refining the analytical techniques for improved precision, speed and broader scope of mycotoxin analysis.

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VALIDATING SAMPLING STRATEGIES FOR VOMITOXIN IN THE MIDWESTERN US

L. Patrick Hart[1], Oliver Schabenberger[2], and Fanzhi Kong[3]

Previously, we investigated the bias and inconsistency due to spatial heterogeneity in toxin distribution in estimating deoxynivalenol (DON) concentrations when sampling kernels from truck loads of wheat with standard seed probes (Schabenberger et al, 1998). A probabilistic model was developed for distributing deoxynivalenol contaminated kernels throughout a lot or bin according to a fixed or random spatial pattern of toxin intensity. Results of a simulation study were compared with data gathered throughout Michigan from fourteen trucks during the 1996 Fusarium Head Blight epidemic. This comparison provided supporting evidence that vomitoxin distribution throughout a truck load may be clustered rather than homogeneous. Therefore, if spatial heterogeneity is not properly represented in the sample, confidence intervals for DON are centered around the wrong value and are less informative for larger sample sizes. In 1998, a more intensive statistical study of sampling was used to validate the 1996 study. Spatial heterogeneity in toxin distribution was greater in 1998 compared to 1996. The importance and possible sources of the differences are discussed. Developing and supervising appropriate sampling strategies remains of critical importance.

INTRODUCTION

Wheat scab, also known as Fusarium Head Blight (FHB) is a serious problem in grain producing regions of the Midwestern U.S. (McMullen et al 1997). An estimated 90 million bushels of wheat were lost in North Dakota during head blight epidemics in 1993.

Contamination of the grain with deoxynivalenol (DON, vomitoxin) is the most serious consequence of infection apart from reductions in grain yield and quality. The toxicological properties and mammalian toxicity of deoxynivalenol (Rotter et al. 1995) prompted the U.S. Food and Drug Administration in 1993 to revise earlier advisory levels for deoxynivalenol in wheat entering the milling process, shifting emphasis to concentrations in finished products (FDA 1993). Since the advisory levels create thresholds for marketing of wheat, detecting the deoxynivalenol contamination level in scab infected wheat accurately and precisely has gained critical importance in terms of food quality and agricultural economics (Hart et al. 1998).

A general recommendation is that manual probes should be inserted to at least 75% of the lot depth. When sampling from large containers such as railcars or ships, this recommendation cannot be followed with seed probes of standard length. In addition, based on the 1996 statistical study (Hart et al, 1998) a minimum of four probes per truck were necessary to predict levels of DON within 1 ppm of the upper limit with ninety-five percent confidence.

In this contribution, we discuss the findings of a 1998 statistical study of sampling designed to validate the 1996 study, and to provide additional information on deoxynivalenol distribution. The following changes were made in the 1998 study: 1) spring wheat from N. Dakota was used in place of winter wheat; 2) five trucks were sampled instead of fourteen; 3) twenty-five

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probes per truck instead of ten were collected, and the contents of each probe were divided into four equal parts based on depth.

RESULTS AND DISCUSSION

Approximately one hundred samples per truck were analyzed by ELISA (Hart et al, 1998). The probe to probe variance for DON distribution for 1996 and 1998 data is shown in Figure 1. The between probe variance for three out of the five trucks sampled in 1998 was outside the range of variances from 1996. Why the variation was greater in the 1998 study is not known, but it could be related to specific factors associated with this particular epidemic, or there may be greater variability associated with spring wheat when compared to winter wheat. Figure two shows the actual distribution of DON within the "Orange" and "Red" trucks as examples of distribution in all of the trucks. To determine coverage probabilities for DON estimates we examined all possible probe combinations of n samples from twenty-five truck probes assuming the overall sample mean DON is the true concentration. When N=4 there are 12,650 possible combinatons, and when N=7 there are 480,700 (Table 1). As in 1996, a minimum of four probes was required to obtain 95% coverage probability, but the interval width was three times higher than in 1996. Therefore, in 1998 at the 95% confidence level the mean is within 3 ppm of the estimated truck mean (1.5 ppm above and below the estimated mean) vs 1 ppm (0.5 ppm above and below the estimated mean) in 1996. The two trucks with mean DON levels below 10 ppm achieved 95% coverage probability for 2 ppm (1 ppm above and below the mean). This is in accordance with the higher variability of the trucks with mean DON concentrations above 10 ppm.

Increasing the number of probes to six per truck increases the 95% confidence level to 1 ppm above and below the mean for four of the five trucks. Economically, six probes may not be an acceptable practice. We have not determined if DON concentrations in a truck can be accurately estimated by pooling wheat from the individual probes, milling the entire sample, and testing a specific volume sub sampled from the pooled wheat. Year to year and regional variability needs to be addressed further. The affect of storage and grain handling may contribute to decreased variability.

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Table 1. Confidence Interval Probabilities (above and below the mean DON concentration for each truck) for N=4, 5, 6, and 7 probes. Standard deviation for DON (stddon) is the probe-to-probe variance for each truck, the DON average (mndon) is the average DON of 25 probes (in ppm) assumed to be the true concentration on that truck.

			Confidence Interval above and below the mean							
Obs	YEAR	TRUCK	0.5	1.0	1.5	2.0	2.5	3.0	mndon	stddon
1	98	Black	0.54	0.87	0.98	1.00	1.00	1.00	11.7	1.43
2	98	Blue	0.55	0.89	0.99	1.00	1.00	1.00	11.8	1.38
3	98	Brown	0.68	0.96	1.00	1.00	1.00	1.00	9.3	1.07
4	98	Orange	0.79	0.99	1.00	1.00	1.00	1.00	5.9	0.87
5	98	Red	0.50	0.84	0.97	1.00	1.00	1.00	14.6	1.55
		Confidence Interval Probabilities for N=5 probes								
1	98	Black	0.61	0.92	0.99	1.00	1.00	1.00	11.7	1.43
2	98	Blue	0.62	0.93	1.00	1.00	1.00	1.00	11.8	1.38
3	98	Brown	0.75	0.99	1.00	1.00	1.00	1.00	9.3	1.07
4	98	Orange	0.85	1.00	1.00	1.00	1.00	1.00	5.9	0.87
5	98	Red	0.57	0.89	0.99	1.00	1.00	1.00	14.6	1.55
		Confidence Interval Probabilities for N=6 probes								
1	98	Black	0.66	0.95	1.00	1.00	1.00	1.00	11.7	1.43
2	98	Blue	0.68	0.96	1.00	1.00	1.00	1.00	11.8	1.38
3	98	Brown	0.81	1.00	1.00	1.00	1.00	1.00	9.3	1.07
4	98	Orange	0.89	1.00	1.00	1.00	1.00	1.00	5.9	0.87
5	98	Red	0.62	0.93	1.00	1.00	1.00	1.00	14.6	1.55
		Confidence Interval Probabilities N=7 probes								
1	98	Blakc	0.75	0.98	1.00	1.00	1.00	1.00	11.7	1.43
2	98	Blue	0.72	0.98	1.00	1.00	1.00	1.00	11.8	1.38
3	98	Brown	0.85	1.00	1.00	1.00	1.00	1.00	9.3	1.07
4	98	Orange	0.93	1.00	1.00	1.00	1.00	1.00	5.9	0.87
5	98	Red	0.70	0.97	1.00	1.00	1.00	1.00	14.6	1.55

Figure 1. Probe-to-probe variances for data from 1996 and 1998.

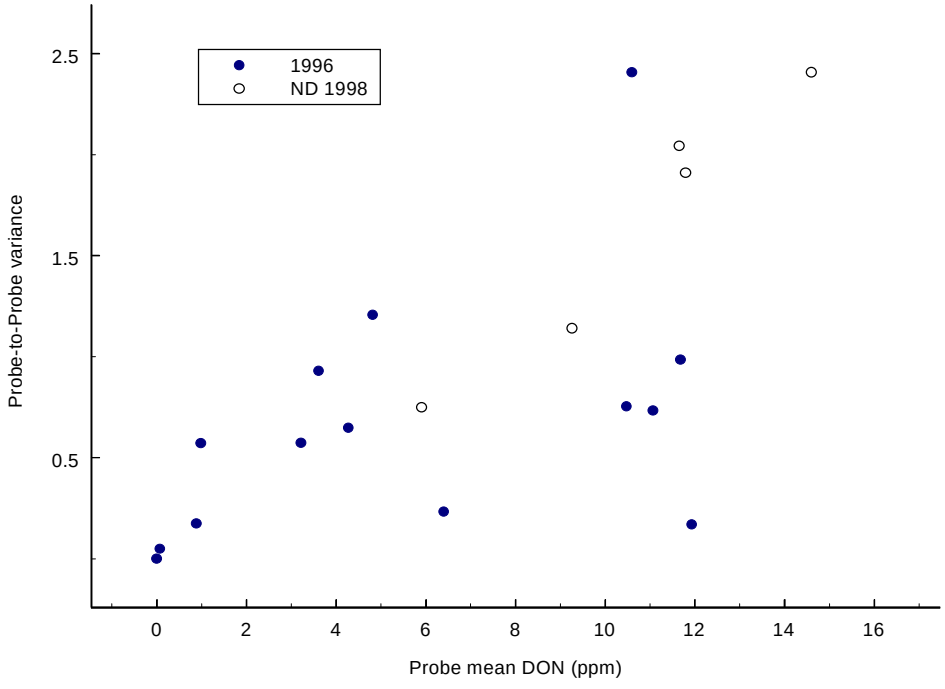
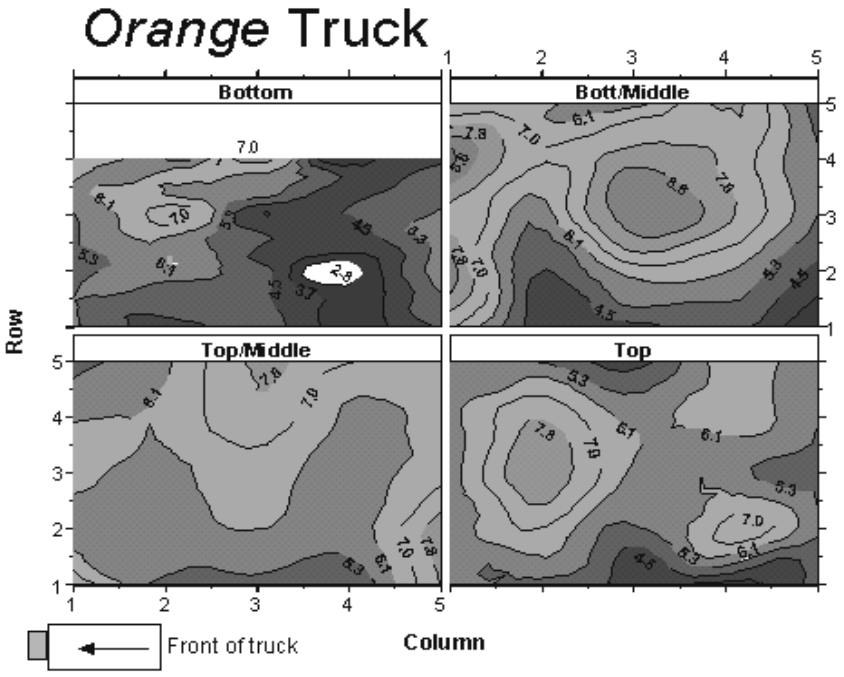
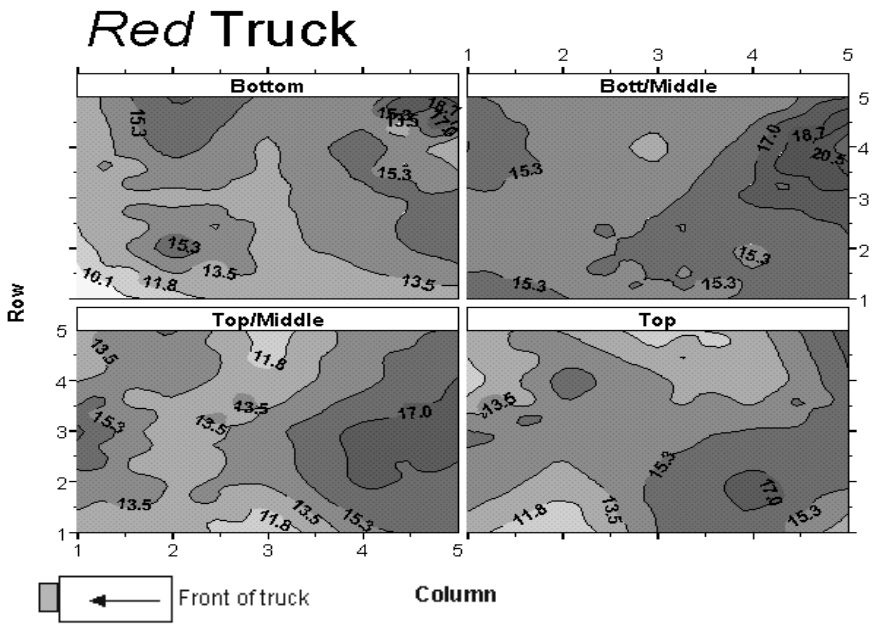


Figure 2. Distribution of deoxynivalenol through four depths, and at different probe insertion points for the "Orange Truck". Deoxynivalenol mean was 5.9 ppm, based on the analysis of one hundred samples (twenty five proes/truck, and four subsample/probe) with a range between 2.8 and 8.6 ppm. "Red Truck" similar to "Orange Truck". Mean deoxynivalenol was 14.6 ppm, with a range between 10.1 and 20.5 ppm.





VOMITOXIN (DEOXYNIVALENOL) INDUCES CYTOKINES AND APOPTOSIS IN HUMAN T CELLS

J.J. Pestka*, R. Uzarski, S.Ross, A.Randell, and G-H. Yang

ABSTRACT

In various well-characterized experimental rodent models, the primary cellular target for vomitoxin (VT) and other trichothecenes produced by *Fusarium* are leukocytes of the immune system. We have hypothesized that the levels of VT and closely related 8-ketotrichothecenes required for toxicity will be identical in mouse and human leukocytes. We have initiated experiments using cloned human Jurkat cells. First, it was observed that VT was indeed capable of inducing apoptosis in this cell line in vitro as determined by changes in cell morphology. Second, it was also observed in T cells that VT could superinduce expression of the cytokines IL-2, IL-4 and IL-5. These results mimic those observed in the mouse model. We are now performing detailed dose response and structure function studies. Third, it was observed that VT rapidly turns on a group of stress-activated protein kinases known as MAP kinases. The results presented here suggest that VT can affect human and mouse leukocyte cell function and viability in a similar fashion. Although the levels required for cytotoxicity (ie. apoptosis induction) were similar, the human Jurkat T cells appeared to be slightly more sensitive to cytokine superinduction then has been previously observed for rodent cell cultures. MAP kinases are involved in signaling cascades that can turn on or off many cellular activities such as apoptosis or cytokine production.

INTRODUCTION

Most food-borne illnesses in developed countries are attributable to microbiological contamination. Not surprisingly, there has been a sharp upsurge in national public interest about microbial and chemical food safety during the past few years. The trichothecene mycotoxins are naturally and frequently-occurring contaminants frequently found in grain-based foods. They are a group of sesquiterpenoid metabolites produced by *Fusarium* and other fungi that include some of the most potent eukaryotic protein synthesis inhibitors known. Concern over the trichothecene mycotoxins is due primarily to (1) their potential adverse effects on human and animal health], (2) their unavoidable capacity to contaminate agricultural, (3) their recalcitrance to degradation during milling or processing , (4) economic losses associated with reduced efficiency of livestock production and through the discarding of highly-contaminated wheat or corn.

In various well-characterized experimental rodent models, the primary cellular target for VT and other trichothecenes produced by *Fusarium* are leukocytes of the immune system. We have hypothesized that the levels of VT and closely related 8-ketotrichothecenes required for toxicity will be identical in mouse and human leukocytes. To examine the effects of trichothecenes on human leukocytes, we have initiated experiments using cloned human Jurkat T cells.

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EXPERIMENTAL

1) Cell lines and Cell Culture. Human Jurkat T cells were obtained from American Type Culture Collection (Rockville, MD). These will be maintained at 37^0C, 5% humidified CO_2 in RPMI-1640 medium supplemented with 10% fetal bovine and 2 mM glutamine, plus penicillin (100U/ml) and streptomycin (100mg/ml). Experiments were carried out in the absence of or graded doses of VT.

(2) Cytotoxicity Assay. Cytotoxicity was evaluated by th eMTT assay. Briefly, cells were cultured for 24 hr with and without activating agent in the presence or absence of VT in 96 well plates (100 μl/well) as described above . MTT (5 mg/ml) will then be added and plates will be incubated for another 5 hr. SDS (10% in 0.01 N HCl) (100 μl/well) will then be added, wells incubated for 16 hr, and then OD590 will be determined using ELISA reader.

(3) Apoptosis. Acridine orange-ethidium bromide staining was used to determine apoptotic and necrotic cells in the above described cultures.

(4) Cytokine Quantitation. Supernatant was collected from appropriate plates at different time points. Cytokine production was determined by ELISA using corresponding human recombinant cytokine standards, purified anti-human cytokine antibodies, and biotinylated anti-human cytokine antibody R&D Systems.

RESULTS

1. VT induced cytotoxic effects at 500 ng/ml (Fig. 1).

2. VT induced apoptosis at 1000 ng/ml (Fig. 2).

3. VT could superinduce expression of the cytokines IL-2, IL-4 and IL-5 (Fig. 3 and 4). These results mimic those observed in the mouse model.

4. It was observed that the VT rapidly turns on a group of stress-activated protein kinases known as MAPkinases. These are involved in signaling cascades that can turn on or off many cellular activities such as apoptosis or cytokine production.

DISCUSSION

Potential regulations that would lower the tolerance level for VT in wheat and wheat products either in the U.S. or other countries could threaten the ability of U.S wheat, barley and resultant products to compete in the national and global economies. Should such regulations be proposed, it is absolutely essential that basic information be available relative to human sensitivity. Despite the importance of this issue, to our knowledge, other than our research group, there are no other labs in the U.S. or world that are currently studying toxicity of VT and related 8-ketotrichothecenes at the cellular/ molecular level or have plans for evaluating its potential effects on humans.

The results presented here suggest that VT can affect human and mouse leukocyte cell function and viability in a similar fashion. Although the levels required for cytotoxicity (ie. apoptosis induction) were similar, the human Jurkat T cells appeared to be slightly more sensitive to cytokine superinduction then has been previously observed for rodent cell cultures. These studies will be repeated using additional dose levels of VT and time points to extend this comparison.

It was particularly striking that we have observed that VT rapidly turns on a group of stress-activated protein kinases known as MAPkinases.

These are involved in signaling cascades that can turn on or off many cellular activities such as apoptosis or cytokine production. Interestingly, MAP kinases or evolutionarily conserved and thus can be found in plant and fungi. The ability of trichothecenes to alter cell function in this manner may have relevance to virulence in plants and to signaling within the fungus.

Figure 1. Effect of VT on MTT Conversion By Human Jurkat T Cells

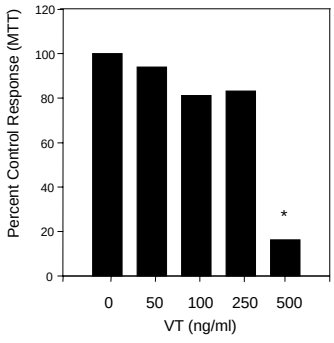


Figure 2. Effect of VT on Apoptosis of Human Jurkat T Cells

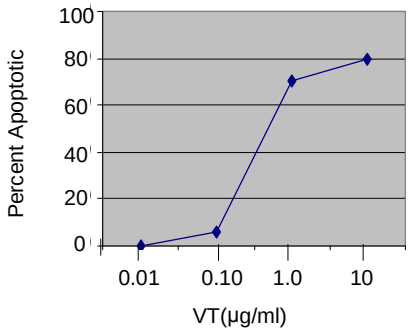


Figure 3. Effect of VT on Interleukin 2 Production By Human Jurkat T Cells

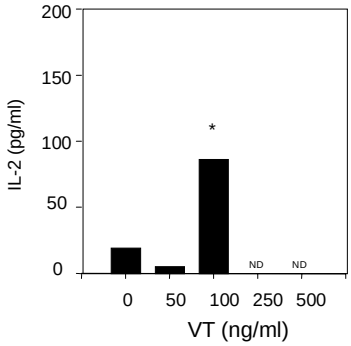
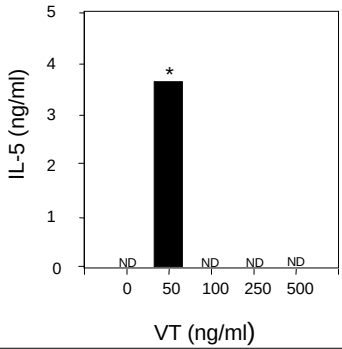


Figure 4. Effect of VT on Interleukin 5 By Human Jurkat T Cells



WHEAT AND BARLEY UTILIZATION RESEARCH, 1999-2000

Paul B. Schwarz

INTRODUCTION

Fusarium Head Blight (FHB) can damage grain quality, and contamination with mycotoxins can lower the acceptability of infected samples for food and feed. The widespread occurrence of FHB since the early 1990's has consequently reduced the amount of wheat and barley available to end-users. The ultimate solution to FHB problems will be development of resistant or tolerant varieties. However, in the interim, methods which can lead to greater utilization of FHB infected grain are of significance.

In 1999, the US Wheat and Barley Scab Initiative provided grants, for utilization research to four laboratories in Michigan, Nebraska and North Dakota. The following provides information as to the objectives and approaches of each of these laboratories.

USE OF FOOD PROCESSING TECHNIQUES TO ELIMINATE/LOWER DEOXYNIVALENOL IN INFECTED WHEAT

Perry Ng, Department of Food Science and Human Nutrition, Michigan State University.

Importance

The three most common treatments to remove/lower deoxynivalenol (DON) in grain are mechanical, heat and chemical treatments, and it appears the chemical treatment is the most promising decontamination process. However, in general, grain treated with chemicals has detrimental effects on the end-use quality, for example in baked products. Thus, new approaches for processes to decontaminate infected grain are needed.

Objective

To explore cleaning and milling techniques and/or techniques for processing of wheat grain into food to eliminate or acceptably lower DON levels of grain infected by *Fusarium.*

Progress

In the preliminary studies, samples of highly infected wheat grain (7.3 ppm of DON) were soaked in sodium bisulfite solutions. Soaked wheat grain was extruded via an extrusion process to produce puffed products. DON levels in the puffed products were reduced to as low as 0.3 ppm via the soaking and extrusion processes. The proposed approach appears viable not only for reducing DON levels, but also for removing moisture and volatile chemicals from the soaking solution, and producing acceptable puffed products.

Planned Activities

The following are studies targeted to meet the objective based on the preliminary work.

- Reducing vomitoxin levels of infected wheat during the cleaning process prior to milling.
- Reducing vomitoxin levels of infected grain during the washing and polishing processes.
- Milling of cleaned and washed wheat; analysis of DON levels of various mill streams.

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- Development of novel processes for utilizing washed and wetted grain, e.g., expand the extrusion conditions used in the preliminary studies to produce better-extruded products and a wider variety of extruded products.
- Utilization of milled materials for baked and steamed-products, analysis of DON levels in the products, and evaluation of product qualities.
- Utilization of chemically soaked grain by extrusion, analysis of DON levels in extrudates, and quality evaluation of extruded products.

FUSARIUM HEAD BLIGHT RESEARCH/ EXTRUSION PROCESSING AS A MEANS OF REDUCING DEOXYNIVALENOL IN CEREAL-BASED FOODS

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Importance

DON is a fairly heat stable compound, and it survives many thermal food processes, such as baking and canning. However, extrusion is a thermal process that can be more destructive to DON than other thermal processes. Extrusion processing reaches higher temperatures and utilizes other destructive forces, such as shear forces and chemical reactions. Using the right set of processing parameters, extrusion processing of scab infected wheat contaminated may result in a processed product with a reduced DON content.

Objective

The purpose of this research is to determine the optimum conditions of extrusion processing that will destroy and detoxify DON in scab infected wheat. Since the same fungus, *F. graminearum*, produces zearalenone (ZEN), some of the work may also address ZEN. Various extrusion parameters will be studied to determine the best combination of extrusion variables (single vs. double screw, screw speed, temperature) and conditions (moisture, additives) for the destruction and detoxification of DON and possibly ZEN.

Progress

- Corn grits were spiked with 4 Fg/g of DON. Spiked corn grits were extruded at 22% moisture, 120, 140 and 160°C. Alpha-Amylase treatment improved recovery of DON from extruded product. The conditions used resulted in no loss of DON.
- Extrusion of a dog food extrusion mixture contaminated with 5.5 Fg/g of deoxynivalenal at 100°C likewise resulted in no loss of DON.

Planned Activities

- Extensive extrusion studies will be done.
- Higher temperatures (160, 180, 200, 220°C) will be studied.
- Screw speeds of 40, 80, 120, 160 rpm will be tested.
- Moisture contents of 18, 22, 26% will be studied.
- Effects of certain food additives and chemicals will be studied.
- Chemicals such as salt, sulfur dioxide, sugars, ammonium and sodium hydroxide, phosphates and others will be studied.

POST-HARVEST CONTROL OF *FUSARIUM* MYCOTOXINS IN GRAIN-BASED FOODS

Charlene Wolf-Hall, Department of Food and Nutrition, North Dakota State University.

Importance

When grains are processed into finished products, the chemical structure of mycotoxins such as DON may be changed due to the heat

and chemical reactions that occur in the food matrix. Chemically altered forms of DON and other mycotoxins may still have toxigenic characteristics. There is a definite need to be able to detect and isolate chemical products from mycotoxin degradation to determine their toxicity, prevalence in human and animal food supplies, and to find means by which this toxicity can be reduced or eliminated. An understanding of the physiology of the mold, which produces mycotoxins, is also important in studies involving pre- and post-harvest contamination of foods and feeds. Several factors, such as the growth substrate, temperature, moisture, and competing organisms have been reported to influence DON synthesis. Most importantly, strain to strain variation in DON synthesis has been observed .

Objective

The long-term goal of this project is to develop methods to control the content of *Fusarium* mycotoxins in cereal grains and their resulting foods or feeds. This may allow the utilization of FHB infected cereals by preventing preformed toxins and toxins formed during processing from ending up in the final foods and feeds. The supporting objectives of this research are to improve mycotoxin detection and quantification procedures for use on cereals and processed cereal products; to determine the fate of these mycotoxins during food processing; to develop treatments to reduce levels of mycotoxins in FHB infected cereals; and to study the metabolism and regulatory mechanisms of *F. graminearum.*

Progress

- *Fusarium* was decreased by 78% with irradiation at 10 kilogray (KGy) with only a 20 % reduction in germination. At 8 KGy there was still significant reduction in *Fusarium* growth, but an actual increase in the germination ability of the treated infected barley.
- Dry heat, steam and microwave treatments were found to significantly reduce *Fusarium*, but the concomitant reduction in grain germination was unacceptable.

Planned Activities

- Evaluate the effects of growth conditions and strain on mycotoxin production.
- Continue to evaluate current analytical methods for DON detection and quanitiation in processed food samples.

UTILIZATION OF FUSARIUM INFECTED MALTING BARLEY

Paul B. Schwarz and Charlene Wolf-Hall Departments of Cereal Science, and Food and Nutrition, North Dakota State University.

Importance

Since 1993, up to 85% of the Midwestern malting barley crop has been contaminated with DON. As FHB infection and DON present a number of potential problems to maltsters and brewers, much of the regional crop has not been purchased or utilized for malting. This has caused regional barley growers significant economic hardship. Resistant or tolerant varieties are not anticipated for several years, and development of methods by which some FHB infected barley could be utilized would be of significance to both barley growers, and to the industry. Problems associated malting and brewing FHB infected barley include, reduced germination, lower malt quality, contamination of beer with my mycotoxins, and beer gushing.

Objective

The objectives of this project are to determine the level of FHB infection that causes irreparable damage to grain quality, and to evaluate physical, chemical and biological treatments for control of *Fusarium* growth during malting.

Progress

- Malting equipment was purchased.
- Dry heat, steam and microwave treatments were found to significantly reduce *Fusarium*, but the concomitant reduction in grain germination was unacceptable.
- Evaluated the impact of *Fusarium* enzymes on malt quality. An increase in soluble nitrogen, due to *Fusarium* proteases, appears to be the principal negative impact on grain quality.

Planned Activities

- Determine the relationship between indicators of *Fusarium* infection and malt soluble protein.
- Evaluate malting process control changes on *Fusarium* growth
- Evaluate chemical biological control agents for control of *Fusarium growth during malting.*

EVALUATION OF PHYSICAL TREATMENTS TO PREVENT FUSARIUM GROWTH DURING BARLEY MALTING

Charlene Wolf-Hall*, Paul Schwarz, Jurgen Schwarz, and James Gillespie

ABSTRACT

Contaminated barley or adjunct grains used in the beer making process may lead to the presence of mycotoxins in the final beer and by-products. This is a problem with Fusarium head blight infected barley. During the germination period an increase in deoxynivalenol (DON) occurs as the mold grows in the moist, warm environment. The objective of this project is to evaluate physical treatments to reduce the amount of DON produced during the barley malting process. The methods compared in this project included dry heat, steam, microwaves, and gamma irradiation. Results, so far, indicate that dry heat, steam heat, and conventional microwaving are too harsh. These treatments tend to kill off the germination prior to affecting the survival of the *Fusarium* in contaminated barley. The irradiation treatments show promise the *Fusarium* growth being decreased by approximately 78% at 10KGy with only a 20% reduction in germination. Further testing is needed to optimize a physical treatment process which will result in killing the *Fusarium* without affecting germination.

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RESISTANCE IN WHEAT CULTIVAR CHOKWANG TO *FUSARIUM GRAMINEARUM*

George Buechley and Gregory Shaner*

Genetic resistance to Fusarium head blight is regarded as an essential component of a management program for this disease. Although a few effective sources of resistance have been identified and are being incorporated into hexaploid wheat cultivars, they do not provide complete control of the disease nor of development of DON in grain. Moreover, nearly all wheat breeding programs in the eastern soft wheat region and in the northern plains spring wheat area of the U.S. are using two related sources of resistance from China: Sumai 3 and Ning 7840. There is no evidence at present for specific virulence in *Fusarium graminearum* toward these sources of resistance, but it is known that isolates of the fungus differ in ability to cause head blight symptoms (Bai and Shaner, 1996). Given the notorious variability within species of *Fusarium*, it is conceivable that if the same source of resistance were to be used widely in the U.S., strains of *F. graminearum* that could overcome this resistance, at least to some degree, might develop. It is prudent to seek other genes for resistance and incorporate these into cultivars intended for commercial wheat production. Additionally, the sources of resistance currently being used (Sumai 3 and Ning 7840) may not provide sufficient protection under conditions conducive for severe disease. Other sources of resistance may contain genes that would interact with the genes from Sumai 3 or Ning 7840 to confer a greater degree of resistance than provided by any of the currently available sources. It is important that sources of resistance other than the few being currently used be identified, characterized phenotypically and genetically, and made available to wheat breeders.

