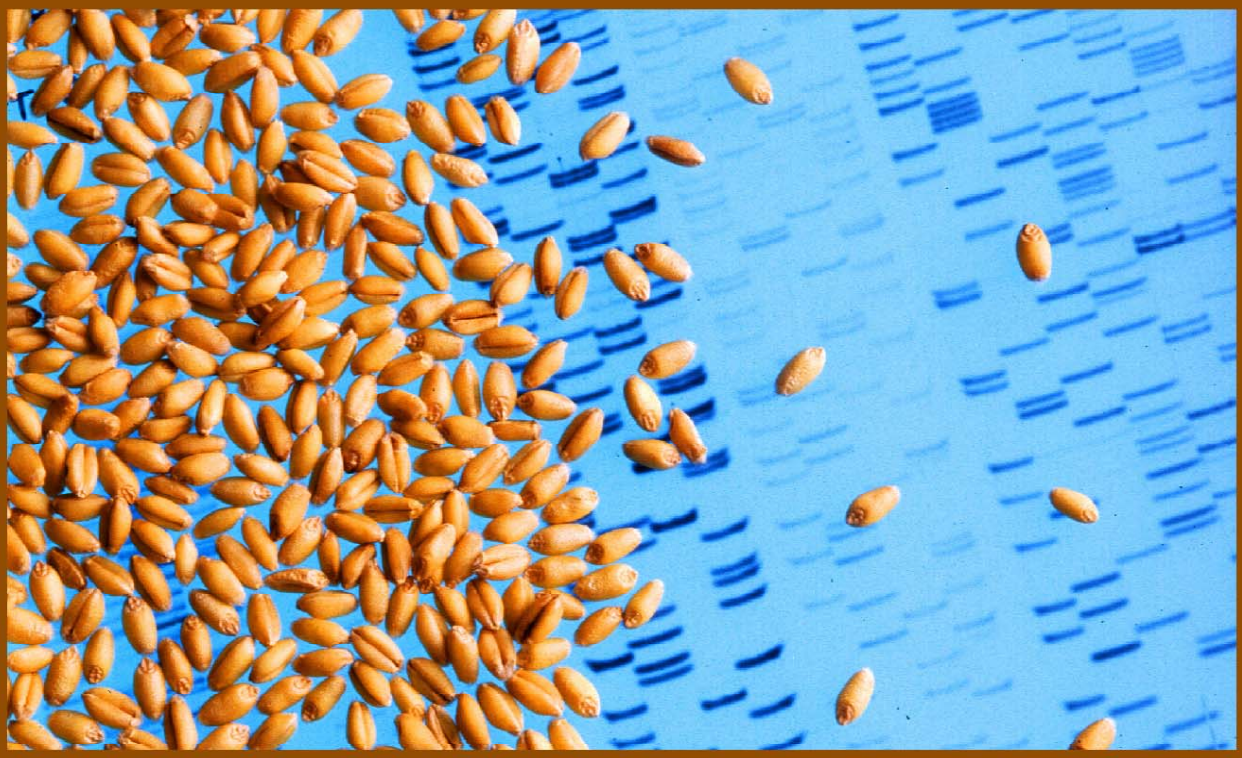
Proceedings of the 2006 National Fusarium Head Blight Forum



Sheraton Imperial Hotel
Research Triangle Park, North Carolina
December 10-12, 2006

Proceedings of the 2006 National Fusarium Head Blight Forum

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Proceedings compiled by: Susan M. Canty, Anthony Clark and David Van Sanford

Photo on cover: Wheat seed scattered on an AFLP gel autoradiograph

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> Buerstmayr, H., Steiner, B., Hart, L., Griesser, M., Angerer, N., Lengauer, D. and Lemmens, M. 2002. "Molecular Mapping of QTLs for Fusarium Head Blight Resistance in Spring Wheat." In: Canty, S.M., Lewis, J., Siler, L. and Ward, R.W (Eds.), Proceedings of the National Fusarium Head Blight Forum; 2002 Dec 7-9; Erlanger, KY. East Lansing: Michigan State University. pp. 22-25.

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PREFACE

PREFACE

The U. S. Wheat and Barley Scab Initiative (USWBSI) was conceived and implemented in 1997 in response to the economic devastation caused by head scab. Since that time, approximately $38,287,433 has been disbursed through USDA-ARS grants to scientists in eight research areas. Each year since 1998, the Initiative has hosted the National Fusarium Head Blight Forum for the purpose of bringing together the community of individuals affected by head scab – wheat and barley growers, processors, consumers and researchers. Since its inception, the objective of the Forum has been to share scientific findings and research results that are aimed at achieving the overarching goal of the USWBSI, i.e., the development and implementation of "*control measures that minimize the threat of Fusarium Head Blight (Scab) to the producers, processors, and consumers of wheat and barley*". The 2006 Forum is no different in that it seeks to bring these individuals together to focus on the research that is most likely to achieve this goal. What distinguishes this forum from previous ones is that the discussion and analysis of research findings will be used to design an Action Plan for the next few years. Recent research results will be presented in poster sessions that are interspersed with breakout and planning sessions. More than ever, the focus will be on research, but the objective will be to design a research strategy that will significantly lower DON levels and reduce the economic impact of scab within a 3 year time frame. As always, stakeholders are invited to the forum to have a voice in shaping the future of the Initiative.

Dave Van Sanford & Tom Anderson
USWBSI Co-Chairs

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OPENING TALKS

THE ECONOMIC AND MARKETING IMPLICATIONS OF EXCESSIVE DON IN WHEAT.

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ABSTRACT

This presentation/paper draws on a number of existing studies on the economic implications of DON on the wheat marketing system. It synthesizes much of this work in a foreword looking presentation. Specific topics include: 1) background on how DON impacts marketing of wheat in the US and other countries; 2) a description of how the current market deals with excessive DON; 3) prospective changes occurring that will impact the demand for reduced DON (notably the impacts of new EU regulations); and 4) discuss challenges and opportunities of new and competing technologies for reducing the incidence of SCAB.

CHEMICAL, BIOLOGICAL AND CULTURAL CONTROL

FIELD EVALUATIONS OF CHEMICAL CONTROLS FOR FUSARIUM HEAD BLIGHT IN MICHIGAN.

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ABSTRACT

Four uniform fungicide trials were planted in Michigan during 3 – 8 Oct 2005. The sites were at the Plant Pathology Farm, East Lansing MI, (inoculated with *Fusarium graminearum* and mist-irrigated); Bean and Beet Farm, Saginaw, MI (inoculated/not-irrigated); Sanilac, Sanilac County, MI and at Williamston, Ingham County, MI (not-inoculated/not-irrigated). Caledonia wheat, treated with Thiram 42S (thiram 42%, 2.0 fl. oz./cwt), was planted at the rate of 24 seeds/ft of row at each site. Head scab developed at all sites but only severe deterioration was measured at the East Lansing (inoculated) site resulting in high DON levels and low yields and test weights. Scab levels across the state were low in 2006. Very little Fusarium developed at the other three sites and DON levels were negligible.

At the East Lansing location, measurable precipitation occurred on only eight days from the period encompassing the first application of fungicide through harvest. Frequent irrigation kept the heads almost continuously wet during early grain maturation. Powdery mildew was evident but only developed to 6.6% foliar area affected by Feekes 10.5.2 in the non-treated control and there were no significant differences among treatments. Stagnospora leaf blotch also developed and by Feekes 10.5.2 the non-treated control had about 5.5% of the foliage affected. Folicur 3.6F (4.0 fl oz) and Caramba 90SL (13.5 fl oz) had significantly less Stagnospora leaf blotch than the non-treated control but were not different from any other treatments. Daily mist irrigation favored FHB development in spite of moderately cool temperatures during and one week following anthesis. Fusarium head blight developed in the trial however there were no differences in incidence or severity among any treatments or the non-treated control. There were no differences in green leaf area (%) remaining at Feekes 11.1 among any treatments or the non-treated control. Only Caramba 90SL (13.5 fl oz) had significantly higher yield than the non-treated control and was also significantly higher than Topguard 1.04SC (14.0 fl oz). No other treatments had significantly higher yield than the non-treated control. Based on analysis of variance, no treatments were significantly different in terms of test weight, percentage damaged kernels, 1000 grain weight or DON levels (3.9-7.0 ppm).

At the Bean and Beet Farm, there were no significant differences among treatments for powdery mildew or Stagnospora leaf blotch (disease ratings were very low), FHB severity, FHB incidence or FHB index. There were no significant differences among the treatments for yield, test weight, 1000 grain weight, FDK or DON levels (0.2-0.4 ppm). At the Sanilac location, leaf rust was evaluated for severity and incidence. All treatments significantly reduced leaf rust compared to the control, but were not significantly different from each other. Powdery mildew and Stagnospora leaf blotch were rated. There were some differences among treatments in 1000 grain weights at Sanilac, but no differences in test weights or yield. DON levels for all treatments were 0 ppm. At the Williamston location, there were no significant differences among treatments for powdery mildew or Stagnospora leaf blotch Fusarium head blight severity and incidence was significantly lower for Prosaro (6.5 fl oz), compared to the untreated control, but not significantly different from other treatments. The Fusarium head blight index was significantly lower than the control for all treatments except Tilt and one of

the Folicur treatments, but there were no significant differences among treatments. Yield, test weight, 1000 grain weight FDK and DON levels (0.1- 0.2 ppm) were not significantly different, based on ANOVA. No phytotoxicity was observed in any of the treatments at any of the sites.

ACKNOWLEDGEMENT AND DISCLAIMER

This material is based upon work supported by the U.S. Department of Agriculture, under Agreement No. 59-0790-6-062. This is a cooperative project with the U.S. Wheat and Barley Scab Initiative. Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the authors and do not necessarily reflect the view of the U.S. Department of Agriculture.

THE USE OF CHEMICAL AND PHYSICAL STRESSORS, 8.5 % NaCl AND 47^{O}C, TO ASSAY POPULATIONS OF A *BACILLUS* STRAIN USED TO CONTROL FUSARIUM HEAD BLIGHT ON WHEAT HEADS IN FIELD PLOTS.

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ABSTRACT

Selected strains of *Bacillus* can be used as biocontrol agents (BCAs), to antagonize *Fusarium graminearum* which causes Fusarium Head Blight (FHB) of wheat and barley. To assay numbers of the biocontrol agent *Bacillus* strain 1BA, after spraying its cells onto wheat heads in field plots, a selective and/or differential growth medium/isolation procedure was developed to allow differentiation of *Bacillus* 1BA from native wheat microflora. The ability of *Bacillus* 1BA to tolerate temperature and salt stresses was exploited to allow most probable number (MPN) estimates of its numbers on inoculated wheat heads over time in field plots. *Bacillus* 1BA grew on Tryptic Soy Agar (TSA) and Nutrient Agar (NA) at various temperatures, ranging from 27^{o}C to 50^{o}C. It also grew on TSA and NA amended with various NaCl concentrations, ranging from 2.5 % NaCl to 10 % NaCl. The elevated temperature and NaCl concentrations that *Bacillus* 1BA could withstand were used in preliminary plate counting of the microflora of wheat heads. Little or no growth of the native microflora occurred with these conditions, which led to continued studies involving the recovery of *Bacillus* 1BA from wheat heads after spray application in the field. To produce BCA inoculum for field plot application, *Bacillus* 1BA was grown in a variety of different broth media, including Field Defined Medium (FDM), FDM + 8.5 % NaCl, Tryptic Soy Broth & Yeast Extract (TSB/YE), TSB/YE + 8.5 % NaCl, half-strength TSB/YE, and half-strength TSB/YE + 8.5 % NaCl. Cell counts of *Bacillus* 1BA in these media were obtained using a MPN procedure, on the day cells were sprayed onto wheat heads at flowering. For the MPNs, a selective agar-solidified medium of TSA + 8.5 % NaCl was used, with an incubation temperature of 47^{o}C . Counts of the BCA on inoculated wheat heads were done at days 0, 3, 6, 9, 12, 15, and 20 after inoculation. In addition a few treatments from Day 15 and Day 20 were heat-pasteurized to help determine if *Bacillus* 1BA propagules were mainly present as vegetative cells or endospores. After heat pasteurization, *Bacillus* 1BA colonies formed on the MPN plates , which confirmed the presence of endospores on wheat heads at Day 15 and Day 20. Inocula of *Bacillus* 1BA produced in different broth media behaved differently over time on wheat heads, with some experiencing little or no population increase, and others showing a dramatic increase in numbers several days after spray application. Most of the inocula from different media experienced a rapid drop-off in numbers soon after spray application to wheat heads. However, most treatments stabilized or increased in numbers on the wheat heads over time, and several treatments showed a dramatic increase in numbers by 9 days after application. This suggested that this biocontrol agent can persist and grow for several days after its application, to antagonize *Fusarium* in the field.

ACKNOWLEDGEMENT AND DISCLAIMER

This material is based upon work supported by the U.S. Department of Agriculture, under Agreement No. 59-0790-5-077. This is a cooperative project with the U.S. Wheat and Barley Scab Initiative. Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the authors and do not necessarily reflect the view of the U.S. Department of Agriculture.

INFLUENCE OF SPRAY VOLUME AND NOZZLE ORIENTATION ON FUNGICIDE EFFICACY FOR CONTROL OF FUSARIUM HEAD BLIGHT.

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ABSTRACT

Enhancing the efficacy of fungicides for control of Fusarium head blight (FHB) has been the goal of researchers studying application technology. Fungicides historically have been reported to reduce FHB and deoxynivalenol concentration (DON) by about 50% as compared to untreated. Initial recommendations on the amount of spray volume to apply fungicide to wheat heads from Extension Service Specialists were in part based on greenhouse studies using a fluorescent tracer dye which showed that coverage on the wheat spike increased as spray volume increased up to 54 GPA. A practical limit of 20 GPA has been recommended to growers for applying fungicides to small grains. Many growers apply fungicide in volumes between 10 and 20 GPA. Studies were conducted on barley, hard red spring wheat (HRSW) and durum over several growing seasons at the North Dakota State University Langdon Research Extension Center to determine the effect of spray volume and nozzle orientation on fungicide efficacy. Fungicide was applied at volumes of 5, 10, and 20 GPA with nozzles oriented forward and backward (F+B) and forward (F) with both orientations angled downward 30 degrees from horizontal. On barley, field severity from a 20 GPA F+B treatment was less than the 5 and 10 GPA F treatment but was not different from a 10 GPA F+B treatment. No differences were found among fungicide treatments determined by FHB incidence, plump or deoxynivalenol (DON) concentrations. On HRSW a 10 GPA F+B treatment had less FHB incidence and better test weight than a 10 GPA F treatment and a better test weight than a 20 GPA F+B treatment. On durum there were no differences due to application method among fungicide treatments in FHB incidence, field severity or DON. There was no evidence to suggest that spray volumes greater than 10 GPA enhanced the efficacy of fungicide for control of FHB.

ACKNOWLEDGEMENT AND DISCLAIMER

This material is based upon work supported by the U.S. Department of Agriculture, under Agreement No. 59-0790-3-079. This is a cooperative project with the U.S. Wheat and Barley Scab Initiative. Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the authors and do not necessarily reflect the view of the U.S. Department of Agriculture.

2006 FHB UNIFORM FUNGICIDE TRIAL ON SPRING AND WINTER WHEATS IN MINNESOTA.

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ABSTRACT

The objective of this trial was to evaluate and compare Fusarium head blight (FHB) control efficacies of non-registered and commercially available fungicide products when applied to spring and winter wheats in Minnesota. Hard red winter wheat (HRWW) 'Jerry' was planted 8 Sept. 2005 and hard red spring wheat (HRSW) 'Steele ND' was planted 6 May 2006 into soybean residue at the Northwest Research and Outreach Center (NWROC). Hard red spring wheat 'Oxen' was planted 12 April 2006 into corn residue at the Southwest Research and Outreach Center (SWROC). The NWROC winter and spring wheat and the SWROC spring wheat experiments were inoculated 22 May, 15 June, and 23 May, respectively with 112 kg ha^{-1} of *F. graminearum* infested corn grain inoculum and misted nightly thereafter to increase disease pressure. Fungicide treatments were applied on HRWW 6 June and HRSW 16 June (SWROC) and 28 June (NWROC) when plants were at the early flowering growth stage (Feekes 10.51). Fungicide treatments were applied using CO_2 backpack-type sprayers adjusted to 40 psi at 18-20 gpa with forward and backward positioned 'XR' Teejet flat fan 8001 VS nozzles. Treatments consisted of: (1) nontreated control; (2) Folicur (tebuconazole) 4 fl oz $acre^{-1}$; (3) Prosaro (tebuconazole + prothioconazole) 6.5 fl oz $acre^{-1}$; (4) Caramba (metconazole) 13.5 fl oz $acre^{-1}$; (5) Topguard (flutriafol) 14.0 fl oz $acre^{-1}$; (6) tebuconazole 2 fl oz $acre^{-1}$ + thiphanate-methyl 8 fl oz $acre^{-1}$; and (7) Tilt (propiconazole) 4 fl oz $acre^{-1}$. A total of 50 spikes $plot^{-1}$ were rated for FHB symptoms from spring wheat tests. Tests were harvested 19 July (HRWW), 28 July (HRSW at SWROC), and 3 Aug (HRSW at NWROC). The tests were arranged in the field as randomized complete block designs with four replicates. ANOVAs were performed with SAS using PROC GLM. Winter wheat results were analyzed using a randomized complete block design while a split plot design was used for HRSW tests (main plot=location, sub plot=fungicide treatments). Fisher's protected least significant difference (LSD) mean comparisons were used to identify differences.

A widespread drought occurred in Minnesota during the 2006 growing season. Disease pressures were light across experiments even under misting. Symptoms of FHB did not develop on HRWW spikes so disease was not rated in that trial. Across locations and treatments, HRSW FHB incidence ranged from 10.8% to 5.0%, FHB severity ranged from 8.5% to 5.9%, and FHB indices ranged from 1.0% to 0.4%. Neither location nor treatment means were significant for FHB incidence, FHB severity, or FHB index. Significant results across wheat classes include yield (HRSW, treatment $P = 0.0198$: HRWW, $P = 0.0215$), thousand kernel weight (HRSW, treatment $P = 0.0311$: HRWW, $P = 0.242$), and deoxynivalenol (DON) content of grain (HRSW, treatment $P = 0.0026$: HRWW, $P = 0.0039$).

Hard red spring wheat yields were greatest with Prosaro (65.3 bu/A), Caramba (62.3 bu/A), and Tilt (60.8/A). Three treatments, Caramba (29.9 g), Tilt (29.4 g), and Folicur (29.2 g) resulted in the largest thousand kernel weights. Two triazole fungicide products, Caramba (0.5 ppm) and Prosaro (0.7 ppm), significantly reduced DON content compared to other treatments.

Hard red winter wheat yields were greatest with Folicur (93.0 bu/A), Caramba (91.0 bu/A), Tilt (86.9 bu/A), tebuconazole + thiphanate-methyl (85.3 bu/A), and Prosaro (83.9 bu/A). Tebuconazole + thiphanate-methyl (34.3 g), the nontreated control (34.7 g), Topguard (34.8 g), and Tilt (35.3) had the smallest thousand kernel weights. Four treatments, Prosaro (0.1 ppm), Caramba (0.1 ppm), Folicur (0.2 ppm), and tebuconazole + thiphanate-methyl (0.3 ppm) resulted in reduced DON levels.

ACKNOWLEDGEMENTS

The authors would like to thank the U.S. Wheat and Barley Scab Initiative and the Northwest Research and Outreach Center for supporting this research; BASF Corp., Bayer CropScience, Cheminova, and Cerex-Agri for supplying fungicides; and the University of Minnesota Mycotoxin lab for providing DON results.

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DISCLAIMER

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

ADJUVANT EFFECTS ON PERFORMANCE OF FOLICUR AND PROSARO FUNGICIDES FOR FHB CONTROL IN DURUM WHEAT AND BARLEY.

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ABSTRACT

Folicur (tebuconazole) and Prosaro (tebuconazole + prothioconazole) are two fungicides that have shown promise in reducing Fusarium head blight (FHB) field severity and deoxynivalenol (DON) levels in small grains. Folicur (tebuconazole) has had special exemptions in some states for use on wheat and barley to suppress FHB, while Prosaro is still an experimental product. The manufacturer of both products, Bayer CropScience, is seeking full registration of both products from the Environmental Protection Agency.

A standard adjuvant recommended for use with tebuconazole is a petroleum-based non-ionic surfactant. Various private companies in the U.S. sell other non-ionic surfactants or have other adjuvants for sale that are silicone-based or are encapsulating products, and these companies also are experimenting with many new formulations of adjuvants. We have conducted extensive studies on hard red spring wheat with adjuvants and Folicur. Results indicated that most adjuvants tested with Folicur reduced FHB severity better than when no adjuvant was applied, while a few products were not as satisfactory as the non-ionic surfactants (McMullen, et al. 2005). Additional testing was needed to determine if durum and barley would react similarly to various adjuvants. Durum wheat and barley cultivars grown in North Dakota have very long and prominent awns compared to most spring wheat cultivars; different types of adjuvants could play a role in deposition and penetration past these awns to the site of infection.

Adjuvants were mixed with 4 fl oz/A rate of Folicur or 6.5 fl oz/A rate of Prosaro and applied at early flowering to durum or early head emergence in barley. Plants were inoculated with a spore suspension (10,000 spores/ 20ml) of *Fusarium graminearum* one hour after application of the fungicides + adjuvants. FHB severities were determined at early soft dough stage. Results with adjuvant testing in the greenhouse indicated that all fungicide + adjuvant treatments significantly reduced FHB field severity, but very few differences were observed among types of adjuvants mixed with Folicur or Prosaro.

ACKNOWLEDGEMENT AND DISCLAIMER

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REFERENCE

McMullen, M., Jordahl, J., and Meyer, S. 2005. Effect of adjuvants on efficacy of Folicur fungicide for FHB control. Page 215 in: Proceedings of the 2005 National Fusarium Head Blight Forum. Dec. 11-13, Milwuakee, WI. U.S. Wheat and Barley Scab Initiative, Michigan State Univ., East Lansing, MI.

UNIFORM FUNGICIDE TRIAL RESULTS ON HRS WHEAT AND BARLEY, FARGO, ND 2006.

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ABSTRACT

Six fungicide treatments were compared to the untreated check for efficacy in reduction of Fusarium head blight (FHB) severity and deoxynivalenol (DON) in 'Tradition' spring barley and 'Steele ND" hard red spring (HRS) wheat, at Fargo, ND. Both crops were planted on May 8 into ground with wheat as the previous crop, and that had been chisel plowed twice prior to planting. Plots were 9'wide and 20' long, with 4 replicates per treatment arranged in a randomized complete block design. Corn grain, inoculated with *Fusarium graminearum*, was spread evenly among each plot two weeks prior to heading. An overhead misting system provided added water to the plots following heading, when the nighttime humidity dropped below 90%. Fungicides were applied at early full head emergence for barley (Feekes 10.4), and at early flowering for wheat (Feekes 10.51), except for the Tilt treatment, which was applied before flowering (Feekes 10.5). Applications were with a backpack-type sprayer equipped with two XR8001 flat fan nozzles oriented toward the grain head at a 30° angle from the horizontal. The fungicides were applied at 18.5 gpa with 40 psi. Disease notes were taken at soft dough stage of development and crops were harvested at kernel maturity. Sub-samples of the harvested grain were ground and analyzed for deoxynivalenol (DON) by the NDSU Veterinary Toxicology Laboratory using gas chromatography and electron capture techniques.

The fungicide treatments included: Folicur 432 SC (tebuconazole – a Bayer CropScience compound) at 4 fl oz/A; Prosaro 421 SC (19% prothioconazole + 19% tebuconazole - a Bayer CropScience compound) at 6.5 fl oz/A; BAS555 1F (metconazole - a BASF compound) at 13 fl oz/A; Topguard 1 SC (flutriafol - a Cheminova compound) at 14 fl oz/A; Folicur 432 SC + Thiophanate-methyl (tebuconazole + CerixAgri product) at 2 fl oz/A tebuconazole + 8 fl oz/A thiophanate-methyl; and Tilt (propiconazole – a Syngenta product) at 4 fl oz/A.

Very high temperatures and no natural rainfall in July resulted in very low disease levels in Fargo in 2006. The untreated FHB field severity was only 0.6 % in barley and 2.0 % in wheat. Despite low disease, all fungicide treatments reduced FHB field severity (P = 0.1) for both crops. DON levels also were low in 2006, 3.0 ppm for untreated barley, and 0.7 ppm for untreated spring wheat. However, most fungicide treatments significantly (P = 0.1) reduced DON in both crops. Compared to other fungicide treatments in barley, the Prosaro treatment resulted in significantly lower DON levels.

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This material is based upon work supported by the U.S. Department of Agriculture, under Agreement No. 59-0790-4-114. This is a cooperative project with the U.S. Wheat and Barley Scab Initiative. Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the authors and do not necessarily reflect the view of the U.S. Department of Agriculture.

EFFECT OF FUNGICIDES ON FHB AND DON IN WHEAT - 2006 UNIFORM FUNGICIDE TRIALS.

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OBJECTIVES

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

INTRODUCTION

FHB, caused predominantly by *Fusarium graminearum* in North America, has had a great impact on every sector of the wheat and barley industries. Wheat growers, millers, bakers, and consumers of wheat products all have been affected by this disease. In addition to causing yield losses associated with reduced kernel size and weight, reduced seed germination, and seedling blight, *F. graminearum* also produces a mycotoxin called deoxynivalenol (DON) (among other toxins) which may accumulate to unacceptable levels in harvested grain. DON levels above 2 ppm may render grain and their by-products unfit for commercialization and consumption. Efforts to minimize the impact of FHB and DON have been centered on the use of management strategies such as host resistance, crop rotation, tillage, and fungicide application. Through collaborative research involving scientists from multiples states, representing various wheat-growing regions, Uniform Fungicide Trials (UFT) have been used to evaluate fungicide effectiveness against FHB and DON. These trials follow standard protocols and have been conducted annually since 1998. The results of the 2006 UFT trials form 17 locations across 8 states are presented herein.

MATERIALS AND METHODS

Each trial consisted of six fungicide treatments and an untreated control in a randomized complete block design, with four replicate blocks (one trial had five block). The core treatments were:

- Non-treated control;
- Folicur 432SC 4.0 fl oz + 0.125% Induce;
- Prosaro 6.5 fl oz/a + 0.125% Induce;
- Caramba 13.5 fl oz/a + 0.125% Induce;
- Topguard 14 fl oz/a + 0.125% Induce;
- Tebuconazole (2 fl oz/a) + Thiophanate-Methyl;
- Tilt 4 fl oz/a + 0.125% Induce.

Treatments were applied at full head emergence (Tilt) and early flowering (all other treatments) using CO_2-pressurized sprayers, equipped with Twinjet XR8002 nozzles or paired XR8001 nozzles mounted at a 60° angle forward and backward.

Planting and crop production practices varied somewhat from trial to trial. See individual trial reports for details. Most plots were planted with a susceptible cultivar. To enhance disease development, plots were either planted into corn or wheat residue and/or artificially inoculated with *F. graminearum*-infested kernels. Many plots were mist-irrigated as a means of enhancing production of, and infection by fungal inocula. In each plot of each trial, percent FHB incidence (INC), diseased-head severity (SEV), index (IND; also known as plot severity), and *Fusarium*-damaged kernels (FDK) were measured as previously described (McMullen, et al., 1999). DON accumulation was measured at one of the two USWBSI-funded DON Testing Laboratories.

For the purpose of data analysis, trials conducted at the same location, but using different cultivars, and trials conducted at different locations in the same state were considered separate studies. Each trial was analyzed separately using a mixed effect model in PROC MIXED of SAS to determine treatment effect on the FHB, DON, yield (bu/ac) and test weight (lb/bu). Linear contrasts were used to make pair-wise comparisons between treatment means and means across groups of treatments. Studies with zero or nominal levels of disease and DON were not analyzed.

RESULTS AND DISCUSSION

Weather conditions in both winter wheat and spring wheat areas were generally unfavorable (dry during flowering) for FHB development. Consequently, non-irrigated trials and a few irrigated trials had nominal disease development. Mean and maximum FHB index, across all replicates of the untreated check plots ranged from 0 to 14.01 and 0 to 26.90%, respectively (Table 1). In 10 of the 17 trials mean index in the untreated check was less that 1% and less than 2% in 13 of the 17 trials.

Fungicide treatment had a significant effect ($P < 0.05$) on FHB in only one of the 17 trials, Fayetteville, AR (Table 1). Treatments 2 (Folicur), 3 (Prosaro) and 4 (Caramba) significantly reduced FHB index relative to the untreated check. Caramba was the most effective treatment, resulting in 65% reduction in IND relative to the check. Based on pair-wise comparisons between treatments means, Caramba was more effective than Folicur but not Prosaro. In three of the other trials with some level of disease (mean IND > 5% in the check) Caramba- and Prosaro-treated plots had the lowest levels of disease, being significantly lower than the check in two of the three cases.

Similar results were observed for DON and other measures of FHB intensity (IND, SEV, INC, and FDK). Since IND is a direct function of INC and SEV (see Paul et al., 2005a,b), only the results for IND are summarized herein. The results for DON are presented in Table 2. The Caramba treatment, treatment 4, was again the most effective. Based on data from Crookston, MN and Fayetteville, AR, this treatment resulted in a significant reduction in DON relative to the untreated check, with percent reduction being between 56 and 64%, respectively. Despite this reduction, however, mean DON levels in Caramba-treated plots still exceeded critical thresholds in the trial conducted at Fayetteville, AR. As was the case with IND, DON levels in Caramba-treated plots was only significantly lower than DON levels in Folicur-treated plots but not Prosaro-treated plots.

A significant reduction in FHB coincided with significant yield increase and higher test weights in Fayetteville, AR. Plots treated with Prosaro and Caramba had significantly ($P < 0.005$) higher yields and test weights than the untreated check plot.

CONCLUSION

In summary, fungicide treatments did reduce FHB intensity and DON relative to the untreated check (based mainly on data from one location). The application of Caramba at a rate of 13.5 fl. oz per acre and Prosaro at 6.5 fl. oz per acre were the most effective treatments overall. Percent control (Hershman and Milus, 2003) was generally higher in trials with low levels of disease than in trials with high levels of disease. This should be interpreted with caution since the ultimate effectiveness of a fungicide treatment should be based on results under high disease pressure. In generally, the overall levels of disease and DON in 2006 were too low for us to make broad conclusions regarding the treatments tested.

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Table 1. Fungicide effect on Fusarium head blight index.

Trial		Wheat	Most effective Treatment[a]				Index (%) Check	
State/PI	Location	Type	Treat	IND (%)	% Control	*P value*	Mean	Max
AR/Milus	Fayetteville	W	4	4.54	65	<0.001	13.64	19.44
IL/Adee	Monmouth	W	NS	…	…	…	0.19	0.75
IL/Paul	DeKalb	W	NS	…	…	…	0.59	1.13
KY/Hershman	Princeton	W	NS	…	…	…	0.05	0.20
LA/Padgett	Macon Ridge	W	NS	…	…	…	0.46	1.40
MN/Hollingsworth	Crookston	S	NS	…	…	…	1.33	2.80
	Lamberton	W	NS	…	…	…	0.50	1.08
MO/Sweets	Columbia 1	W	NS	…	…	…	1.75	2.12
	Columbia 2	W	…	…	…	…	0.48	1.10
ND/McMullen	Fargo	S	4 NS	8.63	26	0.410	14.01	26.90
	Carrington	S/D	3 NS	5.48	61	0.026	11.73	17.75
SD/Draper	Brookings 1	S	4 NS	1.18	79	0.004	5.65	10.54
	Brookings 2	S	NS	…	…	…	1.32	3.03
	Watertown 1	S	…	…	…	…	0.00	0.00
	Watertown 2	S	…	…	…	…	0.11	0.42
	Groton 1	S	…	…	…	…	0.00	0.00
	Groton 2	S	…	…	…	…	0.00	0.00

[a] Treat = the most effective treatment (s) within each trial based on the pair-wise difference between mean IND for each treatment and the check, NS = no significant treatment effect; IND (%) = mean index across plots receiving the most effective treatment; % control = percent control; *P* value = level of significance from *t* test of the difference between mean IND across plots receiving the most effective treatment and the untreated check ($P < 0.05$ ➔ significant different). All tests of significance were done using arcsine-transformed IND.

… = Trials with zero or nominal levels of disease.

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Table 2. Fungicide effect on DON.

Trial		Wheat	Most effective Treatment[a]				Index (%) Check	
State/PI	Location	Type	Treat	DON	% Reduction	*P value*	Mean	Max
AR/Milus	Fayetteville	W	4	4.3	64	<0.001	12.00	13.50
IL/Adee	Monmouth	W	…	…	….	…	…	…
IL/Paul	DeKalb	W	NS	…	…	…	0.03	0.10
KY/Hershman	Princeton	W	…	…	…	…	0.35	0.40
MN/Hollingsworth	Crookston	S	4	0.65	56	0.005	1.47	2.50
	Lamberton	W	…	…	…	…	0.55	0.88
MO/Sweets	Columbia 1	W	…	…	…	…	…	…
	Columbia 2	W	…	…	…	…	…	…

[a]DON data were not available for some trials or available but equally low (below 1 ppm) for all treatments.

[b]Treat = the most effective treatment within each trial based on the pair-wise difference between mean DON for each treatment and the check, NS = no significant treatment effect; DON (ppm = mean DON across plots receiving the most effective treatment; % reduction = percent reduction in DON; *P* value = level of significance from *t* test of the difference between mean DON across plots receiving the most effective treatment and the untreated check ($P < 0.05$ ➔ significant difference). All tests of significance were done using log-transformed.

… = Trials with zero or nominal levels of DON.

