

*Proceedings of the  
2017 National Fusarium  
Head Blight Forum*



**Hyatt Regency Milwaukee  
Milwaukee, Wisconsin, USA  
December 3-5, 2017**

Proceedings compiled and edited by: S. Canty, B. Wiermer and D. Van Sanford

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NOTE: Photo selected in recognition of Dave Van Sanford, who is stepping down as Co-Chair of the USWBSI after serving in this position since 2006.

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# **FHB MANAGEMENT**





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# INTEGRATED FHB MANAGEMENT OF SPRING WHEAT IN IDAHO

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## ABSTRACT

The Fusarium head blight (FHB) management coordinated project has been conducted in southern Idaho for two consecutive years to evaluate the integrated management strategies for reducing FHB and deoxynivalenol (DON) in spring wheat. On May 8, 2017, four varieties (Klasic, Diva, IDO1202S and IDO851) were planted in a split-plot arrangement with variety as the whole-plot and fungicide treatment as sub-plot with 4 replications. Fungicides were applied at anthesis only (Prosaro®) and anthesis + 4 days (Prosaro + Caramba®, Caramba + Folicur® and Proline ®+ Folicur) using a CO<sub>2</sub> backpack sprayer with 8001 VS nozzles at a rate of 20 gal/A. Within 24 to 36 hours of the anthesis fungicide application, a conidial suspension (100,000 macroconidia per liter) was applied on all plots with a CO<sub>2</sub> sprayer with 8003 VS nozzles at a ground speed of 1 second per foot at 40 psi. Using the GLIMMIX procedure (SAS 9.4), the variety by fungicide treatment interaction were determined for FHB index, *Fusarium*-damaged kernels (FDK), test weight ( $P<.0001$ ) and yield ( $P=0.0128$ ). DON analyses will be provided on a later date. Moderately high FHB levels were observed with up to 57.8% FHB index and 11.2% FDK, respectively. Yield and test weight ranged from 59.5 (Klasic) to 108.0 (IDO851) bu/A and 56.5 (Klasic) to 63.0 (IDO1202S) lbs/bu, respectively. Fungicide treatments significantly reduced FHB and increased yields for FHB susceptible (Klasic) and the moderately susceptible varieties (Diva and IDO1202S). However, the efficacy of post-anthesis fungicide applications in significantly reducing FHB index and increasing yield loss compared to anthesis-only application was only observed in Klasic. Resistance is still the most effective strategy to prevent disease and yield loss as shown by the moderately resistant variety, IDO851. It is crucial to validate levels of susceptibility and resistance in the next generation of wheat varieties to determine economic and strategic benefit of anthesis fungicide applications.

## ACKNOWLEDGEMENT AND DISCLAIMER

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# INTEGRATED MANAGEMENT OF FUSARIUM HEAD BLIGHT IN DURUM VARIETIES

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## ABSTRACT

Severe Fusarium head blight (FHB) epidemics occurred in 2014 and 2016 in northwest North Dakota and northeast Montana. This area routinely accounts for over 50% of the durum acreage for the United States. High deoxynivalenol (DON) levels in this production region resulted in severe dockage, and in some cases, grain rejected at point of sale. Given that durum tends to be one of the most susceptible wheat market classes, fungicides are an important management tool to help manage FHB and DON. With funding from the USWBSI, six integrated management trials were conducted on durum in 2016 and 2017. Research locations included Fargo, Langdon, Williston and Nesson Valley. All locations used the standard set of fungicide treatments outlined in the integrated management protocol. The fungicide treatments included prothioconazole + tebuconazole applied at Feekes 10.51 (early-anthesis), prothioconazole + tebuconazole at Feekes 10.51 followed by metconazole 4-7 days later (post-anthesis), metconazole at Feekes 10.51 followed by tebuconazole 4-7 days later, and prothioconazole at Feekes 10.51 followed by tebuconazole 4-7 days later. Some locations also included fungicide treatments that addressed questions commonly asked by growers. At least two durum varieties varying in FHB susceptibility were used at each location. *Fusarium* infested corn spawn was dispersed at each site to enhance FHB development and irrigation was used at Langdon (mist) and Nesson Valley (center pivot). Low to high levels of disease occurred across the locations, and trials were analyzed separately. Adequate disease pressure to separate differences in variety and/or fungicide treatment occurred in three out of the six trials. Results from these trials indicated that most double fungicide applications (early-anthesis and 4-7 days later) resulted in lower DON accumulation when compared to the non-treated control and prothioconazole + tebuconazole applied at early-anthesis. However, a post-anthesis prothioconazole + tebuconazole application was just as effective as the double fungicide programs. The value of a post-anthesis application and multiple fungicide applications in durum needs further investigation to help strengthen FHB and DON management.

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# EFFECT OF A NEW SDHI FUNGICIDE (ADEPIDYN) IN MANAGING FHB AND DON ON SOFT RED WINTER WHEAT IN KENTUCKY

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## ABSTRACT

Currently, the only foliar fungicides recommended for management of *Fusarium* head blight (FHB; caused by *Fusarium graminearum*) and contamination of grain with the associated mycotoxin, deoxynivalenol (DON) belong to the demethylation inhibitor (DMI; also known as triazole) class of fungicides. A new fungicide active ingredient from Syngenta Crop Protection known as adepidyn is currently under the process of registration for use on wheat for management of FHB and DON in the U.S. Adepidyn belongs to the succinate dehydrogenase inhibitor (SDHI) class of fungicides, and will be mixed with propiconazole, a DMI fungicide, and available as Miravis Ace. Miravis Ace was evaluated for its efficacy in managing FHB and DON in mist-irrigated trials on a FHB-susceptible soft red winter wheat cultivar at the University of Kentucky Research & Education Center in Princeton, KY in 2016 and 2017. In 2016, Miravis Ace was applied at Feekes growth stage (FGS) 10.3 or FGS 10.5.1 and compared with Prosaro® fungicide (a mixture of the DMI fungicides prothioconazole and tebuconazole from Bayer CropScience) applied at the same timings and a non-treated control. In this trial, the FHB index was moderately high, with the non-treated control having a FHB index value of 28 (on a scale of 0-100, with 100 being the greatest amount of disease possible). In this trial, Prosaro applied at FGS 10.3 significantly ( $P \leq 0.05$ ) reduced FHB index relative to the non-treated control, but Miravis Ace applied at FGS 10.3 and Prosaro or Miravis Ace applied at FGS 10.5.1 resulted in the significantly lowest FHB index values. In the 2016 trial, DON contamination of grain was relatively high, with grain from the non-treated control having a DON concentration of 6.8 ppm. Both Miravis Ace and Prosaro applied at FGS 10.5.1 significantly reduced DON concentrations in grain compared to the non-treated control, but the lowest DON concentrations were in grain from plots treated with Miravis Ace or Prosaro at FGS 10.5.1. In 2017, Miravis Ace was applied at FGS 10, FGS 10.5.1, or 5 days after FGS 10.5.1. A non-treated control and the comparison treatments of Caramba® (containing the DMI fungicide metconazole from BASF Corporation), Prosaro, or Folicur® (containing the DMI fungicide tebuconazole from Bayer CropScience) applied at FGS 10.5.1 and Prosaro applied at 5 days after FGS 10.5.1 also were included. Overall, disease levels were lower in the 2017 trial, as the non-treated control had a FHB index value of 14 and grain from the non-treated control had a DON concentration of 1.6 ppm. All treatments significantly reduced FHB index relative to the non-treated control, but Miravis Ace or Prosaro applied 5 days after FGS 10.5.1 resulted in the lowest FHB index values. The only treatments that resulted in significantly lower DON concentrations in the grain relative to the non-treated control were Prosaro applied at FGS 10.5.1 and Miravis Ace or Prosaro applied 5 days after FGS 10.5.1. Based on these results, when Miravis Ace becomes registered and available for use, it will provide a new active ingredient from an alternative fungicide class that can be used to help manage FHB and DON. More research is needed to better understand the effective application window for this product and to better understand its efficacy across several geographies and small grain classes.

# MULTI-STATE RESEARCH ON THE EFFECT OF QUINONE OUTSIDE INHIBITOR FUNGICIDES ON DON CONTAMINATION IN WHEAT GRAIN

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## ABSTRACT

Quinone outside inhibitor (QoI; also known as strobilurin) fungicides commonly are used to manage foliar diseases of wheat, and have not been recommended for management of Fusarium head blight (FHB; caused by *Fusarium graminearum*). Reports of low efficacy in managing FHB, and reports of increased deoxynivalenol (DON) associated with late applications (at heading or later) of QoI fungicides are the primary reasons why QoI fungicides are not recommended for FHB management. Despite this, applications of QoI fungicides sometimes are applied at later growth stages (such as Feekes 10.5, “heading complete”) for management of foliar diseases. Many fungicide products registered for wheat contain a mixture of a QoI active ingredient and a demethylation inhibitor (DMI; also known as triazole) active ingredient. Currently, certain DMI fungicides are the only products available that are recommended for FHB control. A multi-state research project was initiated to determine: i) the effects of QoI and QoI + DMI fungicides applied at different growth stages on FHB and DON; and ii) whether the application of QoI + DMI mixtures applied at anthesis or sequential applications of a QoI followed by a DMI counteracted any potential DON increase associated with the application of a QoI alone. Data from trials conducted across seventeen soft and hard wheat-producing states (AR, IA, IL, IN, KY, LA, MD, MI, MN, MO, MT, ND, NY, OH, SD, VA, and WI) were utilized for this research. Although some applications of QoI fungicides did significantly ( $P \leq 0.05$ ) reduce FHB index values compared to the non-treated control, they were not as effective as DMI fungicides in reducing FHB index values. When applied alone, QoI fungicides significantly increased DON values, relative to the non-treated control, when applied at Feekes 10.0 or 10.5. Only one of the three QoI + DMI products applied at Feekes 10.5 significantly increased DON values relative to the non-treated control, while the other two products had no effect on DON. Sequential applications of a QoI fungicide at Feekes 9, followed by a DMI fungicide at Feekes 10.5.1 generally reduced DON relative to the nontreated control; however, the reduction in DON observed with these sequential applications was generally not as great as observed with a solo DMI application at Feekes 10.5.1. In general, this research confirmed that QoI fungicides have the potential to increase DON levels when applied at Feekes 10.0 or 10.5. In addition, following a QoI fungicide with a DMI fungicide applied at Feekes 10.5.1 did not entirely counteract the potential of a QoI fungicide to increase DON levels.

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# FUNGICIDE TIMING AND VARIETY RESISTANCE TO MANAGE FUSARIUM HEAD BLIGHT IN MID- ATLANTIC WINTER BARLEY CROPS

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## ABSTRACT

A three-year field experiment (2015-2017) was conducted to help mid-Atlantic barley producers select FHB-resistant varieties, judge the potential benefit from a fungicide, and choose the optimal timing for a fungicide application should FHB threaten. The split-plot experiment took place in a misted, inoculated nursery in Raleigh, North Carolina, using main plots of three commonly planted six-row barley cultivars (Atlantic, Nomini, and Thoroughbred) and one two-row cultivar (Endeavor). As sub-plots, three fungicide treatments were applied: Prosaro® at 100% spike emergence (P1), Prosaro 6 days later (P2), or no fungicide (UNTR).

Overall, the DON results gave no reason to prefer one of the two fungicide timings: in the first year, both timings had lower DON than UNTR ( $P \leq 0.0005$ ) but in the second year, neither did ( $P \geq 0.14$ ). For disease index (incidence \* severity), P1 was lower than UNTR only in the third year, and P2 was lower than UNTR in the first and third years; however, P2 and P1 switched ranks in those two years. There was no difference in index among the three treatments in the second, low-DON year ( $P \geq 0.14$ ). Considering UNTR means, in both years DON ranking was Atlantic > Nomini = Thoroughbred > Endeavor. The results supported classifying Atlantic as susceptible, Nomini and Thoroughbred as moderately susceptible, and Endeavor as moderately resistant.

Averaging the two fungicide timings, DON was reduced by fungicide application about 32% in comparison with the untreated control across the four cultivars and two years. There was no difference in DON contamination between Prosaro at 100% spikes just fully emerged (P1) and Prosaro at 6 days later (P2), which means growers have a window of nearly a week within which to achieve equivalent benefit from fungicide application. Relative to the S cultivar Atlantic, the DON reduction provided by the MR cultivar Endeavor in the absence of fungicide was 65%. Again relative to untreated Atlantic, DON reduction was 60% from the combination of fungicide and moderate susceptibility (Nomini and Thoroughbred), and 78% for fungicide plus moderate resistance (Endeavor). Taken together, our results indicate that growers concerned about minimizing DON in malting barley should use moderately resistant barley varieties and should apply fungicide if there is scab risk.

## ACKNOWLEDGEMENT AND DISCLAIMER

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# EVALUATION OF FUNGICIDE APPLICATIONS PLUS CULTIVAR RESISTANCE TO REDUCE FHB AND DON INFECTION OF BARLEY IN NEW ENGLAND

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## OBJECTIVE

To evaluate the individual and interactive effects of moderately resistant cultivars and application timings of fungicides on barley yield and the integrated management of *Fusarium* head blight (FHB) and deoxynivalenol (DON) in Vermont.