Several types of resistance to Fusarium head blight in wheat have been described (Mesterhazy, 1995). The two types represented in the resistant sources used commonly in the U.S. are type 2 (resistance to spread of the fungus throughout an infected spike), and to some extent, type 1 (resistance to primary infection). A wide range of variation of type 2 resistance has been found in wheat. The best expression of this type of resistance is found in a related group of cultivars from Nanjing, China (Bai and Shaner, 1996). Type 2 resistance has proven to be less sensitive to environment and easier to manipulate in breeding programs and genetic studies (Bai and Shaner, 1994). It has reasonably high heritability and is conferred by a few genes (Bai et al., 1999; Moreno-Sevilla et al., 1997; Van Ginkel et al., 1996).

The purpose of our work was to identify sources of resistance that may contain genes different from those in Sumai 3 and Ning 7840. We identified a Korean winter wheat cultivar, Chokwang, as having resistance. We originally studied this cultivar because of its partial resistance to *Puccinia triticina*. A recombinant inbred population from the cross Chokwang x Clark was being developed for leaf rust work when we discovered that Chokwang also showed resistance to *Fusarium graminearum*.

MATERIALS AND METHODS

Seed of F_2-derived F_8 families from the cross Chokwang x Clark was planted in flats and allowed to germinate, then flats were placed in a 5 °C coldroom for 65 days to allow plants to

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vernalize. After vernalization, plants were transplanted into 10-cm-diameter pots and placed on a greenhouse bench. High intensity lights supplemented daylight. Plants were fertilized at the tillering stage (GS 23) with Miracle Gro Plant Food 15-30-15 and at stem elongation (GS 31) with Co-Op Farm Gro 9-44-9. The experiment was set up as a randomized complete block on the greenhouse bench, with eight replicates. There were 80 recombinant inbred families, each parent and a Patterson check. Because of powdery mildew, fewer than eight plants were available for inoculation with *F. graminearum* for some families.

Conidia of *F. graminearum* on were produced in Mung bean extract (Bai and Shaner, 1996). After filtration through cheesecloth, conidia were adjusted to a concentration of 10^4 spores per ml. Each plant was inoculated by the point method when it reached the mid flowering stage of growth (GS 63). A droplet of approximately 20 ml was placed into a floret at the middle of the spike. Inoculated plants were incubated in a moist chamber for three successive nights. The chamber was partially open during the day to prevent temperature buildup.

Blighted spikelets on each spike were counted at 5-day intervals, from days 7 through 27 after inoculation. A count of all spikelets on the spike was used to convert number of blighted spikelets to a percentage of blighted spikelets (severity). Area under the curve for the progress of severity over time was the statistic used to characterize resistance (Shaner and Finney, 1977).

RESULTS

There was no association between the mean and standard deviation for area under disease progress curve (AUDPC). Therefore, no transformation was applied to this statistic. The distribution of family mean AUDPCs was bimodal (Fig. 1). Neither mode corresponded to the parental mean, although the higher mode was only slightly below the mean for Clark. The lower mode was considerably higher than the mean for Chokwang. Based upon an analysis of variance, 17 families had an AUDPC not significantly different from the AUDPC for Chokwang. Fifty-three families were not different from Clark. One family had a significantly greater AUDPC than the AUDPC for Clark, but its value barely exceeded the limit established by the LSD.

DISCUSSION

Until the evaluation of the recombinant inbred population is repeated, a thorough analysis of the data, with an attempt to generate genetic models is premature. However, the pattern of inheritance is quite distinct from that observed for an inbred recombinant population derived from the cross Ning 7840 x Clark (Fig. 2). This leads us to conclude that the genes for resistance in Chokwang are different from those in Ning 7840.

Because heterozygosity is largely eliminated by the F_8 generation, our data provide no information about degree of dominance of the genes for resistance. If genes at two loci accounted for most of the resistance in Chokwang, then one-fourth of the families should have the same resistance phenotype as Chokwang. Statistically, 17 of 80 families did not differ from Chokwang, which tends to support a hypothesis of only two independent genes. If these two genes were additive (i.e. if AAbb had the same resistance phenotype as aaBB), there should be three phenotypic classes representing 0, 1, or 2 loci homozygous for the plus allele, in frequencies of 1, 2, and 1, respectively. Clearly, this model does not fit the data (See Fig. 1).

A more complicated model, involving genes of unequal effect, epistatis, or complementation, would seem to be required to explain the distribution. Bai et al. (1999) found evidence that genes for resistance in Ning 7840 were of un-

equal effect. Shaner et al. (1997) were able to discern unequal effects of genes for partial resistance to Puccinia triticina in a recombinant inbred wheat population, and we intend to apply the same method of analysis to the Chokwang x Clark population once the experiment is repeated.

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Fig. 1. Frequency distribution of area under the disease progress curve for Fusarium head blight severity for family means for a recombinant inbred population of Chokwang/Clark. The AUDPCs for Chokwang (4.0) and Clark (13.2) are indicated by arrows.

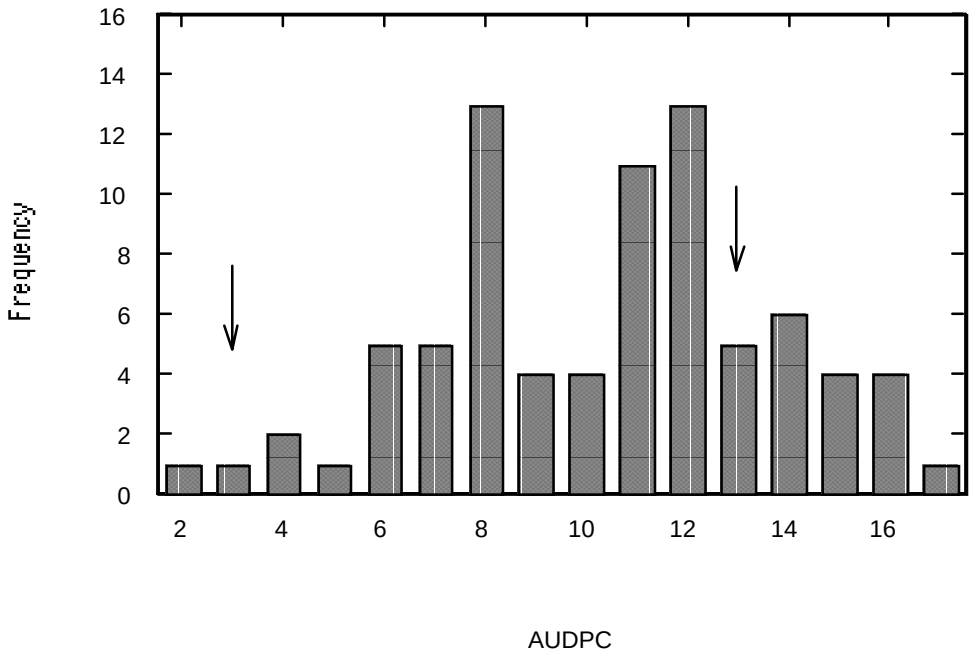
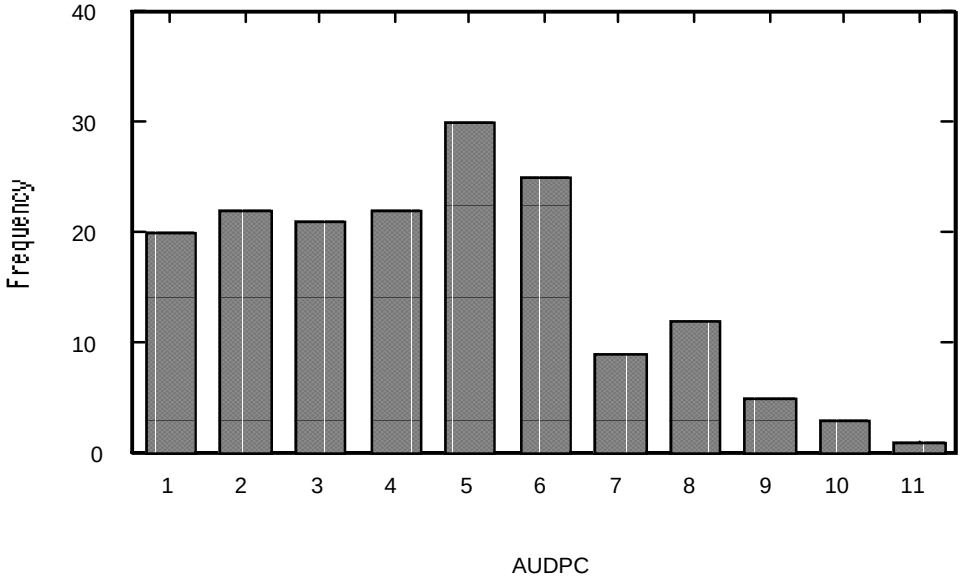


Fig. 2. Frequency distribution of area under the disease progress curve for Fusarium head blight severity for family means for a recombinant inbred population of Ning 7840/Clark.



EVALUATION OF DURUM GERMPLASM FOR RESISTANCE TO FUSARIUM HEAD BLIGHT

Elias M. Elias

Fusarium head blight has been seriously attacking the spring, winter, and durum wheat crop in 12 states in the midwest area. Economic losses in wheat have been in billions of dollars from 1993-1999. Two states, North Dakota and Minnesota, account for two thirds of these dollar losses. North Dakota is the number one producing durum wheat state in the U.S. These losses are disastrous to the farm economy and has direct national impact as alternative sources of supply are sought by importing countries. The search for sources of resistance is essential to insure the development of Fusarium head blight (FHB) resistant durum cultivars. Identified sources of resistance will be incorporated to the currently susceptible durum wheat germplasm to develop resistant cultivars. These cultivars will insure the stability of good quality durum wheat production for the producers, domestic pasta industry, and the international export market.

The main objective of this project is to identify sources of resistance to FHB. Several durum wheat genotypes including durum accessions from the world collection were evaluated for FHB resistance at Prosper, ND and Shanghai China. In 1998, durum wheat from the world collection tested at Prosper, ND were lost because they were out of their adaptation area and were very susceptible to foliar diseases such tan spot *Pyrenophora tritci-repentis* and *Septoria spp*. Therefore the accessions were tested only in China and France in 1999. A total of 500 accessions were sent to the Academy of Agricultural Sciences, Plant Protection Institute (AASPPI) Shanghai, China to be evaluated for FHB in the 1998-99 growing season. Also 50 accessions were sent to Groupment Agricole Essonnois (GAE) in France for evaluation. These evaluations at various sites will allow germplasm exchange and provide international evaluation to a large array of Fusarium strains to determine the effectiveness of incorporated resistance in the germplasm.

The 500 accessions were successfully evaluated at AASPPI. A variation in infection existed among these genotypes, few lines had a very moderate level of resistance to FHB. The identified resistant genotypes will be re-tested in the in the 2000 spring greenhouse to confirm their resistance. Two thousand new accessions were sent to AASPPI to be evaluated in the 1999-00 growing season FHB nursery.

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A POINT INOCULATION METHOD FOR EVALUATING SCAB RESISTANCE IN WHEAT

Y. Jin*, X. Zhang, R. Rudd, and J. Rudd

ABSTRACT

A point-inoculation technique was evaluated in greenhouse and field studies to improve the efficiency of inoculation for evaluating scab resistance. Seeds of foxtail millet (*Setaria italica*) were soaked in distilled water for 24 hours. A thin layer (ca. 1cm) was placed in a glass petri plate (or a larger tray when desired) and autoclaved for 20 minutes. Autoclaved seeds were inoculated with agar pieces of *Fusarium graminearum* cultures. Inoculated plates were incubated for 10-12 days. Colonized seeds were dried and stored at 4°C. Plants were inoculated by placing a colonized seed between lemma and palea of a floret using a pair of fine forceps. Greenhouse experiments were conducted to compare the "millet-inoculation" method with floret-injection using conidial suspension. Five spring wheat cultivars, ranging from moderately resistant to highly susceptible, were used as testing materials. Results indicated that the millet inoculation was as effective as floret injection with or without mist incubation. Both inoculation methods could result in a high level of infections without any mist incubation when relative humidity was high during the incubation period. Soaking the inoculum in sterile water for 20 minutes prior to inoculation promoted infection. An interaction between genotypes and inoculation methods was observed in the greenhouse experiments. This method was also used to evaluate 14 lines in the advanced yield trials at three spring wheat breeding nursery sites where irrigation was not available. Three replications were used at each site. Twenty spikes in each plot were tagged for growth stages and inoculated with colonized millets between the heading and flowering stages. Disease on the inoculated spikes was evaluated 14-17 days after inoculation. Disease on a random sample of 20 non-inoculated spikes was surveyed at the same time. At two of the three locations, scab indices on the inoculated spikes ranged from 33.4% on a resistant genotype to 69.1% on a susceptible genotype, whereas scab indices due to natural infections in these plots ranged from 7.9 to 26.4%. At the third site, scab development was minimal with scab indices ranging from 9.0 to 21.3% on inoculated spikes and 4.0 to 11.6% on non-inoculated spikes. In two winter wheat breeding nurseries where natural scab infections were minimal, inoculated spikes of 12 selected genotypes had scab indices ranged from 5.9 to 39.6%, and 10.3 to 54.0%, respectively, for the two locations. An apparent interaction between genotype and location was observed using this method. Both greenhouse and field experiments suggested that the millet inoculation method could reliably induce scab infection. The advantages of this inoculation seemed apparent: 1) inoculum can be prepared efficiently with minimal preparation and stored for future use; 2) it is portable to be used in breeding nurseries where mist-irrigation is not available; 3) plants can be inoculated anytime between heading and flowering stages; and 4) minimal training is required to carry out the inoculation.

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EVALUATION OF ASIAN, ITALIAN AND BRAZILIAN WINTER WHEAT GERMPLASM FOR TYPES II AND III RESISTANCE TO FUSARIUM HEAD BLIGHT

Anne L. McKendry*, Kara S. Salzman, and Shuyu Liu

INTRODUCTION

Fusarium graminearum Schwabe (teleomorph *Gibberella zeae* (Schwein.), also known as scab, is an increasingly important problem in the north-central region of the United States because of the emphasis on conservation tillage, (Wilcoxson et al., 1988; Bai and Shaner, 1994), rotations with corn (Windels and Kommedahl, 1984), the lack of effective cultural and/or fungicide control (McMullen et al., 1997) and the lack of effective sources of genetic resistance. Yield losses in Missouri alone have exceeded $250 million dollars since 1990. In addition to reduced kernel density and color at harvest, associated deoxynivalinol (DON) accumulation in the grain prevents it from being marketed. Host resistance has long been considered the most practical and effective means of control (Schroeder and Christensen, 1963; Martin and Johnston, 1982), but breeding has been hindered by a lack of effective resistance genes and by the complexity of the resistance in identified sources (Mesterházy, 1997). No source of complete resistance is known, and current sources provide only partial resistance, therefore, the identification of different sources of resistance and their incorporation into adapted wheat varieties is critical to the continued improvement of Fusarium head blight resistance in winter wheat. Screening of germplasm collections has confirmed that accessions from China, Brazil and Europe carry resistance genes (Fedak et al., 1997). Wild and related species of the Triticeae have also been identified as potential sources of resistance genes for wheat breeding (Baier et al., 1980; Liu et al., 1990; Ban, 1997; Rubiales et al., 1996).

OBJECTIVES

This research is a component of the aggressive world-wide search for resistance to scab initiated in 1998 with support from the National Wheat and Barley Scab Initiative. Regions that have been targeted include those geographical areas where resistance has been identified or where environmental conditions are conducive to scab development and include: China, Korea, Japan, Brazil, Italy and Eastern Europe. Approximately 4,200 winter wheat accessions from these target geographical areas were identified in the USDA National Small Grains collection. The purpose of this research was to evaluate, under greenhouse conditions, accessions from China, Korea, Japan, Brazil and Italy for Types II and III resistance (Mesterházy, 1995) to *Fusarium graminearum.*

MATERIALS AND METHODS

In the fall of 1998, 937 accessions representing winter wheat landraces, breeding lines, cultivars and cultivated genotypes from China, Korea, Japan, Brazil and Italy were acquired from the USDA-ARS Small Grains Collection at Aberdeen, Idaho.

Disease Resistance Screening

Vernalized seedlings (4 per accession) were planted in the greenhouse over a two month

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period in the fall and winter of 1998 and 1999, respectively. At first anthesis, plants were inoculated with 10µL of a macroconidial suspension of *Fusarium graminearum* concentrated to 50,000 macroconidia/mL. Inoculum was placed in a single central floret using an Oxford 8100™ repeat dispensing syringe. For all inoculations, a single isolate was used which had been previously determined to be the most aggressive Missouri isolate on our most resistant cultivar, Ernie. Previous research had also determined that this Missouri isolate was more aggressive in causing disease than similar isolates acquired from Indiana, Michigan, Ohio and Virginia. Plants were incubated in a mist chamber (100% relative humidity) for 72 h post-inoculation to promote disease development and then returned to the greenhouse bench. Ratings for Type II resistance (disease spread in the spike) were made at 14 and 21 d after inoculation. At maturity, heads were harvested, kernels were counted and evaluated for the degree of shriveling and the presence of tombstone kernels. Seeds were counted and each was given a value on a 5 point scale as follows: 1 (sound): 2 (slightly shriveled): 3 (moderately shriveled): 4 (very shriveled): 5 (tombstone). Lines meeting the following criteria for resistance are currently being progeny tested to verify resistance.

Definition of Resistance

For the purposes of greenhouse screening, Type II resistance was compared to the resistant checks Sumai 3, Ning 7840, and Ernie. The susceptible check was MO 94-317. Lines are retained for verification that met the following criteria:

1. Disease spread in the head # 2 spikelets.
2. A low kernel quality score based on the 5 point scale outlined above. Lines are retained where the majority of the kernels had a score of 1 on this scale.
3. High kernel retention ($\geq$ 75% compared to an uninoculated head).

Lines were eliminated, regardless of spread, if inoculated heads had low kernel numbers and/or a high kernel quality score.

RESULTS AND DISCUSSION

Table 1 provides information on country of origin and improvement status of accessions screened in the 1998/99 greenhouse season. The majority of the accessions evaluated (627) were from the People's Republic of China with 406 being classified as cultivated accessions. The improvement status of entries in this class is unclear as the term cultivated is used as a "catch-all" to describe those accessions where passport data on improvement status is poor (Dr. Harold Bockelman, USDA-ARS, Aberdeen, Idaho - personal communication). In the greenhouse, many of these "cultivated" accessions had a desirable agronomic type with dense heads, long spikes and multiple seed set across the spikelet. In addition, many were early to mature with 8 weeks of artificial vernalization. Of the 627 accessions from China that were screened, 4% were classified as resistant in the preliminary screen. The frequency of resistant accessions from other countries was lower, however, a total of 6 accessions from diverse geographical origins were also classified as resistant in the preliminary screen. If verified, these may provide breeders with different sources of resistance. Progeny evaluations of each of these accessions are ongoing.

In addition to the 31 accessions classified as resistant, 111 accessions had one or more plants showing high levels of Type II and Type III resistance. Resistant plants from these accessions are also being progeny tested in the greenhouse. Once verification of Type II resistance is completed, inoculated heads will be harvested and kernel quality will be assessed to verify Type III resistance in these lines. Data for completed

verifications will be made available at the 1999 scab forum in South Dakota. Seed harvested from resistant accessions will be increased for distribution and entered into a soft red winter wheat crossing program at the University of Missouri during the winter of 2000. Concurrently, crosses will be made with the susceptible cultivar MO 94-317 to initiate the development of populations for genetic study.

All accessions being verified in the 1999 fall greenhouse have been planted as head rows in the field at Columbia, Missouri for assessment of Type I resistance. Plants will be sprayed at 75%

Table 1. Origin and improvement status of germplasm screened for Type II and III resistances in the greenhouse at Columbia, Missouri during the fall and spring of 1998 and 1999, respectively.

Country	Improvement status	Total number of accessions screened	Number of resistant accessions	Total number of accessions re-screened in 1999/2000
Brazil	Breeding	3	-	-
	Cultivar	5	1	2
	Cultivated	-	-	-
	Landrace	-	-	-
China	Breeding	22	2	5
	Cultivar	75	1	10
	Cultivated	406	13	65
	Landrace	124	9	23
Italy	Breeding	16	-	2
	Cultivar	118	2	11
	Cultivated	28	-	-
	Landrace	13	-	4
Japan	Breeding	9	-	-
	Cultivar	62	2	10
	Cultivated	6	-	-
	Landrace	8	-	1
South Korea	Breeding	-	-	-
	Cultivar	25	1	5
	Cultivated	12	-	2
	Landrace	5	-	2
Totals		937	31	142

† Accessions with one or more plants having excellent Type II (disease spread in the head) and III (kernel quality) including the 38 accessions classified as resistant. Resistant plants in these accessions are being progeny tested in the greenhouse and field in 1999/2000.

‡ Cultivated is a “catch-all” term used to describe improvement status when passport data on the accession is poor. Improvement status is unclear for these accessions.

heading with a macroconidial suspension concentrated to 50,000 macroconidia/mL. Head rows will be maintained under overhead mist irrigation through heading and evaluated for scab incidence and severity18 - 21 d after inoculation. In addition, accessions will be evaluated for winter hardiness, resistance to other relevant diseases, height, maturity and yield under disease pressure.

Field and greenhouse screening of approximately 1000 accessions from Yugoslavia obtained from the National Small Grains Collection is continuing in the 1999/2000 season. This work is being done in cooperation with Dr. Paul Murphy, North Carolina State University.

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EVALUATION OF DIPLOID, TETRAPLOID, HEXAPLOID, AND SYNTHETIC WHEATS FOR TYPE II RESISTANCE TO FUSARIUM HEAD BLIGHT

J. P. Murphy[1*], R. A. Navarro[1], and S. Leath[2]

OBJECTIVES

To evaluate a range of diploid and tetraploid *Triticum* and *Aegilops* species, synthetic (*T. turgidum* sp. *durum* x *Aegilops tauschii*) hexaploids and exotic and adapted cultivated hexaploids for resistance to FHB.

INTRODUCTION

The Southeastern U.S. soft red winter wheat-producing states have, so far, been spared Fusarium Head Blight (FHB) epidemics on the scale experienced in the North-Central and Midwestern States since 1993 (McMullen et al., 1997). Nevertheless, in both the 1997 and 1998 seasons an increase in the incidence of FHB was observed associated, in particular, with minimum/no-till cultural practices in the corn-wheat-soybean rotation that is common in the region. Southeastern breeders, producers, and millers are anxious that proactive measures be taken to avoid a repeat of the Midwestern/North-Central experience with FHB. In order to provide Southeastern breeders with an array of sources of resistance to FHB, we initiated a screening program involving cultivated and related species accessions.

MATERIALS AND METHODS

Sept.-Dec. 98

Seedlings of 83 accessions of 10 diploid and tetraploid species (Table 1) were vernalized in peat pots at 3°C for 65 days in a growth chamber with a 10/14-hour light/dark cycle. Seeds of winter type *T. aestivum* accessions from China, Japan, Italy, The Balkans, and breeding lines and cultivars from the southeastern United States were vernalized in moistened paper towels for 50 days at 3°C. All vernalized and spring growth habit entries were planted in the greenhouse on Sept. 25, 1998. Supplemental lighting to 16-hour days was provided from Oct. 25. Two pots (replications) were planted per entry. Pots were overplanted and later thinned to two plants per pot. Tiller production was high, and three heads per pot, at approximately the same stage of anthesis, were inoculated with 50 ml of a macroconidial suspension (50,000 spores/ml) using a pipette. The inoculation suspension consisted of four aggressive North Carolina isolates of *Fusarium graminearum* identified by Walker et al. (1998). Following inoculation, the plants were placed in a mist chamber for 3 days. The chamber was opened from 9:00 AM to 5:00 PM to prevent excessive heat build-up. Twenty-one days post-inoculation, heads were rated on a 0-5 scale describing infection type as follows: 1.0 - only inoculated floret infected, 2.0 - only inoculated spikelet infected, 3.0 - inoculated spikelet and rachis infected, 3.2 - inoculated and one adjacent spikelet infected, 3.4 - inoculated and two adjacent spikelets infected, 3.6 - inoculated and three adjacent spikelets infected, 3.8 - inoculated and four adjacent spikelets infected, 4.0 - half of spike infected, and 5.0 - whole spike infected.

A square root transformation was utilized and data were analyzed using the PROC GLM procedure in SAS.

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Feb.-May 1999

Sixty-six of the 316 entries that appeared resistant in the first screening were re-evaluated using the protocols described above. Selfed seed from resistant plants were utilized in this second screening to avoid complications that could arise from intra-accession variability.

RESULTS

All diploid and tetraploid accessions exhibited high levels of susceptibility in the first run of the experiment and none were retested. Infection types at 21 days post-inoculation were generally in the 4 to 5 range. Jauhar and Peterson (1998) and Miller et al. (1998) reported on *Thinopyrum junceiforme, Lophopyrum elongatum,* and *T. turgidum* var. *dicoccoides* as sources of resistance to FHB. Although we evaluated relatively few accessions per species in this initial test, we will likely concentrate on the species reported to have resistance to FHB in the immediate future.

Six of the seven Asian cultivated sources with resistance were Chinese in origin (Table 2). The seventh, 'Shinchunaga' was from Japan. Three of the four European cultivated sources with resistance were Italian in origin. The fourth, 'NS 18-99' was from Serbia. The pedigree of 'Mentana' was one-half 'Akagomughi', a Japanese line. The pedigrees of 'Luizia Strampelli', 'Inallettabile 3', and NS 18-99, were unavailable through the Germplasm Resources Information Network (GRIN). The CIMMYT synthetic wheats each had a different *Ae. tauschii* parent, but three of the five lines had the durum cultivar Altar 84 in their pedigree.

The North Carolina breeding lines exhibiting resistance had a mean of 12.5% eastern European germplasm utilized as a source of powdery mildew resistance in their pedigrees. The resistance of these lines to FHB is unknown.

Field and greenhouse screening of eastern European germplasm obtained from the National Small Grains Collection is continuing in cooperation with Dr. Anne McKendry, University of Missouri.

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Table 1. Species, genome designation, and number of genotypes evaluated for Type II resistance to Fusarium Head Blight.

Species	Genome	No. accessions/breeding lines
T. monococcum sp. *monococcum*	A	25
T. monococcum sp. *aegilopoides*	A	4
T. urartu	A	3
Ae. tauschii	D	8
Ae. speltoides	S	7
Ae. sharonensis	s	7
T. timopheevii sp. *armeniacum*	AG	10
Ae. neglecta	UM	5
Ae. cylindrica	CD	11
Ae. triuncialis	UC	3
CIMMYT Synthetic wheats	ABD	77
T. aestivum	ABD	
1997 Uniform Southern Nursery		30
Asian and European accessions		67
Southeastern U.S. breeding lines		59
TOTAL		316

Table 2. Origin and performance of genotypes exhibiting Type II resistance to FHB based on two separate evaluations during the 1998-99 greenhouse season.

	Infection type	
	Untransformed	Transformed
Asian		
Sumai 3	1.3	0.96
Sho Chou	1.5	1.21
Futai 8944	1.8	1.31
Ning 7840	1.9	1.34
Wan Nian #2	2.0	1.40
Shinchunaga	2.0	1.40
JG 1	2.1	1.41
European		
Mentana	1.5	1.21
NS 18-99	2.0	1.40
Luizia Strampelli	3.0	1.71
Inallettabile 3	3.1	1.75
CIMMYT Synthetics		
TA 4064.200	2.6	1.60
TA 4073.200	2.8	1.67
TA 4094.200	2.8	1.64
TA 4054.200	3.0	1.73
TA 4069.000	3.0	1.72
North Carolina		
NC96-13374	2.4	1.55
NC96-13965	2.7	1.62
NC96-14629	2.7	1.63
NC96-13848	3.2	1.79
Checks		
Ernie	2.8	1.64
Roane	3.0	1.71
Freedom	3.1	1.75
NK-Coker 9663	4.3	2.1
Mean	2.5	1.55
LSD (0.05)	--	0.36
CV (%)	--	16.5%

EVALUATION OF SIX-ROWED SPRING BARLEY ACCESSIONS FOR RESISTANCE TO FUSARIUM HEAD BLIGHT

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OBJECTIVE

In the Upper Midwest, six-rowed cultivars are the preferred type for malting. Unfortunately, most of the Fusarium Head Blight (FHB) resistant germplasm identified to date is in a two-rowed genetic background (Prom et al. 1997). To identify additional sources of resistance, accessions of six-rowed spring barley from the USDA-ARS National Small Grains Collection are being evaluated to FHB in the field.

INTRODUCTION

FHB threatens the existence of the malting barley industry in the Upper Midwest (Salas et al. 1999; Steffenson 1998). The deployment of resistant cultivars is the most effective and environmentally sound means of managing the disease. In an early screening effort at the University of Wisconsin, R. G. Shands (1939) identified Chevron, a six-rowed cultivar from Switzerland, as a good source of resistance to FHB. Recent evaluations indicate that Chevron is still the most resistant six-rowed accession to FHB, but is poor in malting quality and agronomic performance. Additional sources of FHB resistance in a six-rowed genetic background need to be identified and exploited in breeding programs.

MATERIALS AND METHODS

The first half of the spring six-rowed barley collection (4035 accessions) were planted in both Langdon and Osnabrock, North Dakota in the spring of 1999. The nurseries were inoculated using methods modified from Prom et al. (1997). Equal amounts of six regional *Fusarium graminearum* isolates (KB171, KB172, KB173, KB176, KB582, and KB672) were applied uniformly to plots in both nurseries. The first inoculation was made when the flag leaves of the earliest maturing plants were expanding. Four successive inoculations were made at weekly intervals to ensure that sufficient inoculum was available for infection of later maturing accessions. To maintain sufficient moisture on the spikes for optimal FHB infection (Paulitz, 1996), an automatic overhead misting system was used, operating twice per day during early morning and early evening. Irrigation began two weeks after the first inoculation and continued until the latest maturing accessions reached the late dough stage of development. FHB severity (average percentage of infected kernels on 5-10 spikes) was assessed on each accession at the mid-dough stage and then two weeks later to record possible changes in the infection level. In addition to FHB assessments, maturity data [Decimal code (Zadok *et al.*, 1974) at five ≤DC15, ≤DC25, ≥DC31] and eight weeks (≤DC15, ≤DC25, ≤DC45, ≤DC45, ≤DC59) and plant height (level 1 = 25cm, level 5 >100cm) at ten weeks post-planting were recorded on accessions in the Langdon nursery. FHB severity and quantitative assessments of these various traits were analyzed statistically to determine possible relationships between disease expression and plant type and development. The country of origin of each accession also was considered. To detect different groups of germplasm based on FHB resistance levels, cluster analysis was performed using

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the between-groups-linkage-method with squared-euclidian-distances-interval in the SPSS statistical package (version 9.0).