2006 UNIFORM FUNGICIDE PERFORMANCE TRIALS FOR THE SUPPRESSION OF FUSARIUM HEAD BLIGHT IN SOUTH DAKOTA.

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ABSTRACT

Fusarium head blight (FHB – scab) has been a serious concern for wheat and barley producers in South Dakota for ten years and a serious epidemic impacted the state's wheat and barley crop in 2005. The objective of this study was to continue to evaluate the efficacy of various fungicides and fungicide combinations for the suppression of Fusarium head blight and other wheat diseases. Two hard red spring wheat cultivars, 'Briggs' and 'Ingot', were planted at three South Dakota locations (Brookings, Groton, and South Shore/Watertown) and Robust barley was planted at Brookings. 'Wesley' winter wheat study sites were also established at South Shore/Watertown and Andover. Studies at both of these sites were conducted under ambient conditions. A misted study with 'Robust' barley was conducted at the Brookings site. Due to drought conditions, FHB only developed at the Brookings site. Only the spring wheat data from that trial is presented in this report. Trial treatments from the Uniform Fungicide Trial treatments list for the suppression of FHB included an untreated check, Folicur (tebuconazole) applied at 4.0 fl oz/A, Prosaro (a premix of prothioconazole and tebuconazole) applied at 6.5 fl oz/A, Caramba (metconazole) applied at 13.5 fl oz/A, Topguard (flutriafol) applied at 14 fl oz/A, and a tank mix of Folicur (tebuconazole) applied at 2 fl oz/A with Topsin-M (thiophanate-methyl) applied at 8 fl oz/A. All treatments included Induce, a non-ionic surfactant, applied at 0.125% v/v. Spring wheat trials were planted in a factorial randomized complete block design with six replications. Winter wheat and barley locations had four replications. Trial treatments were applied at anthesis (Feekes growth stage 10.51). Plots were inoculated by spreading *Fusarium graminearum* (isolate Fg4) inoculated corn (*Zea mays*) grain throughout the field and providing overhead mist irrigation applied for 3 min out of every 20 minutes from 5:00 pm until 9:00 am each day for two weeks following anthesis at the Brookings location only. Other sites had natural inoculum from corn stalk residue and natural moisture conditions. Twenty-one days following treatment, plots were evaluated for leaf diseases, FHB incidence, FHB head severity, and FHB field severity. Samples were collected for Fusarium damaged kernels (FDK), deoxynivalenol (DON), grain yield, and test weight. Spring wheat under dryland conditions at South Shore/Watertown and Groton FHB had negligible FHB. The same was true for winter wheat at both locations. No significant differences resulted from the barley trial. On spring wheat, Prosaro was the only product to significantly reduce FHB incidence, from 11.5% to 5.7%. While there were no measurable differences in FHB severity, both Prosaro and Caramba reduced FHB index from 3.2% on the untreated to 1.3 and 1.8% respectively. All products significantly increased grain yields from about 12-22%, largely due to leaf disease control. Data is not yet available for FDK and DON.

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2006 UNIFORM TRIALS FOR THE PERFORMANCE OF BIOLOGICAL CONTROL AGENTS IN THE SUPPRESSION OF FUSARIUM HEAD BLIGHT IN SOUTH DAKOTA.

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ABSTRACT

Fusarium head blight (FHB – scab) has been a serious concern for wheat and barley producers in South Dakota for ten years and was very severe in parts of SD in 2005. The objective of this study was to continue to evaluate the efficacy of various fungicides and fungicide combinations for the suppression of Fusarium head blight and other wheat diseasesunder SD conditions. Ingot hard red spring wheat and Robust barley were planted at Brookings, South Dakota. Trial treatments included an untreated check; Folicur (tebuconazole) applied at 4.0 fl oz/A; 1BA (*Bacillus subtilus*) from South Dakota State University, Brookings, SD; 1BA + Folicur coapplied, TrigoCor 1448 (*Bacillus* sp.) from Cornell University, Ithaca, NY; and TrigoCor 1448 + Folicur coapplied; C3 (*Lysobacter enzymogenes*) from University of Nebraska, Lincoln, NE; C3 + Folicur coapplied. Additionally, the 1BA isolate was applied after growth in Trypic soy broth + Yeast extract (TS+YE) at full and half strength; Defined broth medium + salt; TS/TE + salt; and ½ strength TS/YE + salt. Anther set of treatments assessed the activity of different surfactants on the activity of 1BA, Induce non-ionic surfactant, Latron CS7, and Agridex crop oil concentrate (COC). Unless otherwise indicated, treatments were grown on site according to specifications from their originating labs and applied at anthesis. Plots were inoculated by spreading *Fusarium graminearum* (isolate Fg4) inoculated corn (*Zea mays*) grain throughout the field at least ten days prior to flowering (wheat) or head emergence (barley) throughout the field and providing overhead mist irrigation applied for 3 min out of every 20 minutes from 5:00 pm until 9:00 am each day for two weeks following treatment. Twenty-one days following treatment, plots were evaluated for FHB incidence, FHB head severity, and FHB field severity. Plots were harvested for yield and test weight and samples were collected for Fusarium damaged kernels (FDK) and deoxynivalenol (DON). Even with amending the environment in 2006, extreme drought limited disease development. In the final analysis, no assessments of disease components revealed significant effects of the treatments in the barley study.

While 1BA appeared to have no effect on yield with or without Folicur, TrigoCor 1448 and C3 both appeared to have synergistic activity when applied with Folicur. In both cases the response was significantly greater that the Folicur treatment alone, which was not different than the untreated in this trial. There were no differences among the treatments for the components of FHB. While there were no significant differences in incidence, there were striking numeric differences when different surfactants were applied with 1BA. This facet of application of BCAs has not been examined and warrants further study.

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USDA-ARS AND THE OHIO STATE UNIVERSITY COOPERATIVE RESEARCH: USE OF FRACTIONAL FACTORIAL FIELD DESIGNS TO ASSESS THE INTEGRATION OF DIVERSE TREATMENTS AGAINST FHB.

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OBJECTIVES

1) Evaluate, *in vitro*, the suitability of various food grade dyes as UV protectants for use with choline metabolizing FHB biocontrol strain OH 221.3; and 2) evaluate the effectiveness of integrating the use of biocontrol strains OH 182.9 [*Cryptococcus flavescens* NRRL Y-30216 (Dunlap et al. 2006), previously reported as *C. nodaensis* nomen nudum] and OH 221.3, Folicur 3.6F, a UV protectant, and a chemical inducer of acquired resistance.

INTRODUCTION

In previous work, we discovered microbial strains that reduce FHB in the greenhouse and field and demonstrated enhanced reduction of FHB via formulation of biocontrol agents with UV protectants (Schisler et al., 2003), and mixing fungicide-tolerant variants of our biocontrol agents with fungicides (Schisler et al., 2002). In more recent work, we have discovered chemical inducers of systemic acquired disease resistance (SAR) that reduce FHB development in greenhouse tests (Zhang et al., 2005) and choline metabolizing strains (CMS) (Schisler et al., 2006) that reduce FHB in greenhouse and field tests. Determining the relative importance of these factors when simultaneously tested and identifying synergies, if any, when multiplexing these factors is crucial to elucidating which of these factors should be included in any recommended IPM program against FHB and which factors are most critical for inclusion in a final FHB biocontrol product. Additionally, further work on identifying inexpensive, nontoxic UV protectants that are effective in enhancing the survival of biocontrol agents in field environments is needed.

MATERIALS AND METHODS

Tests of food grade dyes as UV protectants: Several food grade dyes (Table 1) were tested, *in vitro*, for their ability to aid survival of dried cells of FHB bacterial antagonist OH 221.3 exposed to artificial sunlight supplied from a xenon light source (Suntest Atlas CPS solar simulator, Heraeus DSET Laboratories Inc., Phoenix, AZ). Cells of antagonist OH 221.3 were grown in flasks containing a semidefined liquid medium (SDCL, Schisler 2002), harvested from 24 h growth cultures, combined or not with UV protectants, added as 2 µl droplets of formulated cells (8 reps/treatment) to 96 well microtiter plates, air-dried for 1 h or not, and exposed or not to 6 h of UV light. Cell counts at the time of introduction to microtiter plates were approximately 9×10^9 CFU/ml. Final dye concentrations were 5.00 and 1.25 µM. Wells were rehydrated with 50 µl of weak growth medium, cell growth determined using a spectrophotometer at 620 nm, and qualitative changes in absorbance versus controls determined after set intervals of cell growth (Table 1). Greenhouse tests confirmed that none of the dyes tested adversely affected plant growth (data not shown).

Field Tests using Partial Factorial Designs: Two-level fractional factorial designs (Table 2) were used for field trials in Peoria, IL (insufficient disease development, data not shown) and Wooster, OH in 2006 (Tables 3,4). Biomass of antagonists was pro-

duced in B Braun Biostat B fermentors charged with SDCL medium (1.5 l working volume). Soft red winter wheat cultivars Elkhart (susceptible) and Freedom (moderately resistant) were grown using standard agronomic conditions (Schisler et al., 2006). Treatments were applied at the beginning of wheat flowering at concentrations of 1 x 10^8 and 2 x 10^9 (CFU/ml) for antagonists OH 182.9 and OH 221.3, respectively. UV protectant naphthol yellow (NY) and SAR chemical Na salycylic acid (NaSA) were applied at concentrations of 5.0 µM and 1.6 g/l, respectively and a rate of 80 gal/acre. The fungicide Folicur 3.6F (38.7% tebuconazole) was applied at the recommended AI rate of 4 fl. oz./acre as a chemical control and untreated plants served as an additional control. Corn kernels colonized by *G. zeae* were scattered through plots (~25-40 kernels/m^2) two weeks prior to wheat flowering and mist irrigation was provided periodically for approximately two weeks after treatment application. Heads were scored for disease incidence and severity 21 days after treatment using a 0-100% scale. Analysis of field data obtained from this fractional factorial design was conducted using SAS version 9.1.3 and Design-Expert version 6.0.3 software.

RESULTS AND DISCUSSION

Of the dyes tested for utility as UV protectants for FHB biocontrol strain OH 221.3, NY was the most efficient in enhancing the survival of cells exposed to 6 hours of artificial sunlight. Naphthol yellow did not have a deleterious effect on the growth of fresh cells or dried cells not exposed to artificial sunlight (Table 1).

Treatment component effects were dependent on the wheat cultivar considered. On cultivar Elkhart in Wooster, Ohio, the presence of Folicur 3.6F (P=0.001) and antagonist OH221.3 (P=0.10) significantly reduced disease severity and incidence (Table 3). Antagonist OH 182.9 reduced the DON content (P=0.04) and NY decreased the test weight (P=0.05) of Elkhart grain. NaSA increased DON in Elkhart but reduced DON in Freedom grain (P=0.02, Table 4). No other treatment component significantly influenced test parameters on Freedom. Formulating NY and NaSA to produce a product more resistant to wash-off may be needed to counter the effects of frequent overhead irrigation in field experiments.

Our results using a partial factorial design do not indicate the presence of first order synergistic effects of combining biocontrol agents, a UV protectant, Folicur 3.6F and a SAR chemical. A Dunnett's analysis of individual runs (Table 2) versus untreated controls rarely indicated significant differences (data not shown), suggesting that higher order synergistic interactions between treatment components did not occur. Additional experiments using partial factorial designs in FHB field studies would be necessary to determine if the design can serve as a useful tool for detecting treatment differences while reducing the amount of field area required.

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DISCLAIMER

Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the author(s) and do not necessarily reflect the view of the U.S. Department of Agriculture. Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of others that may also be suitable.

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Table 1. Influence of Food Grade Dyes on Fresh Cell Growth of FHB Antagonist OH 221.3 and the Survival of Dried Cells Exposed or not to 6 h of Artificial Sunlight[a,b]

Treatment	Fresh Cells -UV[c]	Dry Cells -UV[d]	Dry Cells +UV 6h[d]
FD&C Blue #1 (5.00 µM)	-(+)	0	+(-)
FD&C Blue #1 (1.25 µM)	-(+)	+(-)	-(-)
FD&C Yellow #5 (5.00 µM)	0	+(+)	-
FD&C Yellow #5 (1.25 µM)	+(-)	+(-)	+
Fast Green (5.00 µM)	-(+)	-(+)	0
Fast Green (1.25 µM)	0	0	-(-)
FD&C Red #40 (5.00 µM)	+(-)	+	--
FD&C Red #40 (1.25 µM)	0	0	-
Naphthol Yellow (5.00 µM)	0	0(+)	+(+)
Naphthol Yellow (1.25 µM)	0	+	++(+)

[a] Treatment influence on cell survival determined by comparing absorbance of microtiter plate wells containing treated cells with wells containing control cells. Absorbances compared at set intervals of time after 1 ul droplets of fresh cells added to wells and subjected to no treatment (fresh cells –UV), drying (dry cells –UV), or drying and 6 h artificial sunlight (dry cells +UV) and then flooded with 50 uL of growth medium.

[b] Table values are qualitative data and represent major increase , ++; minor increase, +; no change, 0; minor decrease, -; and major decrease, -- in well absorbance compared to the control. Parenthetical values indicate that the reported value is slightly higher (+) or lower (-) than the average qualitative range.

[c] Absorbance (620 nm) determined at 8 to 10 h after growth medium addition to well.

[d] Absorbance (620 nm) determined at 28 h after growth medium addition to well.

Table 2. Fractional factorial, 32 run experimental design for Peoria, Illinois and Wooster, OH field tests integrating multiple factors for reducing FHB (1 indicates presence, -1 indicates absence of the individual treatment factors that make up a treatment "run"). Each "run" was one treated row (Peoria) or plot (Wooster). Design was repeated at each site on FHB moderately resistant cultivar Freedom and susceptible cultivar Elkhart.

Treatment (Run)	Antagonist OH 182.9	Antagonist OH 221.3	UV Protect Naphthol Yellow	Fungicide Folicur 3.6F	SAR chemical NaSalycylic
1	-1	-1	1	-1	-1
2	-1	1	1	-1	1
3	1	-1	1	1	-1
4	-1	1	-1	1	1
5	-1	1	1	1	-1
6	1	-1	-1	1	1
7	1	-1	-1	-1	-1
8	-1	-1	-1	1	-1
9	1	-1	1	1	-1
10	1	1	-1	-1	1
11	-1	-1	1	-1	-1
12	-1	-1	1	1	1
13	1	-1	-1	-1	-1
14	-1	1	-1	-1	-1
15	1	1	1	-1	-1
16	1	1	1	1	1
17	-1	1	1	1	-1
18	-1	1	-1	-1	-1
19	1	1	-1	1	-1
20	1	-1	-1	1	1
21	1	1	1	-1	-1
22	-1	-1	-1	1	-1
23	1	-1	1	-1	1
24	-1	-1	-1	-1	1
25	1	1	-1	-1	1
26	1	1	1	1	1
27	-1	-1	1	1	1
28	-1	-1	-1	-1	1
29	1	1	-1	1	-1
30	-1	1	-1	1	1
31	1	-1	1	-1	1
32	-1	1	1	-1	1

Table 3. 2006 FHB Field Trial Results at Wooster, Ohio: Fractional Factorial Analysis for Main Effects of Wild Type Antagonists OH 182.9, OH 221.3, UV Protectant Naphthol Yellow (NY), Folicur 3.6F, and SAR Chemical Na Salicylic acid (NaSA) on Susceptable Winter Wheat Cultivar Elkhart.

Treatment	% Disease Severity	% Incidence	DON (ppm)	Test Weight (lbs/bu)
+OH182.9	15.1	58.8	11.1	46.5
-OH182.9	15.1	63.6	13.4	46.1
Comparison P	0.86	0.28	0.04*	0.37
+OH221.3	13.5	56.6	12.9	46.5
-OH221.3	16.8	65.8	11.6	46.1
Comparison P	0.07*	0.05*	0.24	0.31
+NY	16.5	64.3	12.2	45.9
-NY	13.7	58.1	12.4	46.7
Comparison P	0.17	0.17	0.83	0.05
+Folicur 3.6F	10.7	51.8	11.9	47.2
-Folicur 3.6F	19.6	70.5	12.7	45.4
Comparison P	0.001*	0.001*	0.46	0.001*
+NaSA	16.1	62.3	13.3	46.0
-NaSA	14.1	60.1	11.2	46.6
Comparison P	0.39	0.62	0.05*	0.20
Overall Model P	0.09	0.07	0.12	0.02

Table 4. 2006 FHB Field Trial Results at Wooster, Ohio: Fractional Factorial Analysis for Main Effects of Wild Type Antagonists OH 182.9, OH 221.3, UV Protectant Naphthol Yellow (NY), Folicur 3.6F, and SAR Chemical Na Salicylic acid (NaSA) on Moderately Resistant Winter Wheat Cultivar Freedom.

Treatment	% Disease Severity	% Incidence	DON (ppm)	Test Weight (lbs/bu)
+OH182.9	2.5	26.7	6.9	51.4
-OH182.9	2.4	26.8	5.8	52.5
Comparison P	0.86	0.98	0.25	0.29
+OH221.3	2.6	28.3	7.0	50.9
-OH221.3	2.3	25.2	5.7	52.9
Comparison P	0.41	0.46	0.31	0.07
+NY	2.7	29.2	5.8	51.4
-NY	2.2	24.4	6.9	52.4
Comparison P	0.32	0.26	0.40	0.37
+Folicur 3.6F	2.1	23.3	6.5	52.6
-Folicur 3.6F	2.8	30.2	6.2	51.3
Comparison P	0.13	0.11	0.72	0.24
+NaSA	2.5	27.7	5.3	51.8
-NaSA	2.4	25.9	7.4	52.0
Comparison P	0.81	0.67	0.02*	0.86
Overall Model P	0.87	0.81	0.47	0.49

2006 RESULTS FROM THE STANDARDIZED EVALUATION OF BIOLOGICAL AGENTS FOR THE CONTROL OF FUSARIUM HEAD BLIGHT ON WHEAT AND BARLEY.

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OBJECTIVE

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

INTRODUCTION

Among the most extensively studied biological agents for control of FHB in the US are strains of *Bacillus* spp, TrigoCor 1448 (Stockwell et al., 2001) and 1BA (Draper et al., 2001), and *Lysobacter enzymogenes* strain C3 (Jochum et al., 2006). Each bacterial strain was effective in some field tests when evaluated separately (Stockwell et al., 2001; Jochum et al., 2006; Khan et al., 2004; Yuen and Jochum, 2004). They were directly compared for efficacy in 2004 and 2005 as part of the USWBSI-funded program for standardized evaluation of biological agents, and because combinations of biological control agents and fungicides were reported to be more effective in controlling FHB than the microorganisms or fungicides alone (DaLuz et al., 2003; Khan et al., 2004; Yuen and Jochum, 2004), standardized evaluations in 2005 also compared these bacterial strains in combination with the fungicide tebuconazole. In the two years testing, however, results were inconclusive as to the effectiveness of the treatments across a range of environmental conditions and crop genotypes (Yuen et al, 2004; Yuen et al., 2005). Experiments in 2006 were conducted, therefore, to evaluate the same agents and to retest the strategy of applying biological agents with a fungicide.

MATERIALS AND METHODS

Five trials were conducted across three states on barley and a range of wheat market classes (Table 1). In each trial, three bacterial biological agents (Table 2) were tested alone or in tank mix with the fungicide tebuconazole (Folicur 432SC, 4.0 fl oz/A). There also was a treatment with tebuconazole alone and a non-treated control. A broth culture of each organism was propagated by the originating laboratory and sent to the researcher in each location. The pre-application population of each agent in the inoculum was determined by the local researcher using dilution plating. All treatment liquids were amended with 0.125% Induce. One application was made per treatment at early flowering (Feekes 10.51) in 20 gal/acre using a CO_2-pressurized sprayer (approximately 40 psi) equipped with flat-fan nozzles oriented forward and backward. The size and number of replicate plots varied among trials. Some of the trials were inoculated with *Fusarium graminearum* and utilized mist irrigation systems to stimulate infection. In all trials, FHB incidence (% heads infected per plot), severity (% spikelets infected per diseased head), and index (% plot severity) were determined from at least 40 heads per plot around 3 weeks after anthesis. The incidence of Fusarium-damaged, kernels (FDK) were determined after harvest. Samples from each plot were sent to the North Dakota State University Veterinary Diagnostic Laboratory, Fargo, ND for analysis of DON content. Analysis of variance was performed on results from each

trial separately. Results from all trials were analyzed together using ProcMixed (SAS), with trials being treated as blocks.

RESULTS AND DISCUSSION

Dry weather conditions resulted in low FHB development in all trials despite misting being provided in some locations. Incidence was less than 10% in three of the trials while severity generally did not exceed 20% in all locations (Table 3A). Accordingly, index measurements and incidence of Fusarium diseased kernels were very low (Table 3B). None of the treatments with a biological agent alone, tebuconazole alone, or a biological agent-tebuconazole combination had a significant effect on any disease parameter compared to the control across the trials (Table 3A&B). The treatments were ineffective in the individual trials except that tebuconzole alone, *Bacillus* 1BA alone, and the combination reduced FDK incidence in the Missouri trial on 'Roane' and treatments involving TrigoCor 1448 reduced disease index on barley in South Dakota (Table 3B). Available DON measurements from Missouri and Nebraska plots indicated no treatment effects as all samples contained less than 0.5 ppm DON (data not shown).

Biocontrol agent numbers in the inoculum suspensions ranged from approximately 10^7 to more than $5X10^8$ colony forming units/ml. There was less variation in inoculum cell concentrations among agents and among locations than observed in previous years. Although the population threshold required for efficacy has not been established for any of these agents, lack of efficacy in the biological treatments in general does not appear to be related in low population numbers being applied in the trials. The primary complicating factor in these trials could have been environmental conditions not favoring sufficient disease development for good separation of treatments. This was evidenced by tebuconzole treatment also displaying little or no effects on disease levels across these trials. Determination of most efficacious biological agent and assessment of benefit of combining biological agents with fungicides will require further testing under higher disease pressure.

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Table 1. 2006 uniform biological control trial locations, crop cultivars, and researchers

State	Crop market class and cultivar	PI and Institution
MO	Soft red winter wheat 'Roane'	L. Sweets, University of Missouri
MO	Soft red winter wheat 'Truman'	L. Sweets, University of Missouri
NE	Hard red winter wheat '2137'	G. Yuen, University of Nebraska
SD	Hard red spring wheat 'Ingot'	M. Draper, South Dakota State University.
SD	Six-rowed barley 'Robust'	M. Draper, South Dakota State University.

Table 2. Biological control agents tested in 2004 uniform trials.

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

Table 3A. 2006 results across five uniform biocontrol trials denoted by state and crop

Treatment	MO 'Roane'	MO 'Truman'	NE Wheat	SD Wheat	SD Barley	LS Mean
INCIDENCE (% heads infected)						
Control	5.3	9.0	28.8	7.0	64.5	22.9
Folicur	3.3	7.0	33.3	6.5	ND	23.3
1BA	6.8	10.0	27.5	6.0	66.0	23.3
1BA + Folicur	7.5	4.5	25.1	5.5	67.0	21.9
TrigoCor 1448	6.0	8.2	29.2	5.5	64.5	22.7
TrigoCor 1448 + Folicur	2.0	2.5	24.2	11.5	60.0	20.0
C3	5.2	5.5	22.1	5.5	68.5	21.4
C3 + Folicur	6.0	6.0	31.2	5.5	66.5	23.0
P	0.101	0.083	0.623	0.284	0.910	0.532
$LSD_{0.05}$	-	-	-	-	-	-
SEVERITY (% spikelets infected)						
Control	8.9	6.1	6.9	11.6	6.5	8.0
Folicur	10.6	14.2	11.4	36.2	ND	17.0
1BA	8.2	15.0	11.3	19.2	7.7	12.3
1BA + Folicur	18.8	8.0	7.7	26.4	5.9	13.4
TrigoCor 1448	10.0	10.5	10.5	21.0	5.9	11.6
TrigoCor 1448 + Folicur	5.5	6.0	10.1	11.6	4.9	7.6
C3	11.0	6.5	9.8	16.2	4.8	9.6
C3 + Folicur	19.0	6.8	12.9	7.6	5.0	10.2
P	0.189	0.070	0.429	0.162	0.044	0.120
$LSD_{0.05}$	-	-	-	-	1.6	-

ND=not determined.

Table 3B. 2006 results across five uniform biocontrol trials denoted by state and crop.

Treatment	MO 'Roane'	MO 'Truman'	NE Wheat	SD Wheat	SD Barley	LS Mean
INDEX (plot severity)						
Control	0.5	0.6	2.2	0.9	4.2	1.7
Folicur	0.4	1.2	4.2	1.6	ND	2.4
1BA	0.6	1.6	3.5	1.2	5.2	2.4
1BA + Folicur	1.3	0.3	2.3	1.4	4.1	1.9
TrigoCor 1448	0.6	1.0	2.9	1.0	3.9	1.9
TrigoCor 1448 + Folicur	0.1	0.2	2.5	1.3	3.0	1.4
C3	0.6	0.3	2.2	0.8	3.3	1.4
C3 + Folicur	1.2	0.4	4.6	0.5	3.3	2.0
P	0.021	0.152	0.390	0.445	0.229	0.0827
$LSD_{0.05}$	0.7	-	-	-	-	-
FDK (%)						
Control	0.6	0	1.6	1.2	ND	0.9
Folicur	0	0.2	0.8	1.5	ND	0.6
1BA	0	0.2	1.6	1.2	ND	0.8
1BA + Folicur	0	0.2	1.2	1	ND	0.6
TrigoCor 1448	0.9	0.2	1	1	ND	0.8
TrigoCor 1448 + Folicur	0.3	0.4	0.9	1	ND	0.7
C3	0.2	0.6	1.5	1.2	ND	0.9
C3 + Folicur	0.3	1.0	1.3	1.2	ND	1.0
P	0.047	0.342	0.999	0.895	-	0.558
$LSD_{0.05}$	0.6	-	-	-	-	-

ND=not determined.

ETIOLOGY, EPIDEMIOLOGY AND DISEASE FORECASTING

EFFECTS OF DON ON BARLEY LEAF TISSUES, SUMMARY OF RESULTS.

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ABSTRACT

DON, a trichothecene toxin produced by *Fusarium graminearum*, is postulated to have a role in pathogenesis in Fusarium head blight of barley and wheat. To understand possible roles of DON, we investigated effects of the toxin in healthy tissues. Portions of epidermis were removed from 1.1 cm barley leaf segments and the segments floated with exposed mesophyll cells in contact with DON solutions. Within 2-4 days, DON at 10-90 ppm had a bleaching effect on chloroplast pigments and damaged cell membranes, including the plasmalemma (Bushnell et al., 2002; 2005). Pending more complete publication of this work (Bushnell et al., 2007), the major findings and conclusions of this project are presented here.

1. The bleaching of leaf tissues was light dependent. Tissues turned white in light as they lost chlorophylls a and b, as well as carotenoids pigments. In the dark, tissues remained green but became flaccid and easily damaged when manipulated with forceps.

2. In both light and dark, DON damaged integrity of the plasmalemma as shown by electrolyte leakage and uptake of Evans blue. Damage was somewhat greater in dark than in light. We conclude that toxicity of DON is not light dependent and that photobleaching is a secondary consequence of damage to cell membranes and not a direct cause of cell degradation.

3. The first membrane to be damaged, as viewed by transmission electron microscopy, was the tonoplast (Bushnell et al., 2005). This allowed cytoplasm to disperse into the vacuole, an irreversible step toward cell death. This preceded dissolution of the plasmalemma, mitochondria, and chloroplasts, as well as the loss of chloroplast pigments.

4. Damage to membranes and chloroplasts apparently is related to the known ability of DON to inhibit cytoplasmic protein synthesis. Cycloheximide, an inhibitor of protein synthesis in cytoplasm of eukaryotes, caused photobleaching of barley leaf segments in our experiments much like the photobleaching caused by DON. Chloramphenicol, an inhibitor of protein synthesis in chloroplasts and prokaryotes, had little or no effect on leaf pigmentation.

5. Ca^{2+} added at 10 mM to test solutions greatly increased toxicity of DON. With Ca^{2+}, DON at 10 ppm bleached leaf segments; without Ca^{2+}, concentrations of 30 ppm or higher were required. The reasons for DON's effectiveness need investigation. In any case, differences in sensitivity to DON among genotypes of barley and wheat or plants at different stages of development, may relate to variations in Ca^{2+} availability within tissues.

6. Fumonisin B_1 (FB_1), a non-trichothecene toxin produced by *F. moniliforme*, caused photobleaching and membrane damage in jimsonweed leaves as reported by Abbas et al. (1992). The results were remarkably

similar to effects of DON in our experiments with barley leaves. FB_1 is known to inhibit ceramide synthesis, disrupting sphingolipid metabolism. Direct comparisons of effects of DON and FB_1 are warranted.

7. As summarized elsewhere (Bushnell et al., 2007), indirect lines of evidence indicate that degradation of cell membranes in DON-treated barley cells is a consequence of programmed cell death (PCD). DON is known to induce PCD (apoptosis) in animal cells. Dissolution of the tonoplast, as occurred in response to DON, is a primary event in plant PCD. Furthermore, Ca^{2+} is required and can enhance plant PCD, much as it enhanced DON toxicity. Both cyclohexamide and FB_1, which cause photobleaching in plants, have induced PCD in animal cells. Finally, anti-PCD genes introduced in plants have provided partial resistance to several necrotrophic pathogens. The role of PCD in response to DON needs additional physiological and molecular investigation, including the apparent link between PCD and inhibition of protein synthesis.

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DURATION OF POST-FLOWERING MOISTURE AFFECTS FHB AND DON IN WHEAT.

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ABSTRACT

Our understanding of how environmental and host genetic influences interact to determine DON concentrations in small-grain spikes is incomplete. High levels of DON have sometimes been observed in the absence of abundant disease symptoms. This multi-year experiment explores the influences of post-flowering moisture duration, infection timing, and cultivar resistance differences on FHB and DON in winter wheat. We conducted two one-year trials of a split-plot experiment in a misted nursery in Kinston, North Carolina. The main plots were four durations of post-flowering misting (0, 10, 20, or 30 days). Sub-plots were soft red winter wheat cultivars with different degrees and putative types of resistance to FHB. In 2005, one susceptible cultivar and six moderately resistant cultivars were planted; in 2006, an additional susceptible cultivar was added to the experiment. Within each irrigation regime, one plot of each cultivar was inoculated with 10^5 *Fusarium graminearum* macroconidia/ml using a backpack sprayer at flowering. In another plot of each cultivar under each irrigation regime, 40 heads received individual inoculation with a spray bottle at each of the following times: 0, 10, or 20 days post-flowering, or never. All treatment combinations were replicated three times. Spike samples were collected at normal harvest time and dissected into glume, rachis, and grain fractions. In addition, samples were randomly gathered from plots backpack-inoculated at flowering at approximately 10-day intervals from two weeks post-flowering through harvest time. Tombstone percentages were determined. DON was assayed in all tissue fractions by ELISA. In 2005, samples from replicated plots inoculated at flowering and misted for 30 days were also assayed for fungal DNA concentration using RT-PCR, and in 2006 this assay is being conducted on samples from all treatment combinations.

In 2005, disease levels were very light. There was no significant relationship between post-flowering moisture duration and either disease or DON levels. No treatment combination was identified in which visual symptoms were low while DON levels were high. In plots backpack-inoculated at flowering and misted for 30 days, the quantity of fungal DNA in each tissue type was positively correlated with the DON concentration. This result is consistent with the hypothesis that the degree of fungal infection within each tissue type determined how much DON was found there at harvest, as opposed (for example) to significant amounts of DON being translocated or leached into grain from infections in glumes or rachis. Further, fungal DNA in each tissue fraction was positively correlated with that in each other tissue fraction, suggesting that the relative degree of resistance to infection in each cultivar was similar among tissue types.

In 2006, a more severe FHB epidemic developed. Both FHB incidence and severity increased significantly ($P < 0.05$) as the duration of post-flowering misting increased up to 20 days. There was no further increase in incidence or severity after 30 vs. 20 days of misting. The disease severity present on individual cultivars did not vary by mist duration in either year analyzed individually, nor in both years combined. The 2006 data suggest that the number of moist days following flowering can significantly increase FHB severity. Assays of DON and RT-PCR for the 2006 samples are currently underway.

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FIELD RELEASE OF *GIBBERELLA ZEAE* GENETICALLY MODIFIED TO LACK ASCOSPORES.

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INTRODUCTION AND OBJECTIVE

Gibberella zeae (asexual state *Fusarium graminearum*) causes wheat head blight (WHB) epidemics worldwide, reducing seed yield and contaminating seed with deoxynivalenol (DON) and other trichothecene toxins. Since 1990, WHB epidemics on wheat and barley have increased in frequency and severity in North America. Reemergence of this plant disease has been associated with the intensification of wheat-maize crop rotations and use of conservation tillage practices that leave large amounts of maize stalk pieces and other crop residues on the soil surface (Parry et al. 1995, McMullen et al. 1997). *G. zeae* is self-fertile and can produce macroconidia (asexual spores) and perithecia with ascospores (sexual spores) during WHB epidemics. The importance of *G. zeae* spores in WHB had been recognized by the late nineteenth century, but the relative contribution of ascospores and macroconidia to WHB epidemics is still debated. On the one hand, ascospores often are recovered at higher counts than macroconidia from traps that sample airborne spores in the field (Shaner 2003). On the other hand, some *Fusarium* species such as *F. avenaceum* and *F. culmorum*, which rarely or never produce ascospores, have produced severe WHB epidemics in Europe and Canada, indicating that macroconidia are sufficient to cause epidemics under some conditions (Parry et al. 1995, Waalwijk et al. 2003).

We previously generated *MAT*-deletion strains that lack ascospores and *MAT*-complemented strains that have regained ascospore production (Turgeon 1998, Desjardins et al. 2004). Both *MAT*-deletion and *MAT*-complemented strains were similar to the wild-type (WT) strain in production of macroconidia and DON in culture. Furthermore, in greenhouse tests, both *MAT*-deletion and *MAT*-complemented strains were virulent when their macroconidia were injected directly into wheat heads at anthesis, and the resulting infected wheat heads were contaminated with DON. The apparent absence of major pleiotropic effects of *MAT*-locus deletion on *G. zeae* radial growth, morphology, pigmentation, macroconidium production, DON production, and virulence on wheat in the greenhouse indicated that these mutants might be suitable for testing under field conditions. The objective of the present study was to investigate the importance of ascospores in WHB epidemics by conducting a series of controlled-release field tests in which *MAT*-deletion strains that lack ascospores were compared to WT and *MAT*-complemented strains that produce ascospores.

MATERIALS AND METHODS

Microbiology. *G. zeae* strain GZ3639 isolated from scabby wheat in Kansas was the WT and progenitor of all transformants (Table 1). All strains were generated and characterized as previously described (Desjardins et al. 2004). For wheat head injection, macroconidium inoculum (5×10^4 spores/ml) was prepared in mung bean liquid medium (Bai & Shaner 1996). Each head was injected with approx. 1000 spores, and controls with mung bean medium. A natural substrate inoculum for field tests was prepared from maize stalk pieces as described (Maldonado-Ramirez & Bergstrom 2000). Dried, mature maize stalks were cut into pieces approx. 15 cm long, sterilized by autoclaving three times, placed in 2.8 L Fernback flasks and inoculated with several blocks of fungal culture material cut from plates of V-8 juice agar medium.

Flasks were incubated for 4 weeks at 20 ± 1°C, under continuous illumination provided by an equal mixture of fluorescent white and black (General Electric BLB, 40 W) light bulbs. After 4 weeks, all fungal strains produced hyphae and macroconidia on the surfaces of the stalk pieces, but only WT and *MAT*-complemented strains also produced perithecia, which were checked microscopically to confirm the presence of mature ascospores. For fungal strain recovery, seeds were surface-disinfested and placed on a *Fusarium* selective medium (Nelson et al. 1983). *G. zeae* colonies from individual seeds were designated as isolates to distinguish them from the single-spored strains that were applied as treatments in the field tests. Because isolates were not purified by single-sporing, they may have contained more than one genotype. Isolates were subcultured to agar medium with hygromycin B (hyg) at 300 μg ml^{-1} or geneticin (gen) at 1 mg ml^{-1} and scored as resistant or sensitive by their radial growth. For selected isolates, DNA was purified and subjected to PCR amplification using standard methods and primers complementary to the *G. zeae Tri5* gene and to the *hygB* gene construct used for transformation (Desjardins et al. 2004).