## INTRODUCTION

Public interest in sourcing local foods has extended into beverages. This had led to a rapid expansion of the northeast malting industry and has given farmers new markets. However these farmers are struggling to produce barley that is not infected with FHB and DON. Hence integrated management strategies are essential for managing yield and quality losses from FHB. Most farmers in New England have experienced significant crop loss from FHB and some farmers have already stopped growing barley. At present few farmers are specifically selecting varieties for resistance to FHB and even fewer are combining host resistance with fungicide applications. Other regions have shown that the use of a well-timed fungicide is an important management tool when suppressing FHB in barley production. In Vermont during 2016, we observed the disease and yield impact of cultivar susceptibility, inoculation with *Fusarium graminearum*, and treatment with fungicides at two timings.

## MATERIALS AND METHODS

The trial was conducted at the Borderview Research Farm in Alburgh, VT in a Benson silt loam soil planted with two spring barley varieties, 'Robust' (susceptible to FHB), 'Conlon' (moderately

resistant to FHB) on 19 April 2016. The experiment was set up as a completely randomized block design with a split-plot arrangement, with cultivar as the main plot and the fungicide treatments as subplots, randomized in four replicated blocks. Fungicide treatments are shown in Table 1. Main plots were sown with barley at 125 lb ac<sup>-1</sup> with a Great Plains grain drill (Salinas, KS). Subplots were 5 x 20 ft including 7 rows with 7-in. row spacing. The first fungicide application was applied at heading (Feekes growth stage, FGS 10.1) on 17 June 2016 including the surfactant Induce at 0.125% V/V. After the fungicide had dried, plots were spray-inoculated with a conidial suspension of *F. graminearum* (40,000 conidia/ml) to augment the development of FHB. The second fungicide application occurred four days after heading on 21 June 2016 including the surfactant Induce at 0.125% V/V, and inoculated with a conidial suspension of *F. graminearum* (40,000 conidia/ml) after the fungicide had dried. Fungicide and *F. graminearum* treatments were applied with a CO<sub>2</sub> backpack sprayer with paired TJ-60 8003vs nozzles mounted at an angle (30° from horizontal) forward and backward, 20-in. apart, pressurized at 30 psi, and calibrated to deliver 20 gal/A. Incidence and severity (percent of symptomatic spikelets on symptomatic heads) of FHB in each plot were rated on 15 July and used to calculate FHB index, where FHB index = (FHB severity \* FHB incidence)/100 (data not shown). Grain was harvested using an Almaco plot combine (Nevada, IA) on 27 July 2016. Grain moisture, plot yield, and test weight were recorded. Yield and test weight were adjusted to bu/A at 13.5% moisture. Analysis of DON content in grain was conducted at the University of Vermont Cereal Grain Testing Laboratory located in Burlington, VT. Treatment

means were calculated, subjected to analysis of variance, and separated by Fisher's protected LSD test ( $P = 0.05$ ).

## RESULTS AND DISCUSSION

Weather conditions in Vermont during the 2016 growing season can be characterized as having below average precipitation and above average temperatures. The incidence of *F. graminearum* even with inoculation was less than 1% in all treatments (data not shown).

The impact of supplemental inoculation with *F. graminearum* was determined by comparing the non-inoculated and inoculum only treatment. Overall, inoculation did not significantly impact DON concentrations or yield as compared with the non-inoculated plots (Table 2).

There was no significant cultivar by fungicide treatment interactions for DON or yield. This indicates that under low disease pressure the varieties responded similarly to the fungicide treatments (data not shown).

When results were combined across cultivars, the fungicide treatments did not significantly impact DON concentrations (Table 2). The barley yields

did respond differently to the fungicide treatments (Table 2). The Prosaro® SC application at heading had significantly higher yields than all other treatments except the Prosaro SC applied 4 days after heading.

Under low disease pressure, there were no significant differences detected in DON concentrations or yield among varieties (Table 3).

Overall low disease pressure led to lack of treatment differences during the 2016 growing season. This underscores the necessity to conduct these types of experiments over numerous years and environments.

## ACKNOWLEDGEMENT AND DISCLAIMER

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**Table 1.** Fungicide treatments, active ingredients and rates applied.

Fungicide treatments	Company	Fungicide active ingredient	Application rates
Control			Water
<i>Fusarium graminearum</i>			40,000 spores/ml
Prosaro SC®	Bayer CropScience	Prothioconazole + tebuconazole	6.5 fl oz ac <sup>-1</sup> + Induce at 0.125% V/V
Caramba®	BASF Ag Products	Metconazole	14 fl oz ac <sup>-1</sup> + Induce at 0.125% V/V
Champ ION <sup>++</sup>	NuFarm	Copper hydroxide	1.5 lbs ac <sup>-1</sup>
Actinovate®	Novozymes	Streptomyces lydicus WYEC	6 fl oz ac <sup>-1</sup>
Sonata®	Bayer CropScience	Bacillus Pumillus strain 108	2 qt ac <sup>-1</sup>

**Table 2.** Main effect treatment on deoxynivalenol (DON) contamination and grain yield at Alburgh, VT 2016.

Fungicide treatment	DON	Yield
	ppm	bu ac <sup>-1</sup>
Non-sprayed, non-inoculated control	0.19	65.7
Inoculated FGS 10.1	0.28	61.4
Actinovate (6 fl oz) at heading	0.23	67.4
Actinovate (6 fl oz) 4 days after heading	0.29	67.3
Caramba (14 fl oz) at heading	0.20	73.1
Caramba (14 fl oz) 4 days after heading	0.35	68.0
ChampION (1.5 lbs) at heading	0.28	65.0
ChampION (1.5lbs) 4 days after heading	0.24	72.0
Prosaro SC (6.5 fl oz) at heading	0.19	82.5
Prosaro SC (6.5 fl oz) 4 days after heading	0.19	78.0
Sonota (2 qt) at heading	0.35	71.6
Sonota (2 qt) 4 days after heading	0.31	67.8
LSD (P=0.05)	NS	9.41

**Table 3.** Main effect of cultivar on deoxynivalenol (DON) contamination and grain yield at Alburgh, VT 2016.

Cultivar treatment	DON	Yield
	ppm	lbs ac <sup>-1</sup>
Conlon	0.24	72.3
Robust	0.28	70.2
LSD (P=0.05)	NS	NS

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# IMPACT OF PREDICTION TOOLS FOR FUSARIUM HEAD BLIGHT IN THE US, 2009-2017

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## ABSTRACT

A multi-state effort to predict epidemics of Fusarium head blight (FHB) was conducted during the 2009-2017 growing seasons. This prediction effort includes web-based tools, which display daily estimates of disease risk for 30 states. Commentary developed by a disease specialist in each state is displayed along with the risk maps. Commentary is also distributed via an FHB Alert System that sends email and text messages to mobile devices. The prediction tools received over 6,345 sessions (10,815 page views) by 3,177 users during the 2017-growing season in the U.S. (March – August). Many of the wheat disease specialists in the 30 states covered by the disease prediction system contributed commentary to the disease prediction effort. More than 80 commentaries were submitted in 2017. The FHB Alert System sent commentary to over 1,100 subscribers in 2017.

Users of the FHB prediction models and the FHB Alert System were surveyed annually in 2010-2014, and then again in 2017. The survey results included input from over 1,600 respondents and indicated that 70% of these users were either farmers or farm advisors. More than 85% of the users applied the information directly on their farm, or used it to make recommendations about disease management to others. Between 2010-2017, greater than 95% of the users considered the information to be of high or moderate value for their farm operations and businesses. A subset of questions targeting the influence of the information suggests 91% of the users experienced moderate or great improvement in their awareness of the disease risk in their area. The results also showed that the information influenced the perception of disease risk for 47% of the respondents, and motivated another 41% to seek advice from others. The 2017 survey asked growers to estimate the monetary value of the information provided to their farm or business. This survey indicates that the median monetary value of the information provided by the prediction system was \$9,500 per user. Combining this figure with use statistics suggests that annual impact of the FHB prediction model exceeds \$30 million. This value is likely influenced by the decreasing value of grain in recent years.

## ACKNOWLEDGEMENTS AND DISCLAIMER

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# UNRAVELING FHB EPIDEMICS IN THE BRAZILIAN SUBTROPICS: LESSONS LEARNED AND CONTROL STRATEGIES

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## ABSTRACT

Fusarium head blight (FHB) of wheat and barley became more frequent and severe in the subtropics of Brazil after the early 1990s. Similar to other regions worldwide, conservation tillage practices and shifts in climate are likely drivers of the FHB resurgence in this region. Epidemiological and control studies conducted for over two decades prior to the resurgence of the disease provided a basis for disease management. However, substantial knowledge of both basic and applied aspects of disease epidemiology, pathogen population biology, and disease control was obtained by combining molecular and epidemiological analysis of disease and pathogen datasets from both experiments and surveys. Our research findings suggest a distinctive condition for FHB in the Brazilian subtropics. Simulation and observational epidemiological/aerobiological studies suggested within-season weather most likely overshadowing any potential effect of previous crop in a zero-tillage region. The predominant random spatial disease patterns and evidence of a wheat kernel-born population that differs from maize stubble-borne population within a field suggests a key role of a well-mixed regional inoculum from both local and distant sources in the epidemics. The molecular identification of >1,000 strains from wheat and barley collected over the last decade uncovered an increased diversity of species and toxigenic genotypes compared to the major wheat regions worldwide, which may pose additional challenge to regional disease management and food security. Besides a dominant deoxynivalenol(DON)-producer (15-ADON chemotype) *Fusarium graminearum* population, nivalenol(NIV)-producing species, *F. meridionale*, among other minor ones within the *F. graminearum* species complex, are important contributors of FHB epidemics in specific regions (~ 30% frequency). A series of controlled-environment studies were designed to compare the biology and epidemiology among the DON and NIV-producing species, including: environmental effects, fertility, growth, spore production, toxigenicity, pathogenicity, cultivar resistance, and fungicide sensitivity. The resurgence of the disease and the recent promulgation of maximum DON limits in wheat grain and byproducts led to an increased interest by researchers, policy makers, and food chain stakeholders. Disease, yield and mycotoxin data have been obtained from both surveys and disease control experiments. For example, a cooperative research network for fungicide evaluation, established in 2010, has provided important field data. These data are currently being used in meta-analyses to best define a cost-effective strategy for optimizing control (fungicide and number of sprays), but also in studies to develop new or improve existing models for predicting yield loss, disease risk and mycotoxin contamination. Although progresses have been made, continuing efforts and more attention to the problem focusing on mycotoxin contamination data are still needed to answer open research questions and improve management.

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# PREVALENCE OF *FUSARIUM GRAMINEARUM* IN NON-CULTIVATED, GRAMINEOUS RESERVOIRS

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## ABSTRACT

Numerous wild grass species are known to harbor *Fusarium graminearum* asymptotically, and their overwintered stems support ascospore production that could contribute to head blight epidemics in wheat and barley. These gramineous reservoirs may also contain diverse pathogen genotypes and serve as sites for sexual recombination. Wild grass inflorescences and stem debris were collected from cereal fields and natural spaces in order to assess the prevalence of *F. graminearum* in these hosts and the likelihood that they are a source of inoculum. Regions of both high- and low-intensity cereal production were included in the survey. A large culture collection from this work includes isolates from new hosts and all locations sampled. Overall, pathogen prevalence was related to local cereal acreage. The incidence of *F. graminearum* in debris is related to disease pressure in the previous year, host species richness, and host density. Wild grasses, particularly those growing in close proximity to cereal fields, were frequent hosts of *F. graminearum*. This is evidence that grasses growing near cereal crops should be treated as potential sources of inoculum. Additionally, *F. graminearum* is the predominant *Fusarium* species found in grasses bordering cereal production, but is relatively less common in natural spaces and areas with low-intensity cereal production.

## ACKNOWLEDGEMENT AND DISCLAIMER

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# INTEGRATED MANAGEMENT STRATEGIES TO LOWER FUSARIUM HEAD BLIGHT AND DEOXYNIVALENOL IN SPRING BARLEY OVER MULTIPLE YEARS AND LOCATIONS

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## ABSTRACT

The disease Fusarium head blight (FHB) is the most devastating spring barley disease in the Midwestern United States. Barley integrated FHB management trials were established at six locations in North Dakota over two years: Fargo, Langdon, Carrington, Williston, Nesson Valley and Prosper. The trials evaluated the effect of fungicide treatment(s) and varietal resistance on the reduction of deoxynivalenol (DON) and protection of yield and test weight. Trials were designed in a randomized complete block with a split-plot arrangement with four replications at all locations. Barley varieties (at least two per location) differing in susceptibility to FHB served as whole plots. Fungicide treatments were the subplots and included prothioconazole+tebuconazole at heading, prothioconazole+tebuconazole at heading + metconazole 4-7 days later, metconazole at heading + tebuconazole 4-7 days later, and prothioconazole at heading + tebuconazole 4 days later. Corn spawn served as the inoculum source at Langdon and Williston, ND. *Fusarium* spores were used at Fargo and *Fusarium* infected residue was used at Carrington and Prosper, ND. Inoculum was applied to all treatments except for the non-treated, non-inoculated checks. The level of FHB severity was evaluated around the Feekes 11.2 growth stage (soft to mid dough) and DON, yield and test weight were obtained after harvest. Data were analyzed using Proc GLM and means were separated with LSD (P=0.05). Results indicated that applying fungicides alone or in combination lowered DON significantly compared to untreated controls. DON levels were lower in the resistant varieties when compared to the susceptible varieties. Both yield and test weight were higher for treatments that included a fungicide application when compared to the non-treated inoculated checks. Results from this study will help strengthen FHB fungicide recommendations for spring barley production.