RESULTS AND DISCUSSION

In general, the disease pressure was higher in Langdon than in Osnabrock. This was likely due to longer wetness periods, earlier sowing date and higher plant densities in Langdon compared to Osnabrock.

1. Field selected germplasm

1.1 Accessions exhibiting high levels of FHB resistance

From 4035 tested accessions in Osnabrock, 12 exhibited a resistance level (less than 20% infection) comparable with Chevron, the resistant check (Table 1). These accessions also were in the same maturity group as Chevron and originated from the United States, Georgia, Mongolia, China, Yugoslavia and Romania. The widely grown susceptible six-rowed malting barley cultivar Foster had a FHB severity of 54% in Osnabrock.

1.2 Additional selected accessions exhibiting FHB resistance in Langdon or Osnabrock.

The level of FHB occurring on barley can be influenced by several traits like maturity and plant height (Steffenson et al. 1996). To avoid the loss of potentially resistant material, especially in early and in late maturing germplasm, 16 additional accessions that exhibited resistance levels marginally below that of Chevron were selected. These accessions were from Ethiopia, China, South Korea, Russia, and central Europe. Nearly one quarter of this group were semi-dwarf types.

1.3 Origin of accessions

The hierarchical cluster analysis based on one FHB severity assessment in Langdon and two assessments in Osnabrock resulted in five groups distinguishable at the 96% probability level:

A) ≤30% FHB infection in Langdon, ≤20% in Osnabrock (diverse origin of germplasm)
B) ≤40% Langdon, ≤20% Osnabrock (mostly European origin)
C) ≤35% Langdon and ≤10% Osnabrock (Europe and USA)

Table 1: Six-rowed barley accessions exhibiting FHB resistance comparable to Chevron in Langdon, ND and Osnabrock, ND in 1999.

CIho or PI Number	Development 10 weeks after planting	Height (cm)	% FHB-severity Langdon*	Osnabrock** 1.	and 2.	Name	Country of Origin
2236	≤DC25	76-100	40		10	Reids Triumph	USA
4095	≤DC25	51-75	40		20		Georgia
4339	≤DC25	≤25	60	20	S***		Mongolia
4530	≤DC25	51-75	40	10	20	7603	China
5809	≤DC25	51-75	30		20	484	
6610	≤DC25	51-75	30		20	Zander 1	USA
6611	≤DC25	51-75	30	10	10	Hietpas 3	USA
6613	≤DC25	51-75	30		10	Seed Stocks 1148-1	USA
7163	≤DC25	51-75	50	10	20	Beaver Dam 8	USA
9114	≤DC25	51-75	40		20	184	Yugoslavia
11526	≤DC25	76-100	10		10	Chevron Sel.	USA
15258	≤DC25	76-100	40		20		Romania
1111	≤DC25	76-100	17	10	14	Chevron	Switzerland
592758	-****	-	-	39	54	Foster	USA

* single reading in Langdon
** two readings in Osnabrock
*** plants were senescent
**** data not available

D) ≤15% Langdon and ≤10% Osnabrock (Ethiopia)
E) ≤60% Langdon and ≤20% Osnabrock (Mongolia, Russia, USA)

2. Database selected germplasm

To increase the genetic diversity for FHB resistance, additional germplasm was selected for further analysis based on FHB severity assessments in both nurseries and country of origin.

Based on the ranked average FHB severity assessments from the Osnabrock nursery, the five most resistant lines from different countries with accessions exhibiting <30% FHB infection were subjected to cluster analysis. Only lines with one FHB reading in Langdon and two FHB readings in Osnabrock were considered; thus lines with late head emergence were excluded. Accessions from Mongolia, Canada, and China had the best FHB resistance (<12% FHB), followed by a major group consisting of western, northern, and central European germplasm (13-15% FHB). Slightly higher levels of FHB infection (>15%) were found for a germplasm group from Australia, United Kingdom, Japan, and Algeria. No significant differences in height and maturity between the grouped accessions were detected. In total, 52 more lines have been selected for further screening.

3. Outlook

Evaluation of the first half of the spring six-rowed barley collection resulted in the identification of 12 accessions with FHB resistance comparable to the standard resistant cultivar Chevron. An additional 80 accessions also were identified that exhibited a level of FHB resistance marginally below that of Chevron. This research will result in the widening of genetic diversity for FHB resistance in barley breeding programs. Data for FHB severity of all accessions will be entered in the Germplasm Information Resource Network (GRIN) to benefit all interested researchers. The resistance performance of the selected germplasm will be re-evaluated under controlled conditions in the greenhouse and also under field conditions in China and North Dakota in 2000. The second half of the six-rowed spring barley collection will be evaluated for FHB resistance in the coming field season.

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SCREENING OF SPRING WHEAT SCAB RESISTANCE FROM THE USDA GERMPLASM COLLECTION

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INTRODUCTION

A few known scab resistant materials have been used as the primary sources of resistance in most breeding programs. The level of the resistance in these materials is moderate, and significant loses can be expected when scab pressure is high. Moreover, the widespread use of these materials will likely result in considerable genetic uniformity, which may lead to potential genetic vulnerability to disease and other biotic or abiotic stresses. Additional sources of resistance are needed to build up higher levels of resistance and to introduce genetic diversity. The wheat germplasm in the USDA National Small Grains Collection (Aberdeen, ID) is a valuable genetic resource for various agronomic traits, disease, and pest resistance for wheat improvement. Over the past two years, we initiated evaluations of scab reaction in USDA spring wheat collections from regions where sources of resistance had been identified. This paper reports the results of germplasm screening in 1998 and 1999.

MATERIALS AND METHODS

Preliminary evaluation of scab reaction in the field nursery. Accessions were first tested in the Preliminary Screening Nursery. The number of accessions and their origin are given in Table 1. Spring wheat cultivars/lines Sonalika (susceptible, early), Wheaton (susceptible, late), Backup (resistant, early), and ND 2710 (resistant, late) were used as checks. Check-to-entry ratio was 1:30. In 1999, checks were planted at three different dates at weekly intervals, starting from the first field plot planting. Materials were planted in one meter single row plots. Growth stage of the test entries was monitored twice a week, and individual plots were tagged when at least 50-75% of the plants reached anthesis. Entries were inoculated with a mixture of 10 *Fusarium graminearum* isolates by spraying 50 ml of conidial suspension (75,000 conidial ml^{-1}) on each tagged plot followed by a second inoculation one week later. Plots were mist-irrigated for two minutes with a 30-minute recess between 8:00 p.m. and 8:00 a.m. Mist-irrigation was continued till the last readings. In addition to conidial suspension inoculation, starting from the jointing stage, 7.5 g of corn and 5 g of oat kernels colonized by *F. graminearum* were spread into each row weekly for three consecutive weeks. Scab reactions were evaluated about 14-17 d after the first spray inoculation. A sample of 20 spikes were evaluated following a 0-9 scale, where 0 indicated free of disease, and 9 indicated over 90% spikletes in a spike were infected. The increment "1" represented 10% disease severity on a spike. Disease incidence represented the percentage of infected spikes in the sample. Resistance to kernel damage of each entry was evaluated at maturity by squeezing the spikes. Entries with good seed set were harvested for further evaluation. After threshing, percent tombstone kernels of each selected entry was evaluated based on a 0-9 scale, where 0 indicated free of tombstone kernels and 9 indicated over 90% of scab infected kernels. Entries or plants within an entry with a low scab index and/or low kernel damage were selected for further evaluation in the greenhouse.

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Greenhouse re-evaluation and characterization

To confirm and characterize scab resistance from the field selections, selected accessions were evaluated by spray and point inoculation in the fall and spring greenhouse seasons. Approximately 40 to 80 plants of each selection were evaluated by point inoculation to assess resistance to spread. A similar number of plants were spray-inoculated to assess resistance to initial infection. At anthesis, flowering spikes were tagged and sprayed with a spore suspension (75,000 spores ml^{-1}). Inoculated plants were incubated in a misting chamber for 48 hr with 8 hr photoperiod, then moved to a greenhouse bench. Disease data were collected 14-17 d after inoculation, depending on the scab development of the susceptible checks (Sonalika and Wheaton). Total number of spikelets and number of diseased spikelets were recorded.

Elite Germplasm Nursery

Based on greenhouse screening, a portion of the preliminary selections was advanced to the field Elite Germplasm Nursery. Entries were replicated three times and arranged in a randomized complete block design. To avoid disease escape due to late maturity, entries with late maturity (later than Wheaton) were pre-germinated in germination paper four weeks prior to planting, and transplanted into the field. Sonalika, Wheaton, Backup, and ND 2710 were used as checks. Check-to-entry ratio was 1:30. The checks were planted at three different dates at a weekly interval. The nursery management was the same as that in the Preliminary Screening Nursery except that all the entries were harvested for seed evaluations.

RESULTS AND DISCUSSION

Preliminary screening for scab resistance

Many entries showed a wide range of reactions to scab between plants. In 1998, based on the field visual disease severity and kernel rating, and uniformity of scab reaction, 57 accessions were selected for greenhouse evaluation. In the 1999 Preliminary Screening Nursery, 55 accessions were selected. In addition, single plant selections were made from 70 accessions. Most of these selections were from South America (primarily from Brazil and Argentina) and Europe (Yugoslavia, Hungary, and Switzerland). The 1999 field selections are being evaluated in the greenhouse to characterize the type and level of resistance. Selections from the greenhouse evaluation will be advanced to the 2000 Elite Germplasm Nursery.

Scab reactions in the Elite Germplasm Nursery

After greenhouse evaluation, 51 accessions from the 1998 Preliminary Screening Nursery were advanced to the 1999 Elite Germplasm Nursery. Most of the test entries in the Elite Germplasm Nursery were found to be moderately susceptible. Five lines, however, consistently exhibited lower disease severity and low kernel damage in various tests (Table 2). Among these lines, Tokai 66 ranked the lowest in severity and very low percentage of damaged kernels (2 on a 0-9 scale). Low kernel damage was observed on 16-52-9 and Mentana. Seed of these selections is available upon request.

Introgression of scab resistance into adapted materials

A total of 17 crosses were made between the newly identified resistant lines and Wheaton or Russ for introgressing the resistance into adapted materials and for developing populations for genetic studies. Backcrossing will continue for those lines that continue to show good resistance.

Table 1. Number and origin of spring wheat accessions screened for scab resistance in 1998 and 1999.

Country of origin	Number of accessions evaluated 1998	1999
Argentina		130
Austria		174
Bosnia and Herzego		22
Brazil	168	11
Bulgaria		12
China	70	3
Czech Republic		28
Greece		43
Hungary		32
Poland		45
Romania		29
Switzerland		291
Italy	113	10
Japan	73	3
Ukraine		26
Uruguay		21
Yugoslavia		62
Others (less than 10 accessions)		39
Total	424	981

Table 2. Average scab index and kernel damage score of two-year evaluation in the field and greenhouse (GH) by spray and point inoculation (point inoc) methods in spring wheat accessions selected for scab resistance.

ID	Accession	Country of origin	Days to flowering	Scab index field	Scab index GH-spray	Scab index GH-point inoc	Kernel damage score§
Tokai 66	PI 382161	Brazil	56	23.2	25.4	10.3	2.3
Sapporo Haru.	PI 81791	Japan	63	29.4	11.9	8.8	2.0
Nobeoka Bozu	PI 382153	Japan	59	31.2	38.4	9.9	5.5
16-52-9	PI 382167	Brazil	64	33.7	37.2	39.0	1.0
Mentana	PI 182416	Italy	61	62.2	20.0	27.0	1.3
ND 2710		ND	55	27.4	22.3	9.0	3.0
Backup		MN	55	40.7	--	--	1.3
Wheaton		MN	58	78.0	90.0	93.3	8.2
Sonalika		Mexico	51	85.7	87.0	76.0	8.2

Days to flowering was estimated in a 1998 field nursery at Brookings, SD.

§ Average of seed scores from the field 1999 Elite Germplasm Nursery.

ASSESSMENT AND REACTION OF SOFT RED WINTER WHEAT GENOTYPES TO *FUSARIUM GRAMINEARUM* AND EFFECTS ON TRAITS RELATED TO YIELD AND SEED QUALITY

Matthew Chappell*, Carl Griffey, Tom Pridgen, Jianli Chen, and Jane Shaw

OBJECTIVES

1) To identify disease-assessment parameters that consistently differentiate resistant and susceptible host response to *Fusarium graminearum* and that are most predictive of subsequent effects on yield and quality related traits.
2) To discern the level of susceptibility or resistance among soft red winter wheat genotypes and the effectiveness of such resistance.

INTRODUCTION

Fusarium head blight (FHB) is responsible for large reductions in wheat yield and quality worldwide. In the 1997-98 growing season, Virginia growers suffered from epidemic levels of scab infection throughout the state. Yield losses for soft red winter wheat caused by scab were estimated to be as high as 33%. Epidemics likely will become more frequent in Virginia due to an increase in wheat acreage under conservation tillage. Virginia's climate is also quite favorable for scab epidemics, with cool moist conditions occurring frequently during flowering. Resistance levels are not high in current varieties grown in the state. However, it is apparent that there are differences among varieties not only in infection levels but also in yield response to the disease. From a grower's standpoint, it would be helpful to know which varieties grown in the region have the least yield and quality reduction under high disease pressure. From a breeder's standpoint, it would be helpful to know what parameters of disease assessment correlate best with losses in yield and quality related traits. Identification of the single most predictable, reliable and hopefully most feasible assessment parameter would allow breeders to focus and rely on a specific disease assessment parameter and, therefore, make field ratings less time consuming.

MATERIALS AND METHODS

Twenty (1997-98) and thirty (1998-99) soft red winter wheat genotypes were grown in replicated 100 ft^2 plots using a randomized complete block design with two treatments. Replications 1-3 comprised the inoculated block and replications 4-6 the non-inoculated control. Planting density was determined based on 1000 kernal weight and a target density of 24 seeds per row foot. All seed was treated prior to planting with Batanò (1.5 oz/100 lbs), Gauchoò (2 oz/100 lbs), and Captanò (3 oz/100 lbs). Baytanò was applied to control powdery mildew and Gauchoò to control aphids and, therefore, Barley Yellow Dwarf virus. Pre-plant fertilizer application included 25N-60P-90K (1997-98) and 25N-100P-100K (1998-99). Plots were planted on October 13, and Harmony Extraò herbicide (.5 oz/acre) was applied on Feburary 11. Spring nitrogen was applied at a rate of 60 lbs/acre along with another application of Harmony Extraò (.5 oz/acre) at growth stage 30, on March 31. Plots were harvested on July 5 with a small plot combine.

Treated plots were inoculated at flowering and again seven days post- anthesis using a conidial suspension of 1L/100 ft^2 at 50,000 spores/mL.

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After inoculation, all field plots received overhead mist irrigation from 8-9:30 A.M. and again from 6-7:30 P.M., unless conditions deemed irrigation not necessary (i.e. rain, heavy dew, or fog). Scab incidence and severity were measured at seven and fourteen days post-inoculation. Grain yield, test weight, 1000 kernel weight, DON toxin content, and percentage of scabby seeds were measured post-harvest. Barley Yellow Dwarf, *Staganospora* glume blotch, root rot, lodging, heading date, and height were also assessed in the field. All data was analyzed using Agrobase software (correlation analysis, LSD, and Anova).

RESULTS AND DISCUSSION

Parameters for Assessing Resistance

Correlations between traits for two growing seasons suggest that scab severity and percentage of scabby seed are the most useful parameters for assessing type IV and type V resistance in wheat varieties and are the main factors in the reduction of yield and test weight. Correlations for scabby seeds and scab severity with yield and test weight were significant over two growing seasons. In contrast, correlations for scab incidence and scab index with yield and test weight were not as significant as scab severity and percentage of scabby seed in 1997-98 and not significant in 1998-99. This indicates that scab incidence and scab index may be less effective tools for assessing resistance and loss. To confirm these findings, correlation values were obtained for yield loss and test weight loss (mean treatment differences calculated for inoculated vs. control) with scab severity, scab incidence, scab index, and percent scabby seeds. Again the correlation values were significant for percent scabby seed and scab severity, but not significant for scab incidence and scab index. This confirms that percent scabby seed and scab severity are the most useful parameters for assessing resistance and loss. DON toxin analysis can also be a useful tool in assessing type IV and V resistance. Correlation values among DON content with yield and test weight were significant. The problem with using DON content as a routine tool for assessing resistance and loss lies in the lack of test facilities and cost of analysis.

A concern in creating an environment that is ideal for *Fusarium* growth is the proliferation of other diseases of wheat, 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, indicating that root rot had no significant impact on yield or test weight. Glume blotch and yield were significantly correlated, 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 IV and V resistance to scab and, therefore must be precisely distinguished and accounted for in all field and lab assessments. Mesterhazy et. Al. have indicated that in field studies *Staganospora nodorum* control can be achieved with an application of Bayleton while plots were in the boot stage. This could be used to reduce or eliminate the correlation between yield and *Staganospora nodorum*, thereby focusing on yield losses due to Fusarium. Lodging also showed a significant correlation to yield and test weight. However, lodging effects can be controlled by application of a growth regulator (Cerone Ò), and should pose few problems in the future in assessing yield and test weight data.

Genotype Reaction and Response to *Fusarium*

Significant differences were observed among inoculated soft red winter wheat varieties with

respect to yield, test weight, scab severity, percentage of scabby seed, and DON concentration. Analysis of variance and LSD indicate that there is a continuum of resistance and yield mean values rather than easily definable group(s). For each parameter a statistically distinct grouping of cultivars was attained from the mean using LSD values and noted as statistically high or low.

In analyzing yield loss data it was found that Agripro Foster and Pioneer 2552, over two years, had lower yield loss than other varieties in the test. Coker 9835 and GA Gore showed consistent and high yield loss. Roane, Coker 9803, and Agripro Foster all showed consistently low test weight loss, whereas Coker 9835 and Madison consistantly had high test weight loss.

In analyzing parameters for assessing resistance, it was concluded that three measurements correlate well with type IV and V resistance. These assessment parameters are scab severity, percentage of scabby seed, and DON concentration. In analyzing scab severity data over two years, Ernie and Roane had the lowest statistically distinct scab severity values. This suggests that Ernie and Roane may be good sources of type IV and V resistance. GA Gore and Coker 9835, over two years, had the highest statistically distinct scab severity values and, therefore, it can be inferred that these cultivars do not possess type IV or V resistance to scab. In analyzing percentage of scabby seed data over two years, P92823A1-1-4-4-5 had a statistically lower percentage of scabby seed, and Agripro Foster, while not statistically lower than the mean, had a low percentage of scabby seed. This suggests that P92823A1-1-4-4-5 and Agripro Foster may also possess type IV and V resistance. As with scab severity, Coker 9835 had the highest statistically distinct percentage of scabby seed, indicating a lack of type IV and V resistance. In analyzing DON concentration over two years, Coker 9803 had statistically low DON values. This suggests that Coker 9803 may contain type IV and V resistance. GA Gore and Coker 9835 had statistically high DON values, which agrees with previous parameter analysis, suggesting that these varieties have little resistance.

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Table 1- SRWW Varieties in the Yield Loss Study and rankings with respect to Scab Severity and Scabby Seed Percentage. Ranks statistically higher or lower than the mean are indicated with a (*).

1997-98 Test			1998-99 Test		
Varieties	**Scab Sev.**	**Scabby Seed**	**Varieties**	**Scab Sev.**	**Scabby Seed**
ERNIE	1 *	4 *	ERNIE	1 *	28 *
P92823A1-1-4-4-5	2 *	1 *	IL94-1549	2	2 *
FREEDOM	3 *	5	IL94-1909	3	25
VA93-54-429	4 *	3 *	AGRIPRO PATTON	4	8
VA96-54-234	5	12	OH 552	5	17
COKER 9803	6	6	ROANE	6	11
PION 2552-B	7	11	P92823A1-1	7	3 *
JACKSON	8	18 *	PION 2552	8	14
PION 2580-B	9	17 *	VA96W-329	9	4
AGRIPRO FOSTER	10	2 *	VA96W-326	10	1 *
VA96-54-216	11	10	AGRIPRO FOSTER	11	5
AGRIPRO MASON	12	8	VA96W-348	12	22
WAKEFIELD	13	7	AGRIPRO MASON	13	6
PION 2684-B	14	15	NY87048W-7	14	7
PION 2643-B	15	9	PION 2643	15	9
FFR555W-B	16	13	VA96W-250	16	21
POCAHONTAS	17	16	COKER 9803	17	19
MADISON	18	14	QUANTUM 706	18	13
COKER 9835	19 *	19 *	FREEDOM	19	18
GORE	20 *	20 *	PION 2580	20	29 *
LSD	8.1	5.1	PION 2684	21	12
			WAKEFIELD	22	16
			FFR 555W	23	27
			MADISON:CH	24	20
			CAYUGA	25	23
			VA96W-247	26	26
			POCAHONTAS	27	10
			JACKSON:CH	28 *	24
			GA GORE	29 *	15
			COKER 9835	30 *	30 *
			LSD	0.07	8.1

HAPLOID PRODUCTION IN TWELVE WHEAT F_1 POPULATIONS VIA THE WHEAT X MAIZE HYBRIDIZATION METHOD

Jianli Chen*, Carl A. Griffey**, Matthew Chappell, Jane Shaw, and Tom Pridgen

ABSTRACT

Wheat x maize hybridization has proven to be efficient for haploid production in wheat. F_1 plants, from 12 crosses between six scab resistant sources and two susceptible soft red winter wheat (SRW) varieties, were pollinated with pollen of maize F_1 hybrid 'Seneca 60'. Mean frequencies of fertilization, embryo formation, embryo germination and haploid green plant regeneration were 83, 20, 45 and 8 %, respectively. Significant differences were found between two SRW parents in F_1 crosses for the efficiency of haploid production, based on the percentage of embryo germination and the percentage of haploid green plants regenerated. A total of 1024 haploids were regenerated from 2254 embryos derived from 13,527 florets pollinated. Improvement of haploid and doubled haploid production and potential use of the wheat x maize hybridization system for studying scab resistance is discussed.

INTRODUCTION

Doubled haploid techniques based on gamete selection provide the advantage of developing immediate homozygosity, which facilitates and improves the precision of genetic and mapping studies and provides greater efficiency of selection in plant breeding. The methods available for haploid production in cereals include anther culture, and wide-hybridization mediated chromosome elimination using *Hordeum bulbosum* or maize as the pollen sources. In wheat, use of the *Bulbosum* method is restricted to KrlKr2 genotypes. Anther culture in winter wheat is very much genotype dependent and offers very low success compared to barley, durum and spring wheat (Laurie and Reymondie, 1991; Fedak et al., 1997).

Since Laurie and Bennett (1986) first reported the method of haploid production in wheat mediated by maize chromosome elimination, this method has been used in wheat haploid production and applied with some success in generating genetic and mapping populations (Laurie and Reymondie, 1991; L. Shugar, K. Buerstmayr and G. Fedak, personal communication). The objectives of this study are: 1) To facilitate gene transfer and pyramiding of resistance to *Fusarium graminearum* from the six best sources screened in our program into soft red winter wheat varieties; 2) To generate the desired genetic and mapping populations for studying the inheritance of diverse resistance sources; 3) To study the feasibility and potential application in wheat breeding.

MATERIALS AND METHODS

Twelve wheat F_1 crosses were used in this study, which were derived from crosses between six scab resistance sources (Ernie, Roane, Shaan85-2, VR95B717, W14 and Freedom) evaluated in our breeding program (Griffey and Chen et al., 1998) and two commercial SRW varieties Pioneer 2684 and Madison. Wheat F_1 plants were vernalized and grown individually in 275 cm^3 pots in the greenhouse. Fertilizer was applied once a week to keep the plants healthy and promote tiller development. To ensure a continuous supply of pollen, maize seed were planted

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twice per week in 11,350 cm^3 pots with one to two seeds per pot. The maize variety Seneca 60 (Laurie, 1989) was supplied by Dr. Fedak of Plant Research Center of Research Agriculture Canada.

Wheat spikes were manually emasculated, with minimal cutting of the glume, one or two days prior to pollination. Fresh pollen was collected from maize and applied to emasculated wheat florets using a small brush. The intact pollinated heads were dipped in 100 mg/L of 2,4-D solution one or two days after pollination. Embryos were excised from fertilized seeds at 12 to 16 days after pollination and placed on Gamborg's B5 basal medium in test tubes. Embryos were maintained in the dark at 20 to 25°C in an incubator until coleoptiles were 1-2 cm long. The tubes were then transferred to a lab bench at 20 to 25°C with 12 hours of light per day provided by fluorescent lamps. Regenerated haploid plants were treated with a 0.1% colchicine solution for 5 hours to double the chromosomes, and then were planted in pots with soil in a growth chamber. Genotype effects were analyzed using Agrobase Software.

RESULTS AND DISCUSSION

Fertilization frequencies obtained for the wheat x maize (Seneca 60) hybridization system were high (mean 83 %) in all F_1 crosses used in this study. The average percentages of embryo formation (20 %), germination (45 %) and haploid production (8 %) were higher than those previously reported (Fedak et al., 1997; Kisana et al., 1993) for haploid production from wheat F_1's.

Less genotype-dependent response was reported for the wheat x maize hybridization system (Fedak, 1997) than for anther culture and the *Bulbosum* method. No significant difference was found for the percentage of seeds and embryos formed among 12 wheat F_1 crosses in the current study. This result agrees with that reported earlier by Matzk and Mahn (1994). However, large differences were observed among crosses for the percentage of embryo germination and haploid regeneration (Table 1). The frequency of embryo germination and haploid regeneration from crosses with Pioneer 2684 were significantly higher than those with Madison (Table 2). This suggests that the efficacy of haploid production could be improved through selection of more responsive parents, such as Pioneer 2684.

Compared to anther culture, the wheat x maize system has three advantages: higher efficacy, less variation and less time consuming. Based on the results previously reported by Kisana et al. (1993), the maize pollen method is about two to three times more efficient than anther culture (5 plants per 100 florets pollinated versus 3 plants per 100 anthers cultured). In the current study, twice as many green plants were regenerated (mean = 7.54%) using the maize pollen method. Kisana et al. (1993) reported that aneuploids or gross chromosomal abnormalities were not observed and confirmed that chromosome variations were not common in wheat x maize-derived plants. He also concluded that this technique could save four to six weeks in obtaining the same age haploid green plants.

The high efficiency of haploid production obtained by the wheat x maize hybridization system, likely will make it very useful for studying wheat scab resistance, as conventional methods of selection have given only incremental improvements to date. The reason for this limited success may be attributed to the complex inheritance of resistance and to limited sources of unique resistance. Results and data presented in this study were obtained by three people (2,400 hours) working over a six month period. The doubled haploid plants produced this spring already have been planted in the 1999-2000 field trails at two locations to evaluate scab resistance and agronomic traits. Type II resistance will be

evaluated in the winter of 2000 in greenhouse tests. This method theoretically will save about 3-5 years compared with the conventional breeding process. Pyramiding resistance genes by conventional selection likely would take a long time with limited success and precision. Producing doubled haploid populations will shorten the time to cultivar release, improve efficacy and efficiency in screening for resistance, and greatly facilitate genetic and mapping studies.

In order to use the wheat x maize system in practical breeding programs, further enhancement of embryo formation, germination, and green-plant regeneration and doubling is needed. Some green plants will die during colchicine-induced chromosome doubling and during transfer; therefore, the final population size may be too small to represent a sufficient number of possible genotypes to make selection effective. In the current study, an average of 20% of the green plants died during the chromosome doubling and then 20% of the surviving plants ceased during transfer stages. Approximately 80 % of the surviving green plants had normal seed set after doubling and transfering stages.

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Table1. Haploid production by wheat x maize hybridization in 12 wheat F1 crosses.								
	Florets	Seeds	Embryos	Green	%			
Pedigree	Pollinated	Developed	Rescued	Plants	B/A	C/B	D/C	D/A
	A	B	C	D				
MADISON/ERNIE	814	704	174	60	86.49	24.72	34.48	7.37
PION2684/ERNIE	1151	958	238	121	83.23	24.84	50.84	10.51
ROANE/MADISON	1569	1286	291	135	81.96	22.63	46.39	8.6
ROANE/PION2684	1730	1518	304	176	87.75	20.03	57.89	10.17
SHAAN85-2/MADISON	957	788	136	60	82.34	17.26	44.12	6.27
PION2684/SHAAN85-2	1040	717	130	76	68.94	18.13	58.46	7.31
VR95B717/MADISON	1300	1146	138	54	88.15	12.04	39.13	4.15
VR95B717/PION2684	1300	1015	159	77	78.08	15.67	48.43	5.92
MADISON/W14	612	452	48	12	73.86	10.62	25.00	1.96
PION2684/W14	729	587	140	61	80.52	23.85	43.57	8.37
ROANE / W14	616	502	109	63	81.49	21.71	57.80	10.23
FREEDOM / PION 2684	1709	1534	387	126	89.76	25.23	32.56	7.37
Total	13527	11207	2254	1021				
Mean					82.87	20.13	45.21	7.54
Crosses with PION2684	5950	4795	971	511	**79.50**	**20.50**	**51.84**	**8.46**
Crosses with MADISON	5252	4376	787	321	**82.56**	**17.45**	**37.82**	**5.67**
DIFFERENCES					**-3.06**	**3.05**	**14.02**	**2.79**
C.V.					7.93	22.76	5.84	21.71
HERITABILITY					0	0.05	0.93	0.59
LSD (0.05)					8.67	5.82	3.53	2.07

Table 2. Analysis of variance for frequency of embryo germination.			
Item	df	Mean Square	
		D/C	D/A
Between crosses with PION 2684 and Madison	1	490.980***	19.404*
Within crosses with either PION 2684 or Madison	4	106.088**	8.352
Error	4	6.864	2.352
Total	9		
* p< 0.05, ** p< 0.01, *** p< 0.001			

DISCOVERY AND DEPLOYMENT OF MOLECULAR MARKERS LINKED TO FUSARIUM HEAD BLIGHT RESISTANCE: AN INTEGRATED SYSTEM FOR WHEAT AND BARLEY

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ABSTRACT

Fusarium head blight (FHB) is a devastating disease of wheat and barley. Quantitative resistance and tremendous expense of screening indicate that molecular markers might expedite the search for resistance. Markers must be polymorphic and informative across populations to be used by breeders. Several groups are mapping genes for FHB resistance in wheat and barley. Although these markers may be validated and made breeder-friendly by labs that developed them, the urgency of the FHB situation requires efforts to accelerate this process. Prospects for a set of regional labs with high throughput characterization facilities, breeder-friendly markers, and doubled haploid technology will be discussed.