Field test sampling and deoxynivalenol analysis. Field tests were conducted under permit from USDA-APHIS. Wheat heads were tagged after heading, and only tagged heads were harvested in order to avoid selection bias. Heads were harvested at maturity, individually hand-threshed, pooled, and weighed to determine yield for each plot. For field test 1, 100 seeds per plot were saved for fungal strain recovery analysis and the remaining seeds were randomly assigned to one of 5 pools for DON analysis. For field tests 2, 3 and 4, heads from each plot were randomly assigned to 1 of 2 pools; half of each pool was ground for DON analysis, and half was sampled for fungal strain recovery analysis. Seed samples were analyzed for DON (the only trichothecene detected at significant levels) by liquid chromatography-mass spectrometry (LC-MS), with triplicate injections of each extract (Plattner & Maragos 2003).

Statistical analyses. A randomized complete block design was utilized in the 2002 and 2003 field tests to compare seed yield, DON contamination, head bleaching symptoms, and fungal strain recovery among treatments. Levene's homogeneity of variance tests at the 5% alpha levels were performed to determine if any data transformations of the dependent variables were necessary. One-way ANOVA tests were performed for each field test to detect differences among treatments in seed yield, DON contamination, head bleaching symptoms, and fungal strain recovery. F-test statistical results were considered significant at $p \leq 0.05$. Duncan's multiple range tests (alpha = 5%) were used as the multiple comparison procedure to find treatment mean differences in dependent variables if a significant F-test statistic was obtained from ANOVA.

RESULTS

Field Test 1 Plan. Field test 1 was a nonreplicated maize stalk treatment test conducted in 2001 at the Christ family farm near Peoria, Illinois, which also was the location for tests 2 and 3. In April, 3 plots of susceptible spring wheat cultivar Wheaton (3 × 3 m) were planted and separated from each other by 3 m of cultivated ground to minimize cross-contamination. For tests 1-4, the field test site was surrounded by a perimeter of 10 m of cultivated ground. From seedling emergence to seed maturity, each plot was mist-irrigated for 30 min, 4 times daily. In May, 2 to 3 weeks before first anthesis, each of the 3 plots was surrounded by wire-mesh fencing and 100 fungal-treated or control maize stalk pieces were placed on the ground in each plot. An additional 100 pieces were placed in each plot 2 weeks later. **Results.** In field test 1, WT strain, one *MAT*-deletion strain, and control were compared (Table 2). During this test, conditions were conducive for development of WHB, with daytime temperatures within the range of 15-30°C for 30 of the 45 critical days from 1 June to mid-July. The WT strain reduced yield by 27% and increased DON contamination by 180% compared to control. The *MAT*-deletion strain did not reduce yield or increase DON when compared to control. Seed infection with *G. zeae* also was higher in the plot infected with the WT strain than in the other 2 plots. *G. zeae* isolates were recovered from 63% of seeds from the plot treated with the *MAT*-deletion strain, but hyg-resistant isolates were recovered from only 1% of seeds in this plot and from no seeds in the other 2 plots. These

data indicate that DON contamination in the plot treated with the *MAT*-deletion strain was due to cross-contamination by the WT strain or by naturally occurring *G. zeae* strains.

Field Test 2 Plan. Field test 2 was a replicated maize stalk treatment test conducted in Illinois in 2002. In April, 12 plots of susceptible spring wheat cultivar Norm (3 × 3 m) were planted, separated from each other by 3 m of cultivated ground, and the entire test site was surrounded by a wire-mesh fence. The test was a randomized complete design with 6 treatments and 2 replicate plots per treatment. Each plot was mist-irrigated for 15 min, 4 times daily and 100 maize stalk pieces were applied to plots twice, as in field test 1. **Results**. In field test 2, 3 ascospore-producing strains, 2 *MAT*-deletion strains, and control were compared (Table 2). During this test, conditions were not conducive for WHB, with daytime temperatures above 30°C for 33 of 46 critical days from mid-June to the end of July. The 3 ascospore-producing strains reduced yield an average of 17%, which was significantly different from controls ($p \leq 0.05$), and caused DON contamination an average of 0.9 μg/g, which was not significantly different from controls. The 2 *MAT*-deletion strains reduced yield an average of 12% and caused DON contamination an average of 0.5 μg/g, neither of which was significantly different from controls. None of the strains increased seed infection with *G. zeae*, which averaged 44% for all treatments. *G. zeae* isolates were recovered from 34% of seeds from the 4 plots where *MAT*-deletion strains had been applied, but hyg-resistant isolates were recovered from only 4% of seeds in these plots and 0.5% of seeds from other plots.

Field Test 3 Plan. Field test 3 was a replicated, combined wheat head injection and maize stalk piece treatment test conducted in Illinois in 2003. In April, 12 plots of cultivar Norm (3 × 3 m) were planted as in field test 2. Two plots were used for injection of macroconidia into heads. The remaining 10 plots were used for maize stalk treatment, in a randomized complete design with 5 treatments and 2 replicate plots per treatment. Each plot was mist-irrigated for 30 min, 4 times daily. After stalk piece treatments were applied, all 12 plots individually were covered with light-shade cloth. Heads at mid-anthesis were injected with macroconidia in 2 replicate plots, each with 5 treatments and 60 heads per treatment. 100 maize stalk pieces were applied to plots twice, as in field test 1. **Results**. In field test 3, WT strain, 2 *MAT*-complemented strains, a *MAT*-deleted strain, and control were compared (Table 2). During this test, conditions were conducive for development of WHB, with daytime temperatures within the range of 15-30°C for 34 of the 45 critical days from June first to mid-July. Following head injection, all 4 strains caused an average head bleaching intensity of 92%, yield reduction of 54%, and DON contamination of 29 μg/g. All strains were significantly different from controls, but the *MAT*-deletion strain was not different from the ascospore-producing strains by any of the parameters tested (data not shown). Following maize stalk treatment, the 3 ascospore-producing strains reduced yield an average of 20% and caused DON contamination an average of 14.3 μg/g, both of which were significantly different from control. The *MAT*-deletion strain caused no yield loss and a DON contamination of 7.2 μg/g, which was not significantly different from control. None of the strains increased seed infection with *G. zeae*, which averaged 55% for all treatments. Despite the high level of seed infection with *G. zeae*, hyg-resistant, gen-sensitive isolates were recovered from only 3% of seeds from the 2 plots where the ascospore-nonproducing strain was applied. In contrast, hyg-resistant, gen-resistant isolates were recovered from an average of 34% of seeds, and accounted for 61% of the *G. zeae*, from the 4 plots where *MAT*-complemented strains were applied. Both classes of antibiotic-resistant isolates were each recovered from an average of 10% of seeds from plots where they were not applied.

Field Test 4 Plan. Field test 4 was a replicated, combined wheat head injection and maize stalk piece treatment test conducted in 2003 at the Purdue University Agronomy Farm near West Lafayette, Indiana. In September 2002, susceptible winter wheat cultivar Patterson was sown in rows spaced 18-cm apart. The entire test site was surrounded by wire-mesh fencing and 10 m of cultivated ground, and was not irrigated. For direct injection of macroconidia into heads, two blocks of 5 plots (each 1 m long and one row wide)

were delineated at opposite ends of the test site. Each plot was used for one treatment, with 50 heads per treatment. At mid-anthesis, heads were injected with macroconidia and groups of heads were covered with a plastic bag for 1 day. For maize stalk treatment, 10 plots (3 × 2.5 m) were delineated and separated from each other by 3 m of cultivated ground. The test was a randomized complete design with 5 treatments and 2 replicate plots per treatment. In April, 100 maize stalk pieces were placed on the ground in each plot.

Results. In field test 4, WT strain, 2 *MAT*-complemented strains, a *MAT*-deleted strain, and control were compared (Table 2). In this test, disease levels were low for both head injection and maize stalk treatments, probably due to the lack of irrigation. Following head injection, the 4 strain treatments caused significant yield reductions, averaging 32%, but did not differ from controls in head bleaching or DON contamination (data not shown). In the maize stalk treatment, the 3 ascospore-producing strains tended to cause more yield reduction (average of 10%) and DON contamination (average of 5.2 $\mu g\ g^{-1}$) than the *MAT*-deletion strain (no yield reduction and 3.8 $\mu g\ g^{-1}$), but none of the treatments were significantly differently ($p \leq 0.05$) from controls. None of the strains increased head bleaching symptoms (data not shown) or seed infection with *G. zeae*, which was <10% for all plots. Hyg-resistant, gen-resistant isolates were recovered from an average of 2% of seeds from plots where *MAT*-complemented strains were applied. Hyg-resistant, gen-sensitive isolates were recovered from an average of 4% of seeds from plots where the *MAT*-deletion strain was applied.

DISCUSSION

The purpose of this study was to test the hypothesis that ascospores are the primary source of inoculum for WHB epidemics caused by *G. zeae*. To test this, we combined ecological and molecular approaches by conducting field tests under conditions that mimic natural WHB epidemics, and by using ascospore-nonproducing strains, generated by transformation-mediated *MAT*-locus deletion, and ascospore-producing strains, generated by *MAT*-locus complementation. In this complex ecological system, we were successful in obtaining WHB epidemics in field tests 1 and 3, but less successful in field tests 2 and 4. Overall, the 4 field tests of fungal-treated maize stalk pieces contained a combined total of 19 plots with ascospore-producing strains, 9 plots with ascospore-nonproducing strains, and 7 control plots. When compared to control plots, ascospore-producing strains caused a significant ($p \leq 0.05\%$) yield reduction in 58% (11 of 19) of plots where they were applied and a significant increase in DON contamination in 47% (9 of 19) of plots. In contrast, ascospore-nonproducing strains caused a significant yield reduction in only 22% (2 of 9) of plots where they were applied and no significant increase in DON contamination in any plots, as compared to control plots. In field tests 1 and 3, in which epidemics developed, the ascospore-nonproducing strains caused significantly less severe epidemics than did ascospore-producing strains. In tests 2 and 4, in which epidemics did not develop, differences between ascospore-producing and nonproducing strains may have been obscured by low levels of disease. This study demonstrates the feasibility of combining molecular and ecological approaches for analysis of a complex agroecosystem. This alternative approach has provided new evidence that ascospores can play a critical role in WHB epidemics, at least in Illinois. Ascospore-nonproducing strains of *G. zeae* could be useful tools to investigate the importance of ascospores in other agroecosystems, especially in regions of Europe where *G. zeae* appears to be displacing *F. culmorum* and *F. avenaceum* as the dominant WHB pathogen (Waalwijk et al. 2003). The importance of ascospores identifies the *G. zeae* sexual cycle as a potential target for control of a plant disease whose reemergence has serious consequences for farm economics and for food and feed safety worldwide.

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Table 1. *Gibberella zeae* strains used in this study.

Strain no.	Strain description	Genotype	Ascospore phenotype
GZ3639	Wild type	*MAT1-1/MAT1-2*	producer
GZ3639MT#39	Mock transformant	*MAT1-1/MAT1-2*	producer
GZ3639 MT#44	Mock transformant	*MAT1-1/MAT1-2*	producer
ΔMAT#14	Gene-deletion mutant	*mat1-1/mat1-2/hygB*^{R}	nonproducer
ΔMAT#40	Gene-deletion mutant	*mat1-1/mat1-2/hygB*^{R}	nonproducer
ΔMAT#73	Gene-deletion mutant	*mat1-1/mat1-2/hygB*^{R}	nonproducer
ΔMAT#78	Gene-deletion mutant	*mat1-1/mat1-2/hygB*^{R}	nonproducer
MAT-comp#9	Gene-complemented mutant of ΔMAT#78	*MAT1-1/MAT1-2/hygB*^{R}*/gen*^{R}	producer
MAT-comp#88	Gene-complemented mutant of ΔMAT#78	*MAT1-1/MAT1-2/hygB*^{R}*/gen*^{R}	producer

Table 2. Disease assessment and strain recovery after treatment with *G. zeae* on maize stalk pieces.

	Treatment		Disease assessment*		Strain recovery**		
Field Test	ascospore phenotype	strain	seed yield	DON	*G. zeae* (%)	HygR GenS	HygR GenR
1	producer	GZ3639	637	35.5	89	0	NA
	nonproducer	ΔMAT#14	855	9.7	63	2	NA
		control	869	12.6	53	0	NA
2	producer	GZ3639	698 c	1.7 c	59	0 a	NA
	producer	MT#39	828 abc	0.7 b	42	5 ab	NA
	producer	MT#44	723 bc	0.4 a	52	0 a	NA
	nonproducer	ΔMAT#40	752 bc	0.6 ab	38	10 b	NA
	nonproducer	ΔMAT#73	858 ab	0.4 a	30	11 b	NA
		control	908 a	0.6 ab	45	0 a	NA
3	producer	GZ3639	728 b	10.8 bc	54	10 a	15 a
	producer	MAT-comp#9	732 b	16.9 d	57	30 b	60 b
	producer	MAT-comp#88	692 b	15.1 cd	56	18 a	61 b
	nonproducer	ΔMAT#78	922 a	7.2 ab	56	6 a	25 a
		control	892 a	6.6 a	51	16 a	24 a
4	producer	GZ3639	790	4.7	10	3 a	20
	producer	MAT-comp#9	945	5.8	8	15 a	15
	producer	MAT-comp#88	835	5.0	10	0 a	25
	nonproducer	ΔMAT#78	960	3.8	8	48 b	14
		control	955	2.5	7	10 a	20

* Disease assessment methods: seed yield per head in mgs; DON = deoxynivalenol (μg/g seed dry weight) by LC-MS. Data are unreplicated (1 plot per treatment) in test 1, and are means of 2 replicate plots per treatment in tests 2, 3 and 4. For tests 2-4, means with the same letter are not different at $p \leq 0.05$; means without a letter indicate that none of the treatments were different. The total number of heads analyzed per treatment was 300 for test 1; approx. 600 for tests 2 and 3; 200 for test 4.

** *G. zeae* (%) = percentage seeds infected with *G. zeae*. HygRGenS = percentage *G. zeae* isolates hyg-resistant and gen-sensitive. HygRGenR = percentage *G. zeae* isolates hyg- and gen-resistant. Number of seeds tested per treatment was 63-100 for test 1, test 3, and control and ascospore producers in test 2; 500-800 for ascospore nonproducers in test 2; 300-450 for test 4. Number of isolates tested for hyg resistance per treatment was 50-82 for test 1; 20-40 for control and ascospore producers in test 2; 220-256 for ascospore nonproducers in test 2. Number of isolates tested per treatment for resistance to hyg and gen was 20-30. NA = not applicable because gen-resistant strains were not released in field tests 1 and 2.

SYSTEMIC COLONIZATION AND PRODUCTION OF DEOXYNIVALENOL THROUGHOUT WHEAT PLANTS FOLLOWING INOCULATION OF CROWN TISSUE WITH *FUSARIUM GRAMINEARUM*.

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ABSTRACT

Fusarium Head Blight (FHB), incited by *Fusarium graminearum (Fg)*, has re-emerged as major disease, causing devastating losses in U.S. wheat producing states for over a decade. FHB poses an additional threat to industry as grain is contaminated with mycotoxins, particularly deoxynivalenol (DON). Crown rot (CR), which can also be caused by *Fg*, is an important and chronic problem in Australia and other similarly arid countries. Although DON has been reported as an aggressiveness factor in FHB, its role in the development of CR is unknown. Experiments, conducted in Australia, with a transgenic isolate of *Fg* (hygromycin resistant), examined the ability of *Fg* to systemically colonize wheat after crown inoculation using the wheat cv. Kennedy (CR susceptible). Following inoculation, *Fg* was recovered from 100% of crowns and 55% of heads. DON was detected at 275ppm in crowns and 7ppm in asymptomatic heads. An experiment, conducted in the U.S., examined three isolates of *Fg*; an highly aggressive (FHB) (B86A11) and competent DON producing isolate; a transgenic isolate (GZ40) lacking the trichodiene synthase (Tri5) gene; and the wild-type isolate (GZ3639) used to produce GZ40. Following crown inoculation of the wheat cv. Wheaton (FHB susceptible) the isolates were recovered from 50-95% of crowns and 5-21% of kernels. The recovery of *Fg* was lowest for GZ40. DON was detected at levels of 91ppm and 23ppm in crowns and 3ppm and 2ppm in kernels, for B86A11 and GZ3639, respectively. DON was detected in a few GZ40 inoculated plants, but below 1ppm, and probably arose from cross contamination in the growth chamber. These experiments demonstrate that crown infection can lead to systemic fungal colonization of wheat and DON production in all tissues, including kernels. The results suggest partial role for DON contributing to the extent of systemic colonization from the crown.

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EXPANDED HOST RANGE OF *FUSARIUM GRAMINEARUM* TO POTATO AND SUGARBEET.

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ABSTRACT

Fusarium graminearum is a well known pathogen of cereals, particularly wheat, barley and corn, where it can cause yield losses, and quality losses due to the production of numerous mycotoxins. Recently, *Fusarium graminearum* was isolated from stored potato tubers and sugarbeet roots showing field dry rot symptoms in North Dakota and Minnesota. The objective of this study was to determine the host range and pathogenicity of *F. graminearum* isolates collected from diseased potatoes and sugarbeet, compared to known *F. graminearum* isolates collected from wheat. Thirty-five isolates (20 from potato, five from sugarbeet and ten from wheat) were tested for the ability to cause disease in potato, sugarbeet and wheat. Potatoes cv. Russet Burbank and sugarbeets cv. Phoenix were inoculated by removing a plug from the tuber/root and replacing it with a mycelial plug of the *F. graminearum* and incubated at 14°C for 4 weeks. Wheat plants cv. Grandin were inoculated at anthesis by spraying the spikelets with a conidial suspension (4×10^4)/ml), incubated for 48 hours and maintained in the greenhouse for three weeks before measuring infection. Disease severity for potato and sugarbeet was estimated by obtaining a ratio of infected tuber/root area to total tuber/root area. Disease severity for wheat was determined using a visual scale for Fusarium Head Blight (FHB). *F. graminearum* isolates were pathogenic to all three crops, regardless of the original host they were isolated from. Typical FHB symptoms were observed in wheat, and both potato and sugarbeet tubers/roots showed typical dry rot symptoms. *F.* graminearum was not pathogenic to sugarbeet seedlings. These findings have major epidemiological implications for crop rotations and other disease management strategies for *F. graminearum.*

DIGITAL IMAGE ANALYSES, RELATIVE CHLOROPHYLL CONTENT, AND MICROSCOPIC EVALUATION OF LEAVES OF FRONTANA AND ALSEN INOCULATED WITH FOUR ISOLATES OF *FUSARIUM GRAMINEARUM*.

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ABSTRACT

Seedlings of spring wheat cultivars Frontana and Alsen were grown in Cone-tainers in the greenhouse to the two-leaf stage. At 14 days post-planting, seedlings were inoculated in a split-plot design, with cultivars as main-plots and fungal isolates as subplots. Four different isolates of *F. graminearum*, obtained from the University of Minnesota Small Grains Pathology Project, St. Paul, were separately inoculated onto the adaxial surface of the middle of each cultivar's primary leaves. Each cultivar-isolate or control combination had ten replicates. Water inoculated primary leaves of seedlings for each cultivar provided control comparisons. Following inoculation, plants were maintained at nearly one hundred percent relative humidity at 23°C for 72 h in an incubation chamber. Lighting was provided under a 12 h light:dark period while plants were incubating. Following incubation, plants were removed to lab benches beneath artificial lighting at temperatures from 21 to 23°C for another 24 h. At 96 h post-inoculation primary leaves were excised at their base near the ligule and placed on a photographic stage adaxial side upwards. Leaves with lesions were photographed using a high-resolution digital camera. Images were analyzed using the Assess digital image analysis software obtained from the American Phytopathological Society Press. Threshold levels of lesion area were established by setting the hue, saturation, and intensity indices of the program to discriminate lesions of inoculated leaves relative to non-inoculated control leaves to provide differentiation of chloroses and necroses versus healthy appearing leaf area. Leaves were then measured for their relative chlorophyll content at the point of inoculation using an Opti-Sciences CCM-200 chlorophyll content meter in two of the six experiments we conducted. Averaged over the inoculation treatments for the six experiments, mean percent lesion area of inoculated leaves of Frontana was 0.23 % and was significantly lower ($P<0.001$) than for Alsen, which was 6.31 %. Significant differences were observed among inoculation treatments ($P<0.001$) of *F. graminearum* and there was a significant cultivar by isolate interaction ($P<0.001$) for percent lesion area assessments. Over the two experiments where relative chlorophyll content was measured on inoculated primary leaves, mean measurements of Frontana showed significantly ($P<0.001$) more chlorophyll content (greener leaves measure with higher numbers) and measured 6.63 whereas Alsen's primary leaves measured 3.27. UV-microscopy and brightfield microscopic observations of inoculated primary leaves of the two cultivars will be discussed.

STRATEGIES TO REDUCE *FUSARIUM* AND MYCOTOXIN CONTAMINATION IN NORWEGIAN CEREALS.

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ABSTRACT

Increasing levels of *Fusarium* toxins have been recorded in Norwegian cereals during the last few years. In 2004, some grain lots with unusually high levels of deoxynivalenol (DON) were recorded, in particular in oats (e.g. 25-30 mg/kg). Alarming levels of T-2 and HT-2 were recorded in oats in 2005. In Norway, only a limited number of check samples of grains used for food and fodder are analyzed for mycotoxin content. To reduce the risk of contaminated cereals entering the food and feed processing chain, a four-year project (2006-2009) was started at *Bioforsk-Norwegian Insititute for Agricultural and Environmental Research.* We are aiming to establish a three-step screening system in order to identify grain lots with high levels of *Fusarium* toxins: **1**- Identify 'high-risk' fields/lots, based on information on cultivation practice and climatic conditions, through the use of a FHB-prediction model. **2**-Analyze the 'high-risk' lots with a rapid test method selected due to its capacity to screen for *Fusarium* toxins in a large number of grain samples at low costs. **3**- Forward selected samples (based on analyzes in step 2) to chemical mycotoxin analyzes.

Fusarium avenaceum , *F. culmorum*, *F. poae* and *F. tricinctum* have been the most frequently recorded *Fusarium* species on cereals in Norway for many years. However, more recently also *F. graminearum* has occurred more frequently, and *F. langsethia* has been detected especially in oats. Investigations will be carried out to clarify if there has been a change in the composition of Fusarium species.

EFFECT OF CORN RESIDUE LEVEL, FUNGICIDE APPLICATION, AND CULTIVAR RESISTANCE LEVEL ON DISEASE INCIDENCE AND SEVERITY OF FUSARIUM HEAD BLIGHT AND DON CONCENTRATION.

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ABSTRACT

Previous cooperative experiments conducted in 2003 and 2004 showed that the presence of corn residue did increase the risk of Fusarium head blight (FHB) in many years, and that 14% or 80% ground cover often resulted in similar levels of disease intensity. The result also indicated that eliminating local sources of inoculum alone may not be enough to provide satisfactory reduction in disease and DON (deoxynivalenol) when disease pressure is extremely high. Experiments were conducted in IN, ND, OH, PA, and SD during the 2005 and 2006 growing seasons to evaluate the effect of corn residue level, fungicide application at flowering, cultivar resistance, and their interactions on FHB. The experiment was a split-split-plot design with three replications at each location. Treatments included two levels of corn residue (approximately 0 and 80% ground cover) as the main plot factor, fungicide treatment [Folicur (Tebucanazole) was applied at 50% anthesis (Zadoks 65)] as the sub-plot factor, and three winter wheat cultivars ('Hopewell', 'Patterson' and 'Truman') as the sub-sub plot factor in IN, OH and PA. The protocol varied at the ND and SD locations. At the SD location, two spring wheat cultivars ('Alsen' and 'Norm') were used. At the ND location, *Gibberella zeae*-colonized corn kernels were used to establish the main plots, and three spring wheat cultivars ('Alsen', 'Argent', and 'Granite') were used as the sub-sub plots; however, the fungicide sub-plot remained consistent. Disease incidence, severity and DON concentration varied between years and locations, and current analysis considers each location and year separately. In 2005, disease incidence, severity and DON concentration varied from 0 to 78% (mean 18%), 0 to 33% (mean 5%), and 0 to 4.1 ppm (mean 0.7 ppm), respectively. Winter wheat locations had very low disease (0-7% mean disease incidence), while spring wheat locations had moderate (35-51% mean disease incidence) disease intensity. Among locations with measurable disease intensity or DON, the effect of cultivar resistance was significant in the majority of cases. Two-way or three-way interactions were observed in several cases indicating that combinations of two or more management practice resulted in better control. This indicates the potential importance of integrating multiple management tactics.

INFLUENCE OF WEATHER ON THE ABUNDANCE OF *GIBBERELLA ZEAE* PROPAGULES WITHIN WHEAT CANOPIES: A LAG REGRESSION ANALYSIS.

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ABSTRACT

The development of Fusarium head blight (FHB) and the accumulation of deoxynivalenol (DON) are largely dependent on the availability and abundance of inoculum of *Gibberella zeae* under the right set of environmental conditions. An understanding of the dynamic associations among inoculum potential, toxin production, weather, and disease intensity would be invaluable to our ongoing efforts to predict the risk of FHB and DON. In an effort to characterize the association between weather variables and inoculum within wheat canopies, spikes were sampled and assayed for propagules of *G. zeae* from plots established in OH, PA, ND, SD and IN from 1998 through 2005. Spikes were collected daily from each field from Feekes growth stage 10 through 11.2, placed in sterile distilled water, and washed to dislodge propagules. Samples of spike wash suspensions were transferred to Petri plates containing Komada selective medium, and *G. zeae* was identified based on morphology of colonies and spores. Inoculum abundance was quantified as the number of colony-forming units per spike (CFU/spike). Ambient weather data were collected and summarized for different periods prior to sampling of spikes. A total of 35 individual weather variables were generated. Polynomial distributed lag regression analysis was used to identify weather variables and the period of time that best predicted spore abundance. Linear mixed models were then used to simultaneously determine the effects of location, year within location, and weather variables on abundance. Inoculum density (based on log-transformed CFU/spike) within wheat canopy was statistically related to weather conditions both on the day of sampling and several days prior to sampling. The response to weather conditions was distributed over nine days, and the functional relationship (linear, quadratic etc) between weather and spore abundance varied with the predictor variable. The most significant predictors of log CFU/spike were average relative humidity, wetness duration, average daily air temperature, and rainfall intensity. Study location also had a major effect on inoculum abundance. Current research focuses on accounting for serial correlation of responses within location-years, and on identifying location-specific determinants of spore density.

ACKNOWLEDGEMENT AND DISCLAIMER

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ENVIRONMENTAL FACTORS INFLUENCING FUSARIUM HEAD BLIGHT OF BARLEY IN THE NORTHERN GREAT PLAINS.

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ABSTRACT

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

Varieties of regionally adapted barley of both 2- and 6-row types were planted at multiple locations in the Northern Great Plains during the 2005 and 2006 growing seasons. At least three varieties were common to each location. Plots were un-irrigated, a minimum of 1.5m x 4.6m in size, and replicated four times in a RCBD. Additional varieties were planted based upon availability and local producer preference. Crop stage was monitored regularly throughout the season and the date at which each plot was at Feekes 10.5 stage was noted. No additional inoculum was introduced into the plots. The incidence and severity of FHB was recorded on a minimum of 25 heads per plot at the soft-dough stage (approximately 21 days after heading). Environmental variables consisting of temperature, relative humidity, and precipitation were recorded with an on-site, or nearby, weather station.

Over the past two seasons, we have successfully collected data for 24 of the 26 locations planted. These represent a range of disease intensities with some varieties at locations in 2005 having almost 100% incidence of infection. Locations in western Minnesota all had relatively low disease (< 3%) in 2005, whereas those in the Dakotas had a much broader range (<1 to 25%). For 2006, a wide-spread drought in the region resulted in negligible disease at most locations. DON data is pending for the 2006 trials, however the concentrations in grain ranged from 0 to 3ppm for 2005.

Correlation analysis was conducted on the limited data set available and the environmental variables that were most associated with disease severity (field index) were pre-heading temperature and measurements of air moisture content (relative humidity, etc). In comparison, only the air moisture content after heading was significantly associated with final DON concentration. Additional results will be presented as data becomes available.

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TIMING OF INFECTION: THE EFFECTS ON FUSARIUM HEAD BLIGHT SEVERITY AND TOXIN ACCUMULATION IN WHEAT KERNELS.

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ABSTRACT

There have been recent reports of wheat grain which appears to be free of Fusarium Head Blight symptoms, but contains significant levels (≥ 2ppm) of the mycotoxin deoxynivalenol (DON). We are investigating the role of infection timing relative to host growth stage in the development of asymptomatic wheat kernels with significant DON. Three hard red winter wheat varieties, Hopewell (moderately susceptible), Valor (moderately susceptible) and Truman (moderately resistant), were used in this field study. The cultivars were subjected to four treatments: (1) ambient conditions (no supplemental moisture), (2) misting during anthesis only, (3) misting during both anthesis and grain-fill stages and (4) misting during grain-fill only. Movable greenhouses and mist chambers were employed to prevent rain or to add moisture as needed. All plots were spray inoculated with four DON-producing *Fusarium graminearum* isolates at both anthesis and late milk stages of growth. Plants were misted overnight for four consecutive nights post-inoculation. Disease incidence and severity were measured in the field prior to harvest. Following harvest, yield and the percentage of *Fusarium*-damaged kernels were also assessed. High Pressure Liquid Chromatography was used to analyze DON content of the samples and statistical analysis was performed using proc MIXED of SAS (version 9.1, SAS Institute, Cary, NC). The disease incidence in treatments 1 and 4 were significantly lower ($P \leq 0.05$) than treatments 2 and 3. Increased moisture during anthesis also resulted in significantly higher ($P \leq 0.05$) disease severity regardless of variety. Although treatments 1 and 4 did not differ in terms of incidence and severity, kernels from treatment 4 contained significantly higher ($P \leq 0.05$) DON than treatment 1. Within treatment 4, Truman (1.5 ppm) contained significantly less ($P \leq 0.05$) DON than the more susceptible cultivars Hopewell and Valor which contained an average of 3.0 ppm and 3.7 ppm DON, respectively. This study suggests that late infections, facilitated by moisture during grain-filling stages of kernel development, may result in low disease intensity yet kernels containing significant levels of DON.

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FOOD SAFETY, TOXICOLOGY AND UTILIZATION OF MYCOTOXIN-CONTAMINATED GRAIN

WHEAT KERNEL BLACK POINT AND FUMONISIN CONTAMINATION BY *FUSARIUM PROLIFERATUM*.

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ABSTRACT

Fumonisins are mycotoxins produced by several *Fusarium* species, especially *Fusarium proliferatum* and *Fusarium verticillioides*, which are common pathogens of maize worldwide. Consumpton of fumonisins has been shown to cause a number of mycotoxicoses, including leucoencephalomalacia in horses, pulmonary edema in swine, and liver cancer and neural tube defects in experimental rodents. Consumption of fumonisin-contaminated maize also has been associated epidemiologically with human esophageal cancer in some areas of the world where maize is a dietary staple. Although *F. proliferatum* is a major cause of maize ear rot, this species also is a minor component of the wheat head blight complex worldwide and has been associated with incidents of black point disease of wheat kernels in the USA. The major aim of the present study was to characterize nine *F. proliferatum* strains from wheat from Nepal for ability to cause wheat kernel black point under greenhouse conditions and for fumonisin contamination of infected kernels. For comparative purposes, the study also included three *Fusarium* strains isolated from US maize: two *F. proliferatum* strains and one *F. verticillioides* strain. Fungal strains were applied by spray or injection of macroconidia to spikes of five wheat cultivars (two soft white spring wheats, one hard red spring wheat, and two durum wheats). All strains produced kernel discoloration and black point, and most strains had some effect on kernel weight and germination. Most strains also produced fumonisins in kernels, but at relatively low levels of less than 10 ug/g (combined fumonisin B1, B2 and B3) as determined by liquid chromatography-mass spectroscopy. However, one strain from Nepal produced high levels of more than 100 ug/g of fumonisins in kernels. These preliminary data indicate a potential for fumonisin contamination of wheat infected with *F. proliferatum*. Surveys are underway to determine the natural occurrence of *F. proliferatum* and fumonisins in US wheat with black-point disease. Those interested in contributing black-point wheat samples for fumonisin analysis are encouraged to contact the corresponding author.

TISSUE DISTRIBUTION AND PROINFLAMMATORY CYTOKINE INDUCTION BY THE TRICHOTHECENE DEOXYNIVALENOL IN THE MOUSE: COMPARISON OF NASAL VS. ORAL EXPOSURE.

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ABSTRACT

Ingestion of the trichothecene mycotoxin deoxynivalenol (DON), a common contaminant of cereal grain, causes adverse effects on growth and immune function in experimental animals and thus poses a health risk to consumers. However, relatively little is known about the risks posed to farmers and grain handlers who are exposed to DON via inhalation of contaminated grain dust. The purpose of this research was to test the hypothesis that DON will distribute in tissues and induce proinflammatory cytokines to a greater extent following nasal exposure than by oral challenge. B6C3F1 mice were treated with a single dose of DON (5 mg/kg) either by nasal instillation or oral gavage. After 0, 15, 30, 60, 120 minutes, mice were euthanized and serum, spleen, liver, lung and kidney samples were collected. An ELISA was devised for the sensitive measurement of DON burden in serum and tissue. In both oral and nasal treatment groups, DON serum and tissue concentrations peaked after 15 minutes and declined by 75 to 90% after 120 minutes. However, DON concentrations were 1.5 to 3.5 times higher in serum and other tissues of mice exposed by the nasal route at all time points assayed as compared to orally exposed mice. The functional significance of different DON tissue concentrations was assessed by measuring the expression of the pro-inflammatory cytokines IL-6, TNF*a*, and IL-1ß in spleen. As expected, oral exposure to DON induced cytokine mRNA expression after 60 and 120 minutes . However, mRNAs for these cytokines were 2 to 3 times higher in spleens of mice exposed to DON by the nasal route. Taken together, these toxicokinetic and functional data indicate that DON is potentially more toxic when inhaled than when ingested. Furthermore, they suggest that adverse human health effects could potentially result from inhalation of DON-contaminated grain dust.

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GASEOUS OZONE TREATMENT OF *FUSARIUM*-INFECTED MALTING BARLEY.