## ACKNOWLEDGEMENT AND DISCLAIMER

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# INTEGRATED MANAGEMENT OF FUSARIUM HEAD BLIGHT IN HARD RED SPRING WHEAT VARIETIES

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## ABSTRACT

North Dakota leads the United States in the production of hard red spring wheat (HRSW). Compared to other small grain market classes, HRSW tends to have the highest amount of Fusarium head blight (FHB) resistance. The use of moderately resistant HRSW varieties coupled with timely fungicide applications can help manage FHB and deoxynivalenol (DON). The number of HRSW varieties growers can select continues to increase, prompting the need to evaluate the effectiveness of fungicides on managing FHB and DON. With funding from the USWBSI, six HRSW integrated management trials were conducted in 2016 and 2017 at three locations. All core treatments in the integrated management fungicide protocol were included at each location. The fungicide treatments included prothioconazole + tebuconazole applied at Feekes 10.51 (early-anthesis), prothioconazole + tebuconazole at Feekes 10.51 followed by metconazole 4-7 days later (post-anthesis), metconazole at Feekes 10.51 followed by tebuconazole 4-7 days later, and prothioconazole at Feekes 10.51 followed by tebuconazole 4-7 days later. Additional fungicide treatments addressing grower based questions were also evaluated at some locations. Data from each research location was analyzed individually due to differences in treatment protocol and disease development. Low to moderate disease pressure occurred at four of the six locations. At locations that evaluated prothioconazole + tebuconazole at early-anthesis and post-anthesis, a post-anthesis application had statistically lower DON levels when compared to the early-anthesis application. Also, with some exceptions, sequential applications of a fungicide often had lower DON levels than one application of prothioconazole + tebuconazole at early flowering. The value of post-anthesis applications warrants further investigation to update fungicide recommendations for FHB and DON management.

## ACKNOWLEDGEMENT AND DISCLAIMER

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# THE SPRAY BEFORE THE STORM: ASSESSING THE RAINFASTNESS OF CARAMBA® FOR CONTROL OF FUSARIUM HEAD BLIGHT

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## ABSTRACT

Fusarium head blight (FHB), caused by *Fusarium graminearum*, is a destructive disease of small grain crops that is responsible for economic losses in the billions. FHB-affected wheat spikes produce fewer, smaller grains that are contaminated with the mycotoxin deoxynivalenol (DON). In order to successfully manage FHB, protective sprays should be applied during anthesis with sufficient time before a rainfall event so that the fungicide can dry and soak into the plant tissue. The rainfastness of a spray describes the minimum time after which rain will not reduce the efficacy of the chemical. Growers are sometimes faced with situations where they need to spray their wheat before an impending rain. In these cases, the rainfastness of their product may determine whether or not they spray. The triazole fungicide Caramba® is marketed to protect against FHB and reduce mycotoxin contamination. The label recommends against applications if heavy rain is predicted, and claims that it is rainfast at one hour. This study explores the effect of heavy rain following an application of Caramba on its efficacy against FHB. The study was conducted in Wooster, Ohio, in the summers of 2014 to 2016. A randomized complete block design was used with four blocks and seven treatments per block. Treatments included a fungicide treated check, an untreated check, and five rainfall treatments at different intervals following the fungicide treatment (0, 60, 105, 150, and 195 minutes). Caramba was applied at anthesis at a rate of 13.5-14 fl oz/A, followed by the application of 4.8 mm of simulated rainfall across the plot. Within two days of the Caramba application, all plots were spray inoculated with a spore suspension of *F. graminearum*. Three weeks after inoculation, FHB incidence (INC) and index (IND) were rated for each plot. Following harvest, *Fusarium* damaged kernels (FDK), DON contamination, yield, and test weight were quantified from each treatment. In the highest disease year, 2016 (mean IND of untreated control = 23%), the mean INC, IND, FDK, DON, YIELD, and TEST WEIGHT from treated plots were all significantly different ( $P < 0.001$ ) from the untreated control but not from the treated control. In the moderate disease year, 2014 (mean IND of untreated control = 15%), mean INC, INDEX, DON, and FDK were generally significantly different ( $P < 0.05$ ) from the untreated control but not from the treated control at 105 minutes. In the lowest disease year, 2015 (no INC or IND collected), there was not a significant effect of treatment on any of the measured responses ( $P > 0.18$ ). These findings suggest that in high disease years, Caramba applications are effectively rainfast immediately, however if there is more flexibility in timing the spray, a dry period of 105 minutes following the spray will consistently achieve best results.

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# EFFECTS OF SAMPLE SIZE ON FUSARIUM HEAD BLIGHT INDEX ESTIMATION AND ITS RELATIONSHIP WITH DEOXYNIVALENOL ACCUMULATION IN WHEAT

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## ABSTRACT

Since Fusarium head blight (FHB) intensity is usually highly variable among spikes within a plot, the number of spikes rated (sample size) for FHB index (IND) quantification must be considered when designing experiments. Poor sampling may result in bias estimates of mean IND with inflated variance, and this could affect interpretation of results in terms of treatment effects and variety reactions, for instance. Moreover, inaccurate estimates of mean IND due to poor sampling may cause the relationship between IND and deoxynivalenol (DON) to break down. A field experiment was conducted to investigate the effects of sample size on the estimation of plot-level mean IND and its relationship with DON. A total of 108 plots of a moderately resistant and a susceptible cultivar were planted and spray inoculated with one of three spore concentrations ( $5 \times 10^4$ ,  $1 \times 10^5$ , and  $2 \times 10^5$  spores.mL<sup>-1</sup>) of *F. graminearum* to generate a range of disease levels. There were four replicate blocks of each cultivar x inoculum density combination. Each experimental unit (plot) was seven rows wide by 6 m long, with a spacing of 19 cm between adjacent rows. IND was rated at 21 days after anthesis by visually estimating the proportion of diseased spikelets per spike. A two-stage cluster sampling approach was used, with an average of 32 spikes evaluated at 10 equally-spaced points in each plot. Mean IND, averaged across spikes, sample points, and blocks was 8.6 (SE=4.0), 10.5 (SE=3.1), and 13.6% (SE=4.5) for the moderately resistant cultivar, and 16.5 (SE=4.6), 22.9 (SE=3.4), 28.5% (SE=5.7) for the susceptible cultivar at low, medium, and high inoculum densities, respectively. These data were used to estimate the predicted relative precision of mean IND for different sample sizes under simple random sampling. The predicted coefficient of variation (CV) for sample sizes of 94, 154, and 248 spikes per plot was 30, 20, and 10%, respectively. Sample sizes below 94 spikes greatly increased CV to a high of 110% for a sample size of 10 spikes per plot. Using the original data from each plot ( $n = 320$ ), simple random samples were drawn without replacement a 1000 times and used to evaluate the effects of the sample sizes of 10, 30, 50, 70, 90, 110, 130, and 150 spikes per plot on mean IND. The sample sizes of 110, 130, and 150 spikes resulted in unbiased estimations of the mean and variance, with relatively high precision. However, sample sizes smaller than 90 spikes resulted in extremely imprecise and biased estimates of mean IND, with inflated variances. In addition, the relationship between mean IND and DON was affected by sample size. Formal analyses will be conducted to identify the best sampling approach to quantify IND using the optimal sample sizes found in this study.

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FIELD EVALUATION, ADOPTION POTENTIAL  
AND AGRONOMIC STUDIES ON *FUSARIUM*  
*GRAMINEARUM* INFESTING MAIZE, *ZEA MAYS*  
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**ABSTRACT**

The efficacy of ethyl acetate extract of botanicals in protecting Maize *Zea mays* against *Fusarium graminearum* causing great loss in maize production was examined in field experiments. A comparative experiment was also conducted using a synthetic insecticide (Redforce). Healthy maize plants were inoculated at 5 Weeks After Planting with Potato Dextrose Agar inoculums of *Fusarium graminearum* that was prepared in the laboratory. 200g/vol ethyl acetate extracts of *Hyptis suaveolens*, *Moringa oleifera*, and *Azadirachta indica* were applied using a hand sprayer in order to control the infested crops immediately after infestation. In a similar set up, synthetic fungicide (Redforce) was prepared according to specification and consequently applied to control infested maize plants. Agronomic practices were observed and spray treatments which commenced immediately after the manifestation of the disease progressed steadily till plant maturity. Remarkable control was established after the treatment of the infected maize with all the botanicals and the most significant efficacy was confirmed with treatment adopted with *Azadirachta indica* extract. Data were collected on maturity rate, extent of damage by *Fusarium*, stem girth, number of leaves and internodes per plant and yield. The experiment forms a basis for the adoptive use of botanicals in ethyl acetate in the control of *Fusarium*.



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# DECREASING DEOXYNIVALENOL (DON) CONTAMINATION IN SOFT RED WINTER WHEAT GRAIN THROUGH AGRONOMIC PRACTICES IN KENTUCKY

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## ABSTRACT

Every year, DON contamination of wheat grain, caused by *Fusarium graminearum*, in soft red winter wheat is a major concern to wheat producers and millers. Current agronomic practices to reduce DON contamination include planting cultivars with moderate resistance to Fusarium head blight and applying efficacious fungicides at beginning anthesis (Feekes 10.5.1). However, it is unclear whether additional agronomic practices can be implemented to reduce DON contamination in Kentucky. The objectives of this study were to 1) investigate the effect of harvesting wheat at different grain moisture concentrations on DON levels and 2) investigate the effect of phosphorus applications at planting on the uniformity of wheat head emergence, flowering, and DON levels. Two field trials, one in which corn kernels infested with *F. graminearum* were spread throughout the experimental area, and the second with no addition of *F. graminearum*-infested corn kernels, were established at the University of Kentucky Research & Education Center in Princeton, Kentucky in the 2016-2017 growing season. The study was conducted as a randomized complete block design. Treatments included two planting dates (October 15<sup>th</sup> and November 22<sup>nd</sup>), two harvest timings (20-22% grain moisture and 13-15% grain moisture), two soft red winter wheat cultivars (moderately resistant to FHB cultivar and susceptible to FHB), and two phosphorous applications applied in furrow at planting (0 kg/ha P<sub>2</sub>O<sub>5</sub> and 47 kg/ha P<sub>2</sub>O<sub>5</sub>). Trials were harvested in June of 2017. The early harvest timings occurred on June 8<sup>th</sup> and 12<sup>th</sup> for the October planting and November planting, respectively. The normal moisture wheat was harvested on June 21<sup>st</sup> for both planting timings. Preliminary results indicate that the 20-22% grain moisture harvest timing had significantly greater yields and reduced *Fusarium* damaged kernels (FDK) than the 13-15% harvest timing. The use of phosphorus increased Fusarium head blight incidence and FDK ratings, and decreased the number of spikelets per head, but did not affect head density. Wheat grain samples for deoxynivalenol analysis are still being processed.



## EFFICACY OF TWO-TREATMENT FUNGICIDE PROGRAMS FOR FHB MANAGEMENT: A MULTI-STATE COORDINATED PROJECT

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### OBJECTIVE

Evaluate the integrated effects of two-treatment fungicide programs and genetic resistance on FHB and DON in all major grain classes.

### INTRODUCTION

For years the recommended fungicide program for FHB and DON management has been a single well-timed application at anthesis. However, recent studies have shown that a “late” application made up to 6 days after anthesis may be just as effective as an anthesis application for FHB and DON management (Bradley et al. 2009; D’Angelo et al. 2014). This has led to questions being asked about the value of combining an anthesis and a late application. We hypothesized that at moderate to high levels of FHB, a “late” or “post-anthesis” application of a fungicide following an anthesis application, coupled with genetic resistance will be more effective at reducing FHB and DON than

an anthesis application alone, resistance alone, or even resistance + an anthesis-only application. We further hypothesize that the benefit of such a program in terms of disease and toxin reduction and yield and test weight increase will be high enough to offset application cost, particularly if Folicur or some other inexpensive generic tebuconazole is used as part of the program. These hypotheses will be tested in all major grain market classes, under a range of weather conditions and baseline levels of FHB and DON.

### MATERIALS AND METHODS

Field experiments were established in 15 US wheat-growing states in 2016 and 2017 to evaluate the effects of cultivar resistance and two-treatment fungicide programs on FHB and DON. Plots were established according to standard agronomic practices for each location. At least three commercial wheat cultivars, classified as susceptible (S), moderately susceptible (MS),

or moderately resistant (MR), were planted in most trials. However, some trials only included two of these resistance categories. Plots were planted in four to six replicate blocks. The standard experimental design was a randomized complete block, with a split-plot arrangement of cultivar as whole-plot and fungicide treatment (Table 1) as sub-plot. All plots were artificially inoculated with either *F. graminearum*-colonized corn kernels spread on the soil surface or spray-inoculated with a spore suspension of the fungus approximately 24-36 hours following the anthesis fungicide treatment. FHB index (plot severity) was assessed during the soft dough stage of grain development. Milled grain samples were sent to a USWBSI-supported laboratory for toxin analysis. For the purpose of this report, percent control of FHB index and DON was estimated for each cultivar x fungicide program combination relative to the untreated susceptible check (the reference treatment) for each trial/environment.