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DIALLEL ANALYSIS OF FHB AND TOMBSTONE KERNELS IN SPRING WHEAT EVALUATED UNDER GREENHOUSE AND FIELD CONDITIONS

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INTRODUCTION

Fusarium head blight (FHB) or head scab caused by *Fusarium graminearum* is one of the most serious diseases of wheat leading to wide spread yield losses worldwide. Provided the favorable weather conditions, this disease is capable of causing severe epidemics, particularly in the warm and humid/semihumid regions (3, 7). Moreover, FHB epidemics in wheat, in recent years, have been associated with above average precipitation, short rotation intervals, and increasing proportion of minimum- or no-till practices by the farmers (4). The losses from this disease are manifold. It not only causes direct loss in grain yield, but also severely affects grain quality by producing various toxic metabolites (1, 7). Under these situations, breeding resistant cultivars, undoubtedly, is the best and sustainable method of control in the long run. However, the limited information on the genetics and inheritance of the disease and the complex evaluation procedures, have slowed down progress. The objective of this study is, therefore, to see if the inheritance of resistance to FHB and tombstone kernels in spring wheat was similar in field and greenhouse environments.

MATERIALS AND METHODS

Five spring wheat genotypes/cultivars of diverse origin with varying degrees of tolerance to FHB (Table 1) were crossed in all possible combinations to produce ten populations. Using the single seed descent method, lines were derived at F5. Fifty $F_{5:6}$ lines, selected randomly, from each population were evaluated for FHB and tombstone kernels in both greenhouse and field experiments. Five hundred lines along with checks and parents were planted in individual hills (one set, replicated over time) in greenhouse in 1998 and 1999. Plants in each individual hill were tagged when they reached anthesis and were inoculated by spraying a conidial suspension (75000 conidia/ml) of isolates of *F. graminearum*. In addition, Fusarium-colonized corn seeds were spread on the ground. An automated misting system (30 seconds every hour from 6 PM-10 AM) was used to maintain a high level of humidity. The same set of materials was evaluated in a field FHB nursery in 1998 and 1999 each with two replications under similar automated misting system.

Visual scores of FHB on the inoculated spikes were recorded after 2-3 weeks using 0-9 scale (0=no infection, 9=100% infection). Plants were harvested at maturity and "tombstone" kernels were rated using 1-5 scale (1= 20%, resistant, 5=more than 80%, susceptible). These scales were expressed in terms of percentage and analyzed using Griffing's diallel method 4 (F1s only); model I (2).

RESULTS AND DISCUSSION

Analysis of variance for all the variables (FHB and tombstone kernels under greenhouse and field environments) revealed highly significant effects for genotypes (data not shown). The mean squares for general combining ability (GCA) were highly significant, and were 2-9 times higher than the mean squares for SCA (Table 2). This indicates GCA as the major

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source of genetic variation, and that the additive effects are of major importance, for the inheritance of resistance to FHB and tombstone kernels in these populations. Similar results were reported earlier (7). Highly significant SCA mean squares, on the other hand, does not rule out the possibility that some non-additive gene effects also exist (6).

CIMMYT 7 with the highest negative GCA effects is the best among five parents in terms of inheritance of resistance to FHB and tombstone kernels under both field and greenhouse conditions (Table 3). On the contrary, parents like Fang 60, with the highest positive GCA effects, appear to have factors for susceptibility. Parents showing highest negative GCA effects would be good sources for the improvement of resistance (5). The presence of highly significant GCA for susceptibility is an important consideration in a breeding program; therefore, highly susceptible parents should be avoided. Sonalika and Seri 82 both have similar and very high per se disease ratings, but the former has higher inheritance of susceptibility (highly significant positive GCA effects) than the latter.

Highly significant correlations (r=0.48-0.90) were observed between FHB and tombstone kernel estimates within field and greenhouse environments (Table 4). FHB and tombstone kernel estimates between field and greenhouse environments were also significantly correlated (r= 0.50).

CONCLUSIONS

- Our results indicate that GCA accounted for much of the genetic variations in these populations and hence the additive inheritance is of major importance.
- Resistance is heritable (e.g., CIMMYT 7 and 2375), and so is susceptibility (e.g., Fang 60). However, inheritance of susceptibility may not necessarily be the same even among the highly susceptible parents.
- Since FHB and tombstone kernels correlate well, any one of the two or both could be used as a tool for selection in the breeding program. Scab index, a weighted measure of FHB and tombstone kernels together, might be an even better method of estimation because of less environmental variation. Similarly, either greenhouse or field should be equally effective for FHB and tombstone kernel evaluation.

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Table 1. Spring wheat lines/cultivars used as parents in diallel cross along with their reaction to Fusarium head blight (FHB), tombstone kernels (TOMB), and maturity.

Lines/ cultivars	Pedigree	Origin	Reaction to		Maturity
			FHB	TOMB	
2375	Olaf/2/Era/Sugamuxi68/12/...	USA	MS-MR	MS-MR	ME
Fang60	Pitic62/Frondosa/3/Pitic62/...	Thailand	VS	VS	ME
Sonalika	II54-388/An/3/Yt54/N10B//Lr64	India	S	VS	E
Seri82	Kvz/Buho//Kal/Bb	Mexico	S	VS	M
CIMMYT7	CS/A.Curv//Glen/3/Ald/Pvn	Mexico	MS-MR	MR-MS	ML

MS-MR=Moderately resistant to moderately susceptible
MR-MS=Moderately Resistant to moderately susceptible
S=Susceptible
VS=Very Susceptible
E=Early
ME=Medium Early
M=medium
ML=Medium Late

Table 2. Analysis of variance for combining abilities (GCA and SCA), Griffing's method-4, model-1; Mean squares for Fusarium head blight and tombstone kernels under field and greenhouse environments.

Source	DF	FHB(FD)	FHB(GH)	TOM(FD)	TOM(GH)	Scab index
GCA	4	57.1**	178.3**	135.4**	96.9**	208.6**
SCA	5	6.4**	49.3**	29.2**	37.5**	43.0**
Residual	980	0.6	3.0	1.3	2.5	1.8

** Significant at 0.01 probability level

FHB(FD)=Fusarium head blight (field)
FHB(GH)=Fusarium head blight (greenhouse)
TOM(FD)=Tombstone (field)
TOM(GH)=Tombstone (greenhouse)

Table 3. GCA effects and mean disease scores (per se) of five spring wheat lines/cultivars for FHB and tombstone kernels under field and greenhouse conditions.

Lines/ Cultivars	GCA Effects (Per se)				
	FHB(FD)	**FHB(GH)**	**TOM(FD)**	**TOM(GH)**	**Scab index**
2375	-3.3**	-1.9	-6.5**	-2.5*	-4.9**
	(68.4)	(56.0)	(59.3)	(51.8)	(86.9)
Fang60	5.4**	11.7**	7.8**	4.1**	10.3**
	(86.2)	(82.1)	(88.6)	(80.8)	(125.3)
Sonalika	2.6**	1.6	4.2**	5.8**	5.1**
	(83.0)	(67.6)	(87.7)	(79.6)	(117.5)
Seri82	0.7	-2.1	2.0*	1.0	0.5
	(83.5)	(70.3)	(82.3)	(80.8)	(117.0)
CIMMYT7	-5.4**	-9.5**	-7.5**	-8.4**	-11.1**
	(65.1)	(56.1)	(53.0)	(44.0)	(81.1)

*, ** Significantly different from 0 at 0.05 and 0.01 probability levels, respectively.

Figures in parenthesis indicate per se disease ratings in percent.

Table 4. Correlation among FHB and tombstone kernels under field and greenhouse environments averaged over ten populations.

Variables	Correlation coefficient (r)
FHB vs Tombstone	
FHB (GH)-Tombstone (GH)	0.478**
FHB (FD)-Tombstone (FD)	0.899**
FHB-Tombstone (Overall)	0.709**
Field vs Greenhouse	
FHB (GH)-FHB (FD)	0.501**
Tombstone (GH)-Tombstone (FD)	0.502**
Scabindex (GH)-Scabindex (FD)	0.583**

** Significant at 0.01 probability level

TRANSFERRING FUSARIUM HEAD BLIGHT RESISTANCE FROM BREAD WHEAT SOURCES TO HIGH YIELDING ADVANCED LINES

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INTRODUCTION

Fusarium head blight is a common disease in high rainfall environments, where it causes severe cereal yield losses. The causal pathogen produces toxins that are hazardous to human and animal health. This last issue is of particular concern in areas where farmers produce grain for food and feed without controlling the level of toxins in the grain.

Though scab resistant wheat germplasm is rather limited, resistance sources have been found in wheats from Brazil, Japan and China. Resistance from these diverse genetic sources has been incorporated into high yielding genotypes in the CIMMYT Bread Wheat Program. However, the sources are mostly tall, late maturing wheat landraces that are low yielding, highly susceptible to leaf, stem and stripe rust, and generally have undesirable agronomic traits. The process of transferring resistance from these sources to high yielding wheats is not an easy one, but over the years the Program has succeeded in developing high yielding lines that show excellent resistance to fusarium head blight.

In this poster we highlight the highest yielding lines with the best resistance to fusarium head blight developed by the Program.

MATERIALS AND METHODS

For many years, the CIMMYT Wheat Program has concentrated on screening and breeding bread wheat germplasm for type I (fungal penetration) and II (fungal spread through the rachis) resistance under field conditions. Before advanced germplasm is evaluated for its response to scab, it is screened for resistance to the three rusts (leaf, stem and stripe) and to foliar blights.

Selection for leaf and stem rust resistance is conducted in the Yaqui Valley, Sonora, in northwestern Mexico, while resistance to stripe rust, *Septoria* spp. and scab is evaluated in Toluca, Mexico State, under artificial inoculation. Additional scab resistance observations are made in Patzcuaro, in the State of Michoacan, and Guadalajara, in the Sierra of Jalisco (State of Jalisco), where natural infestation is very heavy (Fig. 1).

A variety's ability to fill grain properly is very important. To ensure adequate grainfilling, the advanced lines are evaluated for this trait by comparing seed from inoculated plots vs plots protected by fungicide (Folicur) applied every 10 days.

In response to concerns over grain toxicity, grain of advanced lines is screened for toxin content. In this past year levels of DON toxin in lines showing good field resistance were evaluated at CIMMYT using the fluoroQuant method (Romer Labs, Inc).

Details of the methods used by the Wheat Program for inoculum increase and other laboratory and field inoculation procedures are described in Gilchrist et al., 1997.

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RESULTS AND DISCUSSION

The best bread wheat lines obtained in the breeding program combine various resistance responses (lower levels of penetration, reduced spread, decreased DON content and well filled grain) (Table 1). These results are from the last two crop cycles. Data showing resistance reactions were obtained over four cycles.

Table 2 shows progress in the CIMMYT-Shanghai (China) shuttle breeding program, using Sumai 3 as a resistant check. The lines developed through this program show excellent levels of scab resistance (better than Sumai 3) as well as high yield potential and good agronomic type (Fig. 2).

Incorporating fusarium head blight resistance into high yielding wheats is an ongoing process that seeks to broaden the genetic base of resistance through the use of diverse sources. The next step envisioned for this process is to combine the resistance described here with resistance derived from wild relatives of wheat using derivative hexaploid wheats.

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Table 1. Fusarium head blight resistant bread wheat lines developed by CIMMYT s Bread Wheat Program.

Cross	Pedigree	% Damage 1998	% Damage 1999	1998	1999	DON ppm	Test weight losses (%)	Grain quality (0-5)[1]
CATBIRD	CM91045-5Y-0M-0Y-4M-4Y-2M-0M-4M-0Y-3SCM	2.41	0.94	10.34	15.73	1.3	0.85	1
SHA3/CBRD	CMSS92Y00595S-1SCM-0CHN 015Y-3SCM	1.00	0.30	8.95	8.70	0.47	3.06	11
NG8675/CBRD	CMSS92Y00639S-5-5SCM	0.0	0.34	4.40	8.75	0.24	4.40	0-1
MILAN/SHA7	CM97550-0M-2Y-030H-3Y-3Y-0Y-3M-010Y-1SCM-0FUS-1FUS	3.39	0.59	8.77	13.45	0.23	3.56	0-1
GOV/AZ//MUS/ 3/DODO/4/BOW	CM79515-044Y-1M-02Y-07M-3Y-3B-0Y-0PZ-2PZ-0Y-5PZ-010Y-0M-3M-0Y-1SCM	5.30	4.37	11.56	16.48	0.42	0.90	1-2
CHUM18//JUP/ BJY	CM91046-7Y-0M-0Y-4M-8Y -0B-0FC-2FUS-0Y-1SCM	3.29	0.84	10.86	10.63	0.85	18.39	3
CHECKS								
FLYCATCHER (Moderate suscept)	CM43598-II-8Y-1M-1Y-3M-0B-7B-0Y	25.57		21.28		3.2	3.64	4
FRONTANA (Moderate Resistant)		12.90		17.03		2.0	7.71	2

1 0 = Excellent (no differences in appearance with fungicide protected grain) 5 = Poor (shriveled, sunken, highly infected).
2 NG8675 = NIGMAI 4 /OLESON//ALONDRA/YANGMAI 3/3/08181.

Table 2. Fusarium head blight resistant bread wheat lines developed by the Shanghai/CIMMYT shuttle breeding program and tested two years in Atizapan, Toluca, State of Mexico.

Reintroduced lines selected in Shanghai, China	% Damage Type I 1998	Type I 1999	Type II 1998	Type II 1999	DON (ppm)	Grain quality (0-5)[1]
SHANGHAI -0SHG-13GH-0FGR-0FGR	3.97	2.4	3.62	3.33	0.0	0
SHA3/CBRD 0SHG-2GH-0FGR-0FGR	9.90	0.0	8.40	4.28	0.05	0-1
SHA3/CBRD 0SHG-3GH-0FGR-0FGR	10.00	1.13	12.90	1.10	0.13	0-1
SHA3/CBRD 0SHG-5GH-0FGR-0FGR	5.37	2.56	5.04	2.17	0.14	1
SHA3/CBRD 0SHG-1GH-0FGR-0FGR	3.72	0.0	12.41	6.59	0.02	1
SHA3/CBRD 0SHG-16GH-0FGR-OFGR	0.26	5.43	5.65	2.75	0.0	1
NS73/PCI//B143.241.2/3/NING8647 0SHG-8GH-0FGR-0FGR	7.97	2.17	8.33	7.16	NA[5]	4
MIAN YANG81-5[2]//PC B084.985/JIANZIMAI[3] 0SHG-14GH-0FGR-0FGR	6.16	2.45	11.33	4.30	NA	4
PC B084.985/JIANZIMAI//8744 0SHG-4GH-0FGR-0FRG	8.91	3.08	14.53	5.71	NA	2
Checks						
Sumai #3[4] (Resistant)	9.77	1.64	6.06	24.85	0.0	1-2
Flycatcher (Moderate Susceptible)	25.57	7.47	21.28	49.07	3.2	4

1 0 = Excellent (no differences in appearance with fungicide protected grain) 5 = Poor (shriveled, sunken highly infected)
2 MIAN YANG 81-5 = MIANYANG 20 (=70-5858/FAN 6)
3 JIANZIMAI = Landrace
4 SUMAI # 3 = FUNO/TAIWAN WHEAT
5 NA = Not Available

TRANSFERRING FUSARIUM HEAD BLIGHT RESISTANCE TO MALTING AND OTHER TYPES OF BARLEY

L. Gilchrist[1*], H. Vivar[2], P. Hayes[3], C. Velazquez[1], and J. Crossa[1]

INTRODUCTION

Fusarium head blight caused by *Fusarium graminearum* is a disease of barley that reduces grain yield and affects grain quality. The pathogen produces toxins in the grain that are harmful to human and animal health. Swine, in particular, are very sensitive to toxin-contaminated grain.

In the Andean Region (Bolivia, Ecuador, Peru and Southern Colombia) of South America, barley is used for food and feed. In contrast, Mexico, Argentina, Brazil and Uruguay are malting barley producers. Fusarium toxins can affect the quality of both malt and beer by increasing wort nitrogen and reducing rootlet growth, malt recovery and beer gas stability. Beer and malt produced with toxin-contaminated grain will in turn be contaminated and will have a higher propensity to overfoam or gush (Schwarz et al., 1996).

To provide farmers with effective protection against Fusarium head blight, the ICARDA/CIMMYT barley program in Mexico is evaluating new sources of resistance, transferring resistance genes into a malting background, and developing resistant hull-less barley for food and feed.

MATERIALS AND METHODS

Malting barley

A doubled haploid (DH) population (125 lines) was developed in a two-rowed background. Sources of Fusarium resistance (Gob DH 96, Gob DH 24 and CMB 643=Azafran) (Vivar et al., 1997) were crossed with malting quality barley lines [Orca from Oregon (USA), AF 9216 (Brazil) and NE 175 (Uruguay)].

The DH population and parents were evaluated in Atizapan, Toluca, State of Mexico, under field inoculation during the 1999 summer cycle (Fig. 1). The spray and cotton methods were used to screen for type I and type II resistance, respectively (Gilchrist et al., 1997). Twenty spikes at the same growth stage (flowering in the center of the spike) were labeled. Inoculation for type I resistance was done at sunset with a 20-ml suspension (50,000 spores/ml) sprayed over the spikes. The spikes were evaluated after 15 days based on total number of grains as related to the number of visible penetration points, expressed in percentages.

For type II resistance, 20 spikes were inoculated and covered with a glassine bag. The spikes were evaluated after 30 days by counting the total number of infected grains close to the inoculated point and the total number of grains/spike, expressed in percentages.

Statistical categorical data analysis was done to identify the lines with the best Fusarium resistance.

Barley for food and feed

A method similar to the one described above was used to inoculate six-rowed hull-less F6 lines derived from crosses with Chevron (type I

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resistance source). Hull-less barley was reported by Clear et al. (1996) to reduce toxin content in the grain.

RESULTS AND DISCUSSION

Malting barley

Information on parents of the doubled haploid population is presented in Table 1. The disease reaction of the best DH entries are shown in Table 2.

Table 1. Characterization of type I and type II resistance to fusarium head blight present in parents used to produce the doubled haploid population. Atizapan, Toluca, State of Mexico, 1999.

	% damaged grains	
Parents	Type I	Type II
GOB 96 DH	10.7	6.7
Azafran	0.9	10.9
GOB 24 DH	6.7	4.2
Orca	13.7	20.6
NE 175	2.5	38.5
AF9216	7.1	26.3

Table 2. Doubled haploid entries combining the best type I and II resistance to fusarium head blight. Atizapan, Toluca, State of Mexico, 1999.

	% of damage grains	
DH entry number	Type I	Type II
DH 5	7.0	8.9
DH 6	3.7	8.9
DH 24	0.7	9.8
DH 34	9.1	7.4
DH 43	3.7	10.1
DH 60	6.5	6.9
DH 66	8.1	9.2
DH 87	4.9	9.8
DH 112	2.7	9.0
DH 115	4.2	7.1

The statistical analysis gave the range of infection for the group with superior type I (0.7-6.1 % infected grains) and type II (6.9-10.6 %) resistance, with a 95% confidence interval.

When crosses were made, no information was available on fusarium resistance of the malting parents. After field testing, several parents (NE 175 and AF 216) showed a good level of type I resistance, but very high susceptibility to type II (Table 1). These results explain the distribution in the population, which showed 40% type I resistance, 11% type II, and only 8% combining both resistance types (Table 2). Figure 2 shows the range of type I and II infection obtained during the evaluation process.

Barley for food and feed

According to our previous field results, Chevron, a six-rowed barley variety, possesses type I resistance, but not type II. Results presented here show lines to which type I resistance was transferred (Fig. 3) and a susceptible sister line.Only a few lines in this population combine both types of resistance (Table 3).

Table 3. Chevron-derived food and feed barley lines combining type I and II resistance to fusarium head blight Atizapan, Toluca, State of Mexico, 1999.

	% damage	
Cross	Type I	Type II
Petunia 1/Chevron //Tocte CBSS96M00734T-C-1M-1Y-2M-0FGR	1.08 - 6.35	
Petunia 1/Chevron //Tocte CBSS96M00734T-C-2M-4Y-1M-0FGR	6.59 - 22.48	

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RESISTANCE TO FUSARIUM HEAD BLIGHT IN SYNTHETIC HEXAPLOID WHEATS (2n=6x=42, AABBDD)

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INTRODUCTION

Fusarium head blight resistance in bread wheat is thought to be controlled by two to three major genes and several minor genes as modifiers. Head blight resistance can be transferred from alien sources to reinforce and improve the resistance already present in bread wheat.

In general the transfer of alien genes from tertiary genepools is a long process. However, bridge crosses utilizing D-genome synthetic hexaploids (SH) (*Triticum turgidum/Aegilops tauschii*) are a powerful means of swiftly improving bread wheat (*T. aestivum*) for resistance to biotic stresses (Mujeeb-Kazi et al., 1998).

In the past few years, CIMMYT wheat wide crosses has worked in conjunction with wheat pathology on the identification of scab resistant synthetic hexaploid wheats and their bread wheat derivatives.

MATERIALS AND METHODS

Germplasm development

Elite durum wheat (*Triticum turgidum*) cultivars were crossed with several hundred *Aegilops tauschii* accessions following the scheme shown in Fig. 1.

Figure 1. Synthetic Hexaploid Generation

T. turgidum X *Ae. tauschii*

↓

Hybrid
(2n=3x=21 ABD)

↓

Synthetic Hexaploid
(2n=6x=42, AABBDD)

↓

Head Blight Screening

Germplasm screening

Preliminary screening for leaf and stem rust resistance is carried out in the Yaqui Valley, State of Sonora. During the summer cycle, germplasm found to possess leaf and stem rust resistance is inoculated with stripe rust and a mixture of five virulent isolates of *Septoria tritici* in Atizapan, Toluca, State of Mexico.

Lines found to be resistant to the three rusts (leaf, stem and stripe) and *Septoria tritici* are evaluated for head blight resistance. Five spikes from each line are inoculated using the cotton method (Gilchrist et al., 1997). All lines showing type II resistance in this preliminary screening are inoculated the following cycle to confirm the initial observations. In addition, a spore suspension (50,000 spores/ml) is sprayed on each plot to determine type I (penetration) resistance. The

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same plot is harvested and a sample is taken and analyzed for DON content using the fluoroQuant method (Romer Labs, Inc.) Lower DON levels suggest type III resistance.

Grain filling is assessed by comparing test plots inoculated using the spray method with a similar plot sprayed with fungicide (Folicur) every 10 days. Minimum losses in test weight indicate type IV resistance.

RESULTS AND DISCUSSION

Presented in Table 1 are lines showing good fusarium head blight resistance, as demonstrated by their scores for different the resistance components.

Some of the SHs produced so far possess desirable fusarium resistance (type II). These SHs were utilized in crosses with bread wheat cultivars and advanced derivatives that had been tested for resistance (types I, II, III, and IV). The SH diversity has been transferred to bread wheat plant types. The *Ae. tauschii* diversity is revealed by the presence of five accessions (Table 1) in the pedigrees of advanced lines 205, 222, 223, 369, and 447. Micro-satellites of the D-genome are being utilized to elucidate the uniqueness of these accessions.

The best resistance combination is present in the cross Mayoor// TK SN1081/*Ae. tauschii*(222), which has also been identified to possess multiple disease resistance (Mujeeb-Kazi et al., 1999). Studies are currently in progress to determine genetic control, as well as develop an F1-based doubled haploid population in which the bread wheat parent Flycatcher is susceptible to all stresses to which the cross Mayoor// TK SN1081/*Ae. tauschii*(222) is resistant.

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Table 1. Synthetic bread wheat hexaploids with fusarium head blight resistance under artificial inoculation, Atizapan, Toluca, State of Mexico, during the 1998 and 1999 summer cycles.

Lines	% Damage Type I 1998 1999	% Damage Type II 1998 1999	DON (ppm)	Test weight losses (%)	Grain (0-5)
TURACO/5/CHIR3/4/SIREN//ALTAR 84/*Ae. tauschii* (205)/3/3*BUC CASS94Y00034S-24PR-2B-0M- 0FGR-0FGR-0FGR	18.42 - 8.01	9.88 - 8.71	0.58	5.27	2+
BCN//DOY1/*Ae. tauschii*(447) CASS94Y00006S-53PR-2B-0M-0FRG-0FGR-0FRG-0FGR	10.34 - 9.59	10.34 10.07	1	2.64	1
MAYOOR//TK SN1081/*Ae. tauschii*(222) CASS94Y00009S-18PR-3M-0M-0FRG-0FRG-0FRG	7.26 - 0.0	5.81 - 13.96	1.2	6.06	1
MAYOOR//TK SN1081/*Ae. tauschii*(222) CASS94Y00009S-50PR-2B-0M-0FRG-0FRG-0FRG	4.14 - 0.0	9.67 - 14.19	1.2	6.50	0-1
OPATA/5/CPI/GEDIZ/3/GOO//JO69/CRA/4/*Ae. tauschii* (223) CASS94Y00215S-5Y-1M-0M-0FRG-0FRG-0FRG	10.93 - 1.74	27.7 - 22.15	n.a.	6.18	3
MAYOOR/5/CS/*Thinopyrum curvifolium* //GLEN/3 /ALD/PVN/4/SC/ *Leymus racemosus* //2*CS /3/ CNO79 CIGM93.619-3Y-2B-0PR-1Y-0M-0FRG-0FRG-0FRG	10.17 - 0.0	29.46 - 22.58	n.a.	5.35	3
BCN/3/68112/WARD//*Ae. tauschii* (369) CASS94Y00125S-5Y-2M-0M-0FRG-0FRG-0FRG-0FR	12.66 - 11.21	20.60 - 3.76	n.a.	12.17	3-4
SUMAI#3 (resistant check)	4.41 0.86	15.49 10.59	0.27	38.59	3
FRONTANA (moderately resistant check)	12.90 - 11.56	17.03 - 27.85	2.0	7.71	2
MAYOOR (moderately resistant check)	16.94 3.4	17.04 - 27.85	4.5	5.09	3

+Grain 0= Excellent (no differences in appearance with fungicide protected grain).
Grain 5= Poor (shriveled, sunken, highly infected).
n.a. = Not available.

ADVANCES IN RESEARCH ON FUSARIUM HEAD BLIGHT IN THE VIRGINIA WHEAT BREEDING PROGRAM

Carl A. Griffey*, Jianli Chen**, Tom Pridgen, Matthew Chappell and Jane Shaw

OBJECTIVES

1) To optimize techniques for establishment and assessment of *Fusarium* head blight; 2) to identify resistance sources among both exotic germplasm and adapted wheat cultivars or lines; 3) to identify and select scab resistant wheat lines derived from crosses between type II and adapted resistance sources; and 4) to accelerate development of scab resistant cultivars or lines via use of back-crossing, wheat x maize mediated doubled haploid production and molecular marker assisted selection.

INTRODUCTION

Since the early 1990's *Fusarium* Head Blight (FHB) or scab has been one of the most devastating diseases in the U.S. and has increased in importance worldwide. The most tactical approach for controlling this disease is to utilize effective and diverse sources of resistance to develop cultivars possessing multiple types of defense mechanisms. However, success of this endeavor is greatly dependent on first obtaining knowledge of the amount of genetic diversity for resistance, identity of different mechanisms governing resistance, inheritance of resistance, and identifying selectable markers for incorporating and pyramiding resistance genes into wheat cultivars. Several type II resistance sources, such as Sumai 3 and its progeny, have been identified subsequent to the work of Shroeder and Christensen (1963). Recently, sources possessing other types of resistance such as type IV and V have been reported by Mesterhazy (1995 and 1999). Combining type II resistance with other types of resistance will likely lead to the development of cultivars possessing more effective and stable scab resistance.

MATERIALS AND METHODS

Optimization of Techniques for Disease Development and Assessment

Field Tests. Conidial suspension and scabby-seed dispersal methods of inoculation were used in 1997-99 field-tests. Conidial suspensions (378 L/Ac at 50,000 spores/ml) of *Fusarium graminearum* were sprayed on plots at both heading and flowering stages for homozygous genotypes. Segregating populations were sprayed with conidia once at heading and twice during flowering. Scabby seeds (20 Kg/Ac) were dispersed in plots at both the booting and heading stages for homozygous genotypes, and three times in segregating populations, once at booting and twice at heading stages. After inoculation, all field plots received overhead irrigation as a fine mist of water from 8-9 a.m. and again from 6-8 p.m. for 14 to 21 days or until symptoms were observed in most plots. Scab incidence, severity and infection type were assessed three times at 7 day intervals after inoculation. Grain yield, test weight, 1000 kernel weight, percentage of infected kernels and DON content were determined after harvest. All data analyses were performed as for a Randomized Complete Block Design using Agrobase software.

Greenhouse Tests. Single floret inoculation mainly was used in greenhouse tests. A drop

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(about 30ul) of conidial suspension (50,000 spores/ml) was placed into a single floret of the mid-spike using a SAMCO transfer pipette at the beginning of anthesis. Mist irrigation was applied immediately after inoculation at an interval of 45 seconds per 30 minutes for 3-5 days or until the first symptom was visible. Infection type (0-5; Chen, 1989) for each head was assessed 2-3 times at weekly intervals following inoculation.

Germplasm Evaluation and Breeding Tests

Germplasm has been evaluated in greenhouse tests from 1997 to 1999. Three sets of germplasm were used in these studies, which included 31 commercial cultivars or lines, 50 exotic resistance sources and 33 adapted resistant lines. The 31 commercial cultivars or lines were from the Virginia Official Variety Test and a mid-Atlantic Joint Test. The 50 scab resistant sources were from China (17), Italy (2), Brazil (1), Japan (3), Canada (12), France (3) and Austria (12). Three plants of each genotype were tested for type II resistance via the single floret inoculation method and one to two plants were tested for type I resistance via spraying a conidial suspension on the heads. Field tests included a Germplasm Screening Test (GP), Uniform Scab Test (UT) and Yield Loss Test (YT).

Breeding populations were first developed in 1994 with 20 crosses being made between SRW wheat genotypes and six Chinese resistance sources. From these populations, 2450 F_5 head rows were derived and evaluated in Advance Line Tests (AT) in 1999. Presently, more than 572 crosses are included in the Breeding Nursery, and consist of 290 F_1, 8 BC_2F_1, 24 BC_1F_1, 185 F_2, 41 F_3, 11 F_4 and 20 F_5 populations, respectively.

Three replicates of the UT test and one replicate of the GP and AT tests were grown in 20 ft^2 field plots (4-row headrow plots). Yield loss tests consisted of 100 ft^2 plots grown in six replicates, comprised of inoculated (3 reps) and non-inoculated (3 reps) treatments. Segregating populations were grown in individual 125 ft^2 plots.

Accelerated Development of Scab Resistant Germplasm and Populations

F_1 plants derived from 12 crosses between six of the best scab resistance sources and two susceptible cultivars, Madison and Pioneer 2684, were used to produce haploid plants by the wheat x maize hybridization method described by Chen et al. (1999). In addition, F_2 populations of 4 of the12 crosses were produced and are currently being used to study the inheritance and mechanism of resistance. An $F_{2:3}$ population of the cross Madison x W14 is being used to map the scab resistance genes in W14.