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ABSTRACT

Fusarium head blight (FHB) or scab, caused predominantly by *Fusarium graminearum*, is a serious disease problem of barley in the Northern Plains area. The fungus is known to produce the mycotoxin deoxynivalenol (DON) in infected grains which poses safety concerns for human and livestock. The utilization of *Fusarium*-infected grains and the persistence of mycotoxin in malting barley may lead to decreased malt quality. The only effective control is testing and diversion or dilution. Our initial laboratory test of gaseous ozone treatments (GOT) using *Fusarium*-infected grains of malting barley, *Robust*, (with 1.27 g/g DON) at 11 and 26 mg/g O_3 for 15-30 min showed a significant decrease (24-36%) in *Fusarium* survival (Kottapalli et al. 2005). In our present work, we tested the effectiveness of ozone treatment on *F. graminearum* (NRRL R-6574) in culture (potato dextrose broth) at 0 and 2 days after inoculation (dai). We also extended the exposure time to 2 hrs. using naturally infected malting barley grain samples (20 g) with 2 ppm DON and under steeping condition. *Fusarium* conidia did not survive the GOT at 0 dai and no apparent further growth in mycelia (i.e. change in fungal biomass) was observed with GOT at 2 dai even with 5 days of incubation of culture after the O_3 treatment. *Fusarium* survival in ozonated grains with $0 \leq 0.1$ and 2 ppm DON significantly decreased from 23% to 8% and from 49% to 12%, respectively, without affecting germination. Under steeping condition, O_3-treated grains (with 2 ppm DON) that were plated on HPDA and incubated for 3-5 days did not show any *Fusarium* growth. Our initial results strongly suggest that *Fusarium* cannot survive if GOT is prolonged to at least 2 hrs at the same dosage. Hence, the treatment can effectively reduce *Fusarium* survival in stored grains. Moreover, exposure of grains to O_3 during steeping for at least 2 hrs could eliminate *Fusarium*.

GENETIC ENGINEERING AND TRANSFORMATION

TRANSGENIC BARLEY WITH IMPROVED RESISTANCE TO *F. CULMORUM*.

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ABSTRACT

One strategy to reduce the amount of mycotoxins in small grain cereals would be the development of resistant cultivars. Cereal genes conferring resistance to *Fusarium* infection have not yet been identified, but in some wheat cultivars, resistance loci have been mapped. Trichoderma genes encoding chitinolytic enzymes involved in biocontrol have been introduced into several plant species and have been shown to increase the plants' resistance against fungal pathogens.

At Bioforsk we have produced GM-barley where a fungal endochitinase gene, ech42 from *T. atroviride* regulated by the barley promoter *Ltp2*, has been inserted resulting in increased resistance towards *Fusarium culmorum* infection. The advantage of the *Ltp2* promoter is that it permits a gene to be expressed only in the aleurone layer of developing seeds. One of the resulting transformed plant lines, PL9, seemed to be especially promising. The copy number was estimated by the real-time PCR method to be low. Study on the inheritance of the transgenes in T_1 progeny revealed a Mendelian 3:1 segregation pattern.

Some T_1 progenies showed very high *ech42* expression while others had either very low or no detectable expression at all. After inoculation with *F. culmorum*, all *ech42* containing T_1 progenies coming from PL9 showed high resistance. The amount of *F. culmorum* present after point inoculation of the spikes was quantified by real-time PCR analysis. Extremely low amounts or no *F. culmorum* could be detected in seeds located at the same spike close to the point inoculated grains compared to what was found in wild type control plants. The *F. culmorum* resistance was found to be stable when tested in the T_3 generation. Resistance towards *F. graminearum* however, could not be detected in the GM-barley.

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

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ABSTRACT

Wheat and barley scab, also known as Fusarium head blight (FHB) is a devastating disease worldwide, caused mainly by *Fusarium graminearum*. The *Fusarium*-infected grain is contaminated with potent mycotoxins, especially deoxynivalenol (DON), which poses a great threat to human and animal health. DON belongs to the group of trichothecene toxins, which target ribosomal protein L3 at the peptidyltransferase site of eukaryotic ribosomes and inhibit protein synthesis. The goal of our work is to identify mutations in L3 that confer resistance to DON and to determine if FHB resistance can be engineered in transgenic wheat plants by expressing DON resistant L3 genes. In previous studies, we have demonstrated that overexpression of a truncated form of yeast ribosomal protein L3 (L3Δ) in transgenic tobacco plants confers resistance to deoxynivalenol (DON). To determine if expression of the yeast L3Δ in transgenic wheat plants would provide resistance to FHB, the susceptible spring wheat cultivar, Bobwhite was transformed with the yeast L3Δ under the control of the barley floret-specific *Lem1* or the maize constitutive *Ubi1* promoter. Seeds from the homozygous lines containing each construct were able to germinate on media containing DON, unlike the seeds of the wild type Bobwhite plants. To determine if the lines that were resistant to DON were resistant to FHB, five different homozygous lines were evaluated for resistance to FHB in greenhouse tests by inoculating a single spikelet at the central node of the main spike of each plant with a macroconidial spore suspension of *F. graminearum*. All spikelets of inoculated wild type plants turned brown at 21 days after the inoculation. In contrast, only the inoculated spikelets of the transgenic lines turned brown; the uninoculated spikelets remained green in most of the transgenic plants. The disease severity was reduced by 48-56% in four different transgenic wheat lines compared to the untransformed Bobwhite plants. The reduction in disease severity correlated well with the level of expression of L3Δ mRNA. These results demonstrated that transgenic wheat plants expressing the yeast L3Δ showed improved resistance to FHB over the untransformed Bobwhite plants. To determine if resistance to FHB would result in a reduction in DON levels, the mature kernels above and below the inoculated spikelets were analyzed for DON levels. There was a 63-76% reduction in DON levels in the four different FHB resistant transgenic lines. The DON levels in one transgenic line were lower than the DON levels in the resistant line, Alsen. These results provide evidence that resistance to DON correlates with resistance to FHB and results in reduced accumulation of DON in transgenic wheat plants.

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A VIRUS-INDUCED GENE SILENCING SYSTEM FOR THE IDENTIFICATION OF GENES CONTRIBUTING TO FHB RESISTANCE IN WHEAT.

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ABSTRACT

Functional genomics analysis in hexaploid wheat is greatly impeded by the genetic redundancy of polyploidy and the difficulties in generating large numbers of transgenic plants required in insertional mutagenesis strategies. Virus-induced gene silencing (VIGS), however, is a strategy for creating gene knockouts that overcomes both of these impediments: 1) Being a homology-dependent silencing process, it can suppress expression any redundant gene copies that share at least ~85% sequence homology. 2) VIGS is initiated by viral infection, which is a rapid and easy process, unlike regenerating transformed wheat plants. For these reasons we have worked to develop a VIGS system for creating gene knockout phenotypes in hexaploid wheat. Our VIGS system is based on Barley stripe mosaic virus (BSMV). Data will be presented describing the general properties of this silencing system. We are particularly interested in functionally identifying genes that are essential for disease resistance responses in wheat. To this end, we have developed protocols for silencing candidate genes so that we can test if their expression is essential for resistance. We have successfully employed this system to analyze genes required in resistance pathways to leaf rust and several other foliar pathogens of wheat. Very recently, we have developed protocols for silencing genes in the spikes and heads of wheat and are now using BSMV-VIGS to identify genes essential for resistance to Fusarium head blight. The efficacy of this system has been validated in tests in which the wheat line Ning 7840, which has type II resistance to FHB, was first infected with control or experimental VIGS constructs, and then challenged by Fusarium graminearum. It was observed that viral constructs that target no wheat genes or the wheat phytoene desaturase gene, which is assumed to not be involved in FHB resistance, had no effect of Ning 7840's type II resistance. However infection with three different constructs that target wheat chitinase all result in loss of type II resistance.

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ENHANCING RESISTANCE TO *FUSARIUM GRAMINEARUM* BY EXPRESSION OF *ARABIDOPSIS THALIANA* NPR1 IN WHEAT.

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ABSTRACT

Fusarium head blight (FHB)/scab caused by the fungus *Fusarium graminearum* is a destructive disease of wheat and barley. We had previously demonstrated that constitutive expression of *Arabidopsis thaliana NPR1* (At*NPR1*), a key regulator of salicylic acid (SA) signaling, enhances scab resistance in the hexaploid wheat cv Bobwhite (Makandar et al. 2006). The transgenic wheat lines (125A & 192D), which express At*NPR1* from the *Ubi1* promoter, were found to be significantly more resistant to *F. graminearum* in comparison to the non-transgenic control plants in repeated greenhouse experiments. Defense responses (e.g. *PR1* expression) are primed to respond faster in response to challenge by *F. graminearum* in the transgenic *Ubi1*:At*NPR1* plants and the FHB resistant cv Sumai 3, than non-transgenic Bobwhite plants. The enhanced FHB resistance is associated with the faster and stronger expression of the *PR1* gene in *F. graminearum*-challenged spikes of the *Ubi1*:At*NPR1* transgenic plants. Furthermore, *PR1* expression in Sumai 3 and the *Ubi1*:At*NPR1* transgenic wheat was more sensitive to the exogenous application of SA and its analog BTH, than in the cv Bobwhite. Similar to Bobwhite, *PR1* expression in the FHB susceptible cvs Wheaton and Fiedler was also less sensitive to SA, than the FHB resistant Sumai 3 and the *Ubi1*:At*NPR1* transgenic plants, suggesting that sensitivity to SA maybe a useful marker for FHB resistance.

We have concluded two field trials in Manhattan and one in Minnesota in which we have monitored the impact of At*NPR1* on FHB resistance in the *Ubi1*:At*NPR1* transgenic plants. Results of these trials will be presented. In addition, we will present our progress on the introduction of the *Ubi1*:At*NPR1* construct into elite hexaploid wheat and durum cvs.

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Makandar, R., Essig, J. S., Schapaugh, M. A., Trick, H. N. and Shah, J. 2006. Genetically engineered resistance to Fusarium head blight in wheat by expression of *Arabidopsis* NPR1. Mol. Plant-Microbe Interact. 19:123-129.

TRANSGENIC WHEAT WITH ENHANCED RESISTANCE TO FUSARIUM HEAD BLIGHT.

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ABSTRACT

We are developing novel transgenic wheat germplasm resources for resistance to Fusarium Head Blight (FHB). We have developed and are testing transgenic wheat carrying ß-1,3-glucanase, thaumatin-like protein 1 (tlp-1), ribosome-inactivating protein (RIP), lipid transfer protein (LTP), glutathione-S-transferase (GST), thionin, and jasmonic acid inducible Myb transcription factor (JaMyb) genes. Transgenic lines over-expressing these genes were generated using micro-projectile bombardment of the wheat cultivar 'Bobwhite'. Both single and combinations of transgenes were generated. We developed 4, 1, 2, 1, and 4 lines carrying LTP, RIP/tlp-1, TRI 101/tlp-1, TRI 101/ ß-1,3-glucanases, and tlp-1/glucanase respectively. In multiple greenhouse screens of these lines, we identified five (one RIP, two TRI 101/tlp-1, and two tlp-1/glucanase) that exhibited statistically significant reductions in FHB severity compared to the non transgenic controls ($p<0.05$). We also identified 17 lines (1, 2, 1, 6, and 7 transgenic wheat lines carrying the RIP, chitinase/tlp-1, chitinase/RIP, RIP/tlp-1 and chitinase transgenes, respectively) showing reduced severity in comparison with non-transgenic Bobwhite in greenhouse screens. These lines were evaluated in field trials in 2005 and 2006. Three lines (one chitinase, one chitinase/RIP and one RIP) exhibited statistically significant reductions in FHB severity in the summer 2005 field trial compared to the nontransgenic control ($P<0.05$). In 2006, disease severity was extremely low because of the unusually hot and dry weather. We also crossed five transgenic wheat lines (one α-puro-thionin, one TLP and three ß-1,3-glucanase), that exhibited statistically significant reductions in FHB severity in the field, to the type II resistant cv. Alsen. In addition, we developed 9, 13 and 10 transgenic lines carrying LTP, GST and JaMyb genes. Greenhouse screening results will be presented from the transgenic lines derived from the Alsen crosses and the transgenic lines carrying LTP, GST and JaMyb.

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GREENHOUSE FHB REACTION OF DURUM WHEAT EXPRESSING *TRI101* AND A RICE *TLP*.

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ABSTRACT

Fusarium head blight (FHB), caused by *Fusarium graminearum* (Schwabe), is a serious problem in small grain species such as durum wheat (*Triticum turgidum* L.) used for making pasta and semolina. One method being tested to incorporate FHB resistance is gene transfer technology. As previously reported, the cultivar Monroe was transformed by bombarding calli with the pathogenesis-related gene thaumatin-like protein (*tlp*) from rice, and the *Tri101* gene from *F. sporotrichioides*, along with the *bar* gene for selection. PCR and Southern blot analyses identified three insertion events in the 44 regenerated plants and western blot analysis confirmed the expression of both genes in the durum wheat cultivar Monroe. T_2 homozygous lines were identified and three lines from each event were tested for their response to FHB. Replicated greenhouse tests were conducted using spray inoculation with three *F. graminearum* isolates. Spikes from seven of the nine lines showed significantly reduced FHB spread compared to Monroe by 28 days after inoculation, but all had significantly more FHB spread than the resistant bread wheat cultivar Sumai 3. One line with increased FHB at 14 and 21 days after inoculation also showed increased DON. None of the lines showed reduced DON.

HOST PLANT RESISTANCE
AND
VARIETY DEVELOPMENT

DIALLEL ANALYSIS OF FUSARIUM HEAD BLIGHT RESISTANCE IN GENETICALLY DIVERSE WINTER WHEAT GERMPLASM.

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OBJECTIVE

Estimate general and specific combining ability for Fusarium head blight resistance in genetically diverse winter wheat germplasm.

INTRODUCTION

Fusarium head blight (FHB), caused by *Fusarium graminearum* Schwabe [telomorph: *Gibberella zeae* Schw. (Petch)], is a major wheat disease causing significant losses in the Midwestern U.S. and other global wheat regions where weather conditions are warm and humid. Use of FHB resistant wheat cultivars is known to be the best option to reduce damage associated with FHB. Resistant winter wheat varieties released over the past ten years including 'Ernie' (McKendry et al., 1995), 'Freedom' (Gooding et al. 1997), 'Roane' (Griffey et al. 2001), Nc-Neus (Murphy et al., 2004), Truman (McKendry et al., 2005), RCATL33 (Tamburic-Illincic et al., 2006), and Allegiance (Van Sanford et al., 2006) reflect the degree of emphasis given to FHB by wheat researchers in recent years. However, the similarities of these resistant sources with other known sources such as Frontana, Sumai 3, and its derivatives, and their genetic value as parents in breeding programs remain relatively unknown. The objective of this study was to estimate the general and specific combining ability of a number of diverse sources of FHB resistance in winter wheat backgrounds in an effort to help breeders make more informed decisions on which FHB resources to use as parents.

MATERIALS AND METHODS

Twenty genetically diverse winter wheat genotypes (Table 1) were crossed in a 20 by 20 partial diallel to generate the genetic material necessary for this study. Two hundred and ten genotypes (190 F_1 and 20 parents) were developed for analysis. Eight plants per replication per genotype were vernalized at 4°C for 8 weeks and transplanted to the greenhouse. Plants were arranged in a randomized complete block design with 2 replications. The experiment was repeated twice. At anthesis, plants were point-inoculated and scored for type II resistance according to Liu et al. (2005). Data collected included total spikelet number and the number of diseased spikelets on the inoculated head. The Fusarium head blight index (FHBI) was computed as the percentage of diseased spikelets on the inoculated head.

Mean phenotypic FHBI data for each replication as well as that for disease spread were analyzed according to Griffing's Model 1 (fixed effects), Method 2 (parents and crosses) diallel analyses (Griffing 1956). Analyses of variance and correlation analyses were done using SAS (SAS version 9.1, 2005). General combining ability (GCA) and specific combining ability (SCA) were determined using Microsoft Excel (Microsoft, Redmond, WA).

RESULTS AND DISCUSSION

Disease spread and FHBI were highly correlated ($r = 0.98$, $P<0.0001$), as FHBI is derived in part from disease spread data. Analyses were done on each trait independently because FHBI can be confounded by the number of spikelets in the inoculated head. However, results in this experiment were very similar suggesting that FHBI and disease spread could be used interchangeably to describe disease reactions in this set of genetic materials. Analyses of variance indicated no significant effect of environment in this study, thus data were combined over environments for statistical analyses. Effects of parents and crosses were highly significant for both FHBI and disease spread

(P<0.001), however, no significant differences were detected between parents and their F_1s (crosses). GCA and SCA were highly significant (P<0.0001 and P<0.001, respectively), for both FHB and disease spread. FHBI among resistant parents ranged from 6 to 21% while that for susceptible parents ranged from 33 to 70%. The top five resistant parents included Truman, Turda 95, 877-1-2, 870-1-3 and IL9624851 with mean FHBI values of 5.9, 7.7, 8.3, 9.1, and 9.2%, respectively. The most susceptible parents included Coker 9835, MO 94-317 and MO 9965-135 with FHBI values of 69.9, 63.9 and 61 %, respectively (Table 1).

General combining ability of resistant parents ranged from -13.33 to -0.52. For susceptible parents values for GCA ranged from +4.68 to +21.77. Based on GCA, the best parents for use in breeding programs included RR 243, 870-1-3, 816-3-4, Truman, and IL9624851-1 with GCA values of -13.3, -12.01, -11.46, -10.80 and - 9.94, respectively (Table 1). These data suggest that these varieties can impart their FHB resistance to any susceptible variety.

Specific combining ability was significant and estimates for resistant-by-resistant and resistant-by-susceptible crosses are given in Table 2. Low SCA values indicate enhanced levels of resistance for some specific parental combinations and suggest the existence of non-additive gene action (i.e. dominance or epistasis) conditioning FHB resistance. Low SCA values may also suggest the presence of allelic variation among wheat parents. Among the crosses that demonstrate the presence of non-additive gene action are crosses between resistant-by-susceptible parents including Coker 9835 with RR 182 (-16.1), RR 243 (-13.7), 870.1-2 (-13.4), 451.1-2 (-13.3), 816-3-4 (-12.5) (Table 2).

Based on the relative magnitudes of GCA and SCA estimates, additive genetic effects are the major gene effects conditioning FHB resistance in this set of germplasm. Other researchers have also reported significant GCA for FHB resistance in winter (Hall et al., 2003; Buerstmayr et al., 1999) and spring (Mardi et al., 2004) wheat germplasm. The results of our study indicate that the best FHB resistant parents for use in enhancing FHB resistance in U.S. winter wheat should be Truman, and IL96 24851-1 because of their high FHB resistance levels, low GCA values and adaptation in the soft red winter wheat region. A second group of lines including RR 243, 870-1-3, and 816-3-4 are less adapted to the U.S. Midwest but still would make good sources of FHB resistance for breeding programs.

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Table 1. Germplasm, pedigrees, Fusarium head blight resistance data and general combining ability data for a 20 x 20 partial diallel analysis conducted at the University of Missouri in 2006.

Germplasm designation	Cross/Pedigree	FHBI (%)	Disease spread (Spikelet no.)	GCA (FHBI)	GCA (disease spread)	FHB level	Country of origin
Truman	MO 11769/'Madison'	5.9	1.0	-10.8	-1.3	Resistant	U.S.A.
Turda 95	L99 I 1-2/T6-80-86	7.7	1.4	-0.5	0.2	Resistant	Romania
877-1-2	Yang la zi	8.3	1.1	-4.2	-0.8	Resistant	China
870-1-3	Wangshuibai	9.1	1.1	-12.0	-1.8	Resistant	China
IL9624851-1	IL90-6364//IL90-9464/Ning 7840	9.2	1.2	-9.9	-1.4	Resistant	U.S.A
P.97397E1-11	96204//Gfd/INW9824	9.3	1.1	-5.6	-0.7	Resistant	U.S.A
RR 243	Sgv/nobeokabozu/MM/Sumai 3	9.5	1.1	-13.3	-1.9	Resistant	Hungary
816-3-4	Ling Hai Mao Yang Mo	9.6	1.0	-11.5	-1.7	Resistant	China
Fundulea 201 R	F15615-2112/F2076W12-11	10.8	1.2	-1.1	-0.1	Resistant	Romania
451-1-2	Seu Seun 6	12.4	1.2	-7.4	-1.3	Resistant	S Korea
RR 182	Sgv/nobeokabozu/MM/Sumai 3	14.9	2.2	-6.6	-0.7	Resistant	Hungary
Ernie	Pike/MO 9965	20.8	2.1	-4.5	-0.9	Resistant	U.S.A.
Freedom	GR 876/OH 217	21.3	3.0	-2.8	-0.4	Resistant	U.S.A.
MO 960903	IL 85-2872/MO 10501	33.0	4.1	9.0	1.2	Susceptible	U.S.A
Patterson	P691184B8-21-1-1-2-4*2/Caldwell	35.5	4.5	4.7	0.6	Susceptible	U.S.A
Pioneer 2545	Unavailable	49.4	7.0	9.4	1.4	Susceptible	U.S.A
RR 176	Sgv/nobeokabozu/MM/Sumai 3	50.2	8.3	11.3	2.2	Susceptible	Hungary
MO 9965-135	W878//Staddard/01707	61.0	7.7	16.9	2.3	Susceptible	U.S.A
MO 94-317	AP Traveller/Pioneer 2555	63.9	7.3	17.2	2.1	Susceptible	U.S.A.
Coker 9835	Coker 85-20/Pioneer 2550	69.6	9.2	21.8	2.9	Susceptible	U.S.A
$LSD_{0.05}$		7.7	1.1	3.3	0.5		

Table 2. Specific combining ability (SCA) for the Fusarium head blight index estimated for crosses from 13 resistant-by-resistant (upper) and 13 resistant by 7 susceptible (bottom) winter wheat genotypes using partial diallel analysis. The experiment was conducted in 2006 at the University of Missouri.

Germplasm	Ernie	Truman	Freedom	PE97397E1-11	IL9624851-1	816-3-4	877-1-2	451-1-2	870-1-3	Turda 95	Fundulea 201 R	RR 182	RR 243
Ernie		-0.6	2.4	-6.2	-0.6	2.5	12.8	-1.6	2.8	-0.7	9.3	-3.7	0.6
Truman			-2.6	0.4	5.5	11.0	1.0	1.5	5.3	-6.1	10.3	-1.2	6.6
Freedom				3.9	-3.8	-2.3	-6.0	-1.7	2.2	-1.5	-5.7	0.8	-1.4
PE97397E1-11					0.0	-1.0	-2.8	-2.7	0.4	-2.6	-6.5	3.9	2.6
IL9624851-1						4.3	-3.0	5.9	4.7	-2.7	5.0	-1.4	4.6
816-3-4							1.5	9.2	7.3	-3.2	-5.3	3.1	7.6
877-1-2								-3.2	0.1	6.5	-9.1	-4.6	1.7
451-1-2									3.3	-4.1	4.3	-3.3	4.7
870-1-3										-3.1	-4.1	1.0	9.8
Turda 95											13.3	-0.7	-4.1
Fundulea 201 R												-7.5	-0.8
RR 182													2.7
RR 176	-10.0	4.8	1.9	-2.7	-1.0	-0.8	-5.9	-4.3	-5.7	12.5	6.1	-7.7	-10.1
MO 94-317	-7.9	-12.8	10.5	0.3	-6.4	-10.3	2.0	3.9	-6.3	-0.5	15.1	-2.7	-13.0
Patterson	5.9	-2.8	-10.8	4.2	-1.7	-7.8	4.9	-7.3	-4.4	3.9	-1.3	3.1	5.7
Pioneer 2545	7.1	-7.1	-3.5	2.2	-13.0	-8.7	-2.8	2.5	-5.2	1.9	10.6	12.2	-5.8
Coker 9835	7.8	-6.5	13.7	9.6	10.2	-12.5	11.0	-13.3	-13.4	13.6	-8.6	-16.1	-13.7
MO960903	-10.6	-3.8	-2.1	8.2	-12.3	-3.9	11.4	1.4	-8.6	-1.8	-4.1	8.9	-7.5
MO9965.35	-5.94	-8.75	1.35	-3.1	-3.2	-6.9	0.3	-0.3	-3.2	11.0	2.0	6.0	-13.4

LSD $_{0.05}$ ij and ik, 2.23 and LSD $_{0.05}$ ij and kl, 2.17 for comparing SCA of crosses, where i, j, k and l are parental designation.

QTL ASSOCIATED WITH LOW DEOXYNIVALENOL AND KERNEL QUALITY RETENTION IN THE FUSARIUM HEAD BLIGHT RESISTANT CULTIVAR, ERNIE.

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OBJECTIVES

To identify QTL associated with low deoxynivalenol (DON) and kernel quality retention, and determine their relationship with QTL for type II resistance in the soft red winter wheat cross Ernie/MO 94-317.

INTRODUCTION

Fusarium head blight (FHB), caused by *Fusarium graminearum* Schwabe [telomorph: *Gibberella zeae* Schw. (Petch)] reduces grain yield in wheat (*Triticum aestivum* L.) in many regions of the world. Wheat grain produced from the infected head is shriveled, with low test-weight and can have a high percentage of damaged kernels that are contaminated with the mycotoxin deoxynivalenol (DON). Deoxynivalenol is linked to feed refusal in livestock (Meronuck and Xie, 2000) and causes depression of the immune system, nausea, and vomiting in humans (Prelusky et al., 1992). Host resistance is the most cost effective method to reduce both yield and quality losses associated with FHB and DON contamination in wheat. Breeders believe that selection of lines for low FHB may result in low DON and high kernel quality retention. However, reports on the association between FHB resistance and DON are mixed with some reports suggesting the traits are independent (Somers et al., 2003) while others suggest they are interdependent (Wilde et al., 2006).

Ernie, a soft red winter wheat developed at the University of Missouri (McKendry et al., 1995) has a moderately high level of type II FHB resistance. It also has low DON content in inoculated trials with high kernel quality compared to susceptible wheat varieties. Four QTL located on chromosomes 5A, 4BL, 3B and 2B are associated with type II FHB resistance in Ernie (Liu et al., 2006). The current study was designed to identify QTL associated with low DON and kernel quality retention in Ernie and to determine their association with the 4 QTL associated with type II FHB resistance.

MATERIALS AND METHODS

A set of 243 F_3 derived F_8 recombinant inbred lines (RILs), developed at Missouri from the cross Ernie/MO 94-317 were used for this study (Liu et al., 2005; 2006). Type II resistance was determined according to protocols outlined in Liu et al. (2006) in a greenhouse experiment with plants arranged in a randomized complete block design with 3 and 4 replications in 2002 and 2003, respectively. Eight plants per RIL per replication were evaluated in each experiment.

Kernel quality evaluation: Infected heads from each replication were harvested at maturity, and hand threshed to ensure all disease kernels were collected. Kernels from each replication were bulked within line and separated into five groups (sound, slightly, moderately, highly shriveled and tombstones). The number of kernels in each group was counted to precisely determine, the proportion of Fusarium damaged kernels in the head. Kernel quality was determined as the percentage of diseased kernels (i.e. shriveled plus tombstones) to the total number of kernels in the inoculated head. Evaluated kernels were bulked and ground with coffee grinder. Deoxinivalenol was quantified by Dr. Pat Hart at Michigan State University using the mycotoxin extraction kit Veratoxin for DON 5/5 (Veratox®).

Linkage map construction: Polymorphisms between Ernie and MO 94-317 were assessed using 64 *Eco*RI/*Mse*I amplified fragment length polymorphic (AFLP) primer pairs and 420 *Xgwm* and *Xbarc* simple sequence repeat (SSR) markers (Röder et al., 1998, Song et al., 2005). Polymorphic AFLP and SSR makers were used to construct the linkage map with Mapmaker, Version 3.0 (Lander et al., 1987) using the Kosambi mapping function. Markers were grouped with a LOD value of 3.0 and distance less than 37 cM and resulted in 46 linkage groups that were used for QTL analysis.

Statistical and QTL analysis**:** Deoxynivalenol and kernel quality data were subjected to tests of normality (Proc Univariate), homogeneity of variance (Bartlett's test), combined analysis of variance (Proc Mixed), and correlation analysis (Proc Corr) using SAS (SAS version 9.1, 2005). Entry mean-based broad-sense heritability was calculated from the combined analysis of variance. The minimum number of genes was estimated using Cocherham's (1983) modification of Wright's (1968) formula. Composite interval mapping (CIM) was done using WINQTLCART (Version 2.5). One-thousand permutations were performed (Doerge and Churchill, 1996) to determine critical thresholds for significance of QTL.

RESULTS AND DISCUSSION

Both DON and kernel quality data for 2002 and 2003 were continuously distributed indicating the quantitative inheritance of the two traits, but only kernel quality was normally distributed. The DON data were log transformed and reanalyzed (Fig 1). Error variances for both traits were homogeneous (Bartlett's test, $P<0.05$). Genotypic effects among RILs were highly significant ($P<0.0001$) for DON and kernel quality for both individual year and combined data. Mean DON values for Ernie and MO 94-317 were 3.6 and 81.3 ppm, respectively while that for RILs was 37.3 ppm. Mean kernel quality data for Ernie, MO 94-317 and RILs were 27, 85 and 48 % Fusarium damaged kernels. Broad-sense heritabilities for DON and kernel quality estimated from the combined ANOVA were 72% and 77%, respectively, (Table 1) indicating the existence of sufficient genetic variance to make improvement in the two traits. The minimum number of genes conditioning low DON and kernel quality were 3 and 4, respectively. Pearson coefficient of correlation for DON and kernel quality was highly significant ($r = 0.79$, $P<0.0001$).

Three QTL associated with low DON were detected accounting for 29.6 % phenotypic variation. These QTL were located on chromosomes 5A, 4BL and 3B explaining 9.5, 6.1 and 14 % of the total phenotypic variation (Table 1). Four QTL associated with kernel quality retention were detected accounting for 40.3 % of phenotypic variation. These QTL, located on 5A, 4BL, 3B and 2B accounted for 17.2, 6.4, 12.2 and 4.1% of the phenotypic variation in kernel quality. Kernel quality QTL on chromosomes 5A, 4BL, and 3B were co-located with those for DON. Although a fourth QTL for DON was identified on 2B that was co-located with the kernel quality 2B QTL, it was below the LOD threshold for significance.

Based on the QTL position, QTL for DON and kernel quality may be the same. The 4BL QTL for both traits is located at 0.01 cM and is linked with *Xgwm495*. On 5A both QTL are linked to *Xbarc 056* with just 1 cM position difference between the DON QTL and the kernel quality QTL. On chromosome 3B the DON and kernel quality QTL are about 8 cM apart. For both DON and kernel quality, the resistance allele is derived from the resistant parent, Ernie.

In this population, DON and kernel quality were correlated with type II FHB resistance with correlation coefficients of 0.87 and 0.84 ($P<0.0001$), respectively. Liu et al. (2006) identified 4 QTL associated with type II FHB resistance on 5A, 4BL, 3B and 2B which accounted for 19.6, 8.5, 14.3 and 4.0% of the phenotypic variation, respectively. Kernel quality markers identified on 4BL, 5A and 3B were consistent with those identified for type II resistance by Liu et al. (2006). Although the marker on 2B was not the same as that for type II resistance, it was closely linked. For DON, markers on 4BL and 5A were consistent with those identified for type II resistance. Consistent with the findings of Liu et al. (2006) the DON 3B marker was centromeric; however, it may differ from

the marker identified for type II resistance. No significant 2B marker was identified for DON.

Wilde and Miedaner (2006) demonstrated the possibility of selecting wheat lines with low DON content by selecting for FHB severity in the field using spray inoculation with *Fusarium culmorum*. Our results which show associations between QTL for DON and kernel quality with those for type II resistance support their findings and suggest that breeders may select for low DON and kernel quality retention based on type II resistance.

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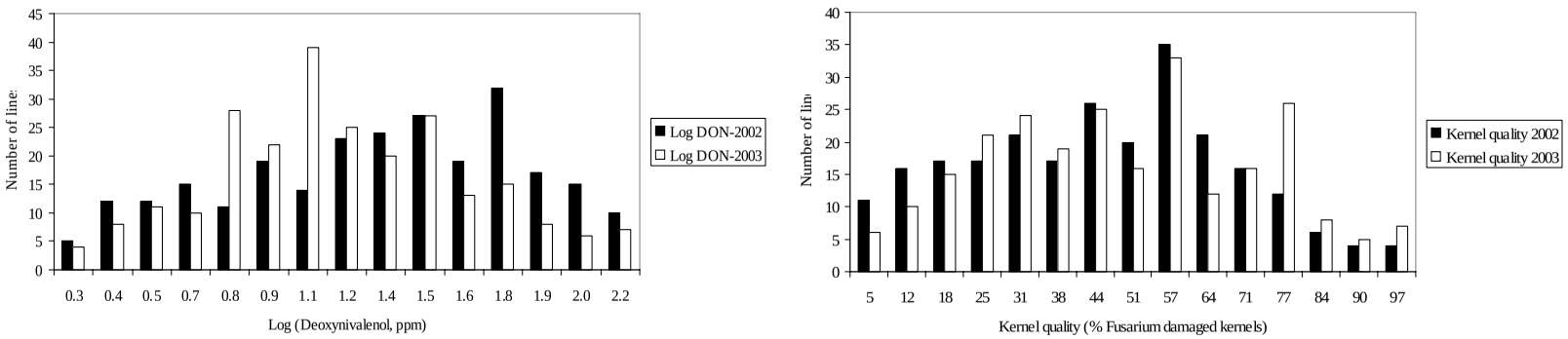

Figure 1. Frequency distributions of logarithm of deoxynivalenol (Log DON) and kernel quality (% Fusarium damaged kernels), in 2002 and 2003.

Table 1. Broad-sense heritabilities, gene number, and QTL associated with low deoxynivalenol (DON) and kernel quality retention in 243 $F_{3:8}$ and $F_{3:9}$ RILs developed from the cross, Ernie/MO 94-317. Data were collected following point-inoculation of RILs with Fusarium graminearum in the greenhouse in 2002 and 2003.

Traits	H^2_{BS} (%)	Number of genes	QTL locations	Peak QTL positions (cM)	Linked marker	LOD value	R2 (%)	Additive effects
			3B	121.70	Xe41m50_6	5.9	14.0	-0.14
			4BL	0.01	Xgwm495	4.5	6.1	-0.10
DON	72	3	5A	45.01	Xbarc056	3.8	9.5	-0.11
			2B	114.01	Xe36m50_3	3.5	4.1	-5.74
			3B	101.31	Xgwm285	8.0	12.2	-7.85
			4BL	0.01	Xgwm495	5.3	6.4	-5.92
Kernel quality	77	4	5A	44.01	Xbarc056	6.8	17.2	-8.63

TRANSFER OF A QTL FOR FHB RESISTANCE INTO HARD WINTER WHEAT USING MARKER-ASSISTED BACKCROSS.