## RESULTS AND DISCUSSION

FHB index and DON results from 36 environments, representing 20 soft red winter, four soft white winter, seven hard red winter, two hard red spring, two hard white spring, two soft white spring wheat classes and three durum are summarized below. Estimated means and percent controls for FHB index and DON for S, MS and MR cultivars treated with a fungicide at anthesis alone or at

anthesis followed by a post anthesis application are shown in Table 2 and 3 and Figure 1. In some environments, DON data were not available at the time of this report. Mean FHB index and DON in the untreated susceptible check ranged from 0 to 63% and 0 to 38 ppm, respectively. Relative to the untreated susceptible or moderately susceptible reference, fungicide treatment applied to MR cultivars resulted in higher mean percent control of FHB index (87%) followed by MS-Treated (73%) and S-Treated (68%). Similarly, mean percent control of DON was 75% for MR-Treated, 73% for MS-Treated and 60% for S-Treated cultivars. Overall, percent controls of both FHB index and DON were highest for fungicide programs that combined an anthesis and a late application (Fig 1) than programs with an anthesis application alone or MS or MR alone. Moderately resistant cultivars alone offered higher mean percent control of both FHB index and DON (75 and 67%, respectively) than MS cultivars alone (65 to 56%, respectively) (Fig 1). Based on these results, there is evidence suggesting that the combination of a “late” or “post-anthesis” and an anthesis fungicide application, coupled with MS or MR cultivars can be more effective at reducing FHB and DON than an anthesis application alone. A more comprehensive analysis of the data as well as a cost-benefit assessment of all FHB management programs evaluated in this study will be conducted.

**Table 1.** Fungicide programs evaluated in the 2016 and 2017 management CP.

Treatment <sup>a</sup>	Product	Rate	Timing <sup>b</sup>
1	Untreated check	...	...
2	Prosaro	6.5 fl oz/A	Anthesis
3	Prosaro	6.5 fl oz/A	Anthesis
	Caramba	14 fl oz/A	4 - 7 days after anthesis
4	Caramba	14 fl oz/A	Anthesis
	Folicur <sup>b</sup> (or generic tebuconazole)	4 fl oz/A	4 - 7 days after anthesis
5	Proline	5.7 fl oz/A	Anthesis
	Folicur <sup>b</sup> (or generic tebuconazole)	4 fl oz/A	4 - 7 days after anthesis
6	Untreated, non-inoculated check	...	...

<sup>a</sup> All treatments will be applied with NIS @ 0.125 v/v

<sup>b</sup> Fungicide application timing varied by trial locations. Post Anthesis treatments were applied at 4, 5 and 7 days after anthesis.

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**Table 2.** Mean FHB index (%) for different fungicide programs applied to FHB susceptible, moderately susceptible and moderately resistant cultivars in 36 environments (ENV) representing different wheat classes (TYPE = SRWW, HRWW, HRSW, HWSW, SWSW and Durum). Results are organized by fungicide treatment (untreated [UT], Prosoar at anthesis followed by Caramba at 4-7 days post-anthesis (II), Caramba at anthesis followed by Tebuconazole at 4-5 days post-anthesis (III) and Proline at anthesis followed by Tebuconazole 4-5 days post-anthesis (IV).

ENV	LOCATION	TYPE	Susceptible (S)				Mod Susceptible (MS)				Mod Resistant (MR)						
			UT	I	II	III	IV	UT	I	II	III	IV	UT	I	II	III	IV
			Check	Fungicide	TRT*	TRT*	TRT*	Check	Fungicide	TRT	TRT	TRT	Check	Fungicide	TRT	TRT	TRT
1	Tallassee 2016, AL	SRWW	63.0	47.6	38.0	55.4	46.3	42.9	37.7	31.4	44.6	24.2	29.5	18.8	24.0	15.4	13.2
2	Tallassee 2017, AL	SRWW	19.9	26.2	11.1	20.2	18.1	20.1	13.9	10.6	24.4	10.2	13.9	5.0	5.0	9.5	7.3
3	Fairhope (A) 2017, AL	SRWW	...	...	...	...	...	32.9	25.3	20.5	58.2	50.5	0.3	1.7	0.0	1.1	0.1
4	Fairhope (B) 2017, AL	SRWW	...	...	...	...	...	13.6	25.2	20.9	3.8	25.8	3.4	0.4	2.0	3.5	3.0
5	Georgetown 2016, DE	SRWW	9.7	3.4	1.8	3.5	1.6	...	...	...	...	...	0.04	0.02	0.02	0.06	0.0
6	Marion 2016, IL	SRWW	13.3	5.8	1.8	1.9	5.6	10.4	0.4	1.5	3.3	5.0	2.8	0.0	0.0	1.5	0.0
7	Marion 2017, IL	SRWW	5.4	2.4	0.3	0.3	3.5	0.0	0.0	0.1	0.3	0.4	0.0	0.0	0.0	0.0	0.1
8	Tippicanoe 2016, IN	SRWW	0.13	0.13	0.0	0.0	0.0	...	...	...	...	...	1.50	0.13	0.0	0.0	0.0
9	Tippicanoe 2017, IN	SRWW	0.33	0.01	0.0	0.01	0.03	...	...	...	...	...	0.04	0.0	0.0	0.0	0.0
10	Princeton 2016, KY	SRWW	8.4	0.4	0.5	1.4	1.0	1.0	0.9	0.0	0.0	0.5	0.5	0.6	0.0	0.0	0.0
11	Princeton 2017, KY	SRWW	5.4	1.0	1.8	0.0	0.5	0.0	0.5	0.3	0.3	0.1	0.0	0.3	0.0	0.0	0.0
12	Aurora 2016, NY	SRWW	0.27	0.00	0.03	...	...	0.00	0.00	0.00	...	...	0.00	0.00	0.00	...	...
13	Aurora 2017, NY	SRWW	2.27	1.03	0.21	...	...	1.17	0.14	0.13	...	...	0.25	0.06	0.08	...	...
14	Wooster 2016, OH	SRWW	18.3	6.0	4.3	4.6	6.8	7.0	1.7	0.5	1.5	1.7	3.6	0.85	0.59	0.72	1.60
15	Wooster 2017, OH	SRWW	11.4	5.1	2.8	4.5	5.4	3.1	1.0	1.0	1.2	1.1	1.0	0.28	0.15	0.20	0.33
16	RECM 2016, TN	SRWW	...	...	...	...	...	8.4	6.1	3.4	7.7	4.4	9.3	4.2	3.4	8.6	2.4
17	RECM 2017, TN	SRWW	...	...	...	...	...	76.8	58.3	57.8	44.6	39.3	0.31	0.02	0.03	0.02	0.06
18	WTREC 2017, TN	SRWW	...	...	...	...	...	85.0	0.8	2.3	2.1	1.6	0.52	0.00	0.00	0.04	0.00
19	TAREC 2016, VA	SRWW	29.9	19.0	14.6	14.2	16.6	...	...	...	...	...	21.5	12.9	9.8	11.4	11.5
20	Arlington 2016, WI	SRWW	1.6	1.8	0.1	3.6	0.4	1.9	1.5	0.0	0.6	1.5	...	...	...	...	...
21	Mead 2016, NE	HRWW	6.5	4.0	2.5	6.8	2.8	...	...	...	...	...	1.25	1.0	1.0	1.0	0.5
22	Lincoln 2017, NE	HRWW	...	...	...	...	...	14.5	10.8	14.5	5.3	12.8	9.0	8.0	12.0	9.8	4.0
23	Volga 2016, SD	HRWW	24.3	14.0	14.3	17.8	11.9	...	...	...	...	...	5.4	3.4	3.9	3.0	3.3
24	Northeast 2016, SD	HRWW	0.04	0.14	0.04	0.11	0.11	...	...	...	...	...	0.00	0.04	0.00	0.00	0.04
25	Volga 2017, SD	HRWW	7.0	4.1	3.5	3.1	4.9	...	...	...	...	...	1.2	0.5	1.0	0.6	1.1
26	Northeast 2017, SD	HRWW	0.23	1.21	0.09	0.27	0.00	...	...	...	...	...	0.21	0.04	0.07	0.07	0.04
27	Prosper 2016, ND	HRWW	...	...	...	...	...	6.6	2.7	0.7	0.6	0.6	3.1	0.3	1.7	0.2	0.1
28	Langdon 2016, ND	HRSW	11.5	0.9	0.3	1.0	0.8	6.0	0.9	0.1	0.2	0.2	...	...	...	...	...
29	Langdon 2017, ND	HRSW	16.3	1.0	0.1	1.2	0.6	16.9	0.4	0.1	0.4	0.0	...	...	...	...	...
30	Aberdeen 2016, ID	HWSW	6.6	5.3	1.1	1.4	2.7	23.9	9.1	2.0	3.5	11.0	...	...	...	...	...
31	Aberdeen 2017, ID	HWSW	57.8	37.7	22.7	37.6	29.6	31.0	14.5	6.0	9.4	10.3	...	...	...	...	...
32	Aberdeen 2016, ID	SWSW	...	...	...	...	...	31.6	15.2	4.7	7.2	12.0	3.83	2.6	0.8	0.9	2.4
33	Aberdeen 2017, ID	SWSW	...	...	...	...	...	28.5	16.3	10.4	14.4	11.7	7.38	4.3	1.3	3.9	2.8
34	Langdon 2016, ND	Durum	...	...	...	...	...	35.8	18.1	2.4	5.4	4.8	36.1	23.0	5.7	14.2	10.7
35	Langdon 2017, ND	Durum	...	...	...	...	...	14.9	1.9	0.5	1.2	0.6	27.9	0.9	0.5	2.3	0.3
36	Wiliston 2017, ND	Durum	...	...	...	...	...	1.2	1.0	0.1	0.2	0.2	1.0	0.2	0.0	0.2	0.1

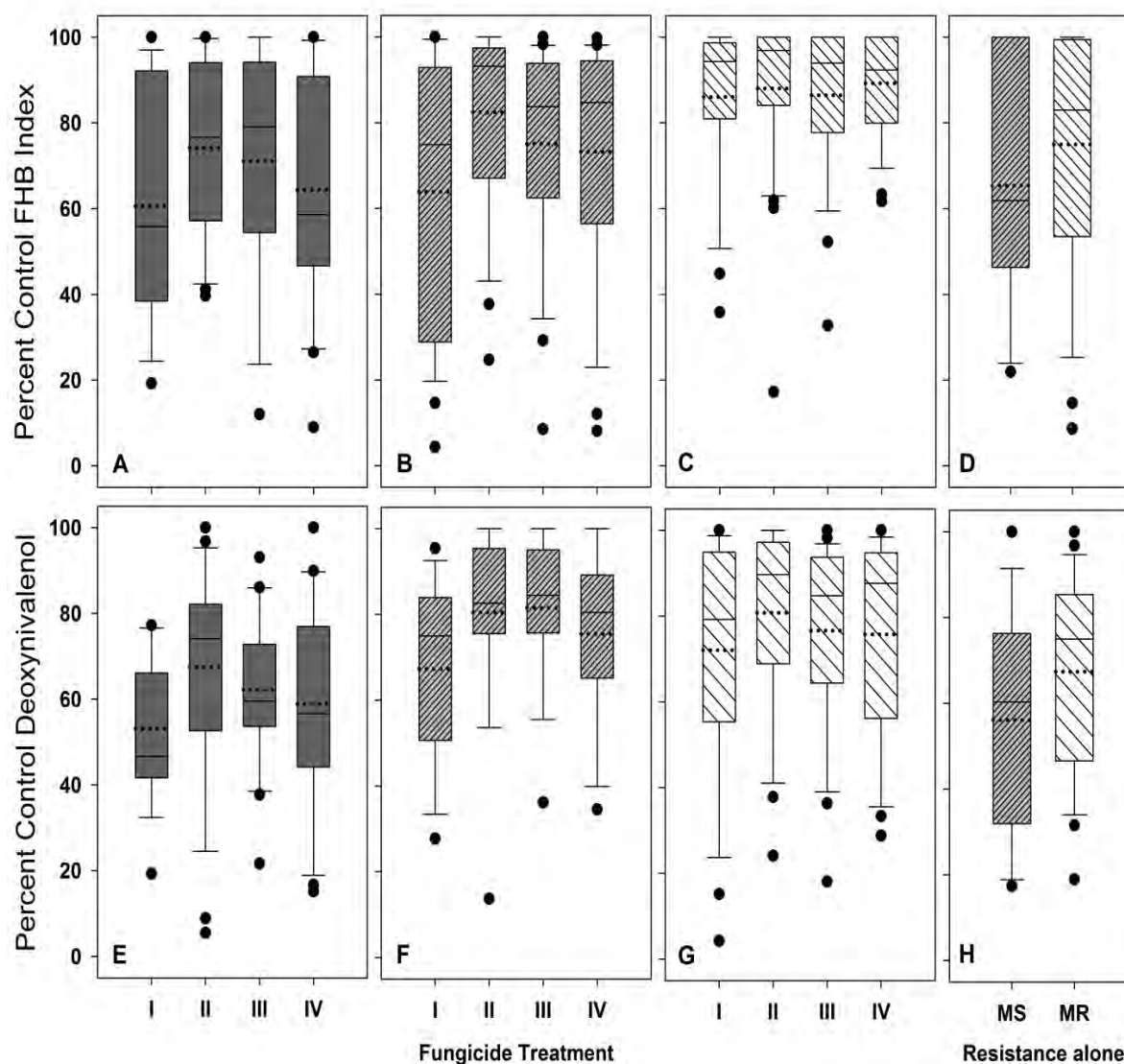
\* See Table 1 for more details

**Table 3.** Mean deoxynivalenol (DON in ppm) for different fungicide programs applied to FHB susceptible, moderately susceptible and moderately resistant cultivars in 34 environments (ENV) representing different wheat classes (TYPE = SRWW, SWWW, HRWW, HRSW, HWSW, SWSW and Durum). Results are organized by fungicide treatment (untreated [UT], Prosoaro applied at anthesis (I), Prosoaro at anthesis followed by Caramba at 4-7 days post-anthesis (II), Caramba at anthesis followed by Tebuconazole at 4-5 days post-anthesis (III) and Proline at anthesis followed by Tebuconazole at 4-5 days post-anthesis (IV)).