RESULTS AND DISCUSSION

Type of Resistance

Schroeder and Christensen (1963) first reported type I (penetration or invasion) and II resistance (spreading or extension) to FHB. Miller et al. (1985) reported type III (toxin accumulation) resistance, and Mesterhazy (1995) reported type IV and V resistance to kernel infection and tolerance, respectively. Few type I resistance sources have been reported, while several type II resistance sources have been identified and offer the most effective component of scab resistance (Bai, 1995; Buerstmayr, 1999; Masterhazy, 1995 and 1999). Results from our studies are in agreement with those of the aforementioned studies.

When conidia were sprayed onto wheat heads, no difference in infection type was found among genotypes, and 100% of the florets of the inoculated heads were infected in 1997 greenhouse tests. In contrast, when the single floret inocula-

tion method was applied, significant differences in resistance were found among genotypes (Tables 1 and 2) evaluated in 1997-1999 tests. While Mesterhazy defines type IV and V resistance independently, data from field tests conducted in 1997-1999 suggests that type IV and V resistance may result from a common mechanism. Significant positive correlations were observed between kernel infection with yield loss and test weight loss (Griffey and Chen, 1998 and 1999). DON content was significantly correlated with scab severity, kernel infection and yield loss. This finding is in agreement with results of most studies (Atlin et al. 1983; Wang and Miller 1988; Chelkowski 1989; Gilbert et al. 1993; Lemmens et al.; 1997; Buerstmayr et al. 1999) except for one by Mesterhazy (1999). Therefore, we conclude that selection of genotypes with a low percentage of scabby seeds is most likely to identify plants with relatively low DON levels. Severity, as a preharvest parameter and scabby seeds as a postharvest parameter are suggested as assessment parameters to evaluate resistance or tolerance to scab.

Techniques for Disease Development and Assessment

Use of precise inoculation methods and reliable assessment parameters are fundamental components for success in FHB research. Among a number of factors affecting disease development under field conditions, inoculum and free-moisture are controllable factors, whereas growth stage and temperature are non-controllable ones. The method used for disease establishment should correspond to specific research objectives and should reduce environmental and experimental variability to a minimum.

Scattering of scabby seeds as the primary source of inoculum is relatively easy, economical and not too laborious and, therefore, has been used in many breeding program to screen large numbers of pure lines and segregating populations. However, the incidence of disease resulting from this inoculation method can be affected greatly by plant height and growth stage (Griffey and Chen et al. 1997,1998) and, therefore, lacks precision in both uniformity and timing of infection. Spraying of conidial suspensions was found to be the most effective inoculation method under field conditions as it minimizes variability due to differences in plant height and growth stage and provides for precise application of uniform inoculum. Because infection rate and efficiency can be more precisely controlled and quantified, this method provides conditions for simultaneous assessment and selection of type II and other types of resistance, which likely represents field resistance.

Floret inoculation can be used effectively in both greenhouse and field evaluations for type II resistance (Griffey and Chen et al. 1997). This inoculation method is not affected by plant height or growth stage and can be conducted under controlled environmental conditions, which are critical for conducting genetic and mapping studies. Because floret inoculation is time consuming, it is not practical for screening large numbers of populations.

To evaluate field resistance, scab severity and percentage scabby seeds were found to be more effective and predictive than scab incidence for evaluating resistance or tolerance to scab. To evaluate type II resistance, mediated by floret inoculation, infection type assessment using a 1-5 incremental scale (Chen, 1989), was used in studies conducted in 1997-99. Infection type is assigned based on a qualified scale, with infection of the rachis being a critical component. Because type I resistance has not been identified in most currently used sources, all plants should produce infection symptoms upon inoculation. Differences among genotypes for type II resistance are expressed after initial infection, and are based on the length of extension time, the number of infected florets and the severity of infec-

tion. Results of our studies indicate that two to three ratings, made at 7-day intervals after inoculation, are necessary to accurately assess disease progress over time and, therefore, type II resistance. Differences between genotypes were observed after the first rating and 21 days after inoculation is usually the optimal time for the last rating. After 21 days, some susceptible spikes exhibit infection of the peduncle. Infection type ratings made 21 days post-inoculation are used in selection of genotypes with type II resistance.

Scab Resistance Sources

Assessments of type II resistance in 32 diverse sources are presented in Table 1. Significant differences were found among genotypes. Type II resistance identified in the germplasm was classified into three groups. Group I consists of highly resistant sources, which mainly includes the Chinese lines. Sumai 3, W14, Shaan 85, Futai 8944, Futai 9002 and Wuhan 1 were included in this group based on data from three years of testing. Based on replicated tests in a single year, Frontana, VR95B717, Shinchunaga, Saikai 165, H821, HC374 and H192 were also classified as highly resistant. Genotypes in this group usually have 1-3 infected florets per inoculated spike with little to no rachis infection, and do not exhibit significant change in infection type from 7-21 days after inoculation. W14, Shaan 85, Futai 8944 and VR95B717 are the four sources being used most extensively in our breeding program because of their resistance, superiority to Sumai 3 for other agronomic traits and/or their good combining ability. Approximately 552 crosses including type II resistant parents have been produced and are represented by $F_1 - F_4$ populations.

Group II includes moderately resistant sources, such as Ning 7840, Ning 9016, Yangmai 6, Yangmai 87158, Fan 1, Changjiang 8809 and VR95B295. The resistance of lines in this group is comparable to that of Funo, Mentana, and Nobeokabouzu-komugi, which are the primary parents of currently available type II resistance sources. Inoculated spikes of plants in this group have 3-5 florets infected, with increased rachis infection. Because some lines in this group have other favorable traits, such as early maturity, good head and/or plant type, these also may be useful sources of type II resistance. A total of 2470 F_5 head rows, with scab resistance derived from such sources, will be assessed for other agronomic traits and performance in field tests this year.

Group III includes sources with little or no type II resistance, such as Wuhan 3, Liang 10, VR95B063, FHB lines and most SRW wheat genotypes (Table 1 and 2). Susceptible genotypes have more than five infected florets per spike, with rachis infection, and exhibit either consistently high infection types or large changes in infection type between 7-21 days post-inoculation. Spikes of susceptible genotypes usually are bleached or white in color with infection of the peduncle in later stages of disease expression. Reaction response of genotypes in group I is more consistent and stable than that of genotypes in groups II and III. This is consistent with findings reported by Mesterhazy (1999) and Buerstmayr (1999).

Type II resistance was minimal or absent in the SRW wheat genotypes evaluated in these studies (Griffey and Chen et al. 1997); however, genotypes possessing other types of resistance or tolerance were identified. Genotypes such as Ernie, Roane, Freedom, P92823A1-1-4-4-5 and VA96W-326, had variable ratings for infection type, but had significantly low ratings for scab severity, yield and/or test weight loss, scabby kernels, and DON content (Table 2). Roane, released in 1998 by the Virginia Wheat Breeding Program, has consistently exhibited tolerance to scab in multi-year tests grown in Virginia and other states. VA96W-326 was found to be comparable to Ernie in maturity and level of resistance in 1999 tests. Roane and VA96W-326,

with high yield potential and resistance to other diseases, are scab-tolerant genotypes we are using in our program to incorporate type II resistance in to. Type II resistance derived from nine different sources was back crossed into 11 different SRW wheat backgrounds. Progeny from eight BC_2F_1 and 24 BC_1F_1 populations possessing type II resistance were crossed to their respective recurrent parents this year. Type II resistance is being transferred into acceptable SRW wheat backgrounds and combined with other types of resistance in some cases.

Future Plans

Because type II resistance has not been found in SRW wheat genotypes, identification and/or release of cultivars with tolerance to scab could provide for immediate reductions in yield and quality losses resulting from scab. SRW wheat genotypes with tolerance to scab will serve as ideal recurrent parents for incorporating type II resistance in to. In this way, type II resistance can be combined with other types of resistance in superior backgrounds. Tolerant SRW wheat genotypes, such as Roane, possess other favorable traits such as high test weight and yield potential, resistance to powdery mildew and Hessian fly, tolerance to Barley Yellow Dwarf Virus, and good general combining.

Development of adapted wheat cultivars with stable and improved levels of scab resistance can be best achieved by pyramiding diverse resistance genes that individually confer only partial resistance and /or act via different mechanisms. The most feasible, and perhaps only possible, means for achieving this goal is to identify molecular markers that are tightly linked to such resistance genes, which can be used to facilitate marker-assisted selection of scab resistance and gene pyramiding. Development of doubled haploid lines via the wheat x maze hybridization system may prove beneficial in accelerating the development of SRW wheat lines with higher levels of combined scab resistance, since pure lines can be obtained in one generation rather than six to eight (Chen and Griffey et al. 1999). These types of research projects are currently being implemented in the Virginia breeding program.

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Table 1. Identification of Type II Resistance to Scab in 32 Diverse Sources via Floret Inoculation in 1997-99 tests.

Genotype	Source	Infection Type (1-5)*		
		1997	1998	1999
Funo	Italy		3.2 - 4	3.0 - 4
Mentana	Italy		3.2 - 3.6	4
Frontana	Brazil		2 - 3.2	
Sumai 3	China	3	2 - 3.4	2 - 3.4
W14	China	1	2	3.4
Shaan 85	China	3	2	2
Futai 8944	China	3	2	2 - 3.4
Futai 8945	China		3.4 - 4	3.4 - 4
Futai 8946	China	3	3.0 - 4	3.8 - 4
Futai 9002	China	2	2	2 - 3.4
Ning 7840	China	2	2 - 3.6	2 - 3.8
Ning 9016	China	3	3.0 - 4	3.8 - 4
Yangmai 6	China		3.4 - 4	3.4 - 3.8
Yangmai 87158	China		3.2	3.6 - 4
Liangmai 10	China		4.0 - 5	4
Fan 1	China	4	3.4	2 - 3.8
Wuhan 1	China	1	3.4	3.2
Wuhan 3	China	5	3.6 - 4	4.0 - 5
Changjiang 8809	China		2 - 3.4	3.6 - 3.8
Nobeokabouzu-komugi	Japan			3.4 - 4
Shinchunaga	Japan			3.4
Saikai165	Japan			2 - 3.4
VR95B717	France			2 - 3.2
VR95B295	France			3.2 - 4
VR95B063	France			4.0 - 5
FHB143	Canada		3.6 - 4	
FHB147	Canada		3.8 - 4	
FHB148	Canada		4.0 - 5	
FHB161	Canada		4	
H821	Canada		2 - 3.2	
HC374	Canada		2	
H192	Canada		2 - 3.4	

* Infection type: (for evaluating type II resistance by floret inoculation)

1.0 = only inoculated floret infected; 2.0 = only inoculated spikelet infected;

3.0 = inoculated spikelet and rachis infected; 3.2 = inoculated and one adjacent spikelet infected;

3.4 = inoculated and two adjacent spikelets infected; 3.6 = inoculated and three adjacent spikelets infected;

3.8 = inoculated and four adjacent spikelets infected; 4.0 = half of spike or over six spikelets infected;

5.0 = whole spike infected.

According to the highest infection type of each individual, resistance can be subdivided into R (1-3), MR (3.2-

MS (3.6-3.8) and S (4-5) reaction classes.

Table 2. Evaluation of tolerance to scab among 18 soft red winter wheat genotypes tested at Blacksburg, Virginia in 1997-99.

LINE	Infection Type (1-5)		Test Weight Loss (%)		Scabby Seeds (%)		Scab Severity (%)		Scab Index		Scab Incidence (%)		Toxin (ppm)
	1997	1999	1998	1999	1998	1999	1998	1999	1998	1999	1998	1999	1998
ERNIE		3.2-4	1.9	2.3	19	19	18	11	8	4	42	31	10.1
ROANE	3.6-3.8	3.0-4	1.8	2.2	20	8	23	14	18	9	79	61	10.6
P92823A1-1-4-4-5			1.8	1.1	14	4	21	15	15	7	67	49	8.1
FREEDOM		3.4-3.8	1.9	3.6	18	10	21	20	18	13	83	64	9.0
AGRIPRO FOSTER		3.6-4	3.6	0.6	19	6	32	17	27	7	83	43	9.0
COKER 9803	5	4.0-5	3.6	1.6	19	11	32	18	20	8	65	44	8.8
GA GORE		4.0-5	11.8	1.7	47	10	85	30	83	17	97	56	20.7
WAKEFIELD		4.0-5	3.8	1.7	23	10	32	21	23	14	71	62	12.3
AGRIPRO MASON	5	4.0-5	5.4	2.8	26	7	37	18	27	9	71	51	12.8
POCAHONTAS		3.8-5	5.7	2.8	29	8	42	26	38	18	93	69	14.2
JACKSON	5	4.0-5	5.6	2.3	39	13	32	29	25	24	77	79	12.8
PIONEER 2552	3.4-3.6	3.8-5	1.8	2.7	23	9	30	15	25	10	84	65	12.8
PIONEER 2684	5	4.0-5	3.6	2.8	32	9	42	21	36	14	87	65	14.1
PIONEER 2643	4	4.0-5	5.4	3.3	25	8	42	18	36	11	86	58	12.8
PION2580	5	4.0-5	3.7	3.4	37	22	29	21	20	11	69	57	13.3
FFR555		4.0-5	7.3	3.4	27	16	46	21	41	12	89	61	14.4
MADISON	5	4.0-5	9.1	5.2	31	12	45	22	34	14	73	63	15.4
COKER9835		4.0-5	7.8	9.1	42	27	52	37	50	33	95	89	17.6
LSD(0.05)					8	6	10	7	13	7	16	17	1.9
Mean					27	11	36	19	30	12	79	58	12.5

EVALUATION OF YUGOSLAVIAN WHEAT GERMPLASM FOR RESISTANCE TO HEAD SCAB OF WHEAT

Anju Gupta[*1], Kimberly G. Campbell[1] and Patrick E. Lipps[2]

INTRODUCTION

Disease: Head Scab has been a huge threat to wheat population in the USA. Grain loss is primarily due to floret sterility, poor to no seed filling causing pink shriveled grains with low test weights. Loss is further amplified by the presence of the fungal mycotoxin, deoxynivalenol (DON).
Host: Wheat (*Triticum aestivum*) is the primary host for the pathogen.
Pathogen: The causal organism is *Fusarium graminearum* Schwabe. (teleomorph = *Gibberella zeae).*

OBJECTIVE

The main purpose of screening Yugoslavian wheat germplasm is to identify new sources of resistance and use these lines as parents in the Ohio 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 cultivar). 210 Yugoslavian winter wheat accessions were selected. 20 seeds per genotype were sown in flats of soil in 1998. 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 the soil. Plants were watered twice a day. The greenhouse temperature varied from 23^0C during the day, with a range of 19^0C to 30^0C and 19^0C at night, with a range of 17^0C to 21^0C.

Inoculum Preparation

Fungal cultures from four aggressive *Fusarium graminearum* isolates were grown in malt extract agarose media 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 suspensions from four different isolates were mixed in equal volume and concentration was adjusted to 10^5 conidia/ml.

Inoculation

The hypodermic syringe inoculation technique as described by Bai et al.(1986) was used. 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. Plants were then returned to the greenhouse bench. The greenhouse temperature varied from 23^0C during the day, with a range of 19^0C to 30^0C and 19^0C at night, with a range of 17^0C to 21^0C.

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Data

Inoculated heads were assessed as percentage of spikelet affected after 10 and 14 days. Disease severity was recorded using a visual assessment scale (Stack et al, 1994, NDSU Extension).

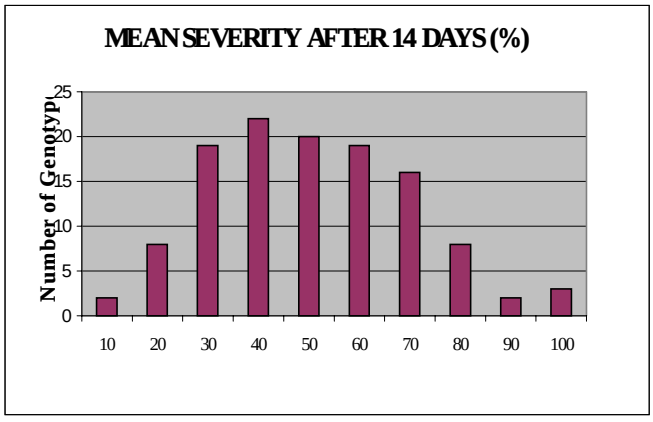
DATA ANALYSIS AND RESULTS

Mean severity for 20 plants for each genotype was calculated using SAS (Release 6.03). One way ANOVA was run over the entire population using general linear model procedure ('PROC GLM'). Genotypes with less than 10 plants were not used in the analysis.

Table I: One way Analysis of Variance for disease severity 14 days after inoculation for 119 genotypes (1148 plants) in the greenhouse.

Source	Degree of Freedom	Sum of Squares	Mean Square	F value	P value	Significance
Model	118	390503.89	3309.29	2.41	0.0001	***
Error	1029	1412829.3	1373.01			
Corrected Total	1147					

Fig. I: Distribution Curve for Mean vs. Frequency of the 119 Yugoslavian Wheat Accessions in the GH.



TEST OF SIGNIFICANCE

Dunnett's one tailed T test was performed for mean severity after 14 days to determine differences from a control mean. The control genotype selected had a mean severity of 91.6 and high number of plants (18) inoculated. Alpha=0.1, Confidence interval=90%, df=1029, Critical value of Dunnett's T=2.879.

CONCLUSION

· The mean severity after 14 days was distributed normally for the 119 genotypes which ranged from highly susceptible to moderately resistant (Fig. 1).

· The significant value of "F" (Table I) implies that there was sufficient evidence for significant differences between the genotype means.

· Dunnette's one tailed T test (<= 0.1) was used to select the genotypes that were statistically significant (more resistant) than the control.

A high alpha level (<= 0.1) was used to minimize the probability of mis- identifying the genotypes as susceptible when they were actually resistant (Type II error).

Table II: Genotypes with significant different mean severity than the control (% mean=91.6) with at least 10 plants inoculated in the greenhouse.

Accession no.	No. of plants per genotype	Mean severity after 10 days	Mean severity after 14 days	Significance, Critical value of Dunnett's T=2.879
17352	12	13.25	18.0	***
17353	10	8.7	21.3	***
184252	14	11.14	26.89	***
184253	12	4	21.58	***
221388	14	20.92	34.92	***
259879	18	14.83	31.88	***
259883	10	10	29.6	***
284657	19	18.05	32.26	***
346801	10	16.14	18.57	***
351263	11	8.72	12.18	***
358332	10	17.2	20.3	***
358337	12	9.25	23.16	***
367245	10	5.11	16.66	***
434650	10	19.37	23.25	***
470104	17	15.82	27.11	***
542442	14	20.14	28.14	***
542444	10	21	25.8	***

FIELD DATA ANALYSIS

These 200 Yugoslavian lines were screened for resistance to the fusarium head blight in the field at OARDC, Wooster, Ohio. Lines were planted in a completely randomized block design with two replications each. Experimental units were 1m long and 30cm apart (0.3 sq. feet). Patterson and Pioneer 2545 were included as susceptible check and Ernie and Freedom as resistant checks. The field was inoculated in the first week of May using colonized corn kernels (Campbell and Lipps, 1998). Heading dates were recorded as early, mid and late. 20 heads from each genotype were rated for %spikelet affected approximately 21 days after anthesis. Data collected were incidence, severity, visual kernel assessment, kernel test weight, % scabby seed and DON level within the harvested kernels. Data was analyzed and compared with the greenhouse data.

FUTURE WORK (1999-2000)

The selected genotypes from this generation with moderately high resistance were planted again in the greenhouse and in the field for evaluation in 2000. The same method for inoculation and disease screening will be conducted as it has been done in the year 1998/9. We will also make crosses between resistant and susceptible lines and with other sources of resistance that are currently available in different breeding program. A pedigree analysis for all the resistant genotypes will be done after completion of disease screening.

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Table III: One way analysis of variance performed on 149 genotypes with different dependent variables.

	DEPENDENT VARIABLE				
SOURCE	FHB incidence (%)	FHB severity (%)	FHB index	Visual kernel rating (%)	Tombstone Kernels (g)
df	148	148	148	100	100
Mean	93.13	32.42	31.08	46.49	23.66
Mean square	233.59	516.6	538.24	2217.09	249.3
F-value	1.86	3.49	3.52	3.19	3.15
P-value	0.0003	0.0001	0.0001	0.0001	0.0001
LSD	25.81	28.02	28.47	62.33	21.14

Table IV: Field data analysis of Yugoslavian wheat germplasm (1998-1999) for Fusarium Head Blight

Genotype	FHB Incidence (%)	FHB Severity (%)	FHB index	Visual Kernel (%)	DON (ppm)	Scabby seed (%)
OH 552	100	32.07	32.07	1.63	2.3	8.03
15893	45	4.1	1.7	30	2.9	18.6
* 17352	70	11.5	7.6	42.5	5.3	13.6
* 184253	94.5	9.7	9.2	6	1.3	12.2
* 184254	82.5	22.5	19.8	5	1.1	5.9
* 284657	85	24.9	24.5	7	3.6	7.5
284667	90	19.5	17.6	2	-	9
284668	80	17.3	14	2	-	6.5
316427	85	24.5	21	5	3.2	4.5
351260	85	16	13.9	7	2.7	8.1
434676	100	9.6	9.6	2	-	3.3
470103	100	19.9	19.9	1.5	0.9	9.6
* 470104	82.5	22.7	18.2	1	5.5	6.1
* 542444	100	22	22	1	-	5

* Genotypes showing strong resistance for head scab in the greenhouse screening.

OH 552 was used as the resistant check in the FHB

SCREENING FOR SCAB RESISTANCE OF WHEAT IN THE GREENHOUSE

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INTRODUCTION

Wheat scab or Fusarium head blight has become the first priority of the Minnesota wheat breeding program. During a 6-year period, greenhouse testing of new sources of scab resistance was conducted beginning in spring, 1994 to present (winter, 1999). About 10,000 wheat genotypes (70,000 plants), including 100 Chinese wheat varieties, have been inoculated and evaluated in the greenhouse.

In 1963, Schroeder (2) observed two types of resistance to scab in wheat cultivars: resistance to initial infection by the fungus (infection-resistance) and resistance to spread of the pathogen within the plant after infection (spread-resistance). Screening for resistance to FHB in the greenhouse is mainly focused on spread-resistance.

MATERIALS AND METHODS

Planting and management in the greenhouse

Greenhouse testing is conducted three times a year: spring (January to April), summer (May to August) and winter (September to December) tests. The days from planting to inoculation (heading) differ for each period. Based on 6-years average, the days from planting to inoculation are as follows: spring test - 42.6 days, summer test - 34 days, winter test - 38.6 days.

Plants are seeded in 14 cm pots, with 5 plants/pot used for evaluation. About 10 to 15 plants per line are used to obtain an accurate measure of spread resistance of advanced lines. In addition, 5 plants of each F5 line are evaluated for spread in the head while the F5 lines are under increase in the winter nursery prior to preliminary yield tests.

About 5 g of Nutricote (14-14-14) fertilizer are applied to each pot one week after planting. About 2 g of Peters (20-15-20) is also given to each pot before heading. Bayleton is used to control powdery mildew. Marathon was applied to the soil to control the aphids with Nicofume used for additional aphid control. Greenhouse temperature was controlled at 20° C with a 16 hour daylength.

Inoculum and inoculation

Pathogen - F. graminearum isolates were collected from the susceptible variety Wheaton from 4 locations in Minnesota: Crookston, Morris, Rosemount and St. Paul (4).
Isolates - Diseased kernels were isolated on PCNB agar first. > 30 isolates were mixed (4).
Inoculum - Inoculum was produced by shaking 4% mung bean soup with the isolates in 1994 and 1995 (3). Since 1996, 4 % mung bean agar has been used to produce conidia spores. Conidia suspension were diluted to 100 conidia per microscope field (100 X).
Inoculation - The tips of the glumes are removed by cutting one spikelet in the middle of the spike. Then 5 microliters of spore suspension

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are injected into a single floret in the middle of the cut spikelet using a microliter pipette (Pipetman).

Incubation - After inoculation, plants were incubated in a dew chamber at 20° C for 3 days, then returned in the greenhouse.

Evaluation of resistance

Two evaluations of disease spread are used:

(1) Scab index - Disease is evaluated 20 days after inoculation using four grades.

Grade 1. Only the inoculated spikelet was infected, **no spread**.
2. Diseases spread to the adjacent **1 or 2 spikelets**.
3. Disease spread to **one half of spike**.
4. Disease spread to the **whole spike**.

Evaluation of resistance:

1.0 - 1.4 = R 2.5 - 2.9 = MS
1.5 - 2.4 = MR 3.0 - 4.0 = S

(2) Severity - % of diseased spikelet.

$$\text{Severity} = \frac{\text{No. of spikelets infected}}{\text{Total No. of spikelets}} \times 100$$

Evaluation of resistance:

< 20 % = R 31-50% = MS
21-30% = MR > 50% = S

RESULTS

About 2500 to 3000 lines were screened each year in the greenhouse from 1994 to 1999. For example, in winter 1997, a total of 1084 F5 lines were evaluated and >50% lines were discarded because of their scab susceptibility. Sixty-five F5 lines from 1997 were selected (5 plants evaluated) based on R reaction and about 89% of the lines selected tested either R or MR in subsequent tests. Therefore, even testing only 5 plants provided reliable data to discard susceptible lines.

During the past 6 years, some Chinese genotypes with high resistance to scab were retested more than 10 times and have been used as resistance sources in the wheat breeding program (Table 1). New crosses with good resistance are presently being tested. Many still depend on Sumai 3 source resistance (Table 2, 3), but other sources are possibly present. For example, cross M5-212 has Yumai 7 as a principle source of resistance and 48 lines from this cross in spring 1999 greenhouse test had a mean scab grade similar to Sumai 3. Ten selected lines from this cross were retested in the summer greenhouse and had about 1/2 the % severity of Sumai 3. Ten lines from 1996-97 M5 have exhibited high resistance for the next three generations (Table 2). Even with high humidity in the summer of 1999, these lines remained similar to Sumai 3. Six advanced lines displayed high resistance in two greenhouse tests and in the field in 1999, with five of these six having Sumai source for resistance (Table 3).

The cooperative breeding and testing effort has released three new varieties with MR to R greenhouse reaction to scab: BacUp (1996), HJ 98 (1998), and McVey (1999).

Using percentage of diseased spikelets to evaluate scab resistance may be more precise than scab index. Percentage of diseased spikelets significantly correlated with greenhouse tests in different years (r = 0.73) and with severity in the field from 1994 to 1997 (r = 0.67-0.87), compared to lower correlations achieved with scab index (r = 0.5).

Table 1. Sources of resistance: (Chinese cultivars and lines)

Variety	Mean scab evaluation* 1995-97	Summer,99	Pedigree	Origin
Sumai 3	1.2(18)	54.4(18)**	Funo/Taiwan wheat	Suzhou, Jiangsu
Wang-shui-bai	1.1(12)	35.0(5)	Local variety	Liyang, Jiangsu
WZHHS	1.3(13)	47.3(4)	Local variety	Weizhou, Funjian
Ning 7840	1.2(9)	42.4(2)	Aurora/Anhui11//Sumai3	Nanjing, Jiangsu
Ning 8306	1.2(10)	35.3(3)	263/Fan5//Ning7302/3/Ning 7084/Yangmai5	Nanjing, Jiangsu
Ning 8331	1.1(5)	58.5(2)	Yangmai4/Ning 7071	Nanjing, Jiangsu
Fujian 5114	1.1(8)	29.0(2)	unknown	Fuzhou, Fujian
Fujian 5125	1.2(8)	37.5(2)	Fufan904/Ning8017	Fuzhou, Fujian
Fujian 60096	1.2(9)	39.2(2)	Jingzhou 2 X Sumai 2	Fuzhou, Fujian
Yumai 7	1.2(15)	39.1(4)	Npegrophar 2/Yanshi 4	Henan
Yan-shi 9	1.3(20)	50.0(2)	Npegrophar 2/Yanshi 4	Henan
Er-mai 9	1.2(19)	37.4(4)	unknown	Hubei
			(Ning7071, Ning7084 & Ning8017 all from Sumai 3)	

Table 2. New resistance lines after three greenhouse test.

Line	Mean scab evaluation* 1997	Summer,99	Pedigree	Sources
212		28.9(10)**	MN94151//Yumai 7/ND673	1998-99 M5
018-5	1.0	29.1(3)	MN90071/SBE0303-19	1996-97 M5
020-7	1.6	57.1(6)	MN90138/Kulm	1996-97 M5
061-2	1.1	54.4(4)	MN92192/SBF608-25	1996-97 M5
074-5	1.0	30.9(3)	MN92390/SBF608-25	1996-97 M5
274II-3	1.2	43.8(6)	SBF608-25/MN92045	1996-97 M5
301-14	1.0	29.5(1)	N90-0666/MN91227	1996-97 M5
307-1	1.4	38.5(3)	MN91227/SBE0303-18	1996-97 M5
308-25	1.3	49.5(3)	MN91227/MN86411	1996-97 M5
327-1	1.0	56.8(5)	MN92197/SBF608-25	1996-97 M5
333-3	1.2	53.0(2)	MN93413/SBE0303-10	1996-97 M5
Sumai 3 (CK)	1.2	54.4(18)		
Roblin (CK)	3.8	91.4(19)		

* 1995-97 scored by scab index, 1999 scored by % severity. ** No. of replicates in the parenthesis.

Table 3. Selected new advanced resistance lines (1999 PY s).

Line	Pedgree	Reaction to scab*			
		Greenhouse		Field,99	
		Winter,98	Spring,99	St. Paul	Crookston
MN 99051	MN91227/Ning8331-4	1.3	1.0	1.5	1.5
MN 99104	Hamer/Fujian60096	1.0	1.5	1.5	1.5
MN 99182	Ning7840-4/MN93505	1.0	1.3	1.5	1.0
MN 99190	Fujian5114-1/2375	1.0	1.0	1.0	1.5
MN 99201	BacUp/Ning7840-4	1.0	1.2	0.5	1.0
MN 99327	Fujian5125-11/MN93434	1.0	1.0	1.0	1.5
Sumai 3 (CK)		1.3	1.2	_	_
Roblin (CK)		3.9	3.8	5.0	5.0

* Scab grade: 1-4 (greenhouse), 0-5(field)

DISCUSSION

Severity, or resistance to spread, is repeatable in greenhouse screening of wheat germplasm and has good relationship to severity in the field. It increases our efficiency because of the ability to screen for resistance year around. Greenhouse severity readings are used as a basis of initial F5 line selection before preliminary yield trials. Susceptible lines are discarded during winter increase before harvest.

Advanced lines are evaluated for scab continuously throughout their development in both the greenhouse and field. Our greenhouse testing procedures do not permit evaluation of resistance to infection, or incidence. Incidence can be evaluated in field testing, but varies due to different environmental conditions (years, location, planting date, etc) (1, 4), as does severity, but severity seems to vary less than incidence.