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ABSTRACT

Epidemics of Fusarium head blight (FHB) can significantly reduce wheat grain yield and quality. Use of resistant cultivars is the most effective measure to control the disease. FHB epidemics have been severe in the central and northern Great Plains of the USA, but most hard winter wheat (HWW) cultivars currently grown in this area are highly susceptible to FHB. Some northern Great Plains HWW cultivars such as 'Darrell', 'Expedition', and 'Arapahoe' which have indigenous, unknown resistance performed better than highly susceptible cultivars in eastern South Dakota and Nebraska, but better level of performance in FHB impacted areas in Kansas, Nebraska, and South Dakota requires combining indigenous and QTL with major effect on FHB resistance into adapted HWW cultivars. Because there are large environmental variations associated with disease evaluation and the disease screening procedure is laborious, time consuming, and costly, progress in breeding for resistant HWW cultivars has been relatively slow using conventional methods. Due to the urgent need of FHB resistant cultivars in the Great Plains areas, a marker-assisted backcross project was initiated for rapid transfer of Chinese FHB-resistance QTLs into HWW grown in the region by use of the USDA high-throughput genotyping facility. Our objectives are to transfer the major QTL from Sumai 3 and other Asian sources into US HWW cultivars and to combine the major QTL with locally adapted minor FHB-resistance QTLs to develop marketable FHB resistant HWW cultivars and/or useful germplasm to minimize FHB damage in the hard HWW region. This is a collaborative project between the USDA Genotyping Center in Manhattan and three public HWW breeding programs in Nebraska, Kansas and South Dakota. The cross ND2928 (Ning 7840/ND706)/Wesley/Wesley was made at the University Nebraska and the crosses Harding/Sumai3/Harding and ND2710/Trego/Trego were made at the South Dakota State University. Using marker-assisted selection, 1000 Bc_1F_3 plants per population were screened for the 3BS QTL using 3 markers (GWM 389,GWM533, GWM493) and the 5A QTL using markers WMC705, WMC150 and Barc 180 (McCartney et al, 2004). About 40 plants per cross were recovered with at least all homozygous marker alleles for 3BS major QTL. Screening for 5AS markers was not very successful because of either non-polymorphism or a missing target band. Selected plants were subjected to AFLP analysis with 20 *EcoR*I/*Mse*I primer pairs to maximize genetic background of the recurrent parents. Five plants per population were selected based on cluster analysis of AFLP data for further backcross to the corresponding recurrent parents in the Genotyping Center. About 100 Bc_2 hybrid seeds from each backcross were harvested and advanced. About 3000 Bc_2F_2 seedlings were screened with 3BS markers early this year and 300 Bc_2F_2 plants homozygous for the 3BS QTL were selected from the three populations. The selected plants will be evaluated in the greenhouse and mist-irrigated fields for FHB resistance and other traits at three locations and by the Genotyping Center. The outputs of this research will facilitate rapid release of adapted FHB-resistant cultivars or new germplasm to help relieve FHB losses in the Great Plains.

EVALUATION OF RESISTANCE AMONG ADAPTED SPRING WHEAT GERMPLASM TO FHB INCITED BY SEVERAL *FUSARIUM* SPECIES.

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ABSTRACT

Though pathogenic races (as described by Flor) are apparently non-existent among fungal isolates able to incite Fusarium Head Blight on wheat, there are several unique species that can produce similar disease symptoms. Common resistance is a recently proposed hypothesis in which resistance levels among wheat lines are observed to be generally quite static when tested against different fungal species. Fusarium graminearum is the most prevalent FHB-causing species in the northern Great Plains; however, F culmorum, F. poae, and others are also present. The objective of this experiment was to test for the presence of common resistance in our region by inoculating several advanced experimental spring wheat breeding lines with four locally acquired Fusarium species. Results will be presented based on disease incidence, severity, and index values from tests performed in the greenhouse using a point inoculation procedure. These experiments will form the foundation by which we will explore whether the common resistance phenomenon is operative on germplasm from within our program.

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USING GENE EXPRESSION ARRAY TO DISCOVER SINGLE FEATURE POLYMORPHISMS FOR MAPPING OF FHB RESISTANCE IN WHEAT.

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ABSTRACT

Although several types of marker systems have been used for mapping of wheat resistance to Fusarium head blight (FHB), single nucleotide polymorphism (SNP) is an ideal future marker system for high-resolution maps, marker-assisted breeding and gene function study because it is the most abundant and informative marker system among all markers currently available. The abundance of SNPs increases the chances of finding markers that are tightly linked to a gene or QTL for resistance to FHB. SNPs found in intragenic regions can be used as perfect markers for selection of the gene/trait of interest. Such markers are powerful tools for marker-assisted breeding and gene isolation. Because different technologies have been available for high throughput genotyping of SNPs, the cost per data point can be very low when they are screened on a large scale. However, discovery of SNP in wheat is still in its infancy, limiting the application of SNP in wheat research. To discover potential SNPs, gene expression arrays have been successfully utilized to discover single feature polymorphisms (SFPs) in Arabidopsis and barley. To explore the possibility of using gene expression array for the discovery of SFPs in the complex wheat genome, we used Affymetrix Wheat Genome Array to screen six wheat varieties (Ning 7840, Clark, Jagger, Encruzilhada, Chinese Spring and Opata 85) of diverse origins. Among the 6 cultivars screened, Ning 7840 is highly FHB resistant and Clark is highly susceptible. A RIL mapping population is available for mapping of new SFPs that may link to FHB resistance. RNA was isolated from leaves and roots of 3-week-old seedlings and cDNAs from the six cultivars were hybridized to the wheat chips. Based on cluster analysis, a total of 396 probe sets with signal intensity of at least 200, p-value of < 1e-10 and overall R^2 >4 were selected for SFP confirmation through DNA sequencing. The result showed that the designed primers from 28 probe sets could amplify one DNA fragment from either Ning7840 or Clark in an agarose gel and these amplified fragments can be scored as dominant markers in the mapping population. To date, DNA sequencing has confirmed that 71 probe sets have SNPs within the probes that coincided with array data. The sequenced fragments were mostly 300-500 nt long and contained up to 20 additional SNPs outside the probe sequence. A total of 288 SNPs representing 90 genes have been discovered so far. Thirty-eight SNPs corresponding to different genes were further verified by SNaPshot analysis. The applicability of wheat SNP markers was demonstrated by genotyping RILs from the population of Ning/Clark. The new SNP markers will be mapped and integrated into an existing genetic linkage map derived from the population to saturate the map and to identify SNPs for high-throughput screening of FHB resistance.

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TRANSCRIPTOME ANALYSIS OF BARLEY AND WHEAT INFECTED WITH *FUSARIUM GRAMINEARUM*.

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ABSTRACT

Fusarium head blight (FHB), caused primarily by *Fusarium graminearum*, is a devastating disease of barley and wheat. The Barley1 and Wheat Affymetrix GeneChip probe arrays provide the opportunity to study in the parallel the expression patterns of 22,000 and 61,000 genes, respectively. Here, we provide a summary of the efforts being employed in our laboratory to explore the host response to *F. graminearum* infection. These analyses are primarily focused on identifying the essential genes and mechanisms involved in providing resistance. We have conducted six RNA profiling experiments including: (1) susceptible Morex barley inoculated with *F. graminearum*; (2) susceptible Morex inoculated with a trichothecene producing and non-producing strains of the fungus; (3) three near-isogenic line pairs containing resistant and susceptible alleles at QTL on barley chromosome 2H bin 8, chromosome 2H bin 13, and chromosome 3H bin 6 inoculated with *F. graminearum;* and (4) a near-isogenic line pair containing resistant and susceptible alleles at a QTL on wheat chromosome 3BS inoculated with *F. graminearum*. Overall, 4.5 million data points of transcript accumulation data have been generated. Other disease parameters such as deoxynivalenol and ergosterol concentration, *F. graminearum* infection histology and disease severity data have been or will be obtained for each of the experiments. An integrated picture of the transcript accumulation patterns along with the disease parameters in wheat and barley during *F. graminearum* infection will be presented. A comparison of the differences in transcript accumulation between the resistant and susceptible genotypes will also be presented. Finally, we will present some preliminary analyses of a comparison of wheat and barley responses to infection.

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RELATIONSHIP OF FUSARIUM HEAD BLIGHT FIELD SYMPTOMS AND KERNEL DAMAGE IN WHEAT.

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ABSTRACT

Fusarium head blight (FHB) is an important disease of small grains, causing a reduction in grain yield, shriveled *Fusarium* damaged kernels (FDK), and low test weight. The two most commonly studied types of resistance in wheat are resistance to initial infection and resistance to spread of infection. However, other types of resistance are hypothesized to exist, including resistance to kernel damage which is characterized by lines exhibiting a lower percent FDK than expected based on observed field symptoms. Twenty-four soft red winter wheat lines were chosen to study resistance to kernel damage. The lines were divided into two groups: 1) twelve lines with similar percent FDK and a range of field symptom ratings; and 2) twelve lines with similar field symptom ratings and a range of percent FDK. In 2006, the lines were grown in a mist irrigated, inoculated FHB nursery at Urbana, IL, and incidence, severity, and kernel quality were assessed for each line. An FHB index from 0 to 100 was used as an overall measure of field symptoms, where 0 is resistant and 100 is susceptible. Kernel quality was evaluated as a visual estimate of the percent FDK in a sample of grain. We observed a range of FHB index values within the set of lines where percent FDK was similar; lines in this group had percent FDK between 1% and 40% with an average of 13.4% FDK but exhibited a range of FHB index values between 3.1 and 60.9. For the second group of lines, lines with similar FHB index values tended to have similar FDK ratings. Based on our results, selection for low percentage of FDK should be possible and, in addition to field symptoms, FDK percentage should be evaluated in breeding for FHB resistance.

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GENETIC AND PHYSICAL MAPPING OF THE BARLEY CHROMOSOME 2(2H) *VRS1* REGION FUSARIUM HEAD BLIGHT RESISTANCE QTLS.

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INTRODUCTION

Developing barley with FHB resistance is problematic in that FHB severity and deoxynivalenol (DON) accumulation are negatively associated with desirable plant traits such as height and days to heading in resistant cultivar CI4196 and others (Urrea et al., 2002). We are helping to solve this problem with three converging approaches i.e. saturation genetic and physical mapping of the major FHB resistance QTLs located on barley chromosome 2H bins 8 and 10, development of isolines containing the resistance QTL in the absence of the undesirable agronomic traits such as tall and late heading, and mutagenesis to isolate variants that are agronomically acceptable such as early and semi-dwarf but retain the FHB resistance genes. In this manuscript we describe our current progress in mapping of the FHB QTL and recent mutant isolation and preliminary characterization. The isoline development is progressing, but accurate phenotyping requires extensive and repeated testing. Thus, definitive results will require another year of FHB testing.

A very strong FHB resistance QTL has been detected on barley chromosome 2(2H) bin 10, explaining 17 to 60% of the variation in Foster x CI4196 crosses (Horsley et al., 2006). A second QTL explaining 3 to 9% of the variation was detected on the same chromosome, bin 8. This general region (bins 5-10) also accounts for 25% of the variation in DON accumulation, with the highest value at the *vrs1* locus. Based on our work in collaboration with Rich Horsley we have chosen to focus on the region containing these loci flanked by the markers ABC306 and MWG882 (bins 8-10).

Horsley et al. also reported that there are two QTLs for plant height flanking the *vrs1* locus (2006). Thus a double cross-over would be required in a relatively short genetic region to isolate a normal height recombinant that still retains FHB resistance. In order to overcome this problem, we have developed backcross (BC) populations that allow us to select one recombinant at a time using molecular markers; these are Morex x FosCIA28 and Morex x FosCIA80. Preliminary results indicate that we have selected normal height 6-rowed recombinants. Testing to determine if they retain the FHB resistance is in progress.

Barley has been and continues to be one of the important species in mutagenesis studies. This is, in part, due to barley's diploid nature and seemingly extraordinary susceptibility to mutagens. Deletions are suitable for identification of the genes because they are not expressed and therefore mRNA isolated from the mutants does not hybridize to the microarray while the wild-type control does. Therefore, the genes residing in the deleted region can be visualized by differential hybridization (Zhang *et al*., 2006).

RESULTS AND DISCUSSION

The genetic and physical map in the chromosome 2H *Vrs1* region, roughly from BF263615 to MWG882, is now well saturated with molecular markers and we have identified multiple BAC clones (Fig. 1; Table 1). In order to saturate this region with markers, 29 rice

chr. 4 bacterial artificial chromosome (BAC) clones with synteny to this region were blasted against the barley expressed sequence tag (EST) database. Currently, 80 markers at 31 unique loci are associated with this region. Of these, 46 have been hybridized to the 6x cv. Morex BAC library and 37 have identified positive BAC clones giving us a physical map consisting of 200 clones (Table 1). These clones are part of 57 different contigs according to the BAC fingerprinting of the Tim Close lab at the University of California, Riverside (http://phymap.ucdavis.edu:8080/barley/index.jsp).

There remains a significant gap in the *Vrs1* distal region from marker BI955972 to MWG503. The reason for this gap is not known at this time. If it represents a region of no polymorphism between CI4196 and Morex or Foster, then it is not likely to harbor FHB resistance genes. Other explanations, however, are also possible.

To obtain additional markers, we mapped 378 DArT markers on the Foster x CI4196 map (unpublished). This map was merged with the existing Foster x CI4196 map and with other DArT barley maps developed by Andrzej Kilian's group resulting in a highly marker enriched barley genome map (Wenzl *et al.*, 2006).

The identification of candidate genes for FHB resistance and morphological characteristics has been emphasized in our studies. Results from the phenotyping of Morex x FosCIA28 and Morex x FosCIA80 suggest that the region between the *Vrs1* locus and the proximal marker ctg9802 (approximately the same location as ABG714B) is not necessary for FHB resistance. However, gene homologues of Far red impaired response, Myb transcription factors, Avr9-Cf9 elicitor, Ring Zn finger, Elicitor response gene 3, NBS-LRR-type, reductase protein, and auxin response factor 10 mapped to this region and may be involved in the undesirable morphological traits of CI4196 including increased height and late maturity.

The region distal of *Vrs1* seems to be low in gene density, based on both our mapping data and the report by Dr. Komatsuda (PAGXIV abstract) that during the cloning of *Vrs1* 4 BACs were sequenced and the only gene found was *Vrs1*. The rice syntenous region contains an AP2 domain transcription factor that is particularly interesting due to the involvement of AP2 type transcription factors in resistance to necrotrophic pathogens. However, this is a gene family and two homologs that we mapped go to chromosome 1(7H) and 7(5H).

Approximately 1 lb of CI4196 seed (from Rich Horsley) was irradiated with 4.5 Gy fast neutrons last spring and grown at Pullman WA (summer '05). Individual M1 heads and bulk M2 seed were harvested (summer '05). A bulk M2 field was grown at Pullman, WA (summer '06) and screened for morphological mutants. Jerry Franckowiak spent a few days at Pullman to help look for mutants. Some potentially useful mutants identified this year include 6-rowed, semi-dwarf, early maturity, lax spike, and upright spike. These have been confirmed as CI4196 based on molecular markers at seven unique loci and will be BCed and mapped to determine if they are from the target region. Those that are will be examined for hybridization to the Barley 1 microarray to identify deleted genes and will be phenotyped for FHB resistance.

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Table 1. All BAC clones currently associated with markers on Figure 1.

Markers	***Associated BAC Clones***
BE194244	52L22, 395n21, 475e12, 476n18, 634h22, 792h1, 792o18, 798j10
BE194424	5d23, 48l7, 67b23, 87b21, 154h7, 316l2, 352d6, 399j5, 449n22, 476f2, 478i15, 499h2, 515j23, 522f23, 601b23, 623c3, 624k7, 674d13, 675o3
BE214081	26m13, 241L2, 539k9, 535k20, 670n8, 698e15, 714c10, 804m20
BE215806	177g21, 178k22, 480c15, 526j3, 534i7, 605n2, 658h23, 771c11
BE216598	216e9, 474o3
BE455758	44b3, 55n21, 87b21, 112b5, 310o19, 352d6, 360o22, 393m6, 399j5, 457h2, 476f2, 499h2, 515j23, 648i4, 675o3, 803o17
BE558794A	385h21
BE558794B	385h21
BE602662	60e22, 183f22, 416L21, 450j21, 461d11, 542i17, 797c10
BF064573	71h20, 266g2
BF254012	287j18, 778k20, 796p6, 21i19, 813i15, 51L22, 206d10, 643h10, 675c2, 769L22
BF254076	22n22, 41j6, 397i12, 523b21
BF263615	459j1
BF267331	715o19, 736f12
BF621513	52a11, 184d13, 256n8, 536e17, 557i19, 792j23
BF622472	512L17, 712p3, 712p6, 715d12, 718g1, 721a23
BF623140	22n22, 41g17, 41j6, 184c17, 397i12, 523b21
BF625659	131n15, 485L14, 706o4, 727j5
BF628601	26h9, 48c3, 49m21, 54o20, 206L22, 236b9, 313n3, 325i12, 342o9, 345n24, 345p23, 351j8, 374k10, 375e12, 375g16, 472a20, 511d22, 523o24, 552b9, 749o7
BF628983	508o22
BG299611	286i1, 703a2
BG300704	384n8, 365e4, 536L11, 672d23
BG365406	287j18, 703a2
BG369432	10m15, 647h2, 134c3
BG369629	102p11, 384i7, 406d16
BG414848	133c13, 814d5
BG416824	112i1, 779a4
BG417014	82i4, 127d9, 559k19, 647k12, 699b21
BG418734	71h20
BI952770	145i1, 185d9, 214o16, 478a12, 606a4, 771c11, 551L22
BI955797	376j4, 465h17, 605b24
BI955972	42a11, 82i1, 116k4, 497h8, 592d23, 785d15, 152L21, 399d9, 523i16, 487f8, 785f2
BI959927	71h20
ctg37907	779a4, 346o12
MWG699	72c21, 75f19, 99m7, 108k15, 224b23, 422L15, 454a7, 559n5, 647k20, 751i5, 768n17
MWG865	485L14, 88f9, 131n15, 727j5, 436p4
myb	131j9, 143j23, 651f16, 813i15

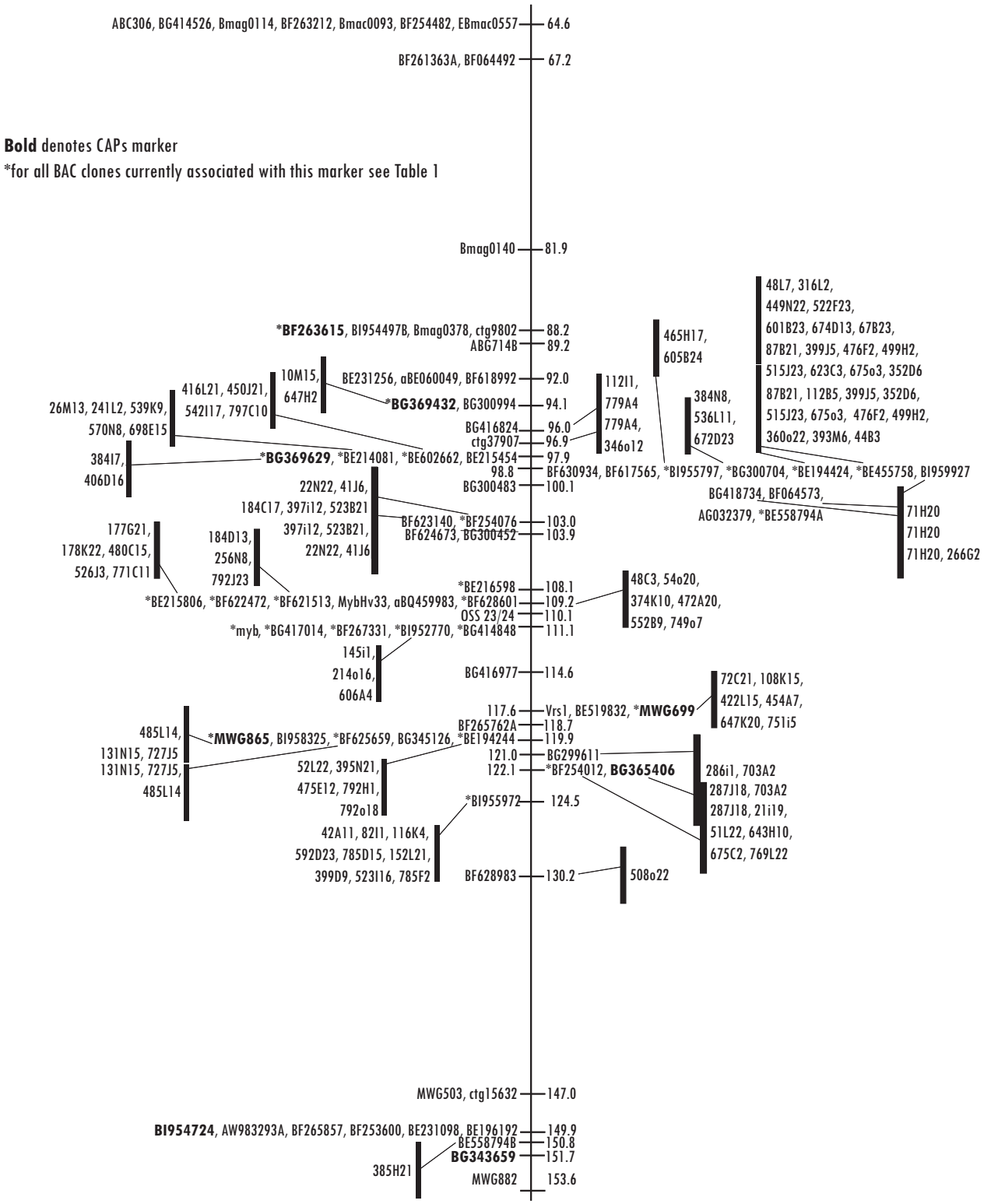

Fig. 1
Chromosome 2(2H) map for bin 8 to 10 with emphasis on the *Vrs1* region. The map was constructed from 56 recombinants selected from the Foster x CI4196 mapping population. The heavy vertical lines indicate BAC clones that have been identified with the markers and the numbers next to them indicate BAC clone addresses. Measurements are in centimorgans and so represent genetic rather than physical distances.

PROGRESS IN DEVELOPMENT AND MAS OF FHB RESISTANT WHEAT CULTIVARS AND GERMPLASM AT VIRGINIA TECH.

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ABSTRACT

To accelerate development of high yielding, FHB resistant SRW wheat lines, we have deployed a combination of top-cross, doubled haploid, backcross and marker assisted breeding methods. During the 2006 crop year, molecular markers linked to FHB resistance QTL located on wheat chromosomes 3BS, 5AS, 3AS, and 6B were used in haplotype analysis of FHB resistance in 56 wheat parents, 120 three-way F_1 progeny, 83 BC_1F_1 progeny, and 145 wheat lines in the 2006 VT FHB Advance and Preliminary Tests. Elite wheat lines having known haplotypes for target marker alleles of validated FHB QTL provide breeding programs with not only a unique source of adapted FHB resistant parents, but also knowledge of selectable markers that can be used to transfer and pyramid such QTL. Haplotyping of parental and advanced lines for known FHB QTL markers is an effective and complementary strategy to phenotypic selection for FHB resistance and, therefore, can be used to accelerate cultivar development.

VA02W-713, a top-cross (Ning7840/Pioneer2691//Roane) derived elite FHB resistant SRW wheat line, ranked 1st in grain yield (77 Bu/Ac) among 54 entries in Virginia's Advance Wheat Test over three locations in 2004. This line also performed well in Virginia's 2005 and 2006 State Variety Trials at six locations and ranked 10th out of 45 entries in the 2006 USDA-ARS Uniform Southern SRW Wheat Nursery over 21 state locations in 2006. Breeder seed of this line is being developed in anticipation of cultivar release in 2008. This line has high grain yield and good FHB resistance with target alleles for markers Xgwm493 on 3BS, Xbarc45 and Xgwm674 on 3AS, and Xgwm508 on 6BS. An additional five lines VA04W-389, VA04W-433, VA04W-474, VA04W-571, and VA04W-592 are potential germplasm releases having good FHB resistance with target marker alleles for at least two QTL on chromosomes 3BS, 5AS, 3AS, and 6B. We also identified two native sources (Massey and VA00W-38) having good FHB resistance with target marker alleles in at least two QTL regions. These and other VT FHB resistant lines are being used as parents in several breeding programs and in pyramiding multiple QTL in adapted wheat backgrounds in our breeding program.

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EVALUATION OF ELITE BREEDING LINES FOR FUSARIUM HEAD BLIGHT (FHB) RESISTANCE.

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ABSTRACT

Thirty six elite breeding lines from breeding programs in the southeast region including 8 from Arkansas, 4 from North Carolina, 6 from Virginia, and 6 from University of Georgia were evaluated with Ernie and Coker 9835 as resistant and susceptible control respectively under misted conditions in Griffin-Campus, Georgia. Ten lines showed similar level of resistance as the resistant control and 24 lines were significantly higher severity than the resistant check, Ernie. A Virginia line, VA05W-500, from a cross of Roane / PIO 2684 // OH 552 showed the best and consistent resistance among all three replications in 36 lines. VA05W-500 showed significantly higher level of resistance than other lines including the resistant control. Many crosses have been made using Sumai 3 or its derivatives as FHB resistant donors. However, FHB resistance could be enhanced significantly through combining the native resistance in soft red winter wheat germplasm. The negative yield drag associated with crosses including exotic germplasm such as Sumai3 or its derivatives could be avoided. Among the ten resistant lines, six lines, GA981621-5E34, GA98401-5E23, GA98401-5E23, AR 97124-4-3, VA05W-498, and LA98090D34-4, were from crosses of native resistant germplasm, and four lines, AR 97002-2-1, ARGE97-1064-11-5, NC03-11465, NC04-27618, were from crosses of exotic resistant germplasm. Native resistant germplasm for FHB resistance should play an important role. Study on the native resistance for FHB is needed for more efficient accumulation of native resistance into local adaptive cultivars.

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COMPLEMENTARY SCREENING TECHNIQUES FOR SELECTION OF BARLEY BREEDING LINES WITH IMPROVED REACTION TO FUSARIUM HEAD BLIGHT.

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ABSTRACT

Selecting malting barley (*Hordeum vulgare*) for resistance to Fusarium head blight (FHB), principally caused by *Fusarium graminearum*, has proven to be a difficult proposition. This can be partially attributed to limited sources of highly resistant germplasm, complex gene action of available sources of resistance and /or linkage to undesirable genes or pleotrophic effects.

These significant genetic constraints aside, we believe the major limitations on progress are related to large environmental effects on the expression of the disease, relatively large error variance associated with subjective visual ratings, and quantitative errors principally associated with sample size for deoxynivalenol (DON) content and mycelium content and the very real possibility of 'escapes'. The need to screen large numbers of breeding lines and the associated cost may limit the number of replicate samples that can be submitted for quantitative analysis from breeding programs. Even if the error variance of a given location were carefully controlled, with replications or other control measures, the large environmental effect generally renders data from a single location less valuable than initially apparent for the selection of lines with improved performance over a broader range of environments. Inoculated and misted nurseries are considered a 'necessary-evil' to ensure a higher likelihood of obtaining some results in years not favorable to disease development. The assumption that inoculated and misted nurseries are representative of natural infection conditions may not always be valid, and there are clearly subtle differences in genotype response depending on the mode of inoculation, such as infected corn spawn vs. conidial suspensions.

The best estimate of a line's reaction to Fusarium is obtained only after several location years of testing. However, it is not uncommon, particularly during preliminary screening, to have only one location of valid data.. In this circumstance, using multiple methods to quantify the variety reaction would appear to offer a better method of identifying improved lines for further screening and use in the breeding program. Beginning in 2003,we have tried to select lines for advancement using an index based on the combined response to three separate estimates of disease; FHB visual scores, grain DON content and grain mycelium content. Data analysis must be conducted with care as the results from these traits are frequently not normally distributed. DON content in particular typically fits a beta-distribution rather than a normal distribution. Correlation coefficients' between these three traits are typically in the 0.15 to 0.65 range. These moderately positive correlations show that the three traits tend to behave similarly but clearly are not equivalent, which confirms that using an index of all three traits may be more appropriate than a single evaluation method alone. This poster will examine a simulation of the ability of multiple methods of disease evaluation at a single site to predict variety performance over a range of environments by comparing the current responses of several advanced lines at the Crookston, MN evaluation nursery in 2006 with their corresponding rankings based on several prior location x years of data.

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

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ABSTRACT

Scab of wheat, caused by *Fusarium graminearum*, is a disease that periodically strikes the US mid-Atlantic region. Breeding for resistance is an effective measure of disease control. The objective of this study was to develop scab resistant soft red winter wheat germplasm adapted to the US mid-Atlantic region using marker-assisted selection. To breed scab resistant germplasm that are adapted to the Mid-Atlantic region, a high-yielding wheat cultivar, McCormick, was used in a backcross program with the Chinese variety Ning7840. Scab resistant germplasm were bred using an accelerated backcross scheme developed to incorporate scab resistance QTLs found on chromosomes 3BS and 5A in the Chinese variety Ning7840. Two rounds of backcrossing were completed using McCormick as the female parent. Progenies from the first round of backcrossing were selected for the presence of the Ning7840 scab resistance alleles at 3BS and 5A, and then for a high background of McCormick alleles. Initially, 600 BC1F1 progenies were screened, 116 had the Ning7840 alleles at marker loci barc147 and gwm533. These loci are linked on chromosome 3BS and separated by 7.4 cM. Additionally, the 116 progenies also had the Ning7840 alleles on chromosome 5A at marker loci gwm304. All three markers showed no segregation distortion. The 116 progenies were further screened with 3BS SSR marker cfd079 and 5A SSR marker wmc705. Cfd079 was observed to be 6.2 cM from gwm533. The two marker loci on 5A, gwm304 and wmc705, were separated by 3.5 cM. Another screened marker, gwm272, was unlinked on chromosome 5DS. Furthermore, additional markers were screened to select progenies that had mostly McCormick background. Two backcross progenies had over 60% McCormick background. Using these two selected BC1F1s, 400 BC2F1s were produced in a second round of backcrossing. Additionally, the two selected BC1F1s were crossed with a wheat line with stripe rust resistance (GA96229-3A41). The BC2F1s are currently being screened with molecular markers to identify those with Ning7840 alleles (on 3BS and 5A) and most McCormick background. Selected BC2F2s populations derived from selected BC2F1 plants will be further screened with 3BS, 5AS, and 2DS markers to select those homozygous resistant (Probability: 16:1,000 for 3 loci). Additionally, we plan to derive near-isogenic lines from an F2 population to identify the effect of each QTL on scab resistance, agronomic and quality traits. We anticipate having a small amount of seed of selected BC2F3s, containing the Ning7840 alleles in the McCormick background, available for distribution to other soft red winter wheat breeders for crossing in the fall of 2008.

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EFFECT OF THE 3BS REGION OF NING 7840 ON AGRONOMIC TRAITS IN SOFT RED WINTER WHEAT.

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ABSTRACT

US Winter wheat breeders are attempting to incorporate scab resistant alleles of the Chinese cultivar Ning7840 into US wheat. The main Quantitative Trait Loci (QTL) on chromosome 3BS of the Ning7840 line that confers resistance to scab can be tracked by three Simple Sequence Repeat (SSR) markers: barc133, gwm493, and gwm533. This study was conducted to determine the effects of the 3BS region of Ning7840 on three agronomic traits: height, heading date, and powdery mildew resistance in crosses with two soft red winter wheat genotypes. In the fall of 2005, 88 F8 recombinant inbred lines of the cross Pioneer 2643/Ning7840 and 66 F8 recombinant inbred lines of the cross Pioneer 2684/Ning7840 were planted in Queenstown, MD. In the spring of 2006, the progenies were scored for heading date, plant height, and resistance to powdery mildew. Transgressive segregation was observed for all three traits, and was especially prominent in powdery mildew resistance. Seeds were set aside for DNA analysis and scored for polymorphisms for the SSR markers barc133, gwm493, and gwm533. Mapping of the SSR markers with Mapmanager software confirmed previous findings that these three markers are closely linked. For the Pioneer 2643/Ning7840 cross, gwm493 and barc133 were 11.2 cM apart and barc133 and gwm533 were 8cM apart. For the Pioneer 2684/ Ning7840 cross a distance of 10.4 cM was observed between gwm493 and barc133, and there was not enough polymorphism to map the gwm533 marker. Linear regression analysis indicated that variation in the three agronomic traits was not significantly affected by the presence of the Ning7840 alleles. Correlation analysis further indicated that the traits and the markers are unlinked. The t-tests of the mean value for each marker class and the three traits were not significant. There appears to be no linkage between the 3 SSR markers and height, heading date, or powdery mildew resistance. Thus, introducing the 3BS region of Ning7840 had no negative effect on these traits. A large number of transgressive segregants, highly resistant to powdery mildew, suggests that other unlinked alleles present on Ning7840 may be beneficial for powdery mildew resistance.

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SINGLE KERNEL SORTING TECHNOLOGY FOR ENHANCING SCAB RESISTANCE AND GRAIN QUALITY.

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ABSTRACT

We developed automated visible and near-infrared (NIR) spectroscopy procedures and instrumentation to select kernels with specific hardness, protein, and color traits to enhance the development of scab resistant hard and soft wheat varieties. The system also shows potential to sort for other characteristics such as scab damage, vomitoxin levels, ergosterol levels, vitreousness, sprout damage as measured by alpha amylase content or falling number, moisture content, selenium content, Karnal bunt-infected kernels, and waxy character. Our single kernel near-infrared system can sort single kernels based on specified properties at a rate of about one kernel/2s (500-1000g/day). We also have high-speed sorting technology that can sort visible defects at rates as high as 80,000 kernels/s (300 bu/hr). This technology is now used routinely for such applications as purifying red or white breeding lines, removing Karnal bunt-infected kernels during routine inspection for the APHIS national surveys, and selecting waxy seeds from segregating populations. While most of our work has been with wheat, we have also shown applications for proso millet, barley, rice, and sorghum. This poster will report the development of this NIR-based sorting system and our sorting accuracies.

A NOVEL APPROACH TOWARDS MOLECULAR CHARACTERIZATION AND PYRAMIDING OF NOVEL SCAB RESISTANCE SOURCES.

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ABSTRACT

Genetic studies on potentially new sources of resistance such Wangshuibai unexpectedly have not yielded novel major resistance QTL regions beside the 3BS QTL mapped in Sumai 3. These type of studies based on Recombinant Inbred Lines populations are time consuming (counting with population development, phenotypic evaluation and molecular mapping) and therefore expensive. Results from genetic diversity studies have been misleading, like in the case of Wangshuibai with no relationship with Sumai3 and completely different haplotype in the 3BS QTL region, yet containing that resistance QTL. In addition the use of unadapted germplasm as source of resistance has delayed the incorporation of resistance genes into high yielding, high quality adapted germplasm. Here, we propose the utilization of family-based genetic analysis, a 'novel' approach in plants, to study two unrelated sources of resistance (SD3942, and SD3934) adapted to the growing condition of the northern plains. This methodology will allow us to identify the genomic regions responsible for the resistance in these lines at the same time that these lines are incorporated into the HRSW breeding program at SDSU. Molecular markers identified as linked to resistance loci will be use in Marker Assisted Selection approaches expedite the development of resistance cultivars by pyramiding the resistance from SD3942 with Sumai 3 derived resistance.

The first year of the project, we will focus in adopting the procedure and the analysis of SD3942. This line has no pedigree in common with Sumai3 or Wangshuibai, yet has better resistance than Alsen or Steele-ND.

The family-based mapping approach is used frequently in human genetics, therefore the data analysis will be straight forward after adapting the methodology to wheat. This approach is based in the co transmission of marker and trait from a heterozygous parent to its progeny. The statistical test is based on a binomial distribution.

Therefore, we expect to identify the genomic region/s responsible for the resistance in SD3942, develop early generation breeding material combining this resistance source with other sources of resistance (ie. Sumai 3), and develop molecular markers linked to the resistance loci in SD3942 to aid in selection.