ENV	LOCATION	TYPE	Susceptible (S)				Mod Susceptible (MS)				Mod Resistant (MR)						
			Fungicide TRT*		Fungicide TRT		Fungicide TRT		Fungicide TRT		Fungicide TRT		Fungicide TRT				
			UT	I	II	III	IV	UT	I	II	III	IV	UT	I	II	III	IV
1	Tallasse 2016, AL	SRWW	38.2	23.0	9.9	13.2	17.0	11.3	9.4	6.9	8.3	9.4	7.8	4.7	4.0	4.7	4.3
2	Georgetown 2016, DE	SRWW	1.46	0.78	1.38	0.46	0.56	...	...	...	...	...	0.78	0.50	0.54	0.54	0.38
3	Georgetown 2017, DE	SRWW	0.45	0.66	0.41	0.51	0.57	...	...	...	...	...	0.14	0.24	0.28	0.22	0.30
4	Marion 2016, IL	SRWW	0.71	0.39	0.29	0.33	0.39	0.56	0.11	0.15	0.11	0.13	0.16	0.14	0.14	0.13	0.21
5	Tippecanoe 2016, IN	SRWW	0.05	0.05	0.00	0.03	0.05	...	...	...	...	...	0.03	0.10	0.00	0.00	0.03
6	Tippecanoe 2017, IN	SRWW	0.24	0.14	0.09	0.10	0.11	...	...	...	...	...	0.26	0.10	0.07	0.14	0.10
7	Princeton 2016, KY	SRWW	1.83	0.62	0.36	0.74	0.43	0.41	0.15	0.09	0.09	0.30	0.27	0.13	0.07	0.13	0.14
8	Princeton 2017, KY	SRWW	0.37	0.25	0.19	0.29	0.22	0.19	0.11	0.09	0.09	0.12	0.05	0.02	0.05	0.06	0.05
9	Aurora 2016, NY	SRWW	0.04	0.01	0.02	...	...	0.01	0.01	0.01	...	...	0.01	0.01	0.01	...	...
10	Aurora 2017, NY	SRWW	3.03	0.71	0.47	...	...	1.70	0.60	0.30	...	...	1.67	0.63	0.31	...	...
11	Wooster 2016, OH	SRWW	8.55	1.95	1.63	2.25	3.98	1.83	0.39	0.15	0.28	0.37	1.25	0.18	0.12	0.15	0.29
12	Wooster 2017, OH	SRWW	8.95	4.98	2.23	3.73	3.13	3.55	1.37	0.59	1.07	0.93	1.93	0.87	0.64	0.78	0.77
13	RECM 2016, TN	SRWW	...	...	...	...	...	0.53	0.35	0.22	0.17	0.29	0.69	0.51	0.26	0.34	0.38
14	TAREC 2016, VA	SRWW	2.87	0.70	0.20	0.20	0.29	...	...	...	...	...	0.42	0.12	0.00	0.13	0.09
15	Arlington 2016, WI	SRWW	0.18	0.08	0.05	0.06	0.13	0.05	0.03	0.00	0.00	0.00	...	...	...	...	...
16	Arlington 2017, WI	SRWW	0.06	0.09	0.06	0.02	0.05	0.00	0.03	0.00	0.00	0.00	...	...	...	...	...
17	East Lansing 2016	SRWW	0.09	0.05	0.02	0.04	0.01	...	...	...	...	...	0.05	0.03	0.00	0.01	0.01
18	East Lansing 2017	SRWW	0.33	0.35	0.16	0.14	0.28	...	...	...	...	...	0.16	0.28	0.19	0.11	0.09
19	Sandusky 2016	SRWW	0.00	0.00	0.00	0.00	0.00	...	...	...	...	...	0.03	0.02	0.02	0.02	0.00
20	Deckerville 2017	SRWW	0.06	0.06	0.01	0.07	0.03	...	...	...	...	...	0.02	0.03	0.01	0.01	0.03
21	East Lansing 2016	SWWW	0.14	0.05	0.03	0.02	0.05	...	...	...	...	...	0.06	0.00	0.01	0.02	0.01
22	East Lansing 2017	SWWW	0.83	0.67	0.54	0.46	0.36	...	...	...	...	...	0.07	0.04	0.01	0.05	0.04
23	Sandusky 2016	SWWW	0.01	0.03	0.00	0.02	0.00	...	...	...	...	...	0.00	0.00	0.00	0.00	0.00
24	Deckerville 2017	SWWW	0.09	0.03	0.00	0.01	0.02	...	...	...	...	...	0.00	0.00	0.00	0.03	0.00
25	Mead 2016, NE	HRWW	0.93	1.30	0.43	0.43	1.03	...	...	...	...	...	1.00	0.30	0.00	0.13	0.00
26	Volga 2016, SD	HRWW	0.00	0.15	0.25	0.27	0.15	...	...	...	...	...	0.18	0.00	0.18	0.00	0.20
27	Northeast 2016, SD	HRWW	0.00	0.00	0.00	0.00	0.00	...	...	...	...	...	0.00	0.00	0.00	0.00	0.00
28	Carrington 2016, ND	HRSW	1.21	0.65	0.34	0.34	0.25	1.00	0.59	0.25	0.34	0.25	...	...	...	...	...
29	Langdon 2016, ND	HRSW	6.20	2.33	1.33	0.93	1.40	3.80	2.75	0.23	0.93	0.83	...	...	...	...	...
30	Aberdeen 2016, ID	HWSW	4.85	3.28	1.50	2.83	2.75	3.63	0.81	0.59	0.62	0.59	...	...	...	...	...
31	Aberdeen 2016, ID	SWSW	...	...	...	...	...	3.55	1.39	0.63	0.80	0.95	0.74	0.25	0.16	0.20	0.23
32	Fargo 2016, ND	Durum	...	...	...	...	...	1.68	1.90	1.45	1.80	1.10	1.15	0.80	0.75	0.60	0.75
33	Langdon 2016, ND	Durum	...	...	...	...	...	10.03	7.25	1.70	2.13	3.58	8.13	7.08	1.60	2.48	3.15
34	Wilfiston 2017, ND	Durum	...	...	...	...	...	0.83	0.50	0.33	0.53	0.48	0.33	0.28	0.63	0.68	0.43

\* See Table 1 for more details





**Fig. 1.** Boxplots showing the distribution of percent control in FHB index (A to D), and deoxynivalenol (E to H) relative to non-treated susceptible (S) or moderately susceptible (MS) for several combinations of fungicide treatment [Prosaro applied at anthesis (I), Prosaro at anthesis followed by Caramba at 4-7 days post anthesis (II), Caramba at anthesis followed by Tebuconazole at 4-5 days post anthesis (III), Proline at anthesis followed by Tebuconazole 4-5 days post-anthesis (IV)] and cultivar resistance. Susceptible in dark gray boxes (A and E), moderately susceptible in gray boxes with patterns (B and F), and moderately resistance in white boxes with patterns (C and G). Dotted and solid lines within the box represent the mean and median, respectively, while the top and bottom lines of the box represent the 75<sup>th</sup> and 25<sup>th</sup> percentiles of the data, respectively. Vertical bars extending beyond the boxes represent the 10<sup>th</sup> and 90<sup>th</sup> percentiles and circles indicate outliers.



## ROBUST MANAGEMENT PROGRAMS TO MINIMIZE LOSSES DUE TO FUSARIUM HEAD BLIGHT AND DEOXYNIVALENOL IN WHEAT

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### ABSTRACT

Wheat grain yield and quality losses due to Fusarium head blight (FHB) and deoxynivalenol (DON) are best minimized by planting a moderately resistant cultivar and applying a DMI fungicide such as Prosaro® or Caramba® at anthesis. However, when conditions are favorable for FHB, growers often deal with local weather and field conditions that prevent them from being able to apply the fungicide at the recommended growth stage. The efficacy of delayed (post-anthesis) Prosaro applications against IND and DON was evaluated as part of a coordinated multi-state study conducted in 2014 and 2015. Cultivars with different levels of FHB resistance (susceptible [S], moderately susceptible [MS] and moderately resistant [MR]) were treated with Prosaro at different times relative to anthesis (untreated or treated at anthesis or 2 to 11 days post-anthesis) and inoculated with spores of *Fusarium graminearum* in 29 trials across 11 states, representing four market classes of wheat. Mean IND and DON in the untreated susceptible check (reference) ranged from 1 to 54% and 0.5 to 33 ppm, respectively. Percent control of IND and DON relative to the reference was estimated from the means and used as a measure of the efficacy of each cultivar x treatment combination. Combinations of Prosaro with a MS (MS\_Proसारो) or MR (MR\_Proसारो) cultivar were consistently more effective than fungicide alone (S\_Treated) or resistance alone (MR\_Untreated) at reducing IND and DON. Percent control ranged from 53 to 99% for IND and from 63 to 89% for DON for MS\_Proसारो and from 82 to 100% for IND and 62 to 100% for DON for MR\_Proसारो. When evaluated across trials, applications made between 4 and 6 days after anthesis were just as effective as anthesis applications, particularly on MR cultivars. Results from a meta-analysis to formally quantify the overall efficacy of each cultivar x Prosaro timing combination on IND and DON will be presented.

## **ACKNOWLEDGEMENTS AND DISCLAIMER**

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## NOVEL FHB CONTROL STRATEGY USING THE VOLATILE TRICHODIENE TO REDUCE MYCOTOXINS

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### ABSTRACT

*Fusarium graminearum* (*Fg*), the primary fungal pathogen responsible for Fusarium head blight (FHB), reduces crop yield and contaminates grain with trichothecene mycotoxins that are deleterious to plant, human and animal health. The first committed step in trichothecene biosynthesis is the formation of trichodiene (TD), which readily escapes into the atmosphere. The volatile nature of TD suggests that it may be a useful signal for coordinating the production of trichothecenes. However, little is known about the potential of TD to regulate genes related to trichothecene biosynthesis. Fumigation of *Fg* cultures and *Fg*-infected wheat heads with TD reduced trichothecene production, downregulated expression of trichothecene biosynthetic genes (*TRI* genes), and upregulated host plant defense genes. To further investigate whether this phenomenon has potential application in FHB control, the trichodiene synthase gene, *TRI5*, was transformed into the previously characterized biocontrol fungus *Trichoderma harzianum* (*Th*) to generate strain *Th+TRI5* as a delivery system for TD, but with the potential added benefit that *Th* itself could provide some control. Wheat plants pre-treated with *Th+TRI5* developed significantly less disease and accumulated less trichothecenes than *Th*-treated or untreated plants. These results indicate that *Th+TRI5* would be an effective management strategy for FHB and trichothecene contamination in wheat.

**FOOD SAFETY  
AND  
TOXICOLOGY**



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TOXIN GENE EXPRESSION ANALYSIS AND  
DEOXYNIVALENOL CONCENTRATION DURING  
POSTHARVEST STORAGE OF WHEAT GRAIN  
FROM A FUSARIUM HEAD BLIGHT  
EPIDEMIC IN NEBRASKA

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**ABSTRACT**

High grain moisture content during storage is conducive to mycotoxin accumulation in grain. This study evaluated the expression of the trichodiene synthase (*Tri5*) gene, the initial step in the deoxynivalenol (DON) biosynthetic pathway, during postharvest storage using qRT-PCR. In the 2014-2015 growing season, field plots of the hard red winter wheat cultivar Overland (moderately resistant to FHB) were treated at anthesis with the fungicides Prosaro<sup>®</sup> and Headline<sup>®</sup>. After 24 hours, wheat heads were inoculated using a spore suspension of *F. graminearum*. Final grain samples had < 2% of FDK after cleaning to remove excessive FDK. Samples were soaked to reach grain moisture contents of 16% ( $a_w = 0.60$ ) and 20% ( $a_w = 0.75$ ). Grain samples were stored for 4 months in a seed cooler at 10°C and 40% environmental relative humidity. Samples were taken at 0, 30, 60, 90 and 120 days after soaking and used to compare the expression of the toxin gene (*Tri5*) and DON concentration. DON was quantified by GC-MS. qRT-PCR on *Tri5* and the housekeeping gene *GAPDH* was performed on a minimum of six biological reps for each combination of fungicide treatment by postharvest time, for a total of 362 reactions. qRT-PCR reactions which efficiently amplified both genes (197) were used for  $2^{-\Delta\Delta CT}$ . Fungicide treatments showed a highly significant effect on DON ( $P < 0.0001$ ), and a significant effect on the ratio *GAPDH/Tri5* ( $P = 0.0223$ ). T-grouping for LS means ( $\alpha = 0.05$ ) showed significant differences with higher levels of DON and  $\log_{10}$  *Tri5* gene expression in Headline<sup>®</sup> compared to Prosaro<sup>®</sup>. Grain moisture content did not influence *Tri5* gene expression under the conditions of this study. Significant reductions in DON and in the level of expression of the *Tri5* gene were registered after 120 days of postharvest storage in grain from Prosaro- treated plots. *Tri5* gene expression showed slight fluctuations over time. DON and *Tri5* gene expression were significantly higher in grain from Headline<sup>®</sup>- than Prosaro<sup>®</sup>-treated plots in postharvest storage. Overall, DON and *Tri5* gene expression decreased over time in stored grain. Data from this study will improve our understanding of how the *Tri5* gene is expressed and thereby predict how much DON is being produced during storage under sub-optimal fungal growth conditions.