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DEVELOPING FHB-RESISTANT WHEAT CULTIVARS FOR THE MIDSOUTH

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OBJECTIVE

To develop FHB-resistant wheat cultivars adapted to the Midsouth.

INTRODUCTION

In the Midsouth (Alabama, Arkansas, Louisiana, Mississippi, and Tennessee) soft red winter wheat is grown on over two million acres annually. Although the incidence of Fusarium head blight (FHB) on wheat in the region is sporadic, the disease can be severe if rainy weather occurs before, during, and after flowering. Environmental conditions were conducive for FHB development in 1990 and 1991 causing widespread, severe disease throughout the region. Currently, none of the wheat cultivars grown in the region have resistance to FHB. Having FHB-resistant cultivars would be an important component of any integrated management program for FHB.

MATERIALS AND METHODS

To develop FHB-resistant cultivars as quickly as possible, advanced Arkansas breeding lines with sources of FHB resistance in their pedigree were selected for FHB evaluation in 1999. Over 200 lines will be evaluated in 2000 for FHB resistance in an inoculated, irrigated screening nursery and for agronomic traits and resistance to other diseases in yield trials at several locations.

To develop cultivars with high levels of FHB resistance, a germplasm enhancement (parent building) program was begun in 1997. Agripro Mason and Pioneer variety 2684 were selected as the adapted parents because of their short vernalization requirements and photoperiod sensitivities which permit more rapid advancement of generations and wide adaptation, respectively. Various sources of FHB resistance (mostly CIMMYT spring wheat lines) were crossed, backcrossed or topcrossed with the adapted parents. Seventy-eight F_3, backcross F_2, or topcross F_2 populations were screened in 1999 for maturity, plant height, yield potential, FHB resistance, and Septoria tritici blotch resistance. Two hundred heads were selected from the best plants in each of the 76 populations that had both good agronomic and resistance characteristics. Heads were threshed individually, and seed from the best 120 heads in each population were planted to the field as headrows and will be screened for FHB resistance in 2000.

RESULTS AND DISCUSSION

Progress is being made toward developing FHB-resistant wheat cultivars for the Midsouth. Arkansas breeding lines with FHB resistance will be advanced to the Wheat Strains Nursery and tested at several locations for yield, resistance to other diseases, and milling and baking quality. Germplasm lines that are selected will be sorted by pedigree, and the best lines within each pedigree will be advanced to a Regional Observation Nursery to be planted by volunteer cooperators who are interested in utilizing the germplasm. Some of the best lines from populations with different sources of FHB resistance will be intercrossed within the Agripro Mason and Pioneer 2684 genepools in order to obtain lines with higher levels of FHB resistance.

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'WINTER NURSERY' FOR WINTER WHEAT

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OBJECTIVE

Accelerate generation advance under field conditions in breeding for resistance to Fusarium head blight.

INTRODUCTION

A primary objective of the U.S. Wheat and Barley Scab Initiative is to develop commercial cultivars (inbred lines) with resistance to Fusarium head blight (FHB) as soon as possible. A major limitation to achieve this objective is the time required to develop inbred lines and to select, with confidence, for FHB resistance. Given that at least certain FHB resistance alleles are codominant (Drake, 1997) and the low heritability of FHB resistance (Van Ginkel et al., 1996), selection for resistance is expected to be more effective with inbreeding.

One can produce two generations of spring wheat, wheat that does not require vernalization, per year seeded in the field and four generations per year if grown in a greenhouse or growth chamber.

At a given location one can produce one generation of winter wheat seeded in the field per year. In southern areas of the U.S. two generations can be grown per year: one seeded in the field and one vernalized in a controlled temperature chamber and transplanted to a greenhouse. In northern locations in the U.S. two generations can be produced per year, but both would need to be vernalized in a controlled temperature chamber and grown in a greenhouse. One could also grow one generation in a greenhouse and one generation transplanted to the field after vernalization in a controlled temperature chamber.

Seed production of wheat plants grown in a greenhouse or transplanted to the field is generally much less than that of plants seeded in the field, seriously limiting the number of progeny. Thus, devising a procedure to increase the number of generations of winter wheat per year under field conditions and maximize seed production per plant, particularly in early generations after a cross, would be valuable to accelerate the development of wheat cultivars with resistance to FHB.

MATERIALS AND METHODS

Wheat crosses were carried out in a greenhouse at Lafayette, IN in March to early April and harvested by 8 May, 1998. F_1 seeds were seeded at Colon, Argentina on 14 May. The seeding rate was approximately 4 seeds per 30 cm in rows 30 cm apart. Approximately 80% of plants had flowered by 23 September at Colon. Plants were harvested by 25 November and carried to Indiana on 27 November. F_2 populations were seeded at Evansville, Indiana on 28 November. A portion of the F_2 seeds were seeded at Kinston, North Carolina on 3 December as a backup. Selected plants were harvested at Kinston by 1 June, 1999 and at Evansville on 16 June. F3 populations were seeded at Lafayette on 1 October, the normal time of seeding.

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RESULTS AND DISCUSSION

All aspects of the generation advance/seed production scheme were successful. The F_1 and F_2 generations were successfully grown, seeding under field conditions. The thinly seeded F_1 plants typically produced 6+ spikes with 60+ well-developed seeds per spike. The F_3 generation was seeded at the normal time of seeding at Lafayette, Indiana. Based on experience in the 1998-1999 seasons, times of seeding and harvest are being shifted to allow earlier seeding of the F_2 generation at Evansville and Kinston. In 1999, the F_1 seeds were seeded at Colon on 7 May. Harvest at Colon is expected to be completed by 18 November, allowing seeding of the F_2 generation at Evansville by 20 November, at least one week earlier than in 1998. Seeding one week earlier at Evansville is important for the establishment of the seedlings for winter survival. The winter of 1998-1999 fortunately was milder than normal.

It is critical to choose a location in the southern hemisphere for generation advance that has sufficient cool temperatures for adequate vernalization, but that has a short wheat dormancy period, so that one can seed the next generation as early as possible in the area of adaptation in the U.S.—especially northern areas like Indiana. The general seasonal temperatures and the latitude near Colon is similar to southern Tennessee, which is at the southern edge of the climate in which wheat adapted to Indiana can be successfully grown.

A benefit of generation advance of winter wheat at Colon is that selection for resistance to certain diseases can be carried out, depending on the season. In 1998, Septoria leaf blotch was moderately severe and a low infection of Fusarium head blight was present. Stem rust was also moderately severe. However, one must take into account differences in pathogen populations in different regions.

One would need to identify a collaborator at the location of the winter nursery. It is important to apply for and obtain all necessary official permits prior to transporting seed into and out of countries. When done on a timely basis, obtaining permission to transport seeds need not be a hindrance to the operation of a program that requires the transport of seed between countries.

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YIELD AND FUSARIUM HEAD BLIGHT RESISTANCE OF HARD RED SPRING WHEAT CULTIVARS

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INTRODUCTION

Fusarium head blight (scab) has increased to epidemic proportions in many regions of the world in recent years. The 1993 epidemic in the North Central wheat-growing belt of the United States stimulated a major research effort to fight this devastating disease. It has been theorized that modern cultivars may be more susceptible than their predecessors and this may have contributed to recent epidemics. This study includes modern and historically significant hard spring wheat cultivars from the Northern Great Plains. Both yield trial data and reaction to scab in an inoculated nursery is included.

MATERIAL AND METHODS

Plant Materials

A set of spring wheat cultivars of historical significance is routinely included in the yield trials of the South Dakota State University spring wheat breeding program. This is generally a non-replicated trial grown at several locations. These historical cultivars serve a dual purpose of demonstrating the evolution of spring wheat cultivars to producers and since they are well characterized, they provide the breeder with a probe of the environment. The plots are planted, maintained, and harvested in the same manor as the breeding yield trial plots. Yield data (bu/a) from 1996 to 1999 were used in this study. These cultivars were also evaluated in 1997-1999 for scab resistance in an inoculated FHB nursery; planted as single row plots (1m) with two replications.

Inoculations and Disease Evaluation

In the mist-irrigated FHB inoculated nursery the rows were tagged when 50 to 75% of tillers in the row were in anthesis. Macroconidical spore suspensions, containing approximately 75,000 to 100,000 conidia/ml, were produced by scraping Fusarium graminearum cultured on acidic potato dextrose agar plates and suspending into water. Approximately 50 ml per row was applied. The first inoculation was done at anthesis and the second one week later. After inoculation the rows were mist-irrigated for 2 min. every 30 min. for a 12-hour period during the night (approximately 24 times per night). The nightly misting continued until readings were taken on the latest maturing cultivar. Percent-infected spikelets (FHB) of 20 spikes per replication were visually estimated 14 days after the first inoculation. After harvest, % tombstone kernels (scab) was determined and grain yield (Inoc Yield) was measured as grams/row.

RESULTS AND DISCUSSION

Table 1 shows the means of the 32 cultivars used in the study. Although the cultivars do differ in their reaction to scab, none are highly resistant. BacUp was the only cultivar with less than 50% FHB. The table illustrates that breeders have made progress for yield, but little progress for scab resistance until recent years. It was not until 1993 that spring wheat breeders began to consciously include reaction to scab into their evaluation criteria.

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Table 2 shows the correlations between the year of release and the four measurements that were taken. All three of the scab assessment measurements were highly correlated with each other. Grain yield and year of release were highly correlated, but there was no relation between year of release and FHB or tombstone kernels. There was no correlation between scab resistance and yield potential, indicating that breeders should be able to make progress on scab resistance and grain yield simultaneously.

Table 1. Means of spring wheat cultivars for three FHB disease assessment measurements in an inoculated FHB nursery and grain yield from non-inoculated yield trials.
Scab= tombstone kernels, FHB= % infected spikelets, Inoc yield= grain yield in the inoculated nursery.

			----------FHB Nursery---------			--Yield Trial--
			Inoc yield	Scab	FHB	Yield
Entry	Name	Release	g/plot	%	%	bu/acre
1	Baart	1900	18.2	51.0	66.1	22.1
2	Marquis	1903	15.2	58.3	64.0	24.7
3	Thatcher	1921	8.3	58.3	73.7	22.9
4	Chris	1965	12.9	69.2	72.1	30.4
5	Era	1971	12.7	64.2	83.3	34.3
6	WS1809	1975	12.4	69.2	66.2	35.9
7	Len	1978	12.8	60.0	80.3	34.2
8	Alex	1981	15.2	60.0	72.6	35.7
9	Marshall	1982	14.7	69.2	75.4	40.1
10	Wheaton	1983	11.4	88.3	84.3	42.9
11	Guard	1983	16.0	67.5	80.1	40.0
12	Stoa	1983	15.2	73.3	72.3	41.9
13	Nordic	1986	15.6	64.2	74.4	40.1
14	Butte 86	1986	23.9	57.5	62.4	41.5
15	2375	1987	24.0	50.8	66.5	39.1
16	Amidon	1988	15.5	65.0	81.3	39.1
17	Prospect	1988	17.8	56.7	78.9	36.7
18	Grandin	1989	27.2	56.7	57.0	40.2
19	Sharp	1990	20.3	56.7	66.5	40.3
20	Kulm	1994	20.8	54.2	68.0	40.0
21	Russ	1995	32.5	50.0	58.3	44.7
22	Norlander	1995	26.1	53.3	55.0	41.5
23	Lars	1995	15.3	70.0	74.7	41.6
24	Hamer	1995	22.3	62.5	69.3	42.8
25	Verde	1995	21.5	55.8	74.9	42.7
26	2398	1995	11.8	80.0	86.6	40.2
27	Trenton	1995	25.6	57.5	69.5	41.2
28	BacUp	1996	26.8	41.7	48.8	31.7
29	Oxen	1996	26.0	60.0	70.2	47.4
30	Keene	1996	17.5	55.0	79.3	42.2
31	Forge	1997	24.7	49.2	65.8	46.2
32	Ingot	1998	36.0	46.7	60.0	45.9
	AVERAGE		19.2	60.4	70.6	38.4
	CV%		28.3	12.6	12.4	6.7
	LSD(.05)		8.9	12.4	14.3	3.1

Table 2. Correlations between year of release, yield in multi-location yield trials (Yield), and three FHB disease assessment measurements in an inoculated FHB nursery. Scab= tombstone kernels, FHB= % infected spikelets, Inoc yield= grain yield in the inoculated nursery.

	Year of Release	Inoc yield	Scab	FHB
Inoc yield	0.40*			
Scab	-0.07ns	-0.79**		
FHB	0.04ns	-0.74**	0.69**	
Yield	0.64**	0.47**	-0.16ns	-0.16ns

*,** significant at 0.01 and 0.05, respectively

RESISTANCE ASSESSMENT IN FIELD AND GREENHOUSE SCREENING OF THE UNIFORM WINTER WHEAT FUSARIUM HEAD BLIGHT NURSERY AT BLACKSBURG, VIRGINIA, 1997-1999

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ABSTRACT

Epidemics of Fusarium head blight in Virginia have been intermittent, but the severity with which they affect the winter wheat crop and subsequently, the Virginia farmer, necessitate rapid discovery of mitigating factors. In the year 1998 alone, farmers saw losses of over 12 million dollars. Research in Virginia has focused on identification and development of resistant cultivars and germplasm, among other aspects of this disease. In the past two years and continuing in this year, Virginia has participated in the Uniform Winter Wheat Fusarium Head Blight Nursery. 1999-2000 will also see the inclusion of a Southern Uniform Winter Wheat Fusarium Head Blight Nursery. In 1997-1998, entries were screened in the field with a conidial suspension and in the greenhouse by the single floret method. Disease was rated by the following parameters: incidence, severity, fhb index (a composite of incidence and severity), percent scabby seed, 1000 kernel weight and DON level. Yield, heading date and flowering date were also recorded. In 1998-1999, Uniform Nursery lines were screened in the field, again with a conidial suspension, and fusarium infection was characterized by incidence, severity, fhb index, percent scabby seed and DON levels. Yield, heading date, flowering date, barley yellow dwarf virus presence and *Stagonospora nodorum* infection were also noted.

Resistance assessment of the seventeen entries included in the Uniform Nursery both years was analyzed in order to show repeatability of types of resistance or relative resistance among entries. There was no correlation between the rankings of entries for any of the disease parameters between the years. In addition, although in each year various disease ratings were correlated to each other or to agronomic traits such as flowering date, not one of these trends was significant in both years. Relative resistance rankings of Uniform Nursery entries in the field and in the greenhouse for 1997-1998 were likewise not correlated. Disease pressure varied greatly between the two growing seasons, as precipitation in 1997-1998 was far above normal and in 1998-1999 was extremely low. This along with variations in inoculation and irrigation methods (as protocols are being refined) may help to explain the lack of similar resistance levels between the years. More Type II and IV/V screening results from this and following years will allow for a more complete analysis. However, the lack of same relationships of disease resistance between lines over years suggests that there is more to be discovered about their various escape mechanisms. In a similar vein, non-repeatable correlations of disease parameters and significant differences between single floret and field disease assessments caution careful identification of quality and quantity in *Fusarium* resistant sources.

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GREENHOUSE AND FIELD EVALUATION OF RESISTANCE TO FUSARIUM HEAD BLIGHT IN SOFT RED WINTER WHEAT

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ABSTRACT

Entries in the Uniform Winter Wheat Scab Nursery along with numerous SRW cultivars and breeding lines were evaluated for resistance to Fusarium head blight in the field and greenhouse in 1999. Type II resistance was assessed in the greenhouse via single floret injections. Type I resistance was measured in a mist irrigated inoculated field nursery, and, to a limited extent in the greenhouse. Seeds from the greenhouse evaluations were plated on selective media to confirm visual evaluation. Field incidence and severity were low, but may have been closer to natural infection levels, since the resistant checks showed some resistance. Visual assessment of seed was misleading in that apparently normal seed, in many cases, were infected with *Fusarium graminearum*.

OBJECTIVES

1) To identify resistance to FHB in greenhouse and field screening trials.
2) To evaluate apparent vs. actual FHB infection by plating out seeds on selective media.

INTRODUCTION

Fusarium head blight 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 non - 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.

MATERIALS AND METHODS

Entries in the 1999 Uniform Winter Scab Nursery along with a number of advanced breeding lines were planted in the field in a randomized complete block design with four replications on 29 October 1998. Each plot consisted of a single 4ft. row. 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.

Isolation of *Fusarium graminearum*

A culture of *F. graminearum* was obtained from scabby wheat seed by surface sterilization and plating onto acidified potato dextrose agar (APDA). To induce sporulation, mycelium from this culture was plated onto carnation leaf agar (CLA). Plating a single-spore onto APDA ensured culture purity. This culture was then increased on PDA to use as the inoculum source for our field and greenhouse screens.

Field Inoculation

The field inoculation protocol was modeled after the method by Fauzi and Paulitz (1994) with some modifications. Fifty-three mason jars containing approximately 500 g of autoclaved corn seed were inoculated with 6 mycelial plugs of *F. graminearum* on April 5, 1999. Ten days later, twenty-five of the mycelium inoculated corn jars were also inoculated with a 10-ml macroconidia solution (400,000 spores/ml). Inoculated corn was maintained at room tem-

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perature for 22 days; jars were shaken daily to help disperse inoculum throughout each jar. Corn kernels were sampled from jars at random and plated onto PDA to verify presence of the pathogen. On April 27, wheat plots were inoculated just prior to heading by spreading 35-40g of the inoculated corn mixture per plot. Plots were mist irrigated daily beginning May 7 for approximately one hour during the early part of the morning, mid-day, and late evening throughout anthesis into early grain fill. Because of extremely dry weather and a delay in irrigation, wheat plots were inoculated a second time with more corn inoculum on May 17.

Disease evaluations were initiated on June 3 when scab symptoms were detected on several of the susceptible cultivars. Incidence was reported as the percentage of scab-infected heads per total number of heads per 2 ft. of row. Disease severity was assessed according to the Visual Scale for Estimating Head Blight in Wheat (Stack and McMullen, 1998). All heads in each row were tagged. Severity was determined by counting the number of infected spikelets and dividing by the total number of spikelets on diseased heads only.

Greenhouse Inoculations

Several advanced breeding lines were evaluated in the greenhouse for Type I (preventing initial penetration and Type II (reducing fungal penetration or spread within the head) resistance. Wheat entries were vernalized on Nov. 13-14, 1998 and potted in the greenhouse on Jan . 8, 1999.

Type II screen (Injections): Macroconidial suspensions were prepared by placing two mycelial plugs from a culture of *F. graminearum* in carboxymethylcellulose (CMC) liquid media. Flasks were placed on a shaker (115 rpm) for 2 weeks at 24 C. Spore suspensions were prepared by filtering the culture through a 3.0-mm Millipore filter system. Macroconidia were resuspended in sterile water and spore concentration was calculated with the aid of a hemacytometer. Inoculation and disease assessment was modeled after the method by Bai et al. (1996). At time of anthesis, a central floret of each spike was marked with a sharpie and inoculated by pipetting 3 µl containing approximately 1,200 spores. After inoculation, plants were placed directly into humidity chambers for three consecutive nights. Chambers were constructed of PVC-pipe tented with plastic, which sat on benches in the greenhouse. A cold-mist humidifier was placed inside each chamber to ensure high humidity. During the day at least one side of chamber was open for ventilation. Plants were moved out of chambers on day 4 and monitored for disease development. The number of diseased spikelets per spike was counted beginning one week after injection and continued every 3 days. The final percentage of infected spikelets per spike was recorded on day 21.

Type I screen (Sprays): Macroconidial suspensions were prepared in sterile water and conidial concentration was adjusted to 400,000 sp/ml. Wheat spikes were sprayed to run-off with a hand–held atomizer and placed in humidity chambers for three consecutive nights as previously described. Twenty-one days after inoculation plants were rated for disease development using a 0-4 scale: 0 = no disease, 1 = 1-25%, 2 = 26-50%, 3 = 51-75%, and 4 = 76-100% of spikelets infected.

Seed Assessment: Wheat seed was collected from both injection and spray test entries. Seeds were surface sterilized for 5 minutes in 0.5% sodium hypochlorite, rinsed three times in sterile distilled water, blotted on sterile filter paper and plated on APDA or PDA containing pentachloronitrobenzene (PCNB) and chloramphenicol. Seed collected from the spray tests were screened first. Total seed number plus the number of visually scabby seed were recorded

for each seed lot. Plates were incubated for 7-10 days at 20 C. Each plate was visually inspected for *F. graminearum* contaminated seed. Seed from the injection test was also assessed for the presence of *F. graminearum*. In this particular test, seed was visually inspected and placed in the following three categories according to appearance: l) normal, 2) small, wrinkled and 3) tombstone. The location and category of each seed was recorded on the top of each petri plate. After incubation, those seed that were positive for the presence of *F. graminearum* were recorded.

RESULTS AND DISCUSSION

Field Screening

For the first time in three years of field screening, incidence and severity in our inoculated, irrigated nursery were rather low (Table 1). This was due to a rare spring drought in Kentucky that we did not fully overcome with irrigation. Nonetheless, the reportedly resistant checks, Ernie and Freedom, actually showed some signs of resistance in our nursery, which was not the case in the previous two years. Thus, the scab pressure that we observed this year may have been closer to what would be expected under a natural infection.

Greenhouse Screening

Even with a large number of replications (15 plants), repeatability of assessment of type II resistance was low (Table 2). The most promising entry, KY 91C-022-36, ranged from 6 to 26% scabby spikelets. With only three plants, however, it is difficult to have confidence in this estimate.

Selective Media

We tend to regard an evaluation of scabby seed after harvest as a confirmation of our assessment of scab on the intact spike. Although visual assessment of seed seems straightforward, plating out the seed on a selective medium revealed some surprises (Tables 3, 4). Seed of Freedom, for example, was visually rated at 17 % scabby, yet plating the seed revealed that 82% was actually infected with *F. graminearum.*

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Table 1. Uniform Winter Wheat Scab Screening Nursery, Lexington, KY 1999.

Cultivar	Average Severity %	Average Incidence %	FHB Index	Height (in)	Yield (bu/a)	Heading Date (Julian)	DON Levels (ppm)
NY87048-7387	1.75	0.65	0.46	40	61.25	134	0.60
Ernie	3.50	1.34	0.09	32	57.82	125	0.78
IL94-1909	4.38	0.68	0.06	41	89.05	129	0.55
OH 544	6.42	1.10	0.10	41	93.68	134	0.55
NY86003-106	8.50	1.75	0.19	37	71.89	133	0.80
M94-1069	11.13	5.63	0.91	35	75.49	128	0.20
Freedom	15.95	4.11	0.72	35	77.90	129	0.63
Geneva	17.08	5.02	1.71	36	56.62	131	0.93
OH 522	17.19	9.92	1.88	35	93.51	127	0.80
NY6003-27	18.79	4.50	2.23	41	68.29	132	1.88
Foster	19.25	2.18	0.93	36	77.90	128	0.50
2545	22.95	9.02	2.00	35	57.99	132	1.70
OH 657	23.38	3.55	1.03	41	80.64	132	0.65
IL96-24078	27.08	1.73	0.49	34	63.48	126	0.75
NY87048W-7405	27.08	8.16	0.98	34	66.23	129	1.00
P92823A1	27.50	5.39	2.58	35	91.28	128	0.55
VA96-54-216	29.45	8.63	2.68	33	102.60	126	0.45
Goldfield	30.21	2.36	0.88	38	107.75	129	0.33
OH 609	30.40	4.98	1.63	36	97.11	127	0.33
Cayuga	31.48	3.97	1.72	42	78.75	135	1.00
Patterson	33.25	5.92	2.15	38	82.36	126	0.88
P88288C1	34.49	7.57	2.94	34	72.23	129	0.55
VA96W-348	38.71	15.27	5.75	32	71.72	127	0.90
M95-3349	43.13	3.15	1.11	37	109.12	128	0.53
IL95-4162	47.50	2.74	1.26	37	101.06	127	0.48
P86958RC2	47.54	3.92	2.35	36	81.33	129	0.95
KY89-895-14	51.20	11.51	5.78	34	88.88	129	0.58
Roane	53.45	18.44	10.06	33	81.84	126	2.38
Location Mean	16.68	4.27	1.06	37	82.29	130	0.80
L.S.D.	21.90	4.24	2.06	2.00	23.63	1.35	0.62
C.V.	71.90	65.99	89.81	4.69	24.91	0.89	59.20

Table 2. Evaluation of fourteen advanced breeding lines in the greenhouse for Type II[a] resistance to scab.

		AUDPC[b]			% Diseased spikelets[c]		
Entry	N	Min	Max	Mean	Min	Max	Mean
91C-092-3	5	0.7	2.3	1.7	6	32	22
91C-092-5	12	0.5	6.5	1.6	5	100	18
91C-092-7	3	0.7	2.8	1.9	6	36	16
91C-092-72	14	0.1	9.1	3.9	5	100	53
91C-092-105	6	0.7	8.2	3.9	7	100	37
91C-092-111	3	1.2	5.3	4.5	17	94	48
91C-019-17	4	0.6	3.2	2.9	5	39	24
91C-022-34	4	0.7	3.2	2.1	6	41	16
91C-022-36	3	0.3	2.5	2.2	6	26	17
91C-022-42	4	0.3	5.0	4.2	6	100	53
91C-046-2	4	0.9	4.9	4.4	7	64	43
91C-261-13	6	0.2	3.4	2.6	5	100	35
91C-261-24	15	0.7	8.8	3.3	6	100	46
92C-432-62	14	0.7	7.4	3.3	6	100	46

[a]Reduction of spread within the spike.[b]Area under the disease progress curve.
[c]Percent of infected spikelets per spike recorded 21 days after injection.

Table 3. Mean number of seed collected and percent of seed infected with *F.graminearum* from fourteen advanced breeding lines screened in the greenhouse for Type II resistance to scab.

	Mean number of seed[a]			Percentage of infected seed		
Entry	Normal	Small/ Wrinkled	Tombstone	Normal	Small/ wrinkled	Tombstone
91C-092-3	32.8	3.8	4.2	5.7	7.7	41.7
91C-092-5	26.6	3.6	1.5	0.3	1.6	33.3
91C-092-7	18.3	0	0	0	0	0
91C-092-72	15.2	15.4	5.5	5.2	14.8	26.1
91C-092-105	15.8	5.5	2.8	5.7	12.2	47.0
91C-092-111	1.0	24.3	7.3	0	3.0	34.2
91C-019-17	24.5	0	4.3	1.8	0	61.9
91C-022-34	12.5	14.3	0.3	4.4	0	0
91C-022-36	8.8	19.6	4.4	2.3	1.0	30.5
91C-022-42	18.8	3.0	3.0	1.0	0	12.5
91C-046-2	12.8	9.0	1.8	0	7.4	11.1
91C-261-13	15.2	5.0	3.1	15.4	7.2	48.4
91C-261-24	10.2	8.3	9.2	9.2	10.2	33.4
92C-432-62	12.0	5.4	2.9	5.1	13.9	50.3

[a] Visual assessment of seed by appearance. [b] Percent of *F. graminearum* contaminated seed per total number of seed by category, recorded 7-10 days after plating on selective media.

Table 4. Evaluation of fifteen advanced breeding lines screened in the greenhouse for Type I resistance to scab.

Entry	N	Disease Score[a]	Total Seed	Percent Visual scabby seed[b]	Percent of seed infected with *F. graminearum*[c]
Glory	3	2.7	19	77	87
KAS EX 108	1	4	6	100	83
FFR 555	5	3.6	21.2	66	70
Foster+Gaucho	4	2.8	16.3	40	92
2552	3	1.7	13.3	34	76
KY 89C-744-44	3	2.3	19.3	37	60
92C-432-62	3	0.3	13	30	18
92C-433-77	1	1	32	19	72
91C-261-3	5	1.2	38.2	15	43
91C-261-3	3	2.7	3	99	100
90C-383-18	1	1	27	100	81
91C-260-6	3	0.7	34.6	6	15
91C271-74	3	1.7	18	51	53
Freedom	2	2	29	17	82
Ernie	3	1	13.4	25	17

[a]Twenty-one days after inoculation plants were rated for disease development using a 0-4 scale: 0 = no disease, 1 = 0-25%, 2 = 26-50%, 3 = 51-75%, and 4 = 76-100% of spikelets infected.
[b]Visual assessment of seed by appearance.
[c] Percent of *F. graminearum* contaminated seed per total number of seed, recorded 7-10 days after plating on selective media.

NCR-184
ARKANSAS STATE REPORT - 1999

Gene Milus

In 1999, Arkansas farmers planted 930,000 acres and harvested 870,000 acres of soft red winter wheat with an average yield of 56.0 bushels per acre (an all-time record!). Fusarium head blight symptoms could be found at trace levels in many wheat fields across the State, but losses due to head blight were negligible. A survey of 91 certified wheat seed lots found *Fusarium* spp. associated 18 to 81% of the seeds, and the most frequently isolated species was *F. graminearum*. The combination of low head blight incidence in the field and high *Fusarium* incidence on seeds was likely caused by the rainy weather between crop maturity and harvest. These data suggest that head blight would have been more severe if the rainy weather had come several weeks earlier.

Personnel working on FHB at the University of Arkansas include Gene Milus, Louis Prom (research associate), and Chris Weight (research specialist) in the Department of Plant Pathology and Robert Bacon (breeder) and John Kelly (research associate) in the Department of Crop, Soil, and Environmental Sciences. Louis Prom accepted a USDA scientist position at Texas A&M, and there will be a research specialist position available in December 1999. Novartis Seeds at Bay, AR, (June Hancock, breeder and Luis Lazo-Anaya, pathologist) and Agripro Seeds at Jonesboro, AR, (Barton Folgleman, breeder) also do some selection for FHB resistance.

Backcross F_2, topcross F_2, and F_3, populations from crosses designed to transfer FHB resistance to the southern soft red winter wheats were selected in the field for heading date, plant height, yield potential, and resistance to FHB, leaf rust, and Septoria tritici leaf blotch. Two hundred heads were selected from the best plants in each of 76 populations. Each head was threshed individually, the seeds were selected for plumpness and low levels of scab, and seed from the best 120 heads per population were planted as headrows in the field for evaluation in an inoculated, irrigated screening nursery in 2000. More than 200 Arkansas advanced breeding lines with sources of FHB resistance in their pedigree were planted as replicated single-row plots for FHB evaluation in 2000 and as yield plots at several locations. See the report in the Proceedings of the Scab Forum for additional details.

The uniform winter wheat scab nursery and the uniform scab fungicide test were evaluated in inoculated, irrigated plots. Significant differences in FHB resistance were found among the winter wheat lines, but no practical level of control was achieved with any of the fungicides. See reports in the Proceedings of the Scab Forum for additional details.