IS THERE VALUE IN QUANTIFYING *FUSARIUM MYCELIUM* FOR BREEDING FHB RESISTANCE?

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ABSTRACT

Previously we described a system of quantifying Fusarium Head Blight (FHB) in barley by ELISA. ELISA had lower variability (lower CV's) than visual scoring or deoxynivalenol (DON) analyses. Thus we tested ELISA, DON, and visual assessment of FHB in 1) selections from a barley doubled-haploid mapping population grown in two environments and 2) the North American barley scab evaluation nursery (NABSEN) grown at four locations. All methods of evaluation had genotype x environment interactions typically found in FHB experiments. Scattergrams of ELISA vs. DON estimates of FHB and DON vs. visual estimates of FHB suggest visual symptomology is not correlated with abundance of *Fusarium* in mature grain or the DON content following harvest. Samples low in ELISA were also low in DON. We conducted laboratory experiments to explain how environmental parameters might affect DON production by *Fusarium graminearum*. In addition we tested for abundance of the antigen specific to the monoclonal antibody used in the ELISA analysis across *Fusarium* species within the B clade (O'Donnell et al., 2004) and in mycelium grown under varying laboratory conditions. There was a temperature by osmotic potential effect on DON production in laboratory-grown cultures of *Fusarium* spp. even though growth of the fungus increased with temperature. Temperature, osmotic potential, or *Fusarium* species had no effect on abundance of antigen in mycelium of the fungi when grown *in vitro*. Therefore, ELISA is a more robust estimate of fungal infestation than FHB or DON individually, and may provide a practical alternative to dual testing for FHB and DON in plant breeding and genetic programs.

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EVALUATION OF SOFT RED WINTER WHEAT LINES FOR RESISTANCE TO MYCOTOXINS AND KERNEL INFECTION: A PROGRESS REPORT.

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OJECTIVES

The objectives of this research are to identify sources of resistance that reduce the levels of deoxynivalenol and/or nivalenol in grain, to develop techniques for efficiently selecting this type of resistance, and to determine the relationships among the different variables used to evaluate resistance to Fusarium head blight.

INTRODUCTION

Cultivars and advanced breeding lines of soft red winter wheat with resistance to Fusarium head blight (FHB) have been developed over the past ten years, but these resistant lines appear to differ in their ability to reduce the level of deoxynivalenol (DON) in the grain. Discovery in the Mid-south of *Fusarium* strains that produces nivalenol rather than DON (Gale et al. 2005) necessitates having resistance to both DON and nivalenol in wheat cultivars adapted to the Midsouth. Resistance to kernel infection and/or late-season infection may be important for achieving low levels of mycotoxins in grain.

MATERIALS AND METHODS

Thirty-five diverse lines with resistance to FHB were planted in the field in a randomized complete block design with four replications at Fayetteville, Arkansas. The experiment was inoculated with infested corn and misted daily to promote disease. Each plot was evaluated for flowering date, FHB incidence, average head severity and average plot severity. After harvest, the percentage of Fusarium-damaged kernels (FDK) was determined and level of DON in the grain was determined at Michigan State University. The percentage of kernels infected by *Fusarium graminearum* will be determined by plating 200 surface-disinfested seeds on peptone-pentachloronitrobenzene (PCNB) agar. Type II resistance (resistance to pathogen spreading in heads) was evaluated in the greenhouse on three pots per line.

RESULTS AND DISCUSSION

Of the 35 evaluated lines, a set of 12 lines (Table 1) was chosen for further evaluation based on local adaptation, level of resistance in the field and greenhouse, and diversity of resistance sources. Having similar flowering dates among lines is critical for FHB evaluations in the field, and these selected lines flowered within a 4-day period. All selected resistant lines were significantly more resistant than the susceptible check (Coker 9835) for most but not necessarily all FHB variables evaluated. Significant differences among resistant lines for some FHB variables suggest that the lines contain different genes that confer different types of resistance. Sources of FHB resistance among the selected lines include native (Freedom, Ernie, Roane, Bess), Chinese (Ning 7840, Sha 3, Ning 8026), CIMMYT (Catbird) and European (Super Zlatno) sources of resistance.

FHB incidence was the best predictor of DON level in the grain (Fig. 1A). Plot severity (Fig. 1B), FDK (Fig. 1C), and level of type II resistance (Fig 1D) were poor predictors of DON level in grain. Although Roane was significantly different from Coker 9835 for plot severity, FDK, and type II resistance, its level of DON was similar to Coker 9835. ARGE97 1047-4-2 had poor type II resistance but was among the lowest for DON level.

A greater understanding of how to select for resistance to mycotoxins is needed to develop cultivars that have low levels of mycotoxin accumulation in grain under conditions favorable for FHB. The 12 lines selected in this study appear to be suitable for identifying sources of resistance to mycotoxins, determining the relationships among different variables used to measure FHB resistance, and for developing efficient methods of selecting resistance to mycotoxins. To determine if lines resistant to DON accumulation are also resistant to nivalenol accumulation, these 12 lines will be inoculated in the greenhouse with two DON-producing isolates and two nivalenol-producing isolates. To determine if aggressive isolates capable of producing high levels of a mycotoxin can overcome resistance to mycotoxin accumulation, the DON- and nivalenol-producing isolates for the experiment will be chosen to represent low and high levels of mycotoxin production. To determine if the DON-bleached floret method is useful for selecting lines that are resistant to DON accumulation, these 12 lines will be inoculated with purified DON as described by Lemmens et al. (2005). To determine if any of these lines have resistance to late-season infection and mycotoxin accumulation, the lines will be evaluated in a separate nursery that will be inoculated and misted near physiological maturity. To determine if any of these lines have resistance to kernel infection, seed from each field experiment will be evaluated for level of kernel infection.

ACKNOWLEDGEMENTS

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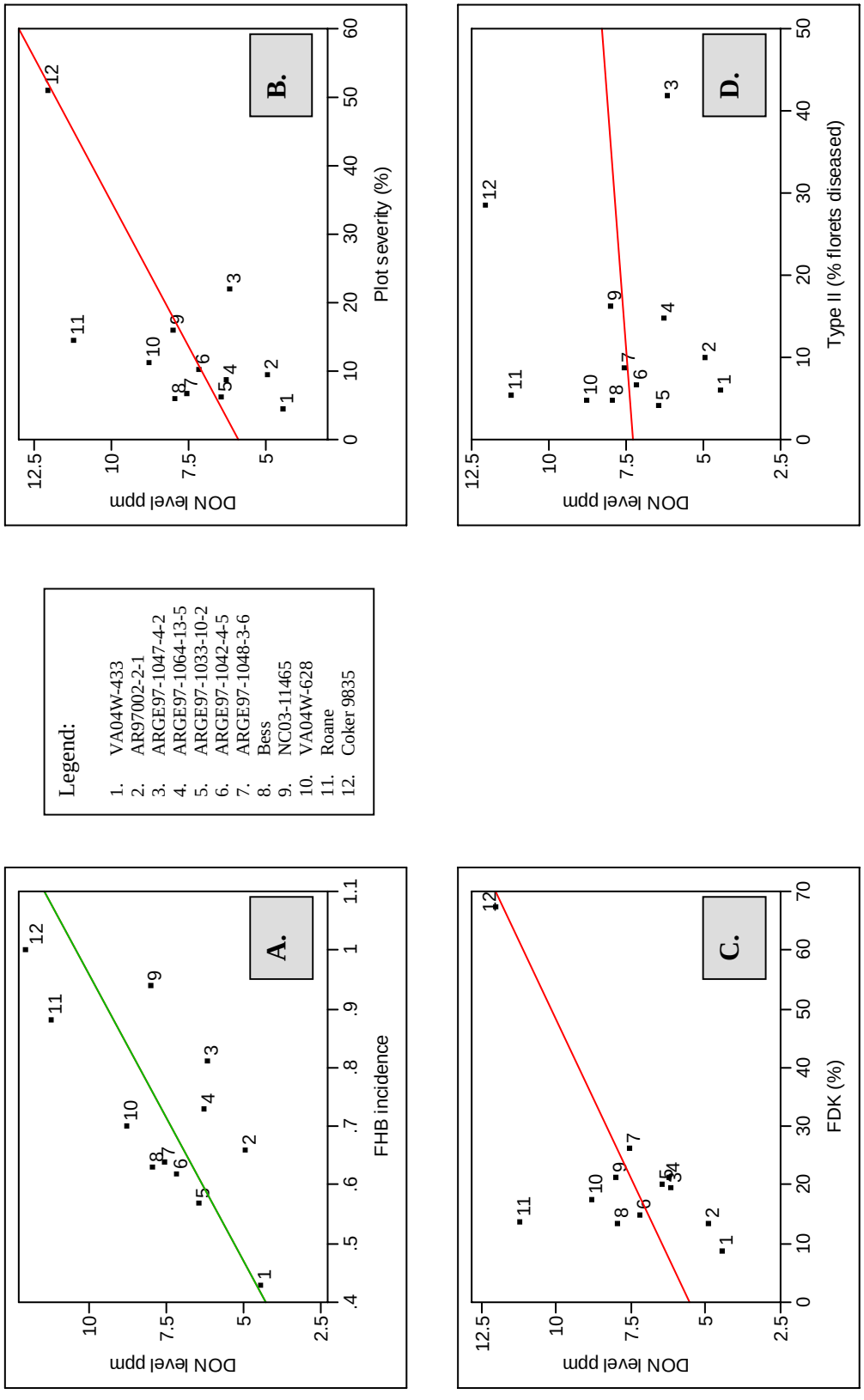

Figure 1. Relationships of DON level to other measures of Fusarium head blight resistance. A) FHB incidence, y= 10.2x + 0.2, P= 0.007, R^2= 0.54; B) Plot severity, y= 0.12x + 5.88, P= 0.019, R^2= 0.43; C) FDK, y= 0.09x + 5.53, P= 0.031, R^2= 0.39; and D) Type II resistance, y= 0.02x + 7.27, P= 0.747, R^2=0.01;

Table 1. Soft red winter wheat lines selected for further evaluation based on local adaptation, level of FHB resistance in the field and greenhouse at Fayetteville, AR, in 2006, and diversity of resistance sources.

Line	Pedigree	DON level ppm[1]	Flowering date in April[2]	FHB incidence[1]	Average head severity[1] (%)	Plot severity[1] (%)	FDK[1] (%)	Type II[3] (% florets diseased)
.04W-433	NING 7840/PION2684//96-54-244 (CK9803/FREEDOM)	4.38	11.9	0.43	9.7	4.6	8.8	6.0
.97002-2-1	AR396-4-2/NING 8026	4.88	13.4	0.66	14.0	9.6	13.5	10.0
.GE97-1047-4-2	P2643 / 3 NING 7840 // PARULA / VEERY # 6	6.13	14.5	0.81	25.2	21.9	19.5	41.8
.GE97-1064-13-5	MASON//FREEDOM/SUPER ZLATNO	6.20	14.9	0.73	12.2	8.8	21.3	14.7
.GE97-1033-10-2	FREEDOM/CATBIRD (G82)	6.38	14.8	0.57	11.3	6.3	20.0	4.2
.GE97-1042-4-5	MASON / CATBIRD	7.13	13.4	0.62	15.9	10.3	15.0	6.7
.GE97-1048-3-6	MASON // SHA 3 / CATBIRD	7.50	15.1	0.64	10.5	6.7	26.3	8.7
ss	MO 11769/Madison	7.90	14.4	0.63	8.9	5.9	13.3	4.8
03-11465	NING 7804/P2643//NC95-22426	7.93	16.1	0.94	16.9	15.9	21.3	16.2
.04W-628	ERNIE//NING7840/ERNIE	8.75	13.5	0.70	15.2	11.3	17.5	4.7
ane	VA71-54-147 (CI17449)/Coker68-15//IN65309C1-18-2-3-2	11.18	15.1	0.88	16.2	14.6	13.8	5.4
ker 9835	Susceptible check	12.00	13.8	1.00	51.1	51.1	67.5	28.6
	LSD (P= 0.05)	4.2	—	0.24	—	8.8	9.6	11.4

[1]Averaged across four replications in FHB nursery at Fayetteville.

[2]Averaged across four replications in each of two fields at Fayetteville.

[3]Averaged across 3 pots with 5 to 8 heads/pot

EVALUATION OF HARD WINTER WHEAT FOR FHB RESISTANCE IN SOUTH DAKOTA.

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ABSTRACT

South Dakota is a primary state in the US Great Plains hard winter wheat region that is threatened by Fusarium head blight (FHB) [caused by *Fusarium graminearum* Schwabe [teleomorph *Gibberella zeae* (Schwein) Petch]. A mist-irrigated field nursery consisting of 257 advanced lines, including the Northern Regional Performance Nursery (NRPN), Crop Performance Testing Variety Trial (CPT), Advanced Yield Trial (AYT), and Preliminary Yield Trial (PYT) was transplanted in May 2006 and evaluated in July 2006. The 28 CPT lines varied significantly ($P < 0.05$) for disease index (incidence percentage*severity percentage/100). Mean disease index in the CPT was 46.5%. The hard red winter wheat (HRWW) 'Darrell' had the lowest disease index (11.6%) followed by SD02279 (18.8%). On the other hand, SD01122 had the highest disease index (89.8%). Darrell was released in 2006. It has the second best FHB rating among all Great Plains HRWW varieties tested in South Dakota during the last six years, next to 'Expedition'. It ranked top for yield in the CPT in 2006 and had an exceptional three-year yield average. It had exceptional performance in the NRPN in 2003 and 2004. It has acceptable milling, good baking quality, and a good diseases package. About 3,800 head-rows and 51 Early Yield Trial (EYT) entries with tagged FHB QTL sources were planted in the '06 – '07 season. Best lines out of the head-row nursery will be included in the EYT in 2008. Resistant lines will be entered into regional nurseries to facilitate development of varieties with broad adaptation to South Dakota and the northern Great Plains.

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IDENTIFICATION OF QTLS FOR TYPE II RESISTANCE TO FHB IN THE NOVEL WHEAT GERMPLASM CJ 9306.

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OBJECTIVES

To identify the QTLs associated with Type II resistance to Fusarium head blight in the novel wheat germplasm CJ 9306.

INTRODUCTION

Fusarium head blight (FHB or scab) caused by *Fusarium* species is one of the most destructive diseases in wheat and barley worldwide. Development of resistant cultivars is the most economic, effective and environment-friendly approach to control this disease. QTL mapping and marker-assisted selection enhance the efficiency of utilizing elite germplasms and breeding resistant cultivars. CJ 9306 is a novel wheat germplasm superior to Sumai 3 in both FHB resistance and agronomic performance (Jiang et al., 2006). It was developed through multiple-parent crossing and recurrent selection combined with modified pedigree methods with the aid of a dominant male-sterile gene *Ta1* (*ms2*). The original parentage included five local superior cultivars and 15 most important resources of resistance to FHB and/or other major diseases from China and other countries (such as Sumai 3, Wangshuibai, Fanshanxiaomai, Wenzhouhongheshang, Emai 9, Zhen 7495, Ning 7840, Nobeokabuzu, Shinchunaga, Frontana, Jinzhou 1, etc.). Recurrent selection has a special advantage in accumulating multiple genes and creating desired gene combinations. Conventional genetic studies indicated that the resistance in CJ 9306 was predominantly inherited as a quantitative trait with both major and minor genes/QTLs (Jiang and Ward, 2006). Because of its excellent resistance, unique history of breeding, and complex parentage, characterization of its FHB resistance by DNA markers is very useful and significant for understanding of the underlying genetic basis and effective utilization of this novel elite germplasm.

MATERIALS AND MEHTODS

A set of 152 $F_{6:7}$ RILs derived from a wheat cross Veery/CJ 9306 and two parents were used to evaluate FHB resistance. The RILs were grown in the greenhouse at Michigan State University in a completely randomized design with two replications. For each line, six plants were planted in two pots, each having three plants per replication. The two parents were planted as the controls many times at an interval of 1 wk. The experiments were repeated three times, sown in December of 2001, January of 2002 and November of 2003, respectively, and designated as Experiment 02, 02a and 04. Single-floret inoculation was conducted immediately before or after initial anthesis. The inoculum was *F. graminearum* isolate PH-1 for Experiment 02 and 02a, and a mixture of two isolates PH-1 and WF-1 for Experiment 04. Six to eight spikes of each RIL were inoculated per replication. For each single batch of inoculation, the checks were included. The inoculated plants/pots were mist-irrigated in a misting chamber at 22-26ºC for three days. Then the pots were transferred to another greenhouse compartment. The number of scabby spikelets (NSS) on the inoculated spikes was visually counted at 5, 9, 13, 17, 21, and 25 days post-inoculation (dpi), respectively. At 25 dpi, the total spikelets were also estimated to calculate the percentage of scabby spikelets (PSS) for each observation. On the basis of PSS data, the area under disease progress curve (AUDPC) was computed.

In 2004, all the RILs and two parents were planted in greenhouse, with each having about 20 seedlings in a

pot. At Zadoks growth stage 11, the leaves were harvested and stored in a freezer at -80ºC for DNA extraction. CTAB extraction was adopted to isolate DNA. A total of about 680 SSR primer pairs were screened for polymorphism between the two parental lines. Polymorphic markers were used to genotype the mapping population with a simple and high throughput polyacrylamide gel electrophoresis system (Wang et al., 2003). The segregating data of 208 SSR markers in total were used to construct a genetic linkage map using JoinMap version 3.0 (van Oijen and Voorrips, 2001) and referring to high-density linkage maps (Shi and Ward, 2004; Somers et al., 2004).

On the basis of replication means, ANOVA was performed for single experiment and over all combination, respectively, and then broad-sense heritability on a line mean basis was estimated. QTL analysis was performed in Windows QTL Cartographer version 2.0 (Wang et al., 2001-2004), based on the genotype means. Single marker analysis (SMA), interval mapping (IM), composite interval mapping (CIM) and multiple interval mapping (MIM) were performed, respectively. A LOD value of 2.5 was set as the threshold value for declaring a QTL. The results of CIM are presented in this paper. For those QTLs/markers with a LOD value of 2.0–2.5, comparison between two groups of RILs with marker allele from Veery and CJ 9306 was conducted to verify the validation.

RESULTS AND DISCUSSION

ANOVA indicated that the differences among RILs were highly significant for both single experiment data and combined analysis over all three experiments ($P < 0.01$). The difference between environments and RIL × environment interaction were also significant

($P < 0.01$). Over all three experiments, the average AUDPC of Veery and CJ 9306 was 9.1 and 1.0, respectively. The average of AUDPC for the RIL population was 5.7 with a range of 0.8–14.2. Frequency distribution was continuous, and transgressive segregation was evident toward susceptibility (Fig. 1). The estimate of heritability in broad sense was 87.2%.

QTL analyses (CIM) indicated that four QTLs were associated with Type II resistance to FHB in CJ 9306. They all showed positive additive effects to increase the resistance (Table 1). The major QTL on 3BS explained 21–26% of phenotypic variation. The explained variation of the QTL on 2DL varied with experiments, from 9.2% to 23.1%. In comparison, the QTLs on 1AS and 7BS showed lower additive effects and explained lower variance. One QTL with negative effects on 5BL was detected by the data of Experiment 04, but not significant for other data sets. In addition, single marker analysis and group comparison of marker alleles suggested two more QTLs, which were located on 5AS and 1B, respectively. The former had positive effects on the resistance, but the latter showed negative effects (Table 2). No significant epistasis was detected.

The major QTL on 3BS has been widely validated in various investigations (Anderson et al., 2001; Mardi et al., 2005; Somers et al., 2003). In our study, comparison of alternative groups of RILs with marker alleles from Veery and CJ 9306 indicated that, on average, selection of the flanking marker Gwm533b or Gwm493 for this major QTL could lead to a decrease of 2.9 in AUDPC. Referring to NSS and PSS, the decrease was 4.0 and 22–24% (Table 2). For the markers linked to QTLs on 2DL, 1AS and 7BS, the average of RILs with allele from CJ 9306 for AUDPC, NSS and PSS was 1.8–2.1, 2.2–2.8 and 14.3–17.2% lower than that of Veery-allele RILs, respectively. The QTL on 5AS could reduce AUDPC by 1.4, or PSS by 10.3%. Chen et al. (2006) suggested that W14, a sister line of CJ 9306, had a major QTL on 5AS, especially for field resistance. In our study, its effects were smaller than the effects of most of other QTLs, and significant only for Experiment 02 and a combined analysis over all three experiments. It may be supposed that this QTL could play a more important role in Type I resistance than in Type II resistance.

In an interval (Wmc272–Barc101) on 2BL, there might be a QTL associated with FHB resistance. Based on the data of markers Barc128 and Gwm120 within this interval, for Experiment 04 and average of all three experiments, the differences in AUDPC between

Veery-allele and CJ 9306-allele RILs (1.4–2.0) were significant, although their LOD did not reach the threshold value. It is suggested that there might be some minor genes/QTLs for FHB resistance in the susceptible cultivar Veery, which are probably located on 5BL, 2BL and 1B. This may provide some underlying elaboration on the evident transgressive segregation described above, and suggest the possibility that a higher level of resistance than CJ 9306 could be achieved by pyramiding QTLs.

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DISCLAIMER

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

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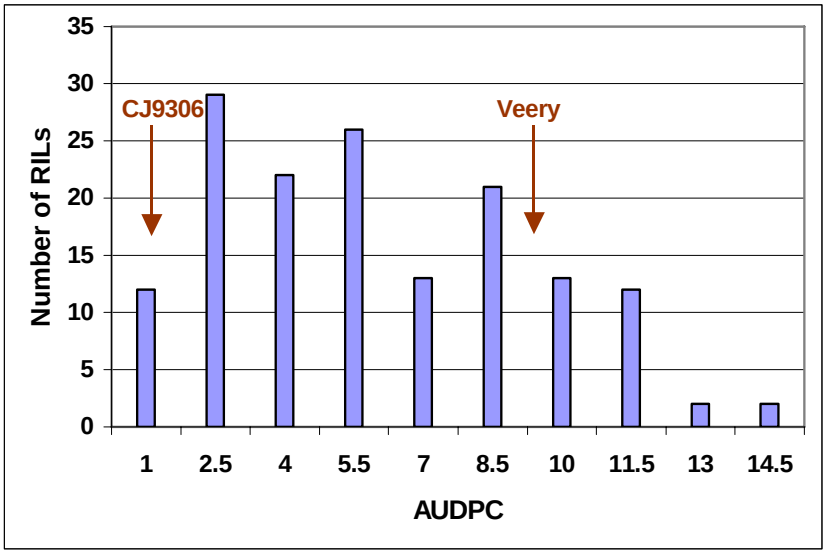

Fig. 1. Frequency distribution of 152 $F_{6:7}$ RILs derived from a wheat cross Veery/CJ 9306 for Type II resistance to FHB (AUDPC over all three experiments).

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Table 1. QTLs for Type II resistance to FHB (ADUPC) in the $F_{6:7}$ RIL population derived from the wheat cross Veery/CJ 9306 (based on CIM).

Interval	Chromosome	Region length (cM)	Experiment	LOD	Additive effects	Explained Variance (%)
Wmc291–Gwm389	3BS	40.0	2002	8.4	1.8	26.3
			2002a	7.7	1.9	20.9
			2004	11.3	2.0	25.6
			Mean overall	9.2	1.6	22.6
Gwm157–Wmc041	2DL	27.1	2002	3.0	1.0	9.2
			2002a	6.4	2.0	23.1
			2004	—	—	—
			Mean overall	3.6	1.1	10.4
Wmc024–Barc148	1AS	15.3	2002	2.6	1.0	8.4
			2002a	—	—	—
			2004	2.3	0.9	4.9
			Mean overall	3.1	1.0	8.6
Gwm400–Gwm573	7BS	29.7	2002	—	—	—
			2002a	2.7	1.2	7.8
			2004	—	—	—
			Mean overall	2.4	0.9	6.8
Barc140–Gwm371a	5BL	31.7	2004	3.3	-1.0	6.1

Table 2. Comparison of alternative SSR markers associated with Type II resistance to FHB (AUDPC) in the $F_{6:7}$ RIL population derived from the wheat cross Veery/CJ 9306 over all three experiments.

Marker	Chromosome	Veery-allele RILs		CJ 9306-allele RILs		Difference
		Number	Mean	Number	Mean	
Gmw533b	3BS	66	7.11 ± 0.42	66	4.24 ± 0.32	2.87 ****
Gwm539	2DL	82	6.58 ± 0.37	62	4.70 ± 0.38	1.87 ***
Barc148	1AS	67	6.48 ± 0.41	49	4.36 ± 0.40	2.13 ***
Gwm400	7BS	99	6.36 ± 0.33	53	4.55 ± 0.42	1.81 **
Gwm425	5AS	60	6.68 ± 0.46	71	5.29 ± 0.37	1.39 *
Barc128	2BL	45	4.54 ± 0.44	49	6.06 ± 0.47	-1.52 *
Barc1160	1B	52	4.82 ± 0.44	95	6.06 ± 0.34	-1.24 *

*, **, ***, ****: Significant at P = 5%, 1%, 0.1% and 0.01%, respectively.

FACILITATION OF INTERNATIONAL *FUSARIUM* NURSERIES AND IMPROVEMENTS OF FHB SCREENING SYSTEM AT CIMMYT.

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ABSTRACT

In March 2006 CIMMYT organized the "CIMMYT Workshop on the Global Fusarium Initiative for International Collaboration". At this workshop it was concluded that two new international spring wheat nurseries were needed for better facilitation of international exchange and evaluation of *Fusarium* relevant spring wheat materials, and the exchange of knowledge generated through the evaluation of these materials.

1. Fusarium International Elite Spring Wheat Nursery (FIESWN).

    a. The specific objective of this nursery is to enable contributors to know the performance of their entries across environments, and allow participants to identify useful sources of resistance in entries from other programs. Regional resistant and susceptible checks from each contributor are important to facilitate interpretation of the results.

    b. The nursery will include two types of entries: Elite FHB/FCR resistant spring wheats (registered or near-registered resistant cultivars) that have performed well in regional FHB/FCR nurseries; Regional FHB/FCR resistant and susceptible reference/standard checks.

2. Fusarium International Preliminary Spring Wheat Nursery (FIPSWN).

    c. The purposes of this nursery include identification of new sources of resistance, examination of stability of QTL for FHB/FCR resistance, surveillance for new and/or problematic pathogen strains, and development of knowledge or solutions in regard to other issues such as negative correlations between resistance QTL and other traits.

    d. The nursery can include: Any materials which address the objectives listed above including NILs of FHB/FCR QTL; Parents of mapping populations.

We have communicated with global communities regarding submission of entries for one or both of these Global Fusarium Initiative international spring wheat nurseries, and we are facilitating the propogation and distribution of these nurseries. The overall objective of these two nurseries is to make useful materials for Fusarium Head Blight (FHB) and Fusarium Crown Rot (FCR) available throughout the world. The current status of these nurseries will be presented.

CIMMYT has also made important changes in FHB screening methodologies for greater precision and accuracy of data collection for wheat and barley, including the relocation of the primary screening site from Toluca to El Batan, Mexico, implementation of an automated fine-misting system, and identification of new isolates for screening. An overview of these changes as well as an initial summary of results will be presented.

PLANT SIGNALING MECHANISMS ASSOCIATED WITH RESISTANCE/ SUSCEPTIBILITY TO *FUSARIUM GRAMINEARUM*.

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ABSTRACT

Fusarium graminearum is the primary agent of Fusarium head blight (FHB) disease of wheat and barley. We had previously demonstrated that constitutive expression of *Arabidopsis thaliana NPR1* gene (At*NPR1*) from the *Ubi1* promoter in transgenic wheat enhances resistance against *F. graminearum* (Makandar et al. 2006). Similarly, overexpression of At*NPR1* in arabidopsis leaves also confers enhanced resistance against *F. graminearum*. NPR1 is a key regulator of salicylic acid (SA) signaling in arabidopsis. Hence, we hypothesized that SA signaling may have a role in plant defense against *F. graminearum*. We have tested this hypothesis by monitoring SA accumulation in arabidopsis and wheat challenged with *F. graminearum*, studying the impact of SA application on growth of *F. graminearum* in arabidopsis and wheat, and monitoring *F. graminearum* growth on arabidopsis mutants deficient in SA accumulation or signaling. These studies confirm an important role of SA in plant defense against *F. graminearum*. In addition, we have also tested the involvement of ethylene and jasmonic acid (JA), two other regulators of plant defense, in plant-*F. graminearum* interaction. Our studies with ethylene and JA-insensitive arabidopsis mutants suggest that ethylene and JA signaling contribute to host susceptibility to *F. graminearum*. In addition, JA application compromises At*NPR1* expression-conferred resistance to *F. graminearum* in the *Ubi1*:At*NPR1* transgenic wheat plants. The *F. graminearum FGL1* gene, which encodes a secreted lipase, was recently shown to have a role in fungal pathogenicity (Voit et al. 2005). Our studies in arabidopsis also indicate a potential role of host lipids, or products thereof, in modulating arabidopsis-*F. graminearum* interaction.

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

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DIALLEL ANALYSIS OF $F_{4:5}$ POPULATIONS FOR SCAB RESISTANCE.

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ABSTRACT

Fusarium head blight (FHB), caused by *Fusarium graminearum*, is an important disease of wheat in South Dakota. This study was conducted to determine combining ability and gene effects in populations derived from mating between spring, winter and facultative wheat genotypes. Six genotypes consisting of susceptible winter wheat 'Nekota' and '2137', moderately susceptible winter wheat 'Harding', moderately resistant spring wheat 'ND2710' and 'BacUp' and resistant facultative wheat 'Ning 7840' were crossed in a partial diallel mating design. $F_{4:5}$ lines were hand transplanted in May 2006 and screened under mist-irrigated field conditions. Artificial inoculation consisted of corn spawn spread at jointing and inoculum suspension spray at flowering stages. Disease index percentage (incidence percentage * severity percentage/100) of the crosses was analyzed using Griffing's method 4 and model 1. General and specific combining abilities were highly significantl ($P < 0.01$). The result showed that both additive and non-additive gene effects were involved in the inheritance of FHB resistance. The ratio of combining ability variation components $[2\acute{o}^2_{GCA}/(2\acute{o}^2_{GCA} + \acute{o}^2_{SCA})]$ was 0.85 indicating that additive gene effects were important.

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BREEDING FOR FUSARIUM HEAD BLIGHT TOLERANCE: INCORPORATING TECHNOLOGY.

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ABSTRACT

Fusarium head blight (FHB) is an episodic disease in the hard winter wheat region of the Great Plains that is known for its diverse and highly variable climate. Fusarium head blight is most commonly found in north and eastern Nebraska, eastern South Dakota, and eastern Kansas. In eastern Nebraska, the predominant rotation is corn-soybeans, but wheat acreage is increasing as the wheat price increases, the general drought continues, and a winter annual is an important rotational crop. In west central NE, the standard rotation is wheat-corn-fallow. However, in west central Nebraska moisture is limiting and FHB rarely occurs except in irrigated production fields. Irrigated wheat is frequently sprayed with fungicide and irrigation is managed to avoid FHB. In order to ensure wheat producers have FHB tolerant varieties, the Nebraska breeding program has used over 70 sources that were previously identified as being FHB tolerant. However, many of those lines were haplotyped and contained the 3BS QTL from Sumai 3 and its derived lines. Rather than use these raw germplasm parents, a decision was made in 2005 to finish out these crosses, but in future to concentrate on using elite germplasm resources that are known to contain the 3BS QTL and often the 5AS QTL. These QTLs will be incorporated with "native" resistance that has been identified in recent releases (e.g. Husker Genetics Brand Overland (formerly NE0143)) and advanced experimental lines (e.g. NE01604, NE02584, NE03490, etc.). The majority of crosses will be relatively simple single crosses (involving elite Nebraska germplasm with the resistance QTLs by elite hard winter wheat germplasm) or three way crosses involving one to two parents with known resistance QTLs followed by marker assisted selection. However, to diversify our FHB germplasm, an alternative crossing strategy will be to cross hard spring wheat by soft winter wheat to hard winter wheat lines that all contain the 3BS QTL and where possible the 5AS QTL. Hence every progeny should minimally have the 3BS QTL and selecting with MAS is only needed for confirmation. The three-way cross will have two hard wheat parents (one soft wheat parent) and two winter wheat parents (one spring wheat parent). It will segregate for spring and winter growth habit, but the spring growth habit plants are easily killed by the Nebraska winter, leaving only winter hardy progeny. However, the population will segregate for hard and soft kernel characteristics. Using optical sorting based on near-infrared spectra, the hard and soft kernels can be readily separated so that predominantly hard kernels are retained. The advantages of using optical sorting in the F_2 or later generations are that: 1. generally large numbers of seed per population are available, 2. relatively large numbers of populations can be screened nondestructively, and 3. that other optical sorts can be run on the selected hard kernel subpopulation. The key to optical sorting is that the trait must be heritable. Preliminary research indicates that sorting for hardness and kernel color is heritable. Ideally the sorted populations could be grown in FHB inoculated fields and sorted for protein content, FHB tolerance, and possibly lower levels of DON. However the latter traits are expected to have low heritability, hence population enrichment is the expected outcome at best.

EVALUATION OF FUSARIUM HEAD BLIGHT RESISTANCE IN SOFT RED WINTER WHEAT.

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ABSTRACT

In 2006, Kentucky wheat producers harvested a record 70 bushels per acre. This made Kentucky 5th in the U.S. for wheat yield. The exceptional yield was bolstered by favorable weather conditions and low disease pressure. Only scattered fields in moderate risk areas suffered severe damage from Fusarium head blight (FHB). Epidemics were successfully created in the FHB nurseries in Lexington, KY and Princeton, KY and progressed to an adequate level for distinguishing resistant and susceptible varieties. In the Lexington FHB nursery, scabby-corn inoculum was introduced three weeks prior to heading and plots were mist-irrigated during the night and early morning. Scabby-corn inoculum was also spread in the non-irrigated Princeton FHB nursery three weeks prior to heading and plants were then treated with conidial suspensions (50,000 spores ml^{-1}) at anthesis and one week post anthesis. Detailed severity and incidence readings on select material were done only in Lexington. Readings were done 24-28 days after flowering because cool weather delayed symptom development. Material from both the Lexington and Princeton FHB nurseries was harvested and analyzed for Fusarium damaged kernels (FDK) by weight and deoxynivalenol (DON) content (ppm). Average disease severity in the Lexington FHB nursery was 41% and the ranged from 2 to 98%. Incidence ranged from 7 to 100% and the average was 38%. The range for FDK from material harvested in Lexington was 0.6 to 76% with an average of 21%. This is significantly higher ($P<0.05$) than FDK range of 0.2 to 58.8% with an average of 6.79 from Princeton FHB nursery material. DON levels were also significantly higher ($P<0.05$) in the Lexington FHB nursery, averaging 11.7 ppm, than at Princeton, where the average was 3.2 ppm. Given the variability of FHB, it is useful to have data from mulitple locations and different environmental conditions. Data from these nurseries enabled selection of breeding material for advancement or use as parents.

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THE 2005-06 UNIFORM SOUTHERN FUSARIUM HEAD BLIGHT SCREENING NURSERY.