## ACKNOWLEDGEMENTS

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# MALTING QUALITY OF FUSARIUM HEAD BLIGHT INFECTED RYE (*SECALE CEREALE*)

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## ABSTRACT

The past decade has seen a dramatic rise in the use of rye grain in malting, brewing and distilling. As there is little to no information on the malting of rye, this project was initiated with the goal of investigating the malt quality of winter rye varieties grown in the USA. A mix of forage, conventional, and hybrid rye varieties were obtained from trials at Cornell University (NY), North Dakota State University (ND) and the University of Minnesota (MN) in 2014 and 2015 (samples=126). *Fusarium* head blight was not an initial concern with this project as many of the samples had no detectable deoxynivalenol (DON), and 75% were below 1.0 mg/kg DON. As such, the samples (n= 117) were micro-malted and analyzed for quality. However, when malts were tested for DON, it was found that 83% of all samples had DON in excess of 1.0 mg/kg. The average DON level on malted rye was an astounding 10.6 mg/kg, and values as high as 43.0 mg/kg were observed. Rye grain samples from NY had the highest average DON levels (2.0 mg/kg), and these increased, on average, by 9-fold following malting. The average increase with the MN samples was approximately 16-fold. In addition, relatively high levels of 3-AcDON, 15-AcDON, nivalenol (NIV), and DON-3-glucoside (D3G) were observed in some of the MN and NY rye malts. While DON levels in the ND samples (2015) were generally below the limit of quantitation (LOQ), DON was found in over 70% of samples (0.2 - 4.0 mg/kg) after malting. Similar results also were seen in a number of the MN and NY rye grain samples with DON levels below the LOQ. When compared to previous reports on FHB and DON during the malting of barley, the results for rye are quite dramatic, and should be of significant concern to maltsters and brewers utilizing rye malt. As *Fusarium* infection is known to alter malt quality, primarily through the action of pathogen produced enzymes, the original intent of this project to define the malt quality of rye varieties became of limited value. Nevertheless, this rye malt quality data does provide some insight into the impact of FHB on malt quality. As previously observed with FHB-infected barley, the wort viscosity,  $\beta$ -glucan and arabinoxylan content of rye significantly decreased in the samples with higher DON levels, while the Diastatic Power, FAN content and wort color tended to increase. However, an interesting result that has not been previously reported involved the phenolic acids in wort (vanillic acid, p-coumaric acids, and total phenolic acids). Phenolic acids in wort were significantly higher in the FHB infected materials. It is speculated to that this may could be related to *Fusarium* infection, the digestion of cell walls, and the release of phenolics as part of this process. Alternatively, it is well known that plant pathogens elicit the production of phenolic acids as a defense response in the host. Total phenolic acids in wort were significantly correlated with trichothecene levels,  $\beta$ -glucan content,  $\alpha$ -amylase, viscosity and extract.

The Pearson correlation analysis of the rye malt DON level with wort viscosity,  $\beta$ -glucan, wort color, vanillic acid, p-coumaric acid and total phenolic contents were -0.64 ( $p<0.0001$ ), -0.34 ( $p<0.001$ ), 0.24 ( $p<0.01$ ), 0.27 ( $p<0.01$ ), 0.70 ( $p<0.0001$ ) and 0.51 ( $p<0.0001$ ) in the across-state samples.

#### **ACKNOWLEDGEMENT AND DISCLAIMER**

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STABLE ISOTOPE DILUTION ANALYSIS FOR  
THE ACCURATE DETERMINATION OF  
DEOXYNIVALENOL IN SORGHUM BY GC-MS

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## ABSTRACT

Sorghum has gained popularity with consumers as a grain source with its gluten-free and high protein dietary characteristics. Sorghum is drought tolerant and an economical and nutritional feed choice for producers. *Fusarium* species produce mycotoxins and are plant pathogens to a wide range of cereal commodities including sorghum. Deoxynivalenol (DON) is the most commonly occurring mycotoxin produced by *Fusarium*. The FDA has established advisory levels for acceptable amounts of DON allowed in finished food products and feed in the United States. A traditional method for the analysis of DON in grains, which utilizes solid phase extraction chromatography with C18 followed by GC-MS, was determined to be ineffective for sorghum due to the complex matrices found with different varieties. The traditional method was modified by incorporating the stable isotope d1-DON as an internal standard. Using this modified method, the unknown amount of DON in a sorghum sample can be accurately and reliably quantitated by basing calculations on the recovery of d1DON.

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# OCCURRENCE OF DEOXYNIVALENOL AND DEOXYNIVALENOL-3-GLUCOSIDE IN HARD RED SPRING WHEAT GROWN IN THE USA

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## ABSTRACT

Deoxynivalenol (DON) is a mycotoxin found in wheat that is infected with *Fusarium* fungus. DON may also be converted to a type of “masked mycotoxin”, named deoxynivalenol-3-glucoside (D3G), as a result of detoxification of the plant. In this study, DON and D3G were measured using gas chromatographic (GC) and liquid chromatography-mass spectrometry (LC-MS) in wheat samples collected during 2011 and 2012 in the USA. Results indicate that the growing region had a significant effect on the DON and D3G ( $p < 0.0001$ ). There was a positive correlation between both methods (GC and LC-MS) used for determination of DON content. DON showed a significant and positive correlation with D3G during 2011. Overall, DON production had an effect on D3G content and kernel damage, and was dependent on environmental conditions during *Fusarium* infection.

# GROWTH OF *FUSARIUM GRAMINEARUM* AND PRODUCTION OF DEOXYNIVALENOL DURING THE MALTING OF WINTER RYE AND TRITICALE

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## ABSTRACT

Previous research had demonstrated a marked propensity for the development of deoxynivalenol (DON) and several other tricothecenes during the malting of FHB infected rye. High levels were, in fact, detected in malts from grain samples that had no detectable DON. This is of concern as there is growing interest in the production of rye for malt, beer, distilled beverages and artisan baking. The objective of this research was to assess the growth of *Fusarium graminearum* and the development of DON during the malting of rye. As part of the study, 26 rye and 6 triticale samples were obtained from several trial locations in Minnesota (2016). DON levels in the unmalted samples ranged from below the limit of quantitation (LOQ) to 1.6 mg/kg. These were malted at two points in time, and there was a “dramatic” increase in DON, with average levels around 10 mg/kg. DON detected in malted triticale samples ranged from 20.9 to 37.2 mg/kg. A smaller subset of 4 rye and 2 triticale samples was selected for additional studies on mycotoxin development and the growth of *F. graminearum* during malting. *Fusarium* species were isolated from 2 rye and 2 triticale samples, and the result showed that *F. graminearum* was the predominant species found on 3 of the 4 samples tested. *F. sporotrichioides*, *F. equiseti*, and *F. poae* were also detected. Six samples were sub-sampled at each day of malting, and were tested by GC-ECD for DON and by LC-MS for DON-3-Glc. The results showed rapid increase in DON starting at the second to third day of germination. *F. graminearum* was estimated by quantifying *Tri5* DNA with RT-PCR. Increases in *Tri5* DNA were observed from steeping through germination day 5. Single kernel analysis was performed on each of the six grain samples, and the resultant six malts. Approximately 40-50 individual grains of each sample were analyzed to help estimate the occurrence of DON before and after malting. In general, DON was not detected on at least 50% of the single rye kernels. A small percentage of single kernels were contaminated with DON, at levels around 10-20 mg/kg, although one single kernel of triticale tested at over 120 mg/kg. However once malted, DON was detected on at least 95% of the single malt kernels from 4 samples of the 6 samples tested with range from 0.05 up to around 150 mg/kg. Only a small percentage of single malt kernels had no detectable DON.



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APPLICATION OF NANOENCAPSULATED CLOVE OILS TO ENHANCE ANTIFUNGAL ACTIVITIES AND INHIBIT MYCOTOXIN PRODUCTION *IN VITRO* IN *FUSARIUM GRAMINEARUM*

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**ABSTRACT**

Fusarium Head Blight (FHB), caused by *Fusarium sp.*, is a devastating disease of barley and wheat in many regions of the world. Deoxynivalenol (DON) is one of several mycotoxins produced by the fungal pathogens in grains during disease development. Food and feed contaminated with DON pose a health risk to humans and livestock. The use of essential oils as natural antifungal compounds in foods has attracted growing interest in recent years, to meet the consumers' requirements in terms of food quality and safety. However, their application in foods was limited due to the hydrophobicity and volatility. This work aimed at investigating the effect of nanoencapsulated clove essential oils on antifungal activity and inhibition of DON against *Fusarium graminearum*. Clove essential oils encapsulated in the Medium-chain triglyceride (MCT) or corn oil nanoemulsion droplets were prepared by high pressure homogenization. The antifungal efficacy of selected stable nanoemulsions against four *F. graminearum* isolates were examined *in vitro* using effective concentrations ( $EC_{50}$ ). The measured antifungal activity was significantly affected by the concentration of clove oils as well as the formulation of the nanoemulsions. For example, Clove essential oils encapsulated in corn oil showed stronger antifungal activity ( $EC_{50}$  = 4.150~5.828 mg/g) than those encapsulated in MCT ( $EC_{50}$  = 5.300 to 5.974 mg/g) and free clove oils. In addition, effects of nanoencapsulated clove essential oils on inhibition of production of deoxynivalenol (DON), 3-acetyl-deoxynivalenol (3ADON), and 15-acetyl-deoxynivalenol (15ADON) by *F. graminearum* isolates were studied in rice cultures. The results showed that rice cultures treated with nanoencapsulated clove oils had significant lower DON contents than the controls. Furthermore, nanoencapsulated clove oils exhibited quicker reductions of mycotoxin production compared to free clove oils. These results provide new perspectives for possibly using this natural antimicrobial compound to control fungal contaminants and extend the shelf lives of foods and/or feedstuffs.



**GENE DISCOVERY  
AND  
ENGINEERING  
RESISTANCE**



DELETION OF *FGNAT1* REVEALS A POTENTIAL  
ROLE OF BENZOXAZINOIDS IN  
SUPPRESSING DON ACCUMULATION

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**ABSTRACT**

The benzoxazinoids 2,4-dihydroxy-1,4-benzoxazin-3-one (DIBOA) and 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA), are a class of compounds produced by wheat, maize, rye, and select barley species (e.g. *Hordeum lechleri*) that spontaneously break down to known phytoalexins benzoxazolinones, benzoxazolin-2-one (BOA) and 6-methoxy-benzoxazolin-2-one (MBOA), respectively. The compounds, collectively referred to as Bx compounds, inhibit the growth of *Fusarium verticillioides*, *F. pseudograminearum*, and *F. graminearum*. These *Fusarium* species contain an arylamine N-acetyltransferase denoted as *NAT1* or *FDB2* which detoxifies BOA with the help of the *FDB1* gene cluster to a form, N-(2-hydroxyphenyl) malonamic acid (HPMA). Previous work in *F. pseudograminearum* identified *FDB2* as necessary for full virulence on wheat in head blight inoculation assays. However, deletion of *NAT1* in *F. verticillioides* had no reduction in virulence in maize seedling assays. Here we deleted the *FDB2/NAT1* homolog in *F. graminearum* and tested its virulence in 15 Northwest wheat varieties for the potential use of increased sensitivity to Bx compounds as a target for breeding that could be aided by RNAi interference (RNAi)-based host-induced gene silencing (HIGS) by targeting *FgNAT1*. No significant virulence reduction in wheat was found with point inoculations of two *FgNAT1* mutants compared to wild-type. However, reduction in deoxynivalenol (DON) was seen with 14 varieties, with reductions as high as 48%. Precedent for this observation is supported in the literature with reductions in DON and *TRI6* expression with increased exposure solely to DIMBOA. If the associated reduction correlates to deletion of *FgNAT1* through detoxification of DIMBOA, then both Bx compounds and detoxification by *FgNAT1* might represent a unique target for breeding and HIGS, respectively, for control of Fusarium head blight.

**ACKNOWLEDGEMENT AND DISCLAIMER**

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## SILENCING EFFICIENCIES OF RNAI VECTORS TARGETING SPECIFIC 50 BP REGIONS OF *TRI6*

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### ABSTRACT

RNAi interference (RNAi)-based host-induced gene silencing (HIGS) has emerged as a viable control for disease including effective silencing of genes for mycotoxin production, thus guarding against mycotoxin accumulation in important crops. For RNAi to be effective, it is not necessary to express an inverted repeat corresponding to a full-length gene target, and identifying short and effective sequences to target will enable construction of shorter vectors (especially useful when targeting multiple genes and limit the potential for off-targeting). Previously, we have shown that targeting *TRI6* with five different length and position fragments have different efficiencies for silencing by RNAi vectors transformed into *Fusarium graminearum*. The factors that determine efficiency of silencing between different RNAi fragments are not fully understood. One of these factors could be the amount of specific small interfering RNA (siRNA) responsible for silencing when bound to argonaute. We previously observed through small RNA sequencing that specific siRNA accumulate at higher levels (peaks) mapping to *TRI6* consistently and regardless of the fragment size, starting position of the fragment, or the genomic position of the transgenic insert. This is likely due to processing by dicer and the propensity of argonaute to bind to siRNA with specific characteristics, such as percentage of GC and the 5' end beginning with uracil. Here we test the silencing efficiency of six very short RNAi vectors targeting 50 bp regions of peak siRNA observed by previous small RNA sequencing. Additionally, a targeted 50 bp low GC% and low siRNA accumulating region hypothesized to have low efficiency in silencing was tested in comparison to the other very short RNAi vectors. These results will dictate our ability to determine silencing efficiency related to observed siRNA accumulation and the possibility for intelligent optimization of RNAi silencing vectors for HIGS or other applications.