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NCR-184 MANAGEMENT OF HEAD SCAB IN SMALL GRAINS ILLINOIS REPORT – NOVEMBER 1999

Frederic L. Kolb*, Larry K. Boze, and Norman J. Smith

ILLINOIS WHEAT PRODUCTION

The estimated wheat yield in Illinois in 1999 was 60 bushels per acre. Acreage harvested was about 1.05 million acres, down about 16 % from 1998. Wheat production in Illinois in 1999 was about 60.6 million bushels. In spite of the lower acreage wheat production increased about 5 percent compared to1998 because yields were higher in 1999 than in 1998. In general, the winter was very mild in Illinois, and the crop developed rapidly in the spring. Wheat was harvested earlier than average in some of the southern regions, but rainy weather delayed harvest in some areas. Scab damage was spotty in 1999 with significant losses in some localized areas and little damage in others. Glume blotch also caused significant losses in yield and grain quality in some areas.

UNIVERSITY OF ILLINOIS RESEARCH

Breeding for Scab Resistance in Soft Red Winter Wheat

Development of scab resistant germplasm and varieties is a major research emphasis in the wheat breeding program. The long-term objective is the development of soft red winter wheat genotypes with excellent resistance to scab combined with resistance to other diseases, high yield potential, and acceptable winter hardiness and milling and baking quality. Our short-term objectives are: 1) to combine genes for resistance to scab from diverse sources; 2) to evaluate the genotypes produced from crosses and identify those with resistance to scab; 3) to identify molecular markers associated with genes for resistance to scab; and 4) to work toward using molecular markers to assist in breeding for scab resistance.

Two Illinois breeding lines entered into the 1999 Cooperative Winter Wheat Scab Screening Nursery were among the most scab resistant lines in the nursery. These lines have potential as parents, represent sources of resistance that are different from the Chinese sources or resistance, and are in soft red winter wheat backgrounds. These lines were made available to other breeders by entering them into the Cooperative Winter Wheat Fusarium Head Blight Screening Nursery.

About 575 breeding lines were evaluated in the misted, inoculated field nursery in 1999. Additional evaluations were conducted in the greenhouse. Material evaluated included germplasm reported to be tolerant/resistant, current varieties, and experimental breeding lines. At least 55 advanced breeding lines with scab resistance equal to or better than Ernie were identified, and the best of these will be evaluated further. Individual heads were selected from 27 segregating populations grown in the field nursery. About 4000 headrows resulting from these selections have been planted this season (1999-2000). About 1700 individual plants from six segregating populations were evaluated in the greenhouse scab screening, and about 210 plants with excellent scab resistance were selected.

We are continuing to select lines from segregating populations, evaluate lines, and increase the number of lines selected from crosses with

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potential scab resistance using both greenhouse and field procedures with misting systems and inoculation. In summary, new lines with scab resistance were identified, and the agronomic performance of previously identified lines was evaluated.

Research on Molecular Markers

Using a population of lines from a cross of resistant and susceptible cultivars, we conducted research on identification of molecular markers linked to scab resistance. About 300 combinations of AFLP (amplified fragment length polymorphism) primers were screened, and eleven AFLP markers showed significant association with scab resistance. These molecular markers were located in one chromosome region. A manuscript on these markers was published in *Phytopathology*, and additional research with these markers is in progress. Two additional QTL have also been identified in this population. This research is in cooperation with Guihua Bai,USDA-NCAUR and Oklahoma State University; Greg Shaner, Purdue University; and Les Domier, USDA-ARS at Urbana, Illinois.

Personnel

A post-doc, Irie Vroh Bi, joined the University of Illinois wheat breeding and research project in August. Irie received his Ph.D. in Genetics and Plant Breeding from the University of Agronomic Sciences, Gembloux, Belgium and is working on molecular markers associated with scab resistance. Two graduate students also joined the wheat breeding program this year. Wenchun Zhou began working toward a Ph.D. in June, and Andrew (A.J.) Stewart began work on his M.S. in August.

Publications

Bai, G-H., F. L. Kolb, G. Shaner, and L. Domier. 1999. AFLP markers linked to a major QTL controlling scab resistance in wheat. Phytopathology 89:343-348.

Bai, G-H., F. L. Kolb, G.E. Shaner, and L.L. Domier. 1999. Using AFLP map to identify scab resistance QTL in wheat. Agron. Abstr. p. 159.

MANAGEMENT OF SCAB OF SMALL GRAINS NCR-184 1999 INDIANA STATE REPORT

Gregory Shaner

SCAB WAS NOT GENERALLY SEVERE IN INDIANA IN 1999, ALTHOUGH SOME RAIN FELL DURING FLOWERING

Scab could be found in many fields, but usually no more than 6% of the heads were affected, and in many fields the incidence of infection was much lower. However, there were limited areas in the state, such as near Sullivan, in which losses due to head blight were as high as 25% in some fields.

CURRENT RESEARCH PROGRAMS

We are continuing our research on the recombinant inbred population of Chokwang/Clark in hopes that the resistance genes will differ from those Ning 7840 and Sumai 3. We have a poster that shows our results of this study (Shaner and Buechley).

We are also continuing the research on approximately 50 selected lines from the USDA NSGC. About two thirds of these lines exhibit type 2 resistance and the other third exhibit type 1 resistance. (Shaner and Buechley)

We are beginning a research project that includes examination of corn stubble for presence of *G. zeae* and development of ascospores in the spring. A new graduate student, Amanda Gevens, is working on this project. (Gevens, Buechley, and Shaner)

The two new FHB low-incidence cultivars, Goldfield and INW9853, performed well and in test plots near Sullivan, IN in which blight incidence in cultivars Patterson, Goldfield, and INW9853 averaged 25, 10 and 8%, respectively. Incidence in plots of cv. INW9824, with one resistance gene from Ning 7840, averaged 15% and 1/3 - 2/3 of the spikelets of infected spikes became diseased compared to 95% of spikelets of infected spikes of Patterson. (Herb Ohm, Xiaorong Shen, David Drake, Ted Kisha, Hari Sharma)

Although natural infections of head blight in nursery plots at Lafayette, IN were low, 2-5 %, single-floret inoculations, both in the greenhouse and field, were successful and allowed selection in limited populations. Resistance from a number of source parental lines is being combined in various crossing schemes and F_3-F_6 populations are in nurseries in the field. Hopefully, effective selection can be accomplished in the field in 2000. (Herb Ohm, Xiaorong Shen, David Drake, Ted Kisha, Hari Sharma)

The venture of growing F1 plants in Argentina for generation advance under field conditions was highly successful in the first attempt in the 1998-1999 season. F_1 seeds produced in the greenhouse in April were seeded in Argentina in early May and harvested in late November. The F_2 generation was seeded in late November at Evansville, IN. Seedlings were established and survived the winter in fine shape. F_3 populations from single F_2 plants were seeded in nurseries at Lafayette and Sullivan, IN at normal seeding dates in October 1999. (Herb Ohm, Xiaorong Shen, David Drake, Ted Kisha, Hari Sharma)

CEREAL CLASSES AND ACREAGE IN INDIANA

Indiana produces soft red winter wheat. In 1999, Indiana farmers harvested 510,000 acres, at an average yield of 66 bu/A, for a total production of 33.6 million bushels.

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ANNUAL NCR-184 REPORT FOR 1999
IOWA

Gary Munkvold* and John Shriver

WHEAT PRODUCTION AND HEAD BLIGHT IN IOWA IN 1999

Wheat for grain was planted on only 31,000 acres and the average yield was 43.0 bu/acre, for a total production of 1.33 million bu. Wheat production is concentrated in the SE portion of the state. Weather in 1999 in this part of the state was characterized by excessive rainfall in the late spring, followed by extremely dry conditions during the mid- to late summer. The result was that Fusarium head blight had a significant effect on yields in some fields, but both yield and disease development were limited by the dry conditions later. My estimate for losses due to scab was approximately 5 bu/acre.

FUSARIUM HEAD BLIGHT RESEARCH

In 1999 we conducted a small spring wheat nursery at Ames in collaboration with Bob Stack, NDSU. Plots were planted in soybean stubble and no irrigation or inoculum was provided. Head blight severity reached about 30% in susceptible checks and was 2-4% in the best experimental lines. Iowa State University does not have an active wheat breeding program. Currently the Department of Agronomy is in the process of hiring a new small grain breeder, but the emphasis is likely to be on oats.

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NCR-184 STATE REPORT
KANSAS 1999

R.L. Bowden

FHB SITUATION IN 1999 IN KANSAS

Kansas wheat generally had high yields and low to moderate disease levels in 1999. There were traces of Fusarium head blight (scab) in central and north central Kansas. Southeast Kansas had a little more, up to 2% in some fields. In contrast, northeast Kansas experienced a significant scab epidemic. Disease incidence in excess of 30% was observed in several fields. Fortunately, the planted acreage of wheat in northeast Kansas was relatively low this year due to excessive moisture during planting time in the fall of 1998. This limited the impact of scab on statewide production. The average estimated loss due to scab in Kansas was 0.2%.

PROGRAMS AND PERSONNEL INVOLVED IN FHB RESEARCH

Breeding program and scab resistance screening

In the spring of 1999, Rollie Sears resigned as the KSU wheat breeder at the Manhattan location and moved to AgriPro. He will remain in Manhattan and continue to breed hard red or white winter wheat for the central and southern Great Plains region. KSU will hire a new wheat breeder to continue the Manhattan breeding program. The KSU wheat breeding program at the Hays location directed by Joe Martin will continue uninterrupted.

Most varieties grown in Kansas are susceptible to FHB. For example, the two most popular varieties in Kansas in 1999 were Jagger and 2137. Both were released by KSU and both are susceptible. In fact, 2137 is very susceptible to scab. Only varieties Karl 92 and Agripro Hondo seem to have useful levels of resistance among hard red winter wheat varieties that have been screened to date. Heyne, a new hard white variety, also has useful levels of resistance. The lack of resistant varieties has contributed to the decline of wheat acres in eastern Kansas by two thirds since 1980.

Gina Brown-Guedira (USDA geneticist) has made some crosses (Heyne/TAM 107) to learn more about the number of genes involved in resistance in our Kansas material. She has also introgressed transgenic chitinase resistance (see below) into Heyne to determine if it improves resistance.

For the second year, a field screening nursery was operated at Manhattan by Bill Bockus, Bob Bowden, and Mark Davis. Plots were inoculated with corn kernels infested with one aggressive isolate of *Gibberella zeae*. Plots were irrigated with fine impact sprinklers for 3 minutes per hour each night starting at heading. Results were better than last year in that we avoided drowning the plants as happened in some plots in 1998. The Uniform Winter Wheat Fusarium Head Blight Nursery was rated at three dates. Soft red varieties OH522, IL95-4162, and NY87048-7387 looked the best in our plot this year. The Kansas Intrastate Nursery was screened and several highly susceptible lines were identified and will be discarded. This nursery will be expanded for the 2000 season. Additions will

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include a commercial cultivar nursery, Hays breeding program nursery, USDA Geneticist nursery, and a special nursery to examine whether polyphenol oxidase (ppo) is related to scab resistance in several populations. This is important because our breeding programs are currently selecting against ppo because it reduces noodle quality. André Rosa, a student in plant breeding, is conducting this project as part of his Ph.D. thesis.

Alien resistance

Bikram Gill (Plant Pathology) and Bernd Friebe (Plant Pathology) are cooperating with Dajun Liu and Peidu Chen (Nanjing Agricultural University) on several projects funded by a McKnight grant. A variety of addition, substitution, and translocation lines from *Roegneria* and *Leymus* into wheat are being characterized cytologically and for resistance phenotype. We are also continuing to look at a set of synthetic hexaploids from Mujeeb-Kazi of CIMMYT.

Transgenic resistance

Bikram Gill (Plant Pathology), S. (Krishnan) Muthukrishnan (Biochemistry), and George Liang (Agronomy) are continuing to cooperate with Wenping Chen, Dajun Liu and Peidu Chen (of Nanjing Agricultural University). Transgenic plants had an increased level of resistance to scab. The following paper was published on expression of chitinase in transgenic plants.
Chen,-W.P.; Gu,-X.; Liang,-G.H.; Muthukrishnan,-S.; Chen,-P.D.; Liu,-D.J.; Gill,-B.S. 1998. Introduction and constitutive expression of a rice chitinase gene in bread wheat using biolistic bombardment and the bar gene as a selectable marker. Theor-appl-genet. 97 (8) 1296-1306.

Cloning pathogenesis-related genes from plants

Wanlong Li, John Fellers (USDA geneticist), Bikram Gill, Peidu Chen and Dajun Liu are continuing to work on a DNA library of pathogenesis-related sequences from infected spikes. John Fellers recently purchased a high-throughput sequencer that will be used to analyze the library.

Pathogen genetics and variability

Jim Jurgenson, Bob Bowden, and John Leslie continued work on a genetic map of a cross between a strain from Kansas and a strain from Japan using AFLPs. To date, 1039 markers have been placed on nine linkage groups. Ron Plattner (USDA mycotoxin unit at Peoria) has preliminary data that toxin type (DON vs. NIV) and amount are segregating in this cross. We are also cooperating with Nancy Alexander (USDA-Peoria) on mapping the trichothecene gene cluster.

A pilot study to compare populations from Kansas and North Dakota using AFLPs is being done by Kurt Zeller in cooperation with Bob Bowden and John Leslie. Analysis of approximately 40 isolates from each location using 80 markers revealed to significant differences between the populations. Samples have been collected or obtained from cooperators in Illinois, Kansas, Minnesota, New York, North Dakota, Ohio, and Virginia. These will be used to look for differences in marker frequencies at the regional level.

The following paper on pathogen genetics was published:
Bowden, R. L., and Leslie, J. F. 1999. Sexual recombination in *Gibberella zeae*. Phytopathology 89:182-188.

NCR-184
1999 KENTUCKY STATE REPORT

Donald Hershman* and David Van Sanford

FUSARIUM HEAD BLIGHT STATUS DURING 1999

Excessively dry conditions during 1999 resulted in very low levels of Fusarium head blight throughout Kentucky.

CURRENT RESEARCH PROJECTS

Field and Greenhouse Screening

Marla Hall, Brenda Kennedy, and Dave VanSanford

Numerous soft red wheat cultivars, breeding lines, and entries in the Uniform "Scab" Nursery were evaluated under mist irrigation in a field nursery near Lexington, KY. Fusarium-infected corn was used to infest the nursery and encourage FHB development. However, difficulty in establishing timely irrigation following distribution of inoculum and lack of rain fall resulted in very low disease levels. Certain lines from this study were evaluated in the greenhouse for Type II resistance.

Inheritance Studies

Marla Hall and Dave VanSanford

A number of populations were synthesized from parents with reportedly different sources of FHB resistance. Several types of genetic analyses will be performed on progeny of these crosses to elucidate inheritance of resistance.

Breeding For FHB Resistance

Dave VanSanford

Numerous crosses have been made and continue to be made to various sources of FHB resistance, within and outside of the soft red winter wheat market class.

Uniform FHB Fungicide Test

Don Hershman and Scott Jones (WheatTech, Inc.)

Tests were performed at two locations in west Kentucky during 1999. The tests relied on natural inoculum and moisture, and because of excessively dry weather befor, during and immediately following crop flowering, very little FHB developed. As a result, none of the fungicide treatments performed better than the non-treated plots.

FHB Survey

Don Hershman, Scott Jones (WheatTech, Inc.), and Phillip Needham (Miles Opticrop)

1999 marked the second year where grower fields were surveyed to determine if there is an association between FHB incidence or severity and the amount of corn surface residue left in a field from the previous crop. Ninety-five and 91 fields were surveyed in 1998 and 1999, respectively. The amount of corn surface residue was significantly correlated with FHB incidence in both years, and with severity in one of two years; however, in all cases, relationships were highly variable as indicated by R^2 values in the low .20s or less. This suggests that other factors are more important than corn residue in determining the incidence and severity of FHB in Kentucky. We hypothesize that the nature of corn production in Kentucky, i.e, small, widely-scattered corn fields throughout the wheat producing areas, results in sufficient FHB inoculum any time weather conditions favor spore production and dissemination. This survey will be extended one final year into the 2000 growing season.

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1999 NCR-184 STATE REPORT
MANAGEMENT OF HEAD SCAB OF SMALL GRAINS

L. Patrick Hart

STATE SITUATION

FHB occurred in Michigan in 1999, but the incidence was less than 1%, and occurrence was generally limited to the central portion of the state. DON was not considered a problem in 1999.

RESEARCH REPORTS

Variability of deoxynivalenol in truckloads of wheat

In a 1996 statistical study on winter wheat, a grain probe sampling protocol was developed to predict levels of deoxynivalenol (DON) in FHB infected grain. The statistical study was expanded in 1998 to examine the variability of DON distribution between probes and within probes in truckloads of spring wheat harvested from FHB infected fields. The variability was greater between probes in the 1998 study compared with the 1996 study. In the 1996 study, the DON average from four probe samples was within 1 ppm of the upper limit of the estimated truck average (95% confidence), or within 0.5 ppm on either side of the estimated truck average (95% confidence). Four probes from the 1998 study predicted the average within 3 ppm of the upper limit (95% confidence), or within 1.5 ppm on either side of the average (95% confidence), thus reflecting the increased variability of DON distribution in the trucks. Two of the five trucks from 1998 had DON means below 10 ppm (5.9 and 9.2 ppm), and four probes predicted the mean (95% confidence) within 2 ppm and 1 ppm, for the upper limit and for either side of the mean respectively.

This statistical study is continuing in 1999 to examine how storage and moving of wheat affects the variability of DON distribution in trucks of wheat. A GIPSA study on DON distribution in barley after removal from storage bins suggested reduced variability compared to variability in our studies using freshly harvested grain. In addition, we would like to examine DON variability in wheat with truck averages in the range of 1-10 ppm.

Identification of peptides that mimic the binding of DON to DON specific antibody

A random 7-mer peptide phage expression library was screened for sequences that bound to the antigen binding region of DON specific monoclonal antibody, and displacing DON-HRP marker enzyme. Two clones were identified with the following sequences:SWGPFPF and SWGPLPF. Both clones were competitive in direct and indirect ELISA for DON. A translation fusion with bacterial alkaline phosphatase was also active in a direct ELISA for DON. The peptide appeared to have 10X greater affinity than DON to the antibody (0.39 µm vs 3.9 µm required to inhibit binding of DON-HRP by 50%, respectively). The peptide also appeared to prevent protein synthesis inhibition by DON in an *in vitro* protein translation system.

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Fungicide trials on wheat to reduce FHB and DON levels

The effect of different applications techniques using Folicure were evaluated to reduce the incidence of FHB, and the levels of DON. Folicur was applied at two rates (4 oz/acre and 2.5 oz/acre), and by two application methods, 1) flat fans at an angle (approximately 60-75^{o} above the horizontal). Disease incidence and severity were not significantly different between treatments and untreated controls. However, DON levels in the treatments were significantly lower than the controls, but not from each other. Average treatment mean for DON was 4.5 ppm compared to 13.4 ppm for the untreated control. These results suggest that standard flat fans placed at an angle can effectively reduce DON levels in grain associated with FHB, but that the apparent incidence and severity are not affected. Reduced rates of Folicur may be as effective as higher rates.

Michigan participated in a Uniform fungicide trial to evaluate different fungicides efficacy on FHB and DON. The fungicides were benlate/ manzate, quadris (2 rates), penncozeb, BASF 500 00F, stratego (2 rates), and folicur. Differences in yield, FHB incidence and severity, and DON levels were not significant between treatments, although folicur had the highest yield and the lowest levels of DON. In the previous two years, application of Quadris to control FHB resulted in higher levels of DON. This year, levels of DON were lower (not significantly) than in the untreated controls.

The relationship between variety (Freedom, Pioneer 2510, and Frankenmuth), and timing of fungicide application (folicur, 4 oz/acre applied at Feekes GS 10.1 or 10.5, except fungicide was applied to Frankenmuth only at GS 10.5) was evaluated for effect on DON levels. Folicur applied at GS 10.5 significantly reduced levels of DON in each variety compared to untreated controls. Folicur only slightly reduced DON in Pioneer 2510 when applied at GS 10.1, but significantly reduced DON in Freedom when applied at GS 10.1. Yields tended to be higher in treated plots, but were not significantly greater than untreated controls.

USEFULNESS OF FINDINGS

Food grain intended for human consumption must be safe and free from harmful chemicals. DON contaminated grain is unsafe for human consumption. The ability to accurately and precisely identify levels of DON in grain is a critical aspect of ensuring food safety. This sampling study has provided a protocol, that if followed, can provide the purchasers of raw grains the means by which to divert grain for appropriate uses, and ensure that DON contaminated grain is not used for human consumption. The incidence of DON like symptoms in school children in 1998 was been associated with burrito tortillas made from wheat. Our past experience with how elevators sample freshly harvested wheat for DON testing suggested that significant errors in accuracy were likely to occur. If implemented by buyers of grain, the protocols developed as a result of this research should reduce the potential of DON contaminated grain being used in the preparation of food.

The peptide mimic of DON may be useful in developing a new generation of analytical tests that are less expensive to produce, more sensitive and capable of detecting lower levels with greater precision, and broader applications. In addition, because preliminary findings suggest the peptide mimic of DON may protect against protein synthesis inhibition, the mimic may be useful in the development of transgenic grain plants with resistance to DON, and therefore result in reduced FHB incidence, severity, and DON levels.

In the absence of other control measures, fungicides have been shown to be an effective means of reducing disease. Our FHB control studies examine fungicide efficacy to provide the wheat industry with appropriate recommendations for reducing FHB and DON. In addition, some studies directed toward application timing, and method of application may result in better control, and possibly in reduced rates of fungicides being applied. This is not only economically beneficial to the grower, but reduces the risk of pesticide residues on the grain.

Because the research on fungicides has been collaborative between states, the ability to draw comparisons across different environments and agro-ecosystems, has resulted in unified recommendations for FHB managment with fungicides. Most states with a potential FHB problem request on a yearly basis a special EPA permit (section 18) allowing the use of Folicur on wheat if the environmental conditions suggest an epidemic could occur. Through educational efforts elevators and other purchasers of grains should be aware of the necessity of applying a specific sampling protocol to trucks of wheat in order to accurately estimate the DON level in the truck.

Immunological approaches to diagnostics, and disease resistance have been major accomplishments over the past five years. Recombinant antibody technology has resulted in the development of antibodies with enhanced affinity to mycotoxins, gene fusions between recombinant antibody DNA and reporter enzyme DNA has resulted in a potentially new way to develop diagnostic tests, and expression of functional recombinant antibody in plants opens the possibilities of using foreign genes to develop new and novel forms of resistance to *Fusarium* plant pathogens that produce mycotoxins. Peptide mimics of toxins may provide an opportunity to study interactions between toxins and their receptor ligands.

WORK PLANNED FOR NEXT YEAR

The statistical study on sampling will be continued for validation of probe collection protocols, and evaluation of how storage and shipment affects the distribution/variability of DON in grain. Work on the peptide mimic will be expanded to the development of transgenic plants and a determination of the possible role the mimic may have in reducing the toxic effects of DON as a virulence factor in wheat and possibly barley. Work will also continue on the development of recombinant antibody to DON, and identification of other peptide mimics that may be useful in elucidating the receptor ligands associated with DON toxicity.

PUBLICATIONS

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NCR-184 COMMITTEE-MANAGEMENT OF HEAD SCAB IN SMALL GRAINS 1999 MISSOURI REPORT

Laura E. Sweets* and Anne L. McKendry

WINTER WHEAT PRODUCTION IN MISSOURI AND THE 1999 FHB SITUATION IN MISSOURI

Winter wheat is grown on all of the Missouri wheat acreage. Most of the acreage is soft red winter wheat with a minimal number of hard red winter wheat acres. Missouri wheat production in 1999 totaled 44.16 million bushels, down 23.2% from last year's production of 57.5 million bushels. Of the 1.02 million acres planted, farmers harvested 0.92 million acres for grain (1.25 million acres were harvested in 1998). Missouri yields averaged 48 bushels per acre, up 2 bushels from last year's average yield of 46 bushels per acre.

1999 was a fairly good year for wheat production and a fairly poor year for Fusarium head blight in most of Missouri. The early part of the season was cool and wet, but most of the state was dry as the wheat crop was flowering resulting in low levels of scab. Localized rainfall lead to scab problems in those areas but the incidence and severity of scab was minimal in Missouri in 1999.

Aphid levels and barley yellow dwarf virus were concerns in the southeastern part of the state. Leaf rust and Septoria leaf blotch came in late in the season and did not move up to the flag leaves until well past heading. Losses from foliage diseases were low for most of Missouri. Missouri did have a Special Local Need Registration (Section 24c Registration) for Tilt which extended the time of application to Feeke's Growth Stage 10.5. However, because of the low level of foliage diseases few growers took advantage of the Tilt label change or the new federal label for Quadris on wheat.

Quality of wheat seed tested by the Missouri Seed Improvement Association was very good this season. There was very little scab, very little black point and little bleaching, shriveling or low test weights. Germinations rates were very good.

There are no official estimates of the number of acres planted to wheat this fall. Seed dealers reported good sales with many selling all their wheat seed. The general feeling is that wheat acres will be up from last year but not perhaps not back to the 1997 level. Increases in wheat acres may be due to several factors including 1) disappointing corn and soybean yields and prices have led to a need for wheat for early cash flow in 2000, 2) corn acreage may be down in 2000 which leads to an increase in wheat with doublecrop soybean, 3) good fall weather meant the soybean crop was harvested in a timely fashion leaving time to plant winter wheat and 4) an increase in acres of pasture wheat to graze this fall or for forage next year to supplement forage supplies decimated by dry conditions during the summer and fall of 1999.

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CURRENT SCAB RESEARCH AT THE UNIVERSITY OF MISSOURI

Uniform Scab Fungicide Trial

The University of Missouri did participate in the Uniform Scab Fungicide Trial coordinated by Dr. Marcia McMullen, NDSU. Nine fungicide treatments were evaluated on Madison. FHB occurred in low levels throughout the plot. There were no statistically significant differences in yield, ppm of DON, % incidence of FHB, % FHB severity or % field severity between the untreated control and any of the nine fungicide treatments. Results of this trial are given in more detail in the report for this initiative project.

Breeding Program

The University of Missouri's Wheat Breeding Program has a major emphasis on accelerating the development of scab resistant soft red winter wheat that was initiated in 1993 and significantly enhanced in the last 2 years with funds from the National Wheat and Barley Scab Initiative. In 1993, an initial screen of genotypes in the breeding program along with those entered into the Missouri Winter Wheat Performance Tests led to the identification of a high level of Type II resistance in the Missouri experimental line MO 12256 which was later released as "Ernie". Resistance in the line has subsequently been verified by several programs and Ernie now serves as the early maturity resistant check in the Uniform Winter Wheat Scab Nursery.

Routine screening of all advanced lines in the breeding program has been introduced in the past 2 years. This screening permits the identification of otherwise unknown sources of resistance in adapted breeding lines, thereby accelerating the release of scab resistant lines to the grower. At the same time it facilitates the elimination of highly susceptible lines from breeding streams. In 1999, 34 advanced lines from among 120 screened were identified that had levels of Type II and III resistance comparable to the resistant check varieties Sumai 3 and Ernie. Once verification is complete, genetic studies will be initiated to investigate the nature of the resistance in lines which differ from Ernie by descent.

Beyond screening, the incorporation of resistance genes identified through germplasm screening programs is essential to the continued improvement of Fusarium head blight resistance in winter wheat. We currently are incorporating genes from Sumai 3, Ning 7840, Frontana, and several CIMMYT sources. In addition, we routinely use soft red winter wheats sources including Ernie, Patton, Goldfield, Freedom and several of our own lines expressing good levels of resistance. Once verified, several Chinese accessions showing good levels of resistance will be added to our crossing block.

Germplasm Evaluation Center

Missouri was identified as a germplasm evaluation center for the National Wheat and Barley Scab Initiative with responsibility for identifying new sources of resistance in winter wheat. During 1998/99, 937 landraces, breeding lines and cultivars from China, South Korea, Japan, Brazil and Italy, were screened for Type II and III resistances. The initial screening resulted in the identification of 240 plants representing 87 accessions with Type II resistance comparable to Sumai 3 and excellent kernel quality. During the fall and winter of 1999/2000, these accessions will be progeny tested and resistance will be verified. Approximately 1000 landraces, breeding lines and cultivars from Yugoslavia will be screened in the greenhouse and field during the 1999/2000 season.

Genotype x Isolate Interaction Study

We are completing a study involving 20 genotypes and 5 geographically diverse isolates to

determine if there are genetic interactions with isolates that might limit the stability of resistance over wide geographical areas. This work has clearly shown a wide range of aggressiveness in isolates currently in use in breeding programs in the eastern United States. In addition, data suggests a possible interaction of isolate with genotype impacting both Type II and III resistances. Work will be completed in the winter of 2000.

Genetic Studies

Studies investigating the inheritance of resistance in Ernie are currently underway utilizing the Missouri breeding line MO 94-317, a widely adapted and highly inbred (F_{12}) line, as the susceptible parent. It has high yield and excellent milling and baking quality but is highly susceptible to scab with a FHBI of $ 0.9 and poor kernel quality under disease pressure.

Conventional Six Generation Means and Variance Analyses

A set of populations (F_1, reciprocal F_1, F_2, BC_1 and BC_2) from the cross Ernie x MO 94-317 is currently under development for conventional genetic analysis of the scab resistance in Ernie. Population development will be completed in 1999/2000 and genetic analyses will be conducted in the fall of 2000. Both Type II and Type III resistance data for each generation initially will be examined for goodness-of-fit (based on P^2 analysis) to simple Mendelian ratios. Where data collected fail to fit a simple dominant/ recessive genetic model, generation means and variance analyses will be conducted.

Monosomic Analyses

Monosomic plants from each of the 21 Chinese Spring monosomics developed at the University of Missouri by Dr. E.R. Sears have been crossed with Ernie in an effort to identify critical chromosomes influencing scab resistance in Ernie. In addition, the results of this study will help focus molecular work aimed at identifying markers associated with genes for scab resistance in this cultivar.

Molecular Analysis

A set of F_3 derived F_7 recombinant inbred lines (RIL's) has been developed from the cross Ernie x MO 94-317 which will be used to map resistance genes in Ernie. Results from screening F_6 RIL's suggest that resistance in Ernie is heritable and relatively simply inherited. Of 1330 lines screened in the spring of 1999, 308 plants were classified as resistant (FHBI # 0.2), 192 as moderately resistant (0.3 # FHBI # 0.5), 295 as susceptible (0.6 # FHBI # 0.8) and 535 as very susceptible (FHBI $ 0.81). Bulks of 10 to 15 lines each of the most highly resistant and susceptible RIL's will be used for bulk segregant analyses. The two bulks and the parents will be screened for polymorphisms initially using 1345 probes currently held at University of Missouri, and if necessary for greater resolution and tighter linkage, by AFLP primer combinations and/or SSR's.

PERSONNEL

Kara Salzman has joined the program as a Research Specialist with responsibilities in the area of germplasm evaluation and introgression of identified resistance genes.