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ABSTRACT

Phenotypic estimates of host resistance to Fusarium head blight (FHB) are greatly confounded by environmental and genotype x environment interaction effects. Thus, multiple evaluations of genotypes are necessary to determine true genetic effects. The objectives of the Uniform Southern FHB Screening Nursery are to provide breeders with a comprehensive set of resistance estimates on advanced generation breeding lines in a timely fashion, and to facilitate the sharing of the best resistant materials throughout the breeding community.

The 2005-06 nursery comprised 34 advanced generation breeding lines and two check cultivars, 'Ernie' (partially resistant) and 'Coker 9835' (susceptible). Six U.S. public programs (Univ. of Arkansas, Univ. of Georgia, Louisiana State Univ., Univ. of Maryland, N. C. State Univ., and VA Tech.), and two private companies (Syngenta, and Agripro) submitted entries. A comprehensive set of field, greenhouse and laboratory results were submitted by 11 U.S., one Romanian and one Hungarian cooperator. Copies of the full report will be available at the 2006 National Fusarium Head Blight Forum and subsequently on line at the USWBSI web site: http://www.scabusa.org/.

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Means Accross Locations 2005-06

	Cultivar/ Designation	FHB Incidence	RANK	FHB Severity	RANK	FHB Index	RANK	FDK	RANK	ISK	RANK	DON	RANK	G'hse Type II	RANK	Heading Date	RANK	Plant Height	RANK	*Fhb1* *Xcfd79* 301.5 bp	*Fhb1* *Xgwm 493* 214 bp	*Fhb1* *Xgwm533.1* 161.5 bp	*Qfhs.ifa-5A* *Xbarc 186* 227.5 bp	*Qfhs.ifa-5A* *Xgwm1304* 332.5
1	Ernie	47	7	24	9	16	9	16	1	20	2	5.7	10	26	9	116	10	33	8	.	.	.		
2	Coker 9835	80	36	50	34	43	36	52	34	58	35	9.7	28	50	19	119	26	34	13	.	.	.		
3	AR 97002-10-2	54	13	27	16	19	16	30	20	33	23	7.4	18	43	17	120	33	36	21	.	.	.		
4	AR 97002-2-1	57	19	23	5	17	11	20	8	28	12	5.4	7	14	3	116	10	33	8	X	.	.	X	X
5	AR 97007-4-1	68	31	37	27	32	29	39	26	45	29	9.6	27	50	19	118	22	35	17	.	.	.		
6	AR 97124-4-1	54	13	28	19	17	11	31	22	30	14	6.0	13	53	22	119	26	38	31	.	.	.		
7	AR 97124-4-2	55	17	30	22	19	16	26	15	31	16	7.4	18	54	25	119	26	38	31	.	.	.		
8	AR 97124-4-3	52	11	25	11	17	11	28	19	32	20	6.8	16	22	6	119	26	39	33	.	.	.		
9	ARGE97-1060-5-5	44	1	23	5	10	1	20	8	18	1	5.7	10	43	17	119	26	41	36	.	.	.		
10	ARGE97-1064-11-5	54	13	25	11	16	9	25	13	27	11	6.9	17	38	13	120	33	39	33	.	.	.		
11	B010973	45	3	25	11	12	3	20	8	24	9	5.9	12	40	15	118	22	31	1	.	.	.		
12	B011260	68	31	44	30	30	27	40	28	48	31	9.9	30	62	34	116	10	37	27	.	.	.		
13	D02-8443	58	23	33	25	22	23	39	26	38	26	9.4	26	54	25	119	26	36	21	.	.	.		
14	D02-8483	60	25	34	26	24	25	24	12	31	16	6.1	14	69	36	116	10	31	1	.	.	.		
15	D02-8486	65	28	45	31	35	30	40	28	45	29	10.4	32	61	32	114	1	33	8	.	.	.		
16	LA95135D54-2-3	76	35	47	33	40	33	51	32	56	34	14.4	36	51	21	119	26	36	21	.	.	.		
17	LA98090D34-4	54	13	24	9	19	16	25	13	26	10	7.9	21	59	29	114	1	36	21	.	.	.		
18	LA99042E-64-B	61	26	40	28	30	27	43	31	41	27	10.5	33	65	35	117	20	37	27	.	.	.		
19	MV6-82-10	57	19	32	24	23	24	31	22	32	20	5.4	7	56	27	114	1	32	4	.	.	.		
20	MV6-82-8	55	17	27	16	19	16	27	18	30	14	6.3	15	61	32	115	6	32	4	.	.	.		
21	NC03-11465	49	8	18	1	13	5	17	3	23	7	2.9	3	38	13	121	35	34	13	X	X	X		
22	NC04-27617	46	5	23	5	14	6	19	6	23	7	3.6	4	21	5	116	10	37	27	X	X	X	X	X
23	NC04-27618	44	1	23	5	15	8	17	3	22	4	2.5	1	23	7	116	10	37	27	X	X	X	X	X
24	NC04-27669	46	5	21	4	12	3	19	6	22	4	2.5	1	13	2	116	10	36	21	X	X	X	X	X
25	VA00W-38	59	24	30	22	21	21	26	15	31	16	7.9	21	11	1	118	22	33	8	X	X	.		
26	VA05W-448	61	26	28	19	21	21	22	11	31	16	5.6	9	33	12	118	22	31	1	.		.		
27	VA05W-491	53	12	26	15	17	11	26	15	28	12	7.5	20	59	29	114	1	35	17	.		.		
28	VA05W-498	50	9	18	1	14	6	16	1	20	2	4.1	5	24	8	116	10	35	17	X	X	.		
29	VA05W-500	45	3	20	3	11	2	17	3	22	4	4.7	6	16	4	115	6	34	13	X		.		
30	VA05W-633	50	9	27	16	18	15	30	20	32	20	8.4	23	60	31	117	20	36	21	.		.		
31	GA96693-4E16	66	29	42	29	35	30	36	25	42	28	10.1	31	53	24	116	10	35	17	.		.		
32	GA961171-4E21	71	33	53	36	40	33	55	36	58	35	9.2	25	29	10	114	1	32	4	.		.	X	
33	GA951231-4E26	66	29	45	31	37	32	53	35	51	33	9.8	29	40	15	115	6	33	8	.		.		
34	GA961567-4A35	71	33	51	35	41	35	51	32	50	32	10.8	34	56	27	116	10	32	4	.		.		
35	GA98401-5E23	57	19	28	19	24	25	40	28	36	25	8.9	24	53	22	115	6	34	13	X		.		
36	GA981621-5E34	57	19	25	11	19	16	31	22	33	23	13.1	35	32	11	122	36	40	35	.		.		
	Sumai 3																			X	X	X	X	X
	Mean	57		31		23		30		34		7.5		42		117		35						
	L.S.D.(0.05)	23		22		22		20		16		6.8		38		5		3						
	CV%	20		35		49.2		33.2		24.3		46.0		46.0		2.3		4.1						

INTROGRESSION AND GENETIC CHARACTERIZATION OF ALIEN FUSARIUM HEAD BLIGHT RESISTANCE IN WHEAT.

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ABSTRACT

Alien species are an important source of genetic variability in wheat (*Triticum* spp.) and carry genes for resistance to numerous pathogens, including *Fusarium graminearum* Schwabe, the causal agent of Fusarium head blight (FHB). The goal of this project was to develop breeder-friendly, FHB-resistant germplasm. Specific objectives were to identify novel sources of FHB resistance derived from relatives of wheat and transfer the resistance to adapted wheat backgrounds. Resistance to FHB was identified in four wheat-*Thinopyrum ponticum* derivatives, using the point inoculation method over three greenhouse seasons. Fluorescent genomic *in situ* hybridization indicated that the four derivatives were partial wheat-*Th. ponticum* amphiploids, each with 56 chromosomes. Conventional hybridization and use of the Ph^I system, which induces meiotic pairing and recombination between homoeologous wheat and *Th. ponticum* chromosomes, facilitated reduction of linkage drag and introgression of *Th. ponticum* chromatin into cultivated wheat. Hybridization of these amphiploids with Alsen, an FHB-resistant wheat cultivar, led to production of wheat lines with reduced amounts of *Th. ponticum* chromatin and favorable agronomic performance. Introgression lines were identified with minimal linkage drag and apparently high levels of FHB resistance. Resistance to FHB was also identified in progeny derived from hybridization of the amphiploids with Reeder, a wheat cultivar noted for FHB susceptibility. These introgression lines could provide wheat breeders access to FHB resistance genes from relatives of wheat, thus promoting development of wheat cultivars with resilient and novel resistance to this disease.

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RNA PROFILING OF SUSCEPTIBLE AND RESISTANT WHEAT VARIETIES IN THE EARLY STAGES OF FHB INFECTION.

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ABSTRACT

To gain a better understanding of the difference in response to *Fusarium graminearum* infection between susceptible and resistant varieties of wheat, gene expression profiling is being performed using the Affymetrix wheat genome array. All profiles are being compiled into a database using Acuity.

So far, we have compared the RNA profiles of three groups of wheat plants: 1) the spring wheat varieties Roblin (very susceptible), Wuhan 1 and Nuy Bay (both resistant, from Chinese and Japanese sources of resistance, respectively); 2) the spring wheat Chinese Spring (susceptible) and the introgression lines 7E and 7ES (both resistant, containing the chromosome 7 from *Thinopyrum elongatum* into Chinese Spring background); 3) the winter wheat Augusta (susceptible) and FHB148 (resistant, derived from Frontana, a Brazilian source of resistance). For group 1 and 2, florets from wheat heads at mid-anthesis were point inoculated with either *F. graminearum* spores or water (mock inoculation). Inoculated spikelets were samples after 0, 1, 2 and 4 days of infection. Two biological replicates were performed for each variety. For group 3, spray inoculation was used and sampling was done at 1, 3 and 6 days after treatment. Microarray hybridization experiments have been conducted using the wheat Affymetrix wheat genome array, comparing mock-inoculated and *Fusarium*-inoculated spikelets from the time course experiments. Northern analysis has also been performed to validate results from the microarray analysis. A preliminary analysis of the major differences observed in the response of susceptible and resistant varieties to *Fusarium* infection will be presented.

DEVELOPMENT AND CHARACTERIZATION OF A WHEAT TRANSLOCATION LINE WITH FUSARIUM HEAD BLIGHT RESISTANCE DERIVED FROM *THINOPYRUM PONTICUM*.

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ABSTRACT

A Robertsonian wheat (*Triticum aestivum*)-*Thinopyrum ponticum* translocation line KS24-2 (7DS-7EL), containing Fusarium head blight resistance QTL (*Qfhs.pur-7EL*) located on the long arm of chromosome 7E, was crossed to Chinese Spring (*ph1b*) to induce homoeologous recombination. An $F_{3:4}$ plant (275-4) was identified, by DNA marker analysis, in which the introgressed segment was reduced. The introgressed chromosome segment of 275-4 was estimated to be the distal one third of the long arm by comparison of the position of DNA markers on the wheat deletion bin map. F_5 plants from 275-4 were crossed with two wheat breeding lines with moderate Fusarium head blight resistance. Segregating populations of the two crosses were screened with DNA markers flanking *Qfhs.pur-7EL*. Analysis of variance for disease response following single-floret inoculation revealed significant differences in disease severity in different genotypes, verifying that the FHB resistance QTL was retained in the shortened chromosome segment. Transmission of the translocation segment of 275-4 was shown to be normal in female gametes, but preferentially transmitted in pollen over wheat chromosome 7D.

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DETERMINING FUSARIUM HEAD BLIGHT RESISTANCE IN SPRING MALTING BARLEY USING DON CONTENTOL OF GRAIN OVER SEVERAL YEARS.

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ABSTRACT

In 2003, researchers at the University of Minnesota Northwest Research and Outreach Center and Busch Agricultural Resources, Inc. (BARI) began collaborating yearly and have evaluated more than 1600 barley lines. Lines of experimental spring malting barley are tested for resistance to Fusarium head blight (FHB) by rating spike symptoms and measuring deoxynivalenol (DON) concentration in grain. Lines are planted into single rows in a misted, corn-spawn inoculated nursery. In 2003, 2005, and 2006, spikes were evaluated for FHB symptoms on a 0-9 scale (9 = susceptible) when symptoms were most apparent during the early dough stage (approx. Feekes 11.1 growth stage). Spikes were harvested when mature and grain samples submitted to the North Dakota State University (NDSU) DON Laboratory for analysis. Spikes were not collected in 2004 because of within field flooding. During 2005-06, both 2-row and 6-row germplasm were included in the tests. Experiments included elite lines (four or more years in yield trials) and advanced lines (two or three years in yield trials) originating from the BARI breeding program; FHB-resistant crosses from an ongoing BARI/ICARDA/CIMMYT collaboration; FHB-resistant lines directly from ICARDA/CIMMYT; as well as standard malting barley checks.

Barley lines with reduced levels of DON accumulation have been identified. Deoxynivalenol levels of 6-row germplasm ranged from 8.0 - 42.5 ppm in 2003, 0.1 - 6.6 ppm in 2005 and 2.7 – 54.6 ppm in 2006. Two rowed barley exhibited lower ranges of DON: 0.03 -2.4 ppm in 2005 and 1.0 – 22.8 ppm in 2006. These data assist in the selection and advancement of BARI advanced and elite breeding lines with reduced DON levels. A BARI/ICARDA/CIMMYT collaboration line (ADV BARI 57) continues to show lowered levels of DON and is now being used in crossing blocks with superior malting parents. Overall, this collaboration has illustrated the need for multi-year data collection and the usefulness of FHB disease nurseries for barley breeding.

CONSIDERATIONS FOR USE OF MAS IN AN APPLIED WHEAT BREEDING PROGRAM.

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ABSTRACT

Marker-assisted selection (MAS) theory focuses on improving population means while breeders are primarily interested in obtaining a new cultivar. My objective was to assess the MAS for improving quantitative traits within the context of a variety development program. A single breeding program was considered and the probability of obtaining a new cultivar was determined based on ten traits. MAS for 1 or 2 genes in early generations was considered. Given certain assumptions, MAS for one gene would require genotyping 46,496 F4 individuals to be 95% certain of releasing of a new cultivar. For two genes (two traits), 14,606 F4 individuals have to be assayed. MAS for a QTL with moderate effect did little to improve the probability of obtaining trait values required for cultivar release. Given these results, backcrossing is an attractive alternative requiring less resources and greater probability of obtaining desired quantitative trait values. Recurrent parents (RP) are often selected late in the development process such that the backcross-improved cultivar reaches commercial production five years after the RP itself. Much of this delay is due to seed increase and accelerated backcrossing has little impact while using considerable resources. A modified backcrossing scheme is proposed. Multiple RP are selected using preliminary phenotypic evaluations, backcrossing is initiated, and each backcross population is advanced or terminated based on the continued phenotypic evaluation of the RP. The backcross derived cultivar is commercially available two years after the RP, few resources are needed, and considerable genetic resources are generated.

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REPORT ON THE 2005-06 NORTHERN UNIFORM WINTER WHEAT SCAB NURSERIES.

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OBJECTIVES

This is a summary of the report on the 2005-2006 Northern Uniform Winter Wheat Scab Nursery (NUWWSN) and the Preliminary Northern Uniform Winter Wheat Scab Nursery (PNUWWSN). A full report will be available on the USWBSI web site prior to the 2006 forum. The objective of these tests is to screen winter wheat genotype adapted to the northern portion of the eastern US for scab resistance.

MATERIAL AND METHODS

The traits assessed and locations that reported data are listed in Table 1. Entries for the NUWWSN came from 14 programs while the PNUWWSN entries came from eight programs (Table 2).

RESULTS

There are eight FHB traits for each trail. Entries with means that were not significantly different than the lowest mean for six or more FHB traits are shown in Tables 3 and 4 (eg entries with at least 6 "l"s). Only two entries had DON < 2 ppm (entries 6 and 7 in the PNUWWSN, see Tables 4 and 5).

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This material is based upon work supported by the U.S. Department of Agriculture, under Agreement No. 59-0790-4-101. This is a cooperative project with the U.S. Wheat and Barley Scab Initiative. Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the authors and do not necessarily reflect the view of the U.S. Department of Agriculture.

Table 1. Traits assessed in the 2005-06 PNUWWSN and NUWWSN tests

Code	Trait	Description	PNUWWSN Locations*	NUWWSN Locations*
SEV	Disease severity from field tests	% of infected spikelets in an infected head.	IL,IN1,IN2,KY,MI,MO, ON,VA	IL,IN1,IN2,KY,MD,MI,NE,NY,OH, ON, VA
INC	Disease incidence	% of heads with at least one infected spikelets	IL,IN1,IN2,KY,MI,MO, ON,VA	IL,IN1,IN2,KY,MD,MI,NE,NY,OH, ON, RO,VA
IND	Disease index	IND = (SEVxINC)/100	IL,IN1,IN2,KY,MI,MO,OH, ON,VA	IL,IN1,IN2,KS,KY,MD,MI,NE,NY, OH, ON,VA
KR	Kernel rating	A visual assessment of the percent infected kernels	IL,MO	IL,KS,MD
PSS	Percent scabby seed	Percent of scabby seed by weight	KY1,KY2	KY1,KY2,MO
ISK	Composite of head and kernel traits	ISK Index = .3 (Severity) + .3 (Incidence)+.4 (% FDK or PSS)	IL,KY,MO	IL,KY,MD,MO
DON	DON (vomitoxin)	PPM of vomitoxin in grain	IL,KY1,KY2,VA	IL,KS,KY1,KY2,MD,VA
GH	Greenhouse severity	Same as SEV except from greenhouse	IL,KY	IL,KY,MO

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

Table 2. Entries in the 2005-06 PNUWWSN and NUWWSN

NAME	**PNUWWSN** PEDIGREE
ERNIE	
TRUMAN	
FREEDOM	
PIONEER 2545	
IL00-8641	IL89-1687 // IL90-6364 / IL93-2489
IL01-16170	IL95-934 / Goldfield_
IL02-18146	Pioneer 25R26 / IL9634-24437 // IL94-1653
IL02-19463	Patton / Cardinal // IL96-2550
IL02-7735	IL94-1653 / IL96-6472
MSU E1009	MSU Line DC076/PIONEER_2555
OH01-6167	OH530/OH585/OH498/34586-20-1
OH01-7653	HOPEWELL/OH601
OH02-15978	PATTERSON/HOPEWELL
OH02-5512	OH569/OH615
OH776	OH513/OH515
P.011034A1-3	9895C1/981251E1//92145E8
P.011035A1-71	981128A1/981477A1//92145E8
P.011050A1-13	981269B1/981251E1//INW0101
P.011099A1-2	92145E8//9388A2/98133A4
P.011151B1-93	INW0101//98135C8/9672B1
MISSING	MISSING
RCAT 32/35B	Ruby/Frontana # 1/AC Ron//25R18/AC Ron
RCAT Akos2290	Zu-Rst
RCAT F13	Maringa x Akos 2196
RCAT TF174/1c	AC Ron x 25R18
VA05W-464	96W-348/P92823A1-1-4-4-5)//McCORMICK
VA05W-510	Roane / Pion 2684//OH 552
VA05W-517	Roane / Ernie//McCORMICK,F6
VA05W-673	Roane*2//W14/Roane,BC3F4
VA05W-681	Roane*2//Futai8944/Roane/3/Roane,BC3F4
M00-3904-9	89D-8096/89D*4763
M02-2152	CLEMENS//SAVANNAH/FL8643-G13-G5
M02*2518	BRADLEY/Pio2552
M03-3002	Winter/Winter FHB bulk population
KY98c-1161-03	Patterson/2540//2552
KY98c-1305-02	Shiloh/2552//2568
KY98c-1169-06	Patterson/2568//2552
KY98c-1164-04	Patterson/2540//25R26
KY98c-1470-02	VA92-51-12/Kristy//2540

NAME	**NUWWSN** PEDIGREE
ERNIE	
TRUMAN	
FREEDOM	
PIONEER 2545	
IL00-8061	P8138I1-16-5-50/Foster//IL93-2489
IL00-8109	P8138I1-16-5-50/Foster//IL93-2489
IL00-8530	IL89-1687 // IL90-6364 / IL93-2489
IL01-11445	IL87-2834-1 / IL95-678
IL01-11934	IL90-6364 / IL94-1909
MSU Line E0001	CLKS_CREAM/MSU LINE D1277
MSU Line E2017	MSU Line D3913/MSU Line D0331
MSU Line E2041	PIONEER_2555/MSU_Line D3743
MSU Line E2042	MSU Line D3743 /PIONEER_2555
MV 6-82	PIO2643/MSY*3/BALKAN//SAL
NE02465	NE95685 (=MO11785/NE87619//NE88492)
NE02584	KS92H363-2
NE03490	WI90-540W/NE93554 (=NE82419/ARAPAHOE)
NH01046	WINDSTAR
NI02425	NE94654 (ARAPAHOE 2*/ABILENE)
OH02-12678	FOSTER/HOPEWELL//OH581/OH569
OH02-12686	FOSTER/HOPEWELL//OH581/OH569
OH02-13567	OH581/IN83127E1-24-5-2-1-31//5088B-D-32-1/OH601
OH02-7217	P92118B4-2/OH561
OH904	ZM10782/FREEDOM//30584-37-2/VA91-54-219
P.0128A1-36	92829A1/Patton/3/Goldfield/X117//Roane/92145
P.0172A1-12	INW9811/Ernie//92823/Ernie/3/92829/Patton
P.0175A1-44	92807A1/92145A2//Freedom/3/INW0411
P.01931A1-5	981227A1/981518//9895/INW0304
P.01946A1-16	981477/981128//INW0304/981250A1
RCAT 202D/ 1	Freedom x Harding
RCAT 32/157	Ruby/Frontana # 1/AC Ron//25R18/AC Ron
RCAT Akos 2234	Ttj-81.F379
RCAT TF 203/2	AC Ron x 25R18
RCAT19/4c	AC Morley x 25R18
VA04W-563	Roane//FUTAI 8946/Roane,BC1F6
VA04W-592	Roane//Er-Mai 9/Roane,BC1F6
VA05W-417	ROANE/3/NING7840/CK9904//PION2552,F7
VA05W-421	ROANE/3/NING7840/CK9904//PION2552,F7
VA05W-452	IL 94-1909/SISSON"S"
M01-4377	Coker 9663/VA91-54-219
COKER 9553	89M-4035A/Pio2580
KY97c-0554-4-6	VA94-54-549/Roane//Kristy
KY97c-0540-1-2	Coker9803/L910097//2552
KY 97c-0388-5-2	2552/VA94-52-25//Pochahontas
KY97c-0304-26-10	Kristy/2628//2540
KY97c-0277-1-8	Foster/VA94-54-549//2552
KS03HW12-6-5	97HW29/97HW131//96HW100-5
KS970085-9-15	HBK0935-125-5-2/VBF0589-1//X960103
MO050101	MO11769/Madison
MO050143	MO11769/Madison
MO050132	MO11769/Madison
MO050194	MO12278/Pioneer2552
MO050207	MO11769/Madison
NY93285-9161	
NY92237-1-sp-9173	
NY94022-9093	
NY93285-9147	
NY93285-9179	

Table 3. Best entries from the 2006 NUWWSN.

ENTRY	NAME	INC	SEV	IND	KR	PSS	ISK	DON	GH	# l	# h
24	OH904	36.9 l	18.9 l	11.3 l	14.1 l	14.6 l	20.8 l	3.7 l	5.0 l	8	0
50	MO050143	45.2 l	22.0 l	11.4 l	19.0 l	9.5 l	23.7 l	6.7 l	7.2 l	8	0
13	MSU Line E2042	36.8 l	28.7 l	9.5 l	24.5 l	13.8 l	22.3 l	5.4 l	17.9	7	0
2	TRUMAN	53.7	20.5 l	12.4 l	21.5 l	5.2 l	24.1 l	6.0 l	3.3 l	7	0
21	OH02-12686	45.8 l	31.3	13.5 l	24.8 l	7.8 l	25.4 l	6.3 l	10.7 l	7	0
28	P.01931A1-5	46.4	24.0 l	13.9 l	11.8 l	4.4 l	19.3 l	3.6 l	11.9 l	7	0
33	RCAT TF 203/2	45.2 l	28.8 l	14.9 l	28.3 l	7.6 l	27.4 l	7.1 l	15.8	7	0
37	VA05W-417	46.2	26.4 l	15.3 l	26.6 l	5.5 l	22.6 l	4.9 l	12.2 l	7	0
51	MO050132	45.5 l	26.1 l	15.4 l	20.7 l	4.9 l	24.4 l	8.3	5.0 l	7	0
5	IL00-8061	44.1 l	25.7 l	15.6 l	14.4 l	5.9 l	21.4 l	5.0 l	14.7	7	0
25	P.0128A1-36	45.2 l	23.8 l	16.2 l	13.2 l	5.8 l	20.1 l	4.0 l	14.7	7	0
35	VA04W-563	50.8	26.9 l	16.5 l	13.4 l	4.2 l	20.3 l	3.3 l	10.8 l	7	0
23	OH02-7217	45.2 l	18.7 l	10.2 l	27.2 l	6.3 l	24.3 l	8.9	19.6	6	0
55	NY92237-1-sp-9173	43.0 l	22.6 l	12.7 l	26.2 l	4.8 l	26.2 l	7.3	22.1	6	0
27	P.0175A1-44	54.5	20.0 l	14.1 l	28.0 l	13.4 l	32.6	6.7 l	3.6 l	6	0
38	VA05W-421	49.2	29.0 l	15.6 l	26.6 l	4.3 l	25.8 l	6.4 l	19.5	6	0
20	OH02-12678	49.8	25.5 l	16.2 l	22.7 l	10.2 l	25.1 l	5.2 l	20.3	6	0
8	IL01-11445	46.5	30.8	16.6 l	19.8 l	1.9 l	25.9 l	6.1 l	13.4 l	6	0
52	MO050194	48.7	26.6 l	17.0 l	27.1 l	14.8 l	30.4 l	7.3	11.9 l	6	0
53	MO050207	47.3	27.2 l	17.2 l	26.3 l	5.8 l	29.9 l	8.6	7.3 l	6	0
36	VA04W-592	52.8	27.0 l	18.2	26.1 l	5.7 l	29.8 l	6.3 l	13.3 l	6	0
1	ERNIE	33.6 l	30.0 l	20.2	19.1 l	4.7 l	25.4 l	7.2 l	24.3	6	0
29	P.01946A1-16	52.3	28.6 l	22.5	25.1 l	10.6 l	30.9 l	5.9 l	7.5 l	6	0
	AVERAGE	49.6	29.0	17.4	26.2	10.8	29.5	7.1	18.9		
	# LOCATIONS	12	13	13	3	3	4	6	3		
	LSD	12.5	11.5	8.5	16.6	19.0	12.7	3.9	10.1		
	R2	0.8	0.7	0.8	0.9	0.5	0.9	0.8	0.6		
	CV	29.0	45.9	58.1	34.5	90.2	27.8	45.9	62.1		

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

Table 4. Best entries from the 2006 PNUWWSN.

Entry	Name	INC	SEV	IND	KR	PSS	ISK	DON	GH	# l	# h
2	TRUMAN	42.5 l	21.1 l	7.1 l	6.5 l	4.8 l	19.9 l	2.2 l	13.7 l	8	0
9	IL02-7735	40.9 l	24.5 l	9.4 l	5.0 l	1.9 l	13.6 l	2.3 l	39.8 l	8	0
38	KY98c-1164-04	44.3 l	24.3 l	11.1 l	22.5 lh	3.2 l	23.6 l	5.6 l	13.5 l	8	1
29	VA05W-673	52.3 l	25.3 l	12.5 l	7.0 l	4.5 l	17.5 l	2.8 l	2.9 l	8	0
18	P.011050A1-13	53.3 lh	23.8 l	13.1 l	11.0 l	5.0 l	17.5 l	3.9 l	12.9 l	8	1
16	P.011034A1-3	48.2 l	30.3 l	13.4 l	24.0 lh	4.9 l	21.1 l	3.2 l	36.4 l	8	1
7	IL02-18146	45.2 l	26.1 l	13.5 l	7.5 l	4.6 l	15.5 l	1.7 l	18.0 l	8	0
5	IL00-8641	51.6 l	23.8 l	13.7 l	8.0 l	8.6 l	18.6 l	4.3 l	24.6 l	8	0
1	ERNIE	50.3 l	23.0 l	14.6 l	12.5 l	3.4 l	20.3 l	5.2 l	26.5 l	8	0
25	RCAT TF174/1c	42.5 l	20.4 l	9.1 l	10.5 l	21.3	22.6 l	4.8 l	8.1 l	7	0
23	RCAT Akos2290	50.5 l	23.5 l	10.1 l	24.0 lh	23.8 h	27.7 lh	4.4 l	9.1 l	7	3
14	OH02-5512	56.5 h	23.0 l	13.1 l	16.5 l	8.3 l	22.2 l	3.4 l	12.0 l	7	1
6	IL01-16170	51.3 l	36.7 h	13.7 l	8.5 l	7.2 l	21.3 l	1.9 l	7.7 l	7	1
28	VA05W-517	52.1 l	23.4 l	14.5 l	20.0 l	2.8 l	22.8 l	3.0 l	47.3 h	7	1
3	FREEDOM	54.3 lh	34.5 lh	14.8 l	24.0 lh	7.2 l	29.2 h	4.5 l	14.4 l	7	4
30	VA05W-681	56.0 h	26.6 l	15.0 l	13.5 l	4.5 l	22.2 l	3.0 l	5.3 l	7	1
19	P.011099A1-2	51.7 l	31.6 l	15.9	17.5 l	2.6 l	22.7 l	3.0 l	26.5 l	7	0
8	IL02-19463	52.5 l	30.1 l	17.3	18.5 l	4.1 l	28.5 lh	4.7 l	32.0 l	7	1
37	KY98c-1169-06	57.0 h	22.4 l	13.2 l	21.0 l	3.4 l	24.0 l	7.4 h	32.6 l	6	2
17	P.011035A1-71	55.3 h	27.9 l	15.7	22.5 lh	9.9 l	22.2 l	4.1 l	26.1 l	6	2
	AVERAGE	54.8	31.3	16.7	22.7	8.9	26.6	5.0	29.4		
	# LOCATIONS	8	8	9	2	2	3	4	2		
	LSD	13.6	14.8	7.9	24.0	15.5	15.5	4.3	37.1		
	R2	0.8	0.5	0.7	0.8	0.7	0.9	0.7	0.7		
	CV	24.4	47.0	49.4	52.2	88.2	35.2	59.6	58.9		

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

Table 5. Summary of results of the 2005-2006 PNUWWSN

Ent	Name	INC		SEV		IND		KR		PSS		ISK		DON		GH		# l	# h
1	ERNIE (MR)	50.3	l	23.0	l	14.6	l	12.5	l	3.4	l	20.3	l	5.2	l	26.5	l	8	0
2	TRUMAN (R)	42.5	l	21.1	l	7.1	l	6.5	l	4.8	l	19.9	l	2.2	l	13.7	l	8	0
3	FREEDOM (MR)	54.3	lh	34.5	lh	14.8	l	24.0	lh	7.2	l	29.2	h	4.5	l	14.4	l	7	4
4	PION. 2545 (S)	62.8	h	46.8	h	27.7	h	46.5	h	15.4	l	38.8	h	8.9	h	58.4	h	1	7
5	IL00-8641	51.6	l	23.8	l	13.7	l	8.0	l	8.6	l	18.6	l	4.3	l	24.6	l	8	0
6	IL01-16170	51.3	l	36.7	h	13.7	l	8.5	l	7.2	l	21.3	l	1.9	l	7.7	l	7	1
7	IL02-18146	45.2	l	26.1	l	13.5	l	7.5	l	4.6	l	15.5	l	1.7	l	18.0	l	8	0
8	IL02-19463	52.5	l	30.1	l	17.3		18.5	l	4.1	l	28.5	lh	4.7	l	32.0	l	7	1
9	IL02-7735	40.9	l	24.5	l	9.4	l	5.0	l	1.9	l	13.6	l	2.3	l	39.8	l	8	0
10	MSU E1009	62.9	h	38.6	h	21.7	h	36.0	h	7.7	l	33.2	h	6.4	h	18.1	l	2	6
11	OH01-6167	52.4	l	36.9	h	16.6		20.0	l	11.5	l	30.4	h	5.6	l	52.3	h	4	3
12	OH01-7653	57.5	h	43.1	h	18.9		35.5	h	8.0	l	30.2	h	6.5	h	58.1	h	1	6
13	OH02-15978	56.3	h	35.1	lh	19.8	h	28.0	lh	13.7	l	30.5	h	5.8	l	47.7	h	4	6
14	OH02-5512	56.5	h	23.0	l	13.1	l	16.5	l	8.3	l	22.2	l	3.4	l	12.0	l	7	1
15	OH776	58.6	h	46.9	h	24.0	h	36.5	h	7.1	l	33.1	h	10.7	h	64.8	h	1	7
16	P.011034A1-3	48.2	l	30.3	l	13.4	l	24.0	lh	4.9	l	21.1	l	3.2	l	36.4	l	8	1
17	P.011035A1-71	55.3	h	27.9	l	15.7		22.5	lh	9.9	l	22.2	l	4.1	l	26.1	l	6	2
18	P.011050A1-13	53.3	lh	23.8	l	13.1	l	11.0	l	5.0	l	17.5	l	3.9	l	12.9	l	8	1
19	P.011099A1-2	51.7	l	31.6	l	15.9		17.5	l	2.6	l	22.7	l	3.0	l	26.5	l	7	0
20	P.011151B1-93	54.3	lh	26.5	l	16.8		18.0	l	2.4	l	21.8	l	7.2	h	45.7	h	5	3
22	RCAT 32/35B	66.9	h	39.6	h	25.4	h	26.5	lh	8.9	l	30.6	h	6.4	h	35.2	l	3	6
23	RCAT Akos2290	50.5	l	23.5	l	10.1	l	24.0	lh	23.8	h	27.7	lh	4.4	l	9.1	l	7	3
24	RCAT F13	63.2	h	29.4	l	20.1	h	40.0	h	38.5	h	43.2	h	7.3	h	23.0	l	2	6
25	RCAT TF174/1c	42.5	l	20.4	l	9.1	l	10.5	l	21.3		22.6	l	4.8	l	8.1	l	7	0
26	VA05W-464	65.9	h	29.9	l	21.9	h	22.5	lh	7.1	l	30.8	h	4.1	l	14.8	l	4	3
27	VA05W-510	61.5	h	33.4	lh	19.9	h	24.5	lh	9.8	l	28.6	lh	3.2	l	48.6	h	5	6
28	VA05W-517	52.1	l	23.4	l	14.5	l	20.0	l	2.8	l	22.8	l	3.0	l	47.3	h	7	1
29	VA05W-673	52.3	l	25.3	l	12.5	l	7.0	l	4.5	l	17.5	l	2.8	l	2.9	l	8	0
30	VA05W-681	56.0	h	26.6	l	15.0	l	13.5	l	4.5	l	22.2	l	3.0	l	5.3	l	7	1
31	M00-3904-9	62.7	h	39.6	h	23.3	h	33.5	h	4.8	l	32.9	h	7.2	h	34.2	l	2	6
32	M02-2152	56.1	h	39.0	h	21.6	h	40.5	h	12.1	l	35.5	h	7.6	h	60.2	h	1	7
33	M02*2518	63.7	h	32.9	lh	19.9	h	26.0	lh	7.1	l	31.7	h	6.8	h	17.8	l	4	6
34	M03-3002	58.2	h	34.4	lh	16.8		27.5	lh	14.5	l	35.2	h	6.0	l	15.6	l	5	4
35	KY98c-1161-03	58.0	h	37.1	h	19.0		24.0	lh	2.8	l	26.7	l	7.4	h	80.2	h	3	5
36	KY98c-1305-02	66.3	h	43.7	h	26.8	h	40.0	h	10.0	l	38.3	h	6.9	h	35.3	l	2	6
37	KY98c-1169-06	57.0	h	22.4	l	13.2	l	21.0	l	3.4	l	24.0	l	7.4	h	32.6	l	6	2
38	KY98c-1164-04	44.3	l	24.3	l	11.1	l	22.5	lh	3.2	l	23.6	l	5.6	l	13.5	l	8	1
39	KY98c-1470-02	61.1	h	42.4	h	21.4	h	36.5	h	15.5	l	38.3	h	6.4	h	60.4	h	1	7
	AVERAGE	55.2		31.5		16.9		22.7		8.8		26.9		5.2		31.0			
	# LOCATIONS	8		8		9		2		2		3		4		2			
	LSD	13.6		14.8		7.9		24.0		15.5		15.5		4.3		37.1			
	R2	0.8		0.5		0.7		0.8		0.7		0.9		0.7		0.7			
	CV	24.4		47.0		49.4		52.2		88.2		35.2		59.6		58.9			