### ACKNOWLEDGEMENT AND DISCLAIMER

This project was funded by USDA-ARS Project 2050-21000-031-00 and by the U.S. Wheat and Barley Scab Initiative, projects FY16-BR-013 and FY16-BA-005. 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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RESPONSE OF WHEAT CONSTITUTIVELY  
EXPRESSING MONOLIGNOL BIOSYNTHESIS  
GENES TO FUSARIUM HEAD BLIGHT

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## ABSTRACT

The goal of this research is to identify Fusarium Head Blight (FHB) resistance in wheat plants through constitutive expression of monolignol biosynthesis genes, a generalized defense response against pathogens. Monolignols are the subunits of the lignin polymer that are secreted into cell walls to provide structural support, and this pathway also is induced upon pathogen attack. The spring wheat CB037 was transformed with constitutive expression constructs containing the gene *SbMyb60*, or a gene encoding a sorghum monolignol pathway enzyme [caffeoyl-CoA 3-O-methyltransferase (*SbCCoAOMT*), 4-coumarate-Coenzyme A ligase (*Sb4CL*), and *p*-coumarate 3-hydroxylase (*SbC3H*)], each under control of the cauliflower mosaic virus (CaMV) E35S promoter. The lead transgenic events, the recipient line CB037, the resistant line Sumai No. 3, and the susceptible line Wheaton, were grown in the greenhouse. At anthesis plants were either spray-inoculated with a conidial suspension of the FHB pathogen to assess Type I resistance (to initial infection), or point-inoculated to assess Type II resistance (to pathogen spread). Disease severity was determined 7, 14 and 21 days after inoculation (dai) for spray inoculations, and 5, 7, 10, 14 and 21 dai for point inoculations and used to calculate Area Under the Disease Progress Curve (AUDPC). Proportion of *Fusarium* Damaged Kernels (FDK) also was determined. Both lead events for *SbCCoAOMT* and *SbC3H* had AUDPC significantly less than that for the susceptible line, Wheaton, and one event, each, had AUDPC similar to that of the resistant line Sumai No. 3. FDK analysis supported these results with both *SbC3H* events and one *SbCCoAOMT* event having FDK similar to Sumai No. 3. AUDPC values for the *Sb4CL* and *SbMyb60* lead events were all significantly greater than that of Sumai No. 3; one event of each had AUDPC similar to that of Wheaton. FDK values for the *Sb4CL* and *SbMyb60* lines were also significantly greater than that of Sumai No. 3. Responses of these lines expressing the *SbMyb60* transcription factor or each of the three enzymes may suggest products from the monolignol pathway that are likely to increase resistance to *F. graminearum*. Further analyses of *SbCCoAOMT* and *SbC3H* expression in wheat may provide novel strategies using monolignol biosynthesis pathway genes to increase resistance to *F. graminearum*.

## ACKNOWLEDGEMENT AND DISCLAIMER

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

## MAPPING FOR FHB QUANTITATIVE TRAIT LOCI IN BARLEY

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### ABSTRACT

*Fusarium* species cause Fusarium head blight (FHB) disease in wheat and barley. Host resistance is controlled by quantitative trait loci (QTL) and previous genetic mapping studies have identified a number of resistant alleles from two- and six-rowed barley accessions. The aim of the current studies are to (1) identify QTL for resistance from an elite barley cultivar, Rasmusson; and (2) fine-map a previously identified QTL on chromosome 6H of barley. For QTL mapping study, a biparental mapping population comprising 93 F<sub>6.7</sub> recombinant inbred lines (RILs) derived from a cross between Rasmusson and PI383933 was developed. Rasmusson is an elite malting barley cultivar released by the University of Minnesota with moderately susceptible response to FHB. PI383933 is a Japanese cultivar with highly susceptible response to FHB and often used as a susceptible check in FHB field trials. Composite interval mapping identified one major QTL associated with FHB and DON resistance on chromosome 7H, which was also associated with plant height, spike length and spikelet density. Minor QTL for FHB and DON were detected on chromosome 6H and chromosome 3H, respectively, and were not associated with agronomorphological traits. The 6hbin7 region harbors coincident QTL for FHB and GPC (grain protein content). Whether the two traits are controlled by linked loci or a single locus remains undetermined. To fine map the 6H QTL region, an F<sub>2</sub> population of 2,082 plants was derived from crossing lines carrying Chevron alleles in the 6H QTL region with the susceptible cv. Lacey. Recombinants identified from the population were genotyped with 34 SNP markers covering the target region (~3.0 cM), which resulted in the identification of 37 recombinants representing 12 recombinant classes. Selected F<sub>2.3</sub> recombinants were tested for FHB response in 2016. Preliminary results suggested that GPC and FHB resistance are controlled by tightly linked loci.

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# FHB RESISTANCE IN DURUM WHEAT BY MEANS OF EPIGENETIC MODIFICATION

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## ABSTRACT

Fusarium head blight (FHB), commonly known as scab, is perhaps the most serious disease affecting wheat in the U.S. and throughout the wheat-growing regions of the world. FHB severely affects wheat by causing yield losses and production of grain infests with hazardous mycotoxins. Enhancing resistance to FHB is a continuous challenge for durum wheat breeding, where most germplasm are susceptible and there is low genetic variation for this trait. Active demethylation of immune-responsive genes in plants has been reported to occur during biotic and abiotic stresses. Our project aims at utilizing this phenomenon to induce heritable demethylation in durum lines as a novel source of FHB resistance.

We treated eight-advanced durum breeding homozygous lines with 5-methyl-azacytidine that removes CG methylation. Over 500 individuals of the treated lines were advanced to the M4 generation without a selection pressure. Thirty two of the 500 M4 lines were selected following preliminary testing against FHB. The 32 selected lines and eight parental checks were further tested for FHB resistance under greenhouse and field conditions. Five of the 32 selected lines tested showed promise, having less than 30% FHB severity as compared with the FHB severities for the parental lines and FHB susceptible lines included as checks which ranged from 50-100%. The analysis of *Fusarium*-damaged kernels (FDK) and deoxynivalenol (DON) on grain harvested from inoculated plants further supported the field and greenhouse disease assessments. The five best performing lines, together with their respective parental lines and some highly susceptible checks, were examined to determine the overall percentages of epigenetic change that were responsible for the enhanced FHB resistance observed. The results of a global methylome level analysis did not show significant differences between the five best performing lines and the parental lines from which they were derived, though it is possible changes occurred in only a few genes providing resistance and that these changes could not be detected by the present method. Transcriptome analysis (with a mean library size of  $\approx 200$  bp, quality scores of  $\geq Q30$  and approximate coverage of 32 - 50 million reads per samples) of the selected lines (treated, parental and susceptible checks) is continuing to discover the candidate gene(s). We have advanced the two most resistant M4 lines by backcrossing, to the parental cultivars, with an aim of testing the stability and inheritance of the resistance. The initial evidence from field-testing indicates there is stability of the resistance observed in the backcross-derived lines. The backcross-derived lines are being further tested in greenhouse.

## ACKNOWLEDGEMENT AND DISCLAIMER

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# CRISPR-EDITING OF SUSCEPTIBILITY GENES IN *ARABIDOPSIS* FOR FUSARIUM HEAD BLIGHT RESISTANCE

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## ABSTRACT

Fusarium head blight (FHB) caused by *Fusarium graminearum* (*Fg*) is a devastating disease of cereal crops such as wheat and barley, resulting in significant yield loss and reduced grain quality. *Fg* produces mycotoxins, which are toxic to humans and livestock upon consumption. Three putative FHB susceptibility genes, *ethylene insensitive 2 (EIN2)*, *homoserine kinase (HSK)* and *2-oxoglutarate Fe(II) oxygenase (2OGO)*, were chosen in this study. It has been shown that *Arabidopsis* mutant and RNAi-suppressed wheat plants deficient in ethylene signaling are resistant to FHB, indicating that *Fg* exploits the ethylene signaling pathway to colonize plants. It has also been shown that *Arabidopsis HSK* and *2OGO* point mutants are more resistant to FHB than wild type plants. While *HSK* and *2OGO* appear to be required for FHB susceptibility, the underlining molecular mechanism is largely unknown. We have successfully edited these three susceptibility genes in *Arabidopsis thaliana* (*At*) using the precise CRISPR/Cas9 gene editing system. Inoculation assay of GFP-tagged *Fg* on the *AtEIN2*, *AtHSK* and *At2OGO*-edited *Arabidopsis* plants showed that fungal colonization were reduced by 50-60%, based on qPCR analysis of the GFP level. We have cloned cDNAs for the barley (cv. Conlon) *HvEIN2*, *HvHSK* and *Hv2OGO* genes. Complementation transformation of the wild type barley FHB susceptibility genes into *AtEIN2*, *AtHSK* and *At2OGO*-edited *Arabidopsis* plants will allow us to identify the functional homologs of these genes and establish their involvement in the interaction between barley and *Fg*. These studies provide a foundation for gene-editing of disease susceptibility genes in barley to improve FHB resistance.



# RESISTANCE TO *FUSARIUM GRAMINEARUM* AND *FUSARIUM MYCOTOXINS* BY EXPRESSION OF *ARABIDOPSIS* AND WHEAT NON-SPECIFIC LIPID TRANSFER PROTEINS IN WHEAT

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## ABSTRACT

Previously we found that overexpressing a non-specific lipid transfer protein (nsLTP), AtLTP4.4 (AT5G55450), in *Arabidopsis* and yeast protects against trichothecene-induced ROS stress.

Protoplasts isolated from *Arabidopsis* expressing the nsLTP had basal ROS levels substantially lower compared to wild type (COL-0) protoplasts. Exposure of the wild type protoplast to deoxynivalenol (DON) induced ROS generation while DON treated protoplasts isolated from the AtLTP4.4-GFP transgenic line did not induce ROS generation. This ROS-protective mechanism of AtLTP4.4 likely accounts for observed protection against trichothecenes, a key *Fusarium graminearum* virulence factor. Our primary research goal is to determine if this trichothecene protection mechanism extends to wheat. We have characterized transgenic wheat expressing AtLTP4.4 and codon-optimized (for wheat) AtLTP4.4 in transgenic Bobwhite, Rollag, Forefront and RB07 lines. We generated elite wheat lines with Ubi:AtLTP4.4:GFP and Ubi:TaLTP3:GFP expression vectors. Confocal and Western analysis showed high level of expression of Ubi:AtLTP4.4:GFP and Ubi:TaLTP3:GFP in Bobwhite and Rb07 wheat. Transgenic wheat lines expressing AtLTP4.4:GFP and TaLTP3:GFP showed reduction in fungal growth in detached leaf assays and substantially less bleaching of the wheat heads relative to non-transgenic controls in response to DON. These lines are being increased for field testing. In addition, we have generated transgenic barley lines with Ubi:AtLTP4.4:GFP and Ubi:TaLTP3:GFP. These lines will also be evaluated for FHB resistance.

## ACKNOWLEDGEMENT AND DISCLAIMER

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DETECTION AND QUANTIFICATION OF *WFHB1-1*  
PROTEIN DURING FHB PATHOGENESIS IN  
WHEAT SHOWS THE ROLE OF THIS  
GENE IN DISEASE RESISTANCE

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**ABSTRACT**

Fusarium head blight (FHB) is one of the major fungal diseases of wheat and barley. This disease not only reduces the yield of these crops but also reduces the quality of the grain. *Qfhb1* has been found as the most important FHB resistance QTL in the wheat. We previously identified wheat gene *Wfhb1-1* as a candidate for the functional genic component associated with this QTL. Western blotting assay with anti-*Wfhb1-1* antibody was conducted to study the differential accumulation of *Wfhb1-1* protein during the early stage of FHB pathogenesis between a pair of near-isogenic wheat lines that has or not have *Qfhb1*, respectively. We will report our results in this poster presentation.

MECHANISTIC STUDIES OF PORE  
FORMING TOXIN GENE  
Nidhi Rawat

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**ABSTRACT**

Genetic resistance is the most economical and sustainable approach of managing Fusarium Head Blight. *Fhb1* QTL located on chromosome 3BS of Chinese landrace Sumai 3, conferring type 2 resistance against Fusarium Head Blight, has been used in breeding programs worldwide. Using map-based cloning approach, *Fhb1* was identified as a Pore-forming toxin-like (PFT) gene. The gene encodes a chimeric lectin with two agglutinin domains and a toxin domain. We have started in vitro experiments to dissect the mode of action of PFT. The results of the experiments will be presented at the 2017 National FHB Forum.