Mr. Shuyu Liu is a doctoral student working on the genetic analyses of scab resistance in Ernie.

FUSARIUM HEAD BLIGHT IN NEBRASKA IN 1999 NCR-184 STATE REPORT

John E. Watkins*[1], P. Stephen Baenziger[2], Amit Mitra[1], Marty Dickman[1], and Tom Clemente[3]

FUSARIUM HEAD BLIGHT INCIDENCE

John Watkins, Plant Pathology

The incidence of Fusarium head blight in commercial wheat fields varied considerably in Nebraska. It ranged from a trace in southwest Nebraska, i.e. near McCook area, to as high as 50+ percent in eastern Nebraska. One third (approximately 600,000 acres) of Nebraska's wheat crop is grown in southwest Nebraska with slightly less than one third grown in eastern Nebraska. The largest wheat-producing region is Nebraska's panhandle where drought is common and Fusarium head blight is rare, except in irrigated fields. There is a growing concern that irrigated wheat may be affected by Fusarium head blight if rains come at flowering because these fields are intensively managed and there is ample moisture provided to support the wheat crop. Hence small amounts of additional moisture from rain may lead to a higher incidence of Fusarium head blight than would normally be expected in this otherwise drought-prone region. Wheat from at least one field in eastern Nebraska was rejected by the elevator because of scabby grain.

Fusarium head blight was present in virtually every field in eastern Nebraska, but in only a few of those fields was the severity high enough to be of concern. The weather at the critical flowering period played a major role in the incidence and severity of Fusarium head blight in Nebraska. Another factor that probably is partially responsible for our variable Fusarium head blight incidence and severity was that many of the wheat fields in eastern Nebraska follow soybeans rather than wheat or corn, which probably helped to keep the initial inoculum level down. In surveys, those fields with the highest incidence of Fusarium head blight usually are ones in which the wheat is planted into corn or sorghum residue. The other factor that affected Fusarium head blight in eastern Nebraska is that many of the popular lines (2137 and Wesley) seem to be more susceptible to Fusarium head blight than other lines which are not as well adapted.

Out of 287 certified seed samples tested by the Nebraska Crop Improvement Association in 1999, 40 samples (14%) tested positive for Fusarium head blight. This was a higher number of samples testing positive for Fusarium head blight than in 1997 and 1998, but would not be considered exceptionally high.

ENHANCED VARIETY DEVELOPMENT OF FUSARIUM HEAD BLIGHT RESISTANT VARIETIES

P. Stephen Baenziger, Agronomy

The Nebraska program is attempting to breed Fusarium head blight resistant lines using conventional plant breeding techniques, specifically: 1. Collecting Fusarium head blight resistance germplasm, 2. Crossing Fusarium head blight resistant germplasm onto elite lines adapted to Nebraska, and 3. Screening the progeny of these crosses for Fusarium head blight resistance.

Fusarium head blight resistant germplasm was collected from Korea and the spring wheat breeding programs. We are especially grateful to Drs. Jackie Rudd and Yue Jin of South Dakota

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State University and to Dr. Bob Busch who have graciously shared their most resistant germplasm. Crosses were made onto this germplasm in our spring, 1999 crossing block. It is to early to screen the progeny of this material. In addition, one elite line which is believed to have a low level of Fusarium head blight tolerance (suggested by Dr. Yue Jin) was submitted to the Uniform winter Wheat Scab Nursery for testing in 1999-2000.

The most important accomplishment was the collection of the germplasm and the identification of a line that may already have a higher level of tolerance than many hard red winter wheat cultivars.

ENHANCED FUSARIUM HEAD BLIGHT RESISTANCE IN WINTER WHEAT GERMPLASM BY PLANT TRANSFORMATION

Amit Mitra & Marty Dickman, Plant Pathology
Tom Clemente, Biotechnology Center

Research efforts have concentrated on transferring novel genes, from diverse sources, with known disease resistance activity into wheat using plant transformation. The four genes, we have attempted to insert into wheat encode: a) CED9, b)IAP (inhibitor of apoptosis), c) lactoferrin and a related derived protein, lactoferricin, and d) oxalyl-CoA-decarboxylase. We also wish to insert both lactoferrin and oxalyl-CoA-decarboxylase in hopes the two genes combined may have enhanced antifungal properties. These four genes were chosen because these genes in transgenic tobacco plants have shown potential for combating economically important fungal diseases of crop plants. In addition, these four genes represent distinctly different target specificities (modes of action). Our interest in CED9 and IAP is that host recognition of a pathogen triggers a cell death pathway resulting in a zone of dead cells around the infection site, a hypersensitive reaction. CED9 and IAP are known regulators of programmed cell death. Lactoferrin is a granule-associated glycoprotein present in mammalian fluids such as milk or tears that has long been reported as a major component of infant defense systems against microbial pathogens. Both lactoferrin and lactoferricin have been shown to be highly antifungal against yeasts and filamentous fungi at concentrations ranging from 3 to 25 g/ml. Oxalyl-Co-A-decarboxylase gene has been cloned from a soil bacterium to specifically degrade oxalic acid which is a pathogenic determinant of certain plant pathogenic fungi such as *Sclerotinia* and *Rhizoctonia*. Although it is not know if oxalic acid is involved in *Fusarium* pathogenesis, this gene might be helpful in providing resistance against the fungus by alteration of pH, chelation and/or neutralization.

Eleven transgenic events were created for IAP (8 using Agrobacterium and 3 using microprojectile bombardment), 24 events for lactoferrin (using microprojectile bombardment), 10 events for oxalyl-Co-A-decarboxylase (using microprojectile bombardment) and 18 events for lactoferrin and oxalyl-Co-A-decarboxylase (using microprojectile bombardment). We were able to create only 2 transgenic plants for CED9. We are unsure of why we have a low frequency of CED9 transformants and will change the construct to see if perhaps there is something unusual with our construct. It is also possible that CED9 is deleterious to transgenic plans and few survive. Seeds from the most advanced lines are being grown in the greenhouse for screening for Fusarium head blight.

The most significant accomplishments of the last year were to put three of our four target genes into wheat and to advance the generations of the advanced lines to a point where we can begin screening R_2 plants for Fusarium head blight resistance. We have also successfully adapted Agrobacterium transformation technology to be routinely used in wheat.

NCR-184 STATE REPORT
NEW YORK 1999

Gary C. Bergstrom

FHB SITUATION IN 1999 IN NEW YORK

Much of New York's cereal production area was under a moderate drought during wheat's stem elongation through grain formation stages in May and June. FHB occurred at only trace levels in winter wheat. No yield losses or contamination of grain by deoxynivalenol were reported. Because there was adequate moisture for early development of the crop and virtually no foliar diseases developed, the crop yielded near record highs. Drought intensified during July and August predisposing corn to extraordinarily high levels of Gibberella stalk rot. We are concerned that this could result in elevated regional levels of airborne *Gibberella zeae* ascospores in May and June of 2000, coming from overwintered, infected corn residues. Incidentally, there were also pockets of severe Gibberella ear rot of corn (with associated deoxynivalenol contamination) in western New York locations that received moisture from scattered thunderstorms during corn silk emergence in July.

PROGRAMS AND PERSONNEL INVOLVED IN FHB RESEARCH

Winter wheat cultivar evaluation

One site of the winter wheat cooperative scab nursery is located at Ithaca, NY. Conditions were abnormally dry from spike emergence through grain fill. Although the nursery was inoculated with infested corn kernels and was irrigated, moisture was still inadequate to produce more than incidental levels of scab. We are considering changing to a mist-type irrigation nozzle for 2000. In addition to the standard 30 cooperative lines, an additional 50 regionally-adapted varieties and lines are also being evaluated. Also, scab reaction of over 75 lines derived from crosses of New York-adapted winter wheat cultivars with Chinese sources of resistance is being assessed.
Personnel: Mark Sorrells and David Benscher (CU Plant Breeding); Gary Bergstrom and Stan Kawamoto (CU Plant Pathology)

Fungicide evaluation

One site of the uniform fungicide trial is located at Aurora, NY. See the overall report by McMullen *et al* in this volume. Even though the plots were inoculated, no scab developed because of the dry conditions from anthesis through grain fill. Treatments had no significant impact on yield (Table 1). Ascospore inoculum was plentiful at this site. These results underscore the need for irrigation to get reliable scab development.
Personnel: Stanley Kawamoto, Christine Stockwell, Gary Bergstrom (CU Plant Pathology); William Cox and Dilwyn Otis (CU Crop and Soil Sciences)

Biological control

Microbial antagonists of *Fusarium graminearum* are being isolated and characterized for potential application to wheat spikes, seed, and crop residue. See the report by Stockwell *et al.* in this volume.
Personnel: Christine Stockwell, Stanley

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Kawamoto, Gary Bergstrom (CU Plant Pathology); Wilmar da Luz (Embrapa Trigo, Passo Fundo, Brazil)

Aerobiology/epidemiology

Remote piloted aircraft are being utilized to study the aerobiology of *Gibberella zeae* ascospores in the lower atmosphere in order to better understand the potential of regional dispersal of airborne inoculum. See the report by Maldonado-Ramirez *et al.* in this volume. Also under investigation are the effects of environmental conditions on the discharge of mature ascospores from perithecia. Research is being conducted in laboratory chambers and under field conditions.
Personnel: Sandra Maldonado-Ramirez, Gary Bergstrom (CU Plant Pathology); Elson Shields (CU Entomology); David Gadoury (CU Plant Pathology, Geneva campus); Don Aylor (Connecticut Ag Experiment Station)

Table 1. Effect of foliar treatment at anthesis on yield and Fusarium infection in Caledonia winter wheat in Aurora, NY in 1999

Treatment and amount/A	% Grain infected by *Fusarium graminearum*	Yield (bu/A @ 13.5 % moisture)
Nontreated	0.8	83.3
Armicarb 100 5 lb	0.3	81.4
BAS500F 2.09EC 452.4 ml & Agridex (0.25 %v/v)	3.3	81.4
Benlate 50WP 0.5 lb & Manzate75DF 1 lb & Induce F (0.25 % v/v)	0.0	82.0
Folicur 3.6EC 6 fl oz	0.3	86.5
GB114 (bacteria) 1.6 lb & Induce F (0.06 % v/v)	1.3	84.0
GB114 (bacteria) 1.6 lb & Folicur 3.6EC 6 fl oz & Induce F (0.06 % v/v)	0.8	81.0
Penncozeb 75DF 2 lb	0.0	80.1
Quadris 2.08SC 9.3 fl oz	0.5	79.8
Quadris 2.08SC 12.4 fl oz	2.8	84.5
Stratego 2.1EC 10 fl oz	0.5	82.6
Stratego 2.1EC 14 fl oz	0.0	73.9
LSD (*P*=0.05)	0.8	NS

NCR-184 REPORT 1999
NORTH DAKOTA

Robert W. Stack

THE FHB SITUATION IN NORTH DAKOTA IN 1999 AND ITS IMPACT ON SMALL GRAINS

Results provided by Marcia McMullen, extension plant pathologist, who conducted a survey of 824 grain crops across ND in 1999. Statewide, Fusarium Head Blight (FHB) was much less severe in 1999 than in the several previous years. Individual severely affected fields of spring wheat and barley could be found in parts of northeastern ND and in fields of durum in north central ND, Several of the worst-affected counties are the same ones which have seen severe FHB problems each year since 1993. Overall, losses to FHB in 1999 were low, about 1 - 3% in eastern and central ND and less than 1% in the western regions.

OVERVIEW OF PRESENT RESEARCH PROGRAMS

The research effort at NDSU continued to be a large one in 1999. Six NDSU departments, three NDSU Research & Extension Centers, and the USDA-ARS Northern Crop Sciences Laboratory located on the NDSU campus, all had research efforts on FHB. Many of the projects receIved funding from the scab initiative and reports from those investigators are included in the forum proceedings. Several of the projects are cooperative efforts between state and federal scientists.

While the principal research emphasis at North Dakota State Univ. continues to be on breeding for, and genetics of resistance to FHB, there is active research in several other areas including epidemiology, soil microbial ecology, physiology and biochemistry, cereal science, disease survey, and chemical control.

FHB resistance is being sought in breeding programs for hard red spring wheat, white spring wheat, durum wheat, and barley. Methods to obtain resistant varieties include both conventional and molecular plant breeding methods. These efforts utilize inoculated-irrigated field nurseries and greenhouse testing. Sources of resistance being used include wheat lines from China, Japan, Hungary, and Brazil. Similar diverse sources are being used for durum and for barley.

UNITS INVOLVED IN FHB RESEARCH

NDSU:
*Dept. of Plant Pathology.
*Dept. of Plant Sciences.
*Dept. of Soil Science.
*Dept. of Cereal Science.
*Dept. of Food Science.
*Dept. of Agricultural Engineering.
*Dept. of Veterinary Science and Microbiology.
*NDSU Extension Service.
*NDAES Research-Extension Centers at Langdon, ND, Carrington, ND, Minot, ND.

USDA
USDA-ARS Northern Crop Sciences Laboratory, Fargo.

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NCR-184 MANAGEMENT OF HEAD SCAB OF SMALL GRAINS: 1999 OHIO REPORT

Patrick E. Lipps*[1], Laurence V. Madden[1], Erick D. De Wolf[1], Michael J. Boehm[1], Kimberly G. Campbell[2], and Anju Gupta[2]

WHEAT PRODUCTION, MARKET CLASS AND YIELD LOSS ESTIMATE, 1999

1,030,000 acres of soft red winter wheat were planted in Ohio in the fall of 1998 to be harvested in 1999. Highly favorable weather conditions for winter survival and production of tillers in the spring lead to a record state average yield of 70 bu/A. There was very little damage from scab in Ohio in 1999. Warm temperatures in April and May advanced the heading of the crop by about 7 to 14 days. The early wheat varieties went into bloom during the second week of May in southern Ohio, but most of the crop flowered during the third week of May throughout the rest of the state. Dry conditions prevailed throughout this time period except in the most northwestern counties in the state. Scab severity ranged from 0 to 25% of heads affected in individual fields in Fulton and Williams Counties in northwest Ohio. However, fields with over 5% of the heads affected were rare in these counties. Average yield loss to scab for Ohio was less than 0.1%.

RESEARCH

Research efforts at OSU were focused on: Disease forecasting, breeding for disease resistance, evaluation of fungicide efficacy and biological control.

Disease forecasting

DeWolf, Madden, Lipps

A) We are participating in a cooperative program with North Dakota, South Dakota, Indiana and Manitoba to monitor inoculum levels, environmental parameters and disease incidence and severity in replicated field plots. Information from multiple sites will be used to develop a disease forecasting system. The cooperative effort is necessary to assess the effect of regional variation in cropping practices, tillage and climate on inoculum levels and subsequent disease levels across the wheat producing regions. Volumetric air sampling and a wheat head bioassay are being used to monitor fluctuations in the levels of inoculum reaching heads. Automated environmental monitoring instrumentation is used to measure temperature, relative humidity, precipitation, solar radiation, wind speed and moisture status of the crop.

B) Erick DeWolf has developed a scab risk assessment model based on historical scab epidemics and environmental information. The model presently incorporates information from Ohio only. We are seeking information from other states for incorporation into the model and for validation. Please contact us if you have information on epidemic levels over several years, heading or flowering dates and corresponding weather data.

C) Erick De Wolf is attempting to evaluate the effect of residue moisture on perithecia development in corn stalks. He is adapting a residue moisture sensor to quantify the moisture status of corn stalks. The work plans to conduct trials in controlled environment chambers and in the field.

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Breeding for scab resistance

Campbell, Lipps and Gupta

A) The main objective of the scab resistance breeding project at Ohio State is to eliminate the most susceptible lines from the advanced breeding material and to incorporate resistance from exotic germplasm. Resistance screening has been accomplished using inoculated, misted field nurseries and greenhouse tests. A series of three nurseries were used to establish early generation scab resistance selections in: F3 bulk populations, head row selections, and progeny from crosses between Fusarium head scab resistance sources and Stagonospora glume blotch resistance sources. We are also interested in evaluating the relationship among scab severity, incidence, scab index, visual kernel damage rating, percent lightweight kernels, yield and DON.

B) Anju Gupta is currently screening germplasm from Yugoslavia obtained from the National Plant Germplasm System to identify new sources of resistance that could be incorporated into the breeding program. She has one year of field and greenhouse data completed and is presenting a poster on this at the Fusarium Head Blight Forum.

C) Anju Gupta has initiated a program to use marker assisted selection for scab resistance. The purpose of this research is to identify regions of the wheat genome linked to scab resistance using simple sequence repeat (microsatellite) markers. She is working with populations derived from a resistant by resistant cross (Ning 7840 x Freedom) and a resistant by susceptible cross (Freedom x OH542). The goal is to identify and combine resistance genes from both Ning 7840 and Freedom.

Fungicide efficacy and dissemination of research information

Lipps and De Wolf

We are currently participating in the Chemical Control Network of the National FHB Initiative headed by M. McMullen and G. Bergstrom. Five fungicides were evaluated in 1999 using procedures and rates directed by the Chemical Control Network. No disease developed in the study plots. Yields across treatments ranged from 70.3 to 76.6 (P = 0.335). It is our goal that once an effective disease forecasting system has been created and effective fungicides have been identified then fungicide recommendations can be disseminated to growers in a timely fashion. Data from inoculum monitoring efforts coupled with environmental information was provided to growers in a weekly update via an electronic information system [Ohio State University Extension's Crop Observation and Recommendation Network (C.O.R.N.)].

Biological Control

Boehm and Lipps

A cooperative research project is being conducted with Dr. David Schisler and Dr. Naseem Kahn (USDA-ARS, Peoria) to determine the efficacy of naturally occurring biological agents for control of head scab. Of over 700 isolates assayed, three reduced the severity of scab in greenhouse bioassays. Disease severity was reduced by 57-93%. Encouraging results were also obtained in the field where treatments reduced disease severity by as much as 43%. Putative biological control treatments will be evaluated again during the 2000 growing season.

NCR-184, MANAGEMENT OF HEAD SCAB OF SMALL GRAINS 1999 SOUTH DAKOTA STATE REPORT

Yue Jin

1999 SCAB DEVELOPMENT IN SOUTH DAKOTA

M. Draper, Y. Jin, and J. Rudd

Significant amount of scab developed in South Dakota. Scab index (statewide average) was 7.5% for spring wheat and 2.5% for winter wheat.

CURRENT RESEARCH PROJECTS

Germplasm introduction and evaluation

Y. Jin

The overall project goal was to identify new sources of scab resistance in spring wheat and to introgress the resistances into adapted materials. Spring wheat accessions from targeted regions of the world and relatives of wheat were evaluated in an inoculated field nursery. Selections from the field screening are being evaluated in the greenhouse to characterize the type and level of resistance. Accessions with acceptable levels of resistance are used for crossing to introgress the resistance into adapted germplasm.

Epidemiology

Y. Jin

Ongoing research on several aspects of scab epidemiology includes effects of soil moisture/ wetness on inoculum (ascospore) production, ascospore survival and accumulation on plant surface, and inoculum potentials from weedy grasses. We serve as a cooperator in the regional collaborative project of inoculum monitoring in the crop season.

Breeding for scab resistance in spring wheat

J. Rudd

Greenhouse and field screening nurseries are used to evaluate early generation and advanced lines for scab resistance. All entries in the advanced yield trials are at least moderately resistant to scab. This is dramatically different from a few years ago when the spring wheat breeding program first began to evaluate for resistance to scab. Several of the moderately resistant lines are equal to or better than existing cultivars for agronomic performance, but no highly resistant lines have been identified that have acceptable agronomic performance.

Breeding for scab resistance in winter wheat

J. Rudd

The first step in developing scab resistant hard winter wheat varieties is to assess the genetic variability for resistance in existing cultivars and advanced breeding lines. Regional and breeding nurseries were screened in a mist-irrigated, inoculated nursery in 1999. Scab resistance sources in the winter crossing block included adapted spring wheats from the SDSU breeding program, Sumai 3 derived spring wheat lines, eastern European winter wheat lines, entries from the 1998 regional winter wheat scab nursery, and adapted hard red and hard white breeding lines. Approximately 200 crosses with scab resistant sources were made and the segregating populations will be evaluated in 2000.

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Fungicide efficacy studies
M. Draper

South Dakota was one of 14 states participating in a set of ten uniform fungicide treatments for scab suppression. Two hard red spring wheat cultivars were planted at two locations each. Nine treatments of the ten treatments were also applied to two hard red winter wheat cultivars planted at a single location. Plots were evaluated for protection of the flag leaf against leaf diseases as well as for average incidence of scab infected heads, average head severity of scab, average plot severity of scab, Fusarium damaged kernels (FDK), deoxynivalenol (DON) content in the harvested grain, grain yield and test weight of harvested grain. The most severe disease occurred at the South Shore, SD location, but scab was also present at the Groton, SD location. All treatments significantly reduced diseased leaf area ($P_{0.05}$) on spring wheat at both locations. Leaf disease on winter wheat was reduced by most treatments. The following treatments significantly reduced scab in spring wheat at both locations: Folicur (6 fl. oz./A), Benlate (0.5#/A) + Manzate (1#/A), BAS 500 (0.25# a.i./A at Feekes 10.3 or 10.51), and Stratego (10 fl. oz./A). The same treatments were effective on winter wheat, as was Stratego (14 fl. oz./A). Winter wheat yields were significantly higher as a result of all treatments except Quadris (0.2# a.i./A). All treatments increased yield on spring wheat at Groton and only the Benlate/Manzate treatment did not increase yield significantly at South Shore. In other treatments, Folicur applied at the 6 fl. oz./A rate outperformed the 4 fl. oz./A rate on average plot severity of scab in spring wheat at both locations, but was not significantly different from the 4 fl. oz. rate on winter wheat. Even when scab was reduced numerically by the higher rate of Folicur, no significant increase in yield was attained.

Molecular biology and DNA markers for scab resistance
Y. Yen

The goal of this project is to understand the molecular biology of scab resistance in wheat while identifying breeder-friendly, PCR-based DNA markers for indirect selection in breeding. We have evaluated some newly introduced Chinese spring wheat lines and their parental lines for scab resistance. Our preliminary data indicated that Yiyuan 2, Chuanyu 12 and 3854 may have some degree of Type II and Type III resistance. Yiyuan 2 and 3854 were crossed with susceptible lines and the hybrid populations have been advanced to F2. A silver-staining-based AFLP protocol was optimized for screening user-friendly DNA markers. Our preliminary assay of parental lines with 64 PCR primer sets revealed 1 to 35 polymorphic bands, respectively, between Yiyuan 2 and Wheaton with an average of 13.3 polymorphic loci per primer set.

Biocontrol of wheat diseases
B. Bleakley

Four different strains of *Bacillus* isolated from South Dakota wheat foliage and residue which antagonize the fungi causing tanspot and head blight of wheat have been used in laboratory, greenhouse, and field trials. Cell-free ethyl acetate extracts of *Bacillus* broth culture supernatants have been analyzed by thin layer chromatography. Each strain produced a different fingerprint of ninhydrin-positive spots, which will individually be checked for activity against *F. graminearum*. During summer of 1999 whole cells of one of the *Bacillus* strains were applied to wheat in field plots (in cooperation with Marty Draper) to see if the bacteria afforded protection against head blight or other diseases. The summer was dry and did not favor extensive disease development, but data were collected and will be analyzed.

PERSONNEL

Researchers/Project

Y. Jin/Small Grain Pathology; M. Draper/Extension Plant Pathology; J. Rudd/Spring and WinterWheat Breeding; B. Bleakley/Soil Microbiology; Y. Yen/Cytogenetics-Molecular Biology.

Supporting staff

X. Zhang (Research Associate, Pathology); Terrence Hall (Research Assistant Pathology); R. Rudd (Research Assistant, Pathology/Breeding

NCR 184: VIRGINIA 1999 STATE REPORT ON FUSARIUM HEAD BLIGHT

Carl Griffey*, Erik Stromberg, Jianli Chen, Matthew Chappell, Jane Shaw, and Tom Pridgen

The incidence of Fusarium Head Blight (FHB) in Virginia was nil during the 1998-99 season due to drought conditions in the mid-Atlantic and Northeastern U.S., which extended from the flowering through grain ripening stages. During this period, total precipitation received in most of the wheat production regions of Virginia was very low. Only 0.03 inches (0.076 cm) of precipitation were recorded at the Eastern Virginia Agricultural Research and Extension Center in Warsaw, Virginia during this period.

Research aimed at developing a means to control FHB in wheat with the application of a single fungicide, multiple fungicides, or a biological agent on wheat heads prior to or during anthesis was conducted this past year. Effectiveness of fungicide treatments on the control of FHB could not be assessed this past year due to the absence of FHB development under the excessively dry conditions. This was despite planting plots no-till into chopped corn stubble and over seeding them with corn seed inoculated with *Fusarium graminearum*.

The ultimate goal of the breeding program is to pyramid different types of FHB resistance into superior soft red winter (SRW) wheat backgrounds. Among over 200 wheat lines tested in both greenhouse and field tests, we have identified or confirmed the presence of type IV (kernel infection) and type V (yield loss) resistance in SRW wheat cultivars such as Roane (Virginia), Freedom (Ohio), Ernie (Missouri), and INW9824 (Indiana). Yield losses due to scab among 20 SRW wheat genotypes tested in an inoculated, irrigated nursery varied from 4 to 48% and percentage of scabby seeds varied from 14 to 47%. Additional SRW wheat lines possessing these types of resistance have been identified in multi-state, cooperative, winter wheat screening nurseries. Also, we have identified and confirmed high levels of type II (invasion) resistance in 7 wheat lines from China, 5 from Canada, and 2 from France. Resistance from these sources is being incorporated into SRW wheat via traditional breeding methods and also is being back-crossed into several diverse SRW wheat cultivars. To date, more than 350 crosses involving scab resistant parents have been produced and 2500 advanced lines are being evaluated for resistance and agronomic performance.

To improve the precision in breeding and selection for scab resistance, genetics and mapping studies have been initiated and the wheat by maize hybridization system has been used to develop doubled-haploid populations. A 10 parent diallel including parents with diverse types of resistance has been produced for studying the inheritance of scab resistance. Preliminary genetic studies indicate that type II resistance in the cross W14 x Madison is governed by two complementary genes and, therefore, should be feasible to transfer. The $F_{2:3}$ population from this cross will be used to screen and select resistance-related molecular markers, which can be applied to marker-assisted selection and gene pyramiding.

A website for information on small grains disease control has been established as a primary means of distributing information on the biology, de-

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scription, cultural and chemical means of control of small grain diseases to county extension agents within Virginia, small grains producers in Virginia, the Agri-business community and others. This website can be assessed at: http://www.ppws.vt.edu/stromberg/smallgrain/sgrain.html

MANAGEMENT OF FUSARIUM HEAD BLIGHT IN SMALL GRAINS NCR-184 1999 STATE REPORT FOR WISCONSIN

Heidi F. Kaeppler

PRODUCTION

Growing conditions varied throughout Wisconsin in 1999. In general, enough moisture was received during the spring and early summer to produce good stands. Drier conditions during the later part of the summer resulted in good harvest conditions. Wisconsin farmers harvested 120,000 acres of soft red winter wheat in 1999. Yield per acre was 60 bushels, and total production was 7.20 million bushels, which was 3 percent lower that in 1998. Spring barley was harvested from 65,000 acres with an average yield of 52 bushels per acre. Total barley production of 3.38 million bushels was similar to that in 1998. Spring wheat acreage remained at approximately 7000 acres. The average yield of spring wheat was 40 bushels per acre, and production was 280,000 bushels, up 33% from 1998. Incidence and severity of head scab in wheat and barley were low in 1999, as in 1998. Estimates from the Wisconsin Department of Agriculture Trade and Consumer Protection rated incidence at trace to 1% in several counties, and not detected in many others. Disease severity, where detected, was estimated at around 1%. The low occurrence and severity of head scab in Wisconsin was similar to that observed in neighboring states in 1999.

Head Scab Research: Research is being conducted in several labs at the University of Wisconsin toward increased understanding and management of head scab in small grains. Brief descriptions of current research projects are provided.

Berne Jones, USDA-ARS and collaborator Anja Pekkarinen, VTT, Helsinki, are conducting research to identify barley compounds that inhibit proteases produced by *Fusarium graminearum*. To date, several *Fusarium* proteases have been isolated and purified, and results from recent experiments provide evidence that there are compounds in barley that inhibit the proteases. Dr. Jones and Dr. Pekkarinen are now working to isolate and purify the inhibiting compounds for further molecular characterization. In addition to this research, Dr. Jones has obtained two new malting machines to aid in high throughput malting quality screening of scab tolerant breeding lines being developed by breeding programs. With the new malting units, up to 40% more samples can be evaluated than previously. The increased efficiency will allow the lab to quickly test the usual numbers of standard breeding lines, plus additional lines from scab resistance breeding efforts.

Heidi Kaeppler and Ronald Skadsen are conducting collaborative research in the genetic engineering of wheat and barley for enhanced resistance to head scab. Two antifungal genes (permatin and hordothionin) have been transformed into barley and are being delivered into wheat. The permatin gene was cloned from oat by Ronald Skadsen and the hordothionin gene was cloned from barley by Dr. Skadsen in collaboration with Berne Jones. Additionally, two pathogen response genes which act as regulators of several resistance genes are being delivered into wheat. Transgenic barley lines are being evaluated for presence and expression of the transgenes, and resistance response to inocula-

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tion with *Fusarium graminearum*. Collaborators in preliminary testing of transgenic barley lines include Lynn Dahleen, USDA-ARS, Fargo, ND, and Steve Leath, NC State Univ. Research is also underway to investigate tissue specific expression of the resistance transgenes, and inter/intracellular targeting of the proteins for optimal expression. Postdoctoral research associates Sathish Puthigae and Jianming Fu, and graduate student Maria L. Federico are conducting the studies. To date, two promoters have been cloned from genes displaying tissue specific expression. The promoters have been fused to a synthetic green fluorescent protein (gfp) gene and are being tested for floral specific expression in transient and transformation assays. Research is also being conducted to study the timing and mode of infection of *Fusarium graminearum* in barley using a strain of *Fusarium* transformed to express gfp. Microscopic analysis are being conducted to determine what tissues are infected by *Fusarium* and how the fungus enters the tissue and grows. Information gained from this research will be used for designing optimal expression strategies for resistance transgenes.

Fun Sun Chu, UW Food Res. Inst., is conducting research aimed at improving ELISA methods for mycotoxin detection and developing rapid immunochemical methods for monitoring key enzymes and regulatory proteins involved in the biosynthesis of DON and related mycotoxins. Dr. Chu's research involves improvement of monoclonal antibody-based ELISA for DON. Additionally, his group is working on developing monoclonal antibodies for permatin, trichodiene synthase, and the protein encoded by the Tri6 gene (a regulatory gene controlling DON formation). Antibodies and ELISA methods developed in Dr. Chu's lab will be utilized for analysis in several of the studies described above.

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