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

Table 6. Summary of results of the 2005-2006 NUWWSN

ENT	NAME	INC		SEV		IND		KR		PSS		ISK		DON		GH		# l	# h
1	ERNIE (MR)	53.7		30.0	l	20.2		19.1	l	4.7	l	25.4	l	7.2	l	24.3		5	0
2	TRUMAN (R)	33.6	l	20.5	l	12.4	l	21.5	l	5.2	l	24.1	l	6.0	l	3.3	l	8	0
3	FREEDOM (MR)	55.0		29.0	l	16.4	l	35.7		17.5	l	38.4		8.8		13.3	l	4	0
4	PION. 2545 (S)	67.4	h	47.8	h	31.9	h	52.5	h	42.1	h	57.9	h	12.9	h	38.2		0	7
5	IL00-8061	44.1	l	25.7	l	15.6	l	14.4	l	5.9	l	21.4	l	5.0	l	14.7		7	0
6	IL00-8109	53.8		33.6		19.7		22.8	l	15.0	l	29.9	l	4.4	l	24.1		4	0
7	IL00-8530	55.3		33.6		22.3		15.8	l	6.7	l	27.8	l	6.1	l	22.0		4	0
8	IL01-11445	46.5		30.8		16.6	l	19.8	l	1.9	l	25.9	l	6.1	l	13.4	l	6	0
9	IL01-11934	52.7		27.7	l	18.5		20.3	l	14.5	l	25.4	l	5.1	l	17.5		5	0
10	MSU Line E0001	47.5		32.2		12.3	l	36.3	h	11.0	l	36.1		9.0		49.1	h	2	2
11	MSU Line E2017	53.3		35.4		17.3	l	35.3		7.9	l	33.1		8.4		30.3		2	0
12	MSU Line E2041	66.7	h	39.0	h	25.7	h	40.5	h	23.0		46.4	h	12.2	h	39.1		0	6
13	MSU Line E2042	36.8	l	28.7	l	9.5	l	24.5	l	13.8	l	22.3	l	5.4	l	17.9		7	0
14	MV 6-82	65.4	h	48.2	h	31.7	h	36.6	h	7.8	l	40.8		5.6	l	51.3	h	2	5
15	NE02465	60.4	h	45.1	h	26.5	h	34.6		24.2	h	43.6		9.1		48.0		0	4
16	NE02584	57.6		33.0		19.7		47.1	h	23.7	h	44.6		8.3		47.6		0	2
17	NE03490	53.6		31.7		15.6	l	34.0		17.9	l	35.0		12.7	h	30.0		2	1
18	NH01046	47.9		36.5		16.6	l	23.5	l	22.8		34.3		9.4		38.4		2	0
19	NI02425	57.5		42.7	h	23.8	h	44.6	h	25.0	h	44.0		8.2		40.8		0	4
20	OH02-12678	49.8		25.5	l	16.2	l	22.7	l	10.2	l	25.1	l	5.2	l	20.3		6	0
21	OH02-12686	45.8	l	31.3		13.5	l	24.8	l	7.8	l	25.4	l	6.3	l	10.7	l	7	0
22	OH02-13567	48.9		25.4	l	14.2	l	27.3	l	4.6	l	27.7	l	7.5		14.6		5	0
23	OH02-7217	45.2	l	18.7	l	10.2	l	27.2	l	6.3	l	24.3	l	8.9		19.6		6	0
24	OH904	36.9	l	18.9	l	11.3	l	14.1	l	14.6	l	20.8	l	3.7	l	5.0	l	8	0
25	P.0128A1-36	45.2	l	23.8	l	16.2	l	13.2	l	5.8	l	20.1	l	4.0	l	14.7		7	0
26	P.0172A1-12	54.0		32.6		21.3		15.8	l	12.0	l	31.1	l	3.7	l	12.5	l	5	0
27	P.0175A1-44	54.5		20.0	l	14.1	l	28.0	l	13.4	l	32.6		6.7	l	3.6	l	6	0
28	P.01931A1-5	46.4		24.0	l	13.9	l	11.8	l	4.4	l	19.3	l	3.6	l	11.9	l	7	0
29	P.01946A1-16	52.3		28.6	l	22.5		25.1	l	10.6	l	30.9	l	5.9	l	7.5	l	6	0
30	RCAT 202D/ 1	61.7	h	37.1		21.3		45.8	h	13.1	l	42.5		8.9		22.5		1	2
31	RCAT 32/157	49.9		33.1		14.7	l	23.6	l	16.0	l	31.6	l	9.5		55.0	h	4	1
32	RCAT Akos2234	46.8		32.6		15.9	l	29.9		21.6		28.2	l	6.4	l	21.5		3	0
33	RCAT TF 203/2	45.2	l	28.8	l	14.9	l	28.3	l	7.6	l	27.4	l	7.1	l	15.8		7	0
34	RCAT19/4c	47.0		33.0		14.8	l	30.0		27.6	h	31.0	l	6.6	l	45.5		3	1
35	VA04W-563	50.8		26.9	l	16.5	l	13.4	l	4.2	l	20.3	l	3.3	l	10.8	l	7	0
36	VA04W-592	52.8		27.0	l	18.2		26.1	l	5.7	l	29.8	l	6.3	l	13.3	l	6	0
37	VA05W-417	46.2		26.4	l	15.3	l	26.6	l	5.5	l	22.6	l	4.9	l	12.2	l	7	0
38	VA05W-421	49.2		29.0	l	15.6	l	26.6	l	4.3	l	25.8	l	6.4	l	19.5		6	0
39	VA05W-452	63.1	h	37.6		23.8	h	33.4		7.1	l	36.4		7.3		46.5		1	2
40	M01-4377	57.0		37.3		22.9		31.2		4.7	l	33.8		6.2	l	12.2	l	3	0
41	COKER 9553	64.5	h	41.4	h	29.7	h	44.8	h	9.0	l	41.4		10.4		50.5	h	1	5
42	KY97c-0554-4-6	64.2	h	33.5		22.0		23.7	l	15.6	l	33.8		5.7	l	12.5	l	4	1
43	KY97c-0540-1-2	67.6	h	35.7		23.6	h	38.1	h	10.5	l	42.2		8.4		34.6		1	3
44	KY97c-0388-5-2	67.5	h	44.4	h	30.4	h	34.1		27.2	h	46.9	h	9.0		58.6	h	0	6
45	KY97c-0304-26-10	63.0	h	42.4	h	23.5	h	48.4	h	18.0	l	47.5	h	8.6		56.1	h	1	6
46	KY97c-0277-1-8	64.9	h	38.0		25.8	h	36.3	h	23.8	h	46.1	h	8.9		22.0		0	5
47	KS03HW12-6-5	56.5		25.3	l	16.3	l	33.1		17.6	l	36.3		12.2	h	28.6		3	1
48	KS970085-9-15	70.3	h	43.7	h	30.7	h	41.0	h	17.6	l	43.4		8.5		24.1		1	4
49	MO050101	54.9		30.3		21.2		23.4	l	10.3	l	32.2		6.9	l	9.8	l	4	0
50	MO050143	45.2	l	22.0	l	11.4	l	19.0	l	9.5	l	23.7	l	6.7	l	7.2	l	8	0
51	MO050132	45.5	l	26.1	l	15.4	l	20.7	l	4.9	l	24.4	l	8.3		5.0	l	7	0
52	MO050194	48.7		26.6	l	17.0	l	27.1	l	14.8	l	30.4	l	7.3		11.9	l	6	0
53	MO050207	47.3		27.2	l	17.2	l	26.3	l	5.8	l	29.9	l	8.6		7.3	l	6	0
54	NY93285-9161	39.7	l	31.1		12.5	l	29.7		17.9	l	27.5	l	6.5	l	31.2		5	0
55	NY92237-1-sp-9173	43.0	l	22.6	l	12.7	l	26.2	l	4.8	l	26.2	l	7.3		22.1		6	0
56	NY94022-9093	59.4	h	49.7	h	28.3	h	52.5	h	19.9	l	48.4	h	16.1	h	44.4		1	6
57	NY93285-9147	40.3	l	33.9		12.6	l	37.6	h	14.6	l	30.7	l	6.7	l	27.1		5	1
58	NY93285-9179	39.3	l	36.9		14.1	l	39.2	h	10.3	l	29.4	l	6.7	l	30.7		5	1
	AVERAGE	52.4		32.1		18.7		29.4		12.9		32.5		7.4		24.9			
	# LOCATIONS	12		13		13		3		3		4		6		3			
	LSD	12.5		11.5		8.5		16.6		19.0		12.7		3.9		10.1			

PLANT BREEDING AND VARIETY DEVELOPMENT: A VITAL CAPACITY FOR U.S. NATIONAL GOALS.

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ABSTRACT

Investment in plant breeding in the U.S. is declining in the public and private sector. Many university plant breeding education programs are at risk. A factual and compelling response is needed. Significant efforts have been made; for example, the National Plant Breeding Study (NPBS) of the 1990's. However, the NPBS relied on intensive time and energy of a few individuals, who eventually were unable to continue. As a result, NPBS recommendations have seen little follow-up. Follow-up from subsequent efforts, such as the 2005 plant breeding education workshop organized by Michigan State University, faces the same risk. A sustainable effort to respond to the decline in plant breeding investment requires a means whereby multiple individuals can distribute and coordinate effort over time. To meet this need, the Plant Breeding Coordinating Committee (CC) is being established. The CC will serve as a long-term forum for leadership regarding issues, problems, and opportunities of strategic importance to the public- and private-sector U.S. national plant breeding effort. It will be the first and only regular opportunity for U.S. plant breeders from all crops and sectors to coordinate their leadership efforts. It will allow plant breeders to develop 'indigenous' leadership on strategic issues, learn from experience, and build alliances across the general society. As an initial approach, the CC will analyze the role of plant breeding in achieving widely-popular national goals:

- Excellence in science and technology.
- A competitive agricultural system in the global economy
- Competitiveness, sustainability, & quality of life in rural America
- A safe and secure food and fiber system
- A healthy, well-nourished population
- Harmony between agriculture and the environment

The work of the CC will enable plant breeders to make the value of their work more visible to the non-technical public, leading to positive outcomes for the future of plant breeding. (The start-up workshop for the Plant Breeding CC will take place in Raleigh, NC, on Feb 8-9, 2007; www.plantbreedingworkshop.ncsu.edu).

QTLS FOR THREE TYPES OF RESISTANCE TO FUSARIUM HEAD BLIGHT IN A WHEAT POPULATION OF WANGSHUIBAI/WHEATON.

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ABSTRACT

Fusarium head blight (FHB), caused by *Fusarium graminearum*, can significantly reduce both grain yield and quality of wheat. Growing FHB resistant cultivars is an effective means to reduce the losses caused by the disease. Currently used FHB resistance sources are mainly 'Sumai 3' and its derivatives. Use of FHB resistance sources other than 'Sumai 3' may enrich the genetic diversity of FHB resistance sources. 'Wangshuibai' is a FHB-resistant Chinese landrace unrelated to 'Sumai 3'. To map quantitative trait loci (QTLs) for resistance to initial infection (type I), FHB symptom spread within a spike (type II), and deoxynivalenol accumulation in infected grain (type III), 139 F_6 derived recombinant inbred lines (RILs) were developed from a cross between 'Wangshuibai' and an FHB-susceptible cultivar, 'Wheaton'. More than 1300 simple sequence repeat (SSR) and amplified fragment length polymorphism (AFLP) markers were analyzed in this population. Five QTLs for type I resistance were detected, on chromosomes 3BS, 4B, 5DL, 3AS, and 5AS; seven QTLs for type II resistance were located, on chromosomes 3BS, 1A, 5AS, 5DL, 7AL, and 3DL; and seven QTLs for low DON content were detected, on chromosomes 3BS, 5AS, 1A, 5DL, 1BL, and 7AL. These QTLs jointly explained up to 31.7%, 64%, and 52.8% of the phenotypic variation for the three types of FHB resistance, respectively. The QTLs on the distal end of 3BS, 5AS and 5DL contributed to all three types of resistance. Two QTLs, on 7AL and 1A, as well as one QTL near the centromere of 3BS (3BSc) showed effects on both resistance type II and III. The broad-sense heritabilities were low for type I resistance (0.36), but high for type II resistance (0.75), and type III (0.71). The result suggested that selection for type II resistance may simultaneously improve type I resistance and reduce DON content as well. The QTLs for FHB resistance identified in 'Wangshuibai' have potential to be used to enhance FHB resistance by pyramiding FHB resistance QTLs from different sources.

ACKNOWLEDGEMENT AND DISCLAIMER

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

GRAIN SHATTERING AND FHB-RESISTANCE QTLS LINKAGE IN WHEAT.

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ABSTRACT

Grain shattering can cause substantial loss in wheat (*Triticum eastivum* L.). Recently, the grain shattering problem has resurfaced with the introduction of Fusarium head blight (FHB, caused by *Fusarium graminearum* Schwabe) resistant germplasm, 'Sumai3', a susceptible wheat genotype to grain shattering. This study was designed to elucidate the relationship between grain shattering and FHB resistance based on mapping quantitative trait loci (QTL) governing resistance to the two traits. A recombinant inbred line population was developed from the cross 'Sumai3' (PI 481542)/'Stoa'(PI 520297) by single seed descent method was used to achieve the objectives of this study. Stoa, a hard red spring wheat cultivar released by North Dakota State University, is resistant to shattering but susceptible to FHB. The RILs and their parents were evaluated fro grain shattering across four North Dakota (ND) environments in 2004 and 2005. Similarly, the same material was evaluated for FHB reaction in the hard red spring wheat (HRSW) breeding scab nursery at Prosper, ND in 2004, and 2005. In order to detect QTL's for grain shattering, ten most resistant and ten most susceptible lines for grain shattering were used. Simple interval mapping analysis of the grain shattering data showed that two QTL's on chromosomes 7A and two on chromosomes 3B are involved in grain shattering. On the other hand, four QTL's were detected on chromosomes, 7A, 3B, and 2B for FHB. Among the QTL for grain shattering on chromosome 7A, one is 7.6 cM away from the one FHB QTL. Similarly, one QTL for grain shattering on chromosome 3B was located 1.5 cM away from a FHB QTL. These close positions between QTL's of grain shattering and resistance to FHB confirm the linkage between the two traits observed by breeders within the populations involving Sumai3 FHB source of resistance. However, based on the distance observed between these QTL's, the linkage can be broken if appropriate breeding method are applied. The HRSW breeding program at North Dakota State University and many other wheat breeding programs were successful to break this undesirable linkage by releasing cultivar/germplasm with resistance to both traits.

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MOLECULAR MARKER CHARACTERIZATION OF FUSARIUM HEAD BLIGHT RESISTANT GERMPLASM.

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ABSTRACT

Molecular markers linked to Fusarium head blight (FHB) resistance QTLs have been identified in the wheat genome. Marker-QTLs linkages validated in different genetic backgrounds or by different research groups can be used to postulate the presence of resistant alleles in other germplasm. The objective of this research was to postulate the presence of 3 FHB QTLs in FHB resistant germplasm using DNA markers. One hundred and sixty eight wheat lines from the USDA spring wheat germplasm collection which had moderate to high levels of FHB resistance based on field visual disease levels, visual scab kernel, and DON levels were used in this study. Marker allele type was determined for *Fhb1* (using marker STS3B-256) from Sumai 3 on chromosome 3BS; *Qfhs.ndsu-3AS* (dupw227) from Frontana; and *Qfhs.ifa-5A* (barc186) from Wuhan 3 and Sumai 3. Thirty-two accessions displayed Sumai 3 type of *Fhb1* allele of marker *STS3B-256* (Table 1). All the FHB resistance germplasm originating from Japan, except PI 81791, had the *Fhb1* allele. The second largest group of *Fhb1* Sumai 3 haplotype was from South America. Only five FHB resistant European lines and one Chinese line had the *Fhb1* haplotype. The one Chinese line with *Fhb1* was a modern cultivar. The Chinese lines without *Fhb1* were landraces originated from a spring wheat production region different from where Sumai 3 was grown, which explains the low frequency of *Fhb1* haplotype in this set of Chinese materials. Twenty-four accessions had the Frontana *dupw227* haplotype. Twelve accessions with the Frontana haplotype in the Chinese germplasm were landraces. Only four lines with the Frontana *dupw227* haplotype were of European origin, and the others were from South America. Seventeen accessions from Europe and 11 from South America displayed Wuhan 3 and Sumai 3 type of *Qfhs.ifa-5A* QTL allele using *barc186.* This allele was most common in the Japanese FHB resistant germplasm. About half of the accessions did not display any of the known QTL alleles indicating that they may have novel FHB resistance alleles. There are bound to be false positives and negatives with these data, especially dupw227 and barc186 markers, because relatively little is known about their allele diversity and the diagnostic potential of the markers. Nevertheless, accessions postulated to not contain these QTL should receive high priority for future genetic characterization, mapping, and introgression to complement the resistance genes already present in breeding populations.

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Table 1. Geographical Distribution of DNA markers alleles of three Fusarium head blight (FHB) resistance QTLs in FHB resistant germplasm.

		No. of accessions with the same marker allele as the QTL donor			
Country/ Region	No. accessions tested	*Fhb1* (STS3B-256)	*Qfhs.ndsu-3AS* (dupw227)	*Qfhs.ifa-5A* (barc186)	None
China	23	1	12	4	9
Europe	81	5	4	17	45
Japan	14	13	0	10	1
S. America	50	13	8	11	33
Total	168	32	24	42	88

PATHOGEN GENETICS AND GENOMICS

THE IDENTIFICATION OF A GENE IN *FUSARIUM GRAMINEARUM* THAT CONTRIBUTES TO BUTENOLIDE SYNTHESIS.

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ABSTRACT

The development of expressed sequence tag (EST) databases, directed transformation and a sequenced genome have facilitated the functional analyses of *Fusarium graminearum* genes. Extensive analysis of 10,397 ESTs, derived from thirteen cDNA libraries of *F. graminearum* grown under diverse conditions, identified a novel cluster of eight genes (gene loci *fg08077 – fg08084*) located within a 17 kb region of genomic sequence contig 1.324. The expression of these genes, as detected by Northern analysis and qPCR, is concomitantly up-regulated under growth conditions that promote mycotoxin production. Gene disruption experiments followed by metabolite analysis of the transformants indicated that one of the genes, *fg08079*, is directly involved in butenolide synthesis, a secondary metabolite derived from glutamic acid. The mycotoxin butenolide is produced by several *Fusarium* species and has been suggested, but not proven, to be associated with tall fescue toxicoses in grazing cattle. To confirm that this gene is involved in butenolide biosynthesis, the complete, intact gene was added back to the disruption mutants. The add-back transformants were once again able to synthesize butenolide. As expression of these genes can be detected very early in wheat and barley infection, butenolide may play a role in plant infection. However, greenhouse testing for FHB (Fusarium Head Blight) using disruption mutants of *fg08079*, showed that this gene did not contribute significantly to virulence in wheat heads. We will continue to exploit genomic and proteomic tools to identify genes that are involved in FHB disease.

HAPLOTYPE NETWORKS FROM *FUSARIUM GRAMINEARUM* REVEAL PATTERNS OF EVOLUTION.

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ABSTRACT

DNA sequences of two nuclear genes (*MAT1-1-3*, and *Tri101*) were examined from over 500 isolates of *Fusarium graminearum* collected from South American and Korean wheat, maize and sorghum. Haplotype networks were developed for each gene that illustrate the relationships between the DNA sequences of isolates in this study based on the minimum number of base pair changes that separate the isolates. Some lineage diagnostic single nucleotide polymorphisms (SNPs) are not conserved in this strain set. The lack of dichotomous branching suggests that the lineages did not evolve in a stepwise fashion. The haplotype networks cannot be resolved without cycles, which is consistent with recent gene flow between the lineages.

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EMERGENT POPULATIONS OF *FUSARIUM GRAMINEARUM SENSU STRICTO* IN THE UPPER MIDWESTERN U.S. DISPLAY GRADIENT OF FREQUENCY AND A HIGH MYCOTOXIN POTENTIAL.

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ABSTRACT

Based on preliminary analyses of 4,957 fungal cultures gathered from wheat in Minnesota (MN), North Dakota (ND) and South Dakota (SD) in 2003 and 2004, we previously reported that *Fusarium graminearum sensu stricto* in the Upper Midwest consists of genetically divergent populations (Proceedings of the 2005 National Fusarium Head Blight Forum, page 158). Besides the common Midwestern 15ADON population, chemotyping using PCR and VNTR analysis revealed the presence of two additional and genetically divergent populations (emergent populations) that have increased dramatically in frequency in ND and northwestern MN over a short time period. Plotting the frequency of populations according to sample location clearly reveals a gradient of decreasing frequencies of the emergent populations toward southern regions of MN and ND and with highest frequency being observed at locations close to the Canadian border (Manitoba). This distribution implies that introduction and spread of these emergent populations probably has a North to South direction. Potential spread further south is currently being examined by a collection from 2006 consisting of approximately 1,200 wheat samples from 36 locations in ND, MN and SD that concentrated on the southern range of distribution of these emergent populations. Knowledge of the distribution and frequency of these emergent populations is important as greenhouse experiments conducted in spring 2006 that included a total of 60 strains (with three repetitions) from the three known populations clearly indicated that the susceptible cultivar Norm accumulated substantially more deoxynivalenol when inoculated with strains from the two emergent populations than when inoculated with strains from the common and widespread MW 15 ADON population. Whether higher mycotoxin levels also accumulate under field conditions to at least partially explain the increase in frequency of these emergent populations in some regions needs to be determined further.

ACKNOWLEDGEMENTS

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DISCLAIMER

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

REAL-TIME QUANTITATIVE EXPRESSION STUDIES OF THE ZEARALENONE BIOSYNTHETIC GENE CLUSTER IN *FUSARIUM GRAMINEARUM*.

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ABSTRACT

Zearalenones are estrogenic mycotoxins produced by several *Fusarium* species and can cause reproductive problems in animals. We have previously described the use of *Fusarium pseudograminearum* to identify the polyketide synthase gene *PKS4* as having a major role in zearalenone (ZON) production (Lysøe et al. 2006. Appl. Environm. Microbiol. 72: 3924-3932). An *Agrobacterium tumefaciens*-mediated transformation protocol was used to replace the *PKS4* gene with a *hygB* resistance gene in a high zearalenone-producing *F. graminearum* strain. PCR and Southern analysis of transformants identified isolates with single insertional replacements of *PKS4*, and HPLC analyses were used to confirm the lack of the ability of this mutant to produce ZON. Barley root infection studies showed no alteration in the pathogenicity of the wild type and the *PKS4* mutant. Also others have proved the involvement of *PKS4* as well as the neighboring gene *PKS13* in the synthesis of ZON (Gaffoor et al 2005. Eukaryot. Cell 4: 1926-1933; Kim et al. 2005. Mol. Microbiol. 58: 1102-1113). Expression experiments of the genes located in the cluster where *PKS4* and *PKS13* are positioned, however, have to date been limited. This study focuses on the real-time expression of seven genes in the cluster in ZON-producing and ZON-deficient mutant strains, under inducing conditions on inoculated sterile rice and during wheat infection. The two polyketide synthase genes *PKS4* and *PKS13* and the alcohol oxidase *FG12056* showed similar gene expression pattern as the putative transcriptional regulator *FG02398*, while the non-ribosomal peptide synthase *FG02394*, the K^+ channel ß subunit *FG12015* and the protein kinase *FG02399* displayed a somewhat different expression pattern. The expression of *PKS4, PKS13, FG12056* and *FG02398* genes were quite consistent in relation to each other between the two experiments, while the others varied. *PKS13* had slightly higher gene expression than *PKS4* in both experiments, possibly suggesting individual enzymatic activity as opposed to an enzyme complex. *FG02398* and *FG12015* showed higher gene expression in wheat than on parboiled rice, suggesting a role during wheat infection. Based on these expression data, the knowledge that ZON is able to bind to the estrogen receptor, and literature studies, we suggest a potential role of ZON in *Fusarium*.

TWO MITOGEN-ACTIVATED PROTEIN KINASE SIGNALING CASCADES REGULATE SENSITIVITY TO ANTIFUNGAL PLANT DEFENSINS IN *FUSARIUM GRAMINEARUM*.

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ABSTRACT

Two cysteine-rich antifungal defensins, MsDef1 and MtDef4, from *Medicago* spp., share 41% amino acid sequence identity and inhibit the growth of the fungal pathogen *Fusarium graminearum* at micromolar concentrations. However, the molecular mechanisms by which these defensins inhibit the growth of this fungus remain largely unknown. In order to determine the fungal signaling cascades that are modulated by these defensins, we have screened 4,800 insertional mutants of *F. graminearum* and isolated several mutants that selectively exhibit hypersensitivity to MsDef1, but not to MtDef4. The molecular characterization of two of these mutants, designated *enhanced sensitivity to defensin (esd)*, has revealed that the Mgv1 and Gpmk1 MAP kinase signaling cascades play a major role in regulating sensitivity of *F. graminearum* to MsDef1, but not to MtDef4. The Hog1 MAP kinase pathway, which is responsible for adaptation of this fungus to hyperosmotic stress, does not participate in the fungal response to these defensins. Significantly, the *esd* mutants also exhibit hypersensitivity to other defensins used in this study except MtDef4 and are highly compromised in their pathogenesis on wheat heads and their ability to cause infection in wounded tomato fruits. The studies reported here for the first time implicate two MAP kinase signaling cascades in a plant defensin-mediated alteration of fungal growth. Based on our findings, we propose that specific MAP kinase signaling cascades are essential for protection of a fungal pathogen from the antimicrobial proteins of its host plant.

SPATIAL PATTERNS OF TRICHOTHECENE GENOTYPES OF *GIBBERELLA ZEAE* IN WHEAT FIELDS.

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ABSTRACT

Grain infected with *Gibberella zeae* often contains the trichothecene mycotoxins deoxynivalenol (DON) and nivalenol (NIV), threatening the health of humans and domesticated animals. Isolates of *G. zeae* that produce DON may also produce two acetylated derivatives, 3-ADON and 15-ADON. These derivatives may vary in toxicity, and NIV is considered to be ten times more toxic to animals than DON. Little is known about the spatial distribution of trichothecene genotypes of *G. zeae* (3-ADON, 15-ADON, and NIV) in wheat fields. We collected GPS-referenced FHB samples from individual wheat fields in Virginia, New York, and North Carolina. Singleplex and multiplex PCR assays were used to evaluate trichothecene genotypes of *G. zeae* in these geographically-referenced populations. Spatial patterns of trichothecene genotypes were visualized by contour plots of genotype counts over entire fields. Knowledge of the spatial patterns of trichothecene genotypes of *G. zeae* in wheat fields may aid in developing and/or excluding strategies for disease management. Little or no testing for NIV is currently performed in the eastern United States. Should the NIV genotype be present in wheat fields in the eastern United States, it would be essential to implement appropriate assays for detecting NIV contamination in these regions.

TRICHOTHECENE GENOTYPES IN ATMOSPHERIC POPULATIONS OF *GIBBERELLA ZEAE*.

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ABSTRACT

Gibberella zeae (*Fusarium graminearum* sensu stricto) is the principal causal agent of Fusarium head blight (FHB) of wheat and barley in the USA. Grain infected with *G. zeae* often contains the trichothecene mycotoxins deoxynivalenol (DON) and nivalenol (NIV), threatening the health of humans and livestock. Isolates of *G. zeae* that produce DON may also produce two acetylated derivates, 3-ADON and 15-ADON. These derivates may vary in toxicity, and NIV is considered to be ten times more toxic to animals than DON. We used singleplex and multiplex PCR assays to evaluate trichothecene genotypes (3-ADON, 15-ADON, and NIV) in atmospheric populations of *G. zeae* collected over three years in New York, USA. Results indicated that the majority of the isolates were of the 15-ADON genotype. Knowledge of the distribution and spread of trichothecene genotypes of *G. zeae* in the atmosphere may be used to infer sources of inoculum for regional epidemics of FHB, and may aid in the development of strategies for disease management. Immigrant strains of *G. zeae* with altered toxin profiles, if transported long distances through the atmosphere, have the potential to spread rapidly across North America and displace native strains.

GENE EXPRESSION ANALYSIS OF CONIDIUM AND ASCOSPORE DEVELOPMENT IN *FUSARIUM GRAMINEARUM*.

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ABSTRACT

To understand the infection cycle of the head blight pathogen *F. graminearum*, gene expression profiles were monitored during both ageing and germination for conidia and ascospores. Ascospores and conidia were treated in a similar manner, either aged under desiccating conditions or suspended in liquid germination medium. RNA was extracted from spores and cultures and used to query the 18K feature *F. graminearum* Affymetrix GeneChip. Overall, a slightly greater number of probe sets corresponding to genes were detected in ascospores (9,207) than in conidia (8,815; detection p value <0.001). However, the large majority of probe sets (8,068) were shared between conidia and ascospores. While a similar number of genes were detected at most stages of development, the biggest difference among spore types was upon desiccation where the number of probe sets detected in ascospores (6,801) was more than twice the number detected in conidia (2,916). These results indicate that ascospores remain more metabolically active than conidia upon ageing. Peroxisomal proteins and genes involved in lipid beta-oxidation are strongly up-regulated both in fresh conidia and in ascospores. After suspending conidia or ascospores in liquid germination medium, numerous genes involved in transcription, RNA splicing, protein synthesis, and amino acid and nucleotide metabolism were highly induced. Up-regulation of proteasome components and secretory proteins were observed as spores established polarized growth after 8h of incubation. Comparing gene expression in spores with expression in hyphae under a variety of environment regimes indicates that a total of 328 probe sets were specific for ascospores and another 150 were specific for conidia. Spore-specific gene expression may be used to develop hypotheses concerning spore maturation, dormancy and initiation of germination that ultimately may serve as the underlying basis for novel disease control strategies.

ACKNOWLEDGEMENT AND DISCLAIMER

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

A NOVEL G-BETA LIKE PROTEIN IS ESSENTIAL FOR PATHOGENESIS IN THE WHEAT SCAB FUNGUS *FUSARIUM GRAMINEARUM*.

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ABSTRACT

Fusarium head blight (FHB) or scab caused by *Fusarium graminearum* is an important disease of wheat and barley. In addition to yield losses, infected grains are reduced in grain quality and contaminated with mycotoxins. In our previous study, we identified several mutants with reduced virulence by random insertional mutagenesis. In one of these mutants, the transforming vector was inserted in a predicted gene named *TBL1* (for transducin beta-like gene 1). *TBL1* is homologous to the mouse *TBLR1*, which encodes a putative nuclear receptor corepressor. The Tbl1 protein contains three WD40 repeats and an N-terminal LisH domain, which is involved in protein-protein interactions. We generated the *tbl1* deletion mutant by the gene replacement approach. The •*tbl1* mutant was non-pathogenic and significantly reduced in conidiation. It was defective in colonizing flowering wheat heads and more sensitive to a plant defensin MsDef1. Conidium germination was delayed in the "*tbl1* mutant. The "*tbl1* mutant accumulated a red pigment that may be related to the upregulation of the aurofusarin synthesis cluster based on our microarray analysis. Interestingly, *TBL1* is the only LisH domain-containing gene in the *F. graminearum* genome. To determine its function, we generated a *TBL1*$^{\text{"LisH}}$ allele and transformed it into the "*tbl1* mutant. Phenotype analysis of the resulting transformants expressing *TBL1*$^{\text{"LisH}}$ suggests that LisH is essential for the *TBL1* function and plant infection. We also generated a *TBL1*-GFP fusion construct and examined its expression and localization. Our results indicate that *TBL1* plays a critical role in conidium germination, response to plant defense compounds, and colonization of wheat tissues.

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OTHER PAPERS

THE U.S. WHEAT AND BARLEY SCAB INITIATIVE WEB SITE.

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ABSTRACT

The U.S. Wheat and Barley Scab Initiative (USWBSI) web site (http://www.scabusa.org) is an integral part of the USWBSI. The site is an important information resource for all aspects of the Initiative, including Research, News, Forums, Literature, and Contact Information.

The site includes several publicly searchable databases that provide information on all Projects, Grants, Institutions, Documents, Committees, and Contacts associated with the Initiative. This year, several new features have been introduced as part of a goal of continual improvement. These include an online bibliography database (http://scabusa.org/refbase/) for *Fusarium* related publications, an RSS news feed (http://scabusa.org/rss.html), and a community blog (http://scabusa.org/wordpress/) where users can relate the latest news and information as it pertains to the Initiative. The bibliography database is a user supported project. Submissions of papers to be included are encouraged and should be sent to Sue Canty.

Additionally, a beta version of an electronic pre-proposal submission system (EPSS) has been introduced. A content management system named eGroupWare (http://www.egroupware.org/) was chosen as the foundation on which to build the electronic pre-proposal submission system. Current features include user defined virtual folders for data storage, sophisticated user rights management, and a customizable portal interface. A web-based file editing software package was developed in-house and incorporated into the eGroupWare package to allow users to collaboratively edit documents required for the electronic pre-proposal submission system. This system's performance is currently being analyzed. After appropriate changes are made, the system will be finalized and available to all those who submit pre-proposals next year.

Future goals are to integrate the USWBSI web site more closely with GrainGenes (http://wheat.pw.usda.gov), an online database of molecular and phenotypic information for the Triticeae, and to finalize the interface for a new online calendar of events (http://scabusa.org/cal.html and http://scabusa.org/webcal/month.php). This poster provides an overview of the USWBSI web site and solicits ideas and suggestions for future improvements to the site.

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DISCLAIMER

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