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# TARGETING WHEAT GENES ASSOCIATED WITH SUSCEPTIBILITY TO *FUSARIUM GRAMINEARUM* FOR ENHANCING FHB RESISTANCE

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## ABSTRACT

Our work in wheat and *Arabidopsis* has identified the involvement of 9-lipoxygenases (9-LOXs) as susceptibility factors in plant interaction with *Fusarium graminearum*, the principal causative agent of FHB in wheat and barley. Knockout of 9-LOXs in *Arabidopsis* and RNA-interference (RNAi)-mediated knockdown of 9-LOXs in the hexaploid wheat cv Bobwhite resulted in enhanced resistance against *F. graminearum* (Nalam et al. 2015). Resistance in these LOX-silenced wheat RNAi lines is characterized by the lack of spread of infection from the inoculated spikelet. The goals of the proposed work are to establish whether (i) the FHB resistance promoting effect of knockdown of *Lpx3*, a wheat 9-LOX, is also effective in wheat backgrounds other than Bobwhite, (ii) one or more *Lpx3* homeolog(s) in wheat contribute towards susceptibility to *Fusarium graminearum*, and (iii) nonsense and/or missense *Lpx3* variants can provide a non-GMO strategy that in the future can be utilized by breeding programs to enhance FHB resistance in wheat. We are utilizing a TILLING approach to address these goals.

## ACKNOWLEDGEMENT AND DISCLAIMER

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## LOSS FUNCTION OF *TAHRC* IN THE *FHB1* REGION INCREASED WHEAT FHB RESISTANCE

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### ABSTRACT

Fusarium head blight (FHB), mainly caused by *Fusarium graminearum*, is a devastating wheat disease worldwide. Use of genetic resistance has been proved to be one of the best approaches to minimize the disease damage. Many quantitative trait loci (QTLs) have been reported for FHB resistance in different sources, but only *Fhb1* on the chromosome 3BS of Sumai3 and Ning7840 shows a consistent major effect on reducing FHB symptom spread within a spike (type II resistance) in different genetic backgrounds. Recently, several *Fhb1* candidate genes have been reported, including a *GDSL lipase* (*GDSL*, Schweiger et al. 2016), a *pore forming toxin-like protein* (*PFT*, Rawat et al. 2016), and a *histidine-rich Ca-binding protein* (*TaHRC*, Su et al. 2006). Among these genes, *GDSL* and *PFT* are characterized as presence (resistance allele) and absence (susceptible allele) of the genes in contrasting genotypes, whereas *TaHRC* has a 752 bp deletion in the resistant genotype, and the susceptible genotype does not carry the deletion. To determine most possible candidate for *Fhb1*, we genotyped an association-mapping panel of 142 accessions using the three candidate genes and other markers in *Fhb1* region and phenotyped the population for FHB resistance, and found that only the accessions with Ning 7840 haplotype showed FHB resistance and they contain all resistance alleles (Sumai3 alleles) at the three loci; whereas all other accessions with various haplotypes were FHB susceptible. Among the susceptible haplotypes, Dahongpao haplotype contains both Sumai3 alleles of *GDSL* and *PFT* genes. Sequence analysis of the accessions with Dahongpao and Ning 7840 haplotypes found that the three candidate genes share identical sequences between the two haplotypes except that Dahongpao haplotype has the 752 bp deletion in *TaHRC*. RNA interference and gene editing experiments confirmed that loss of function of *TaHRC* significantly enhances FHB resistance. Screening 1165 wheat landraces and cultivars from 53 countries for the three genes found that *GDSL* and *PFT* are present together (70 accessions) or independently (61 accessions) in wheat landraces from many countries where *Fhb1* has never been detected. In Chinese landraces, *GDSL* and *PFT* were detected in many landraces collected nationwide. The deletion mutation in *TaHRC*, however, is present only in the accessions from Japan and China, not from any other area in the world. In China, the deletion in *TaHRC* is detected only in southern China where FHB has been a historic problem. Haplotype and function analyses, and geographic distribution of candidate gene alleles reveal that *TaHRC* is the key candidate for *Fhb1* and the *Fhb1* resistance allele

was evolved from a single deletion in the Dahongpao haplotype. *Fhb1* resistance allele is relatively new that was most likely evolved in southern China.

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

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# DEVELOPMENT OF WHEAT VIRUSES AS EXPRESSION VECTORS FOR HIGH-THROUGHPUT SCREENING OF ANTIMICROBIAL PEPTIDES IN WHEAT AGAINST FUSARIUM HEAD BLIGHT

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## ABSTRACT

Plant viruses have been used to express a variety of specialty products and to study functions of host genes. However, information on the use of monocot-infecting viruses as viral expression vectors is scanty. This study developed and evaluated a reverse genetics system for *Wheat streak mosaic virus* (WSMV) and *Triticum mosaic virus* (TriMV), naturally infecting distinct wheat viruses of the family *Potyviridae*. *In vitro* transcripts from cDNA clones of WSMV and TriMV were infectious on wheat with symptoms similar to respective wild-type viruses. The ability of WSMV and TriMV to express foreign genes in wheat was examined by engineering sequences encoding for a green (GFP) or red fluorescent protein (RFP) genes into the genomes of both viruses. GFP- or RFP-tagged WSMV and TriMV infected wheat and efficiently expressed fluorescent proteins in leaves, stems, roots, and wheat heads, specifically florets and the lemma, awns, and anther filaments and stigma. Both WSMV and TriMV retained the GFP or RFP sequences for more than 120 days and for 6 serial passages at 14-day interval. Since GFP- or RFP-tagged WSMV and TriMV systemically infected wheat heads, these viruses can be utilized for transient expression of antimicrobial peptides (AMPs) with activity against *Fusarium* head blight (FHB). This system can be employed for rapid screening of numerous AMPs for their activity against FHB to identify the 'candidate AMPs' prior to generation of transgenic wheat. Thus, reverse genetics system of WSMV and TriMV will make an ally from an enemy for *in planta* screening of AMPs, followed by development of resistance to FHB and DON production in wheat through transgenic approach.

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# **PATHOGEN BIOLOGY AND GENETICS**



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MICROBIAL CORRELATES OF *FUSARIUM*  
BIOMASS AND DEOXYNIVALENOL  
CONTENT IN INDIVIDUAL WHEAT SEEDS

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**ABSTRACT**

Manipulating the microbiome of wheat seeds and heads may contribute to control of *Fusarium* head blight and mycotoxin accumulation in grain, which creates a food safety hazard. With the aim of identifying novel management targets, we looked for correlations between *Fusarium* biomass or deoxynivalenol (DON) content and characteristics of the microbial communities inhabiting wheat grain. Individual seeds were sampled in an attempt to approach the fine spatial scale at which microbial communities are organized. Seeds were collected from a common wheat variety planted across a mist irrigated nursery in St. Paul, Minnesota, in each of two successive years. Across 96 seeds in 2016, DON content varied by c. 470 fold (from 5.4 to 2517  $\mu\text{g}$  DON  $\text{g}^{-1}$  seed), and *Fusarium* biomass varied over 1 million fold (from  $1.9 \times 10^0$  to  $2.3 \times 10^6$  copies *TRI5*  $\text{mg}^{-1}$  seed). The relationship between *Fusarium* biomass and DON content was strong (for log transformed values; adjusted  $R^2=0.63$ ,  $P<0.001$ ). However, fit with DON content was improved in an additive model including both *Fusarium* biomass and bacterial diversity (Hills index; adjusted  $R^2=0.82$ ,  $P_{\text{Hills}}<0.001$ ). Similarly, additive models including the relative abundance of particular bacterial taxa sometimes improved fit with DON content, compared to the model including *Fusarium* biomass alone. For the samples presenting the greatest deviation from the expected relationship between *Fusarium* biomass and DON content, bacterial communities could be readily distinguished between seeds with lower vs. higher than expected DON content. These data suggest that bacterial communities associated with wheat seeds may substantially impact the development of *Fusarium* head blight and the accumulation of mycotoxins in grain. With rigorous development, the microbiome of wheat heads may become a target for agricultural management.

# FUSARIUM SPECIES COMPLEX CAUSING FUSARIUM HEAD BLIGHT ON OAT IN MANITOBA

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## ABSTRACT

Fusarium head blight (FHB) is caused by a complex of *Fusarium* species in North America. *Fusarium graminearum* is the predominant species causing FHB on wheat. It produces several toxic secondary metabolites, among which deoxynivalenol (DON) and zearalenone (ZEN) are the most closely monitored due to their high detection rates and strong toxicity. FHB on oat was initially associated with *F. graminearum* in Canada. However, *F. poae* has become more frequently isolated in commercial oat fields in recent years. *F. poae* can produce a wide range of type A and B trichothecene mycotoxins as well as several non-trichothecene mycotoxins. To date, relatively little is known about the prevalence of *F. poae* and its impacts on the commercial oat production in western Canada. In this study, we surveyed *Fusarium* species infecting commercial oat fields in Manitoba from 2014 to 2016. Samples were collected from 124 commercial fields and *Fusarium* biomass in contaminated grains was assessed by real time qPCR using primer sets specific to *Fusarium* species commonly found in Western Canada. Our preliminary results indicate that *Fusarium* species infecting oat are, in fact, more diverse than *Fusarium* species infecting wheat and mycotoxins other than DON need to be considered.

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# CELLULAR AND SUBCELLULAR CHANGES OF *FUSARIUM GRAMINEARUM* DURING DON MYCOTOXIN BIOSYNTHESIS

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## ABSTRACT

The ascomycete fungus *Fusarium graminearum* causes scab disease on wheat and barley and contaminates grains with trichothecene mycotoxins (e.g. DON). To understand how DON biosynthesis is spatially regulated in the fungal cell, the subcellular localization of DON biosynthetic enzymes was determined and cellular changes of hyphae during toxigenesis were studied *in vitro* and *in planta* on wheat. Morphological changes of DON producing cells and dynamics of organelles, such as the ER, nuclei, vacuoles, and lipid bodies in *F. graminearum* during DON biosynthesis were documented and quantified, using organelle specific dyes, including ER-Tracker, CMAC, and BODIPY 493/503 and fluorescence microscopy. By fluorescence tagging of fungal proteins, which catalyze early and late steps in DON biosynthesis, including hydroxymethylglutaryl CoA reductase (Hmr1), trichodiene oxygenase (Tri4), calonecetrin oxygenase (Tri1), as well as Tri14, a protein of unknown function, we observed co-localization with the endoplasmic reticulum (ER) of DON producing cells (Menke et al., 2013, Boenisch et al., 2017). Applying superresolution microscopy and 3D imaging on DON producing cells, we further demonstrate that the ER shifts during DON induction from being reticulate to being thickened with pronounced perinuclear and peripheral ER both *in planta* and *in vitro*. Transmission electron microscopy of DON producing cells revealed that organized smooth ER membranes (OSER) are formed upon DON induction (Boenisch et al., 2017). OSER are stacks of smooth ER membranes, with a ~10 nm cytoplasmic space between each ER sheet (Snapp et al., 2003; Boenisch et al., 2017). Since enzyme active sites for Tri1, Tri4, and Hmr1 are predicted to be on the cytoplasmic side of the ER membrane, we conclude that DON is likely synthesized in the cytoplasmic spaces within OSER. Thus, DON biosynthesis might be facilitated and sequestered from targets of DON inhibition, such as ribosomes and mitochondria. Knowledge about cell biological mechanisms, which may facilitate or even enable DON biosynthesis and/or to protect the fungus from toxic effects of DON, might open new possibilities to interfere with fungal DON biosynthesis and prevent DON contamination of cereals by *F. graminearum* in the future.

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# FUNGAL G-PROTEIN COUPLED RECEPTORS PROMOTE FUSARIUM HEAD SCAB ON WHEAT

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## ABSTRACT

*Fusarium graminearum* is a globally important cereal pathogen and mycotoxin producer. When *F. graminearum* spores land on a plant, the pathogen must decide if it is suitable, where to infect, and when to deploy different virulence strategies. But how *F. graminearum* senses the “touch and taste” of its environment through external receptors is largely unknown.

Cell biology and transcriptomics of *F. graminearum* have shown the spatial temporally regulation of a complex array of characterised and putative virulence mechanisms during wheat infection. This includes the dramatic up-regulation of trichothecene mycotoxin biosynthesis, plus other secondary metabolite gene clusters, during symptomless infection. Distinct groups of putative secreted effector proteins were up-regulated either during symptomless or symptomatic infection. The induction of secreted lignocellulolytic enzymes followed a two-step mechanism, resulting in elevated expression during the development of disease symptoms and the degradation of dead plant cells. Collectively, this implies that *F. graminearum* must sense alterations in the microenvironment within the host and coordinate virulence accordingly.

G-protein coupled receptors (GPCRs) are the largest class of extracellular receptors in eukaryotes and *F. graminearum* possesses more than other model fungi. Interestingly, a subset of GPCRs were highly expressed during the establishment of symptomless infection. The generation of a collection of *F. graminearum* mutants lacking individual GPCRs has now reveal their involvement in promoting the establishment of wheat infection, and their potential as targets to intervene in the establishment of Fusarium Head Scab.

## ACKNOWLEDGEMENTS

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# THE ROLE OF THREE SMALL SECRETED CYSTEINE-RICH PROTEINS OF *FUSARIUM GRAMINEARUM* ON FUNGAL GROWTH AND PLANT INFECTION

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## ABSTRACT

*Fusarium graminearum* (*Fg*) secretes secondary metabolites as well as proteins during colonization of the host plants. Among the secreted proteins are small cysteine-rich proteins (SCPs) that show sequence homology to the SCPs with pathogenicity roles in other host-pathogen interactions. With an objective of elucidating their role in fungal growth and plant infection, we developed knockout (KO) and *in locus* overexpression (OX) *Fg* transformants for the genes that encode three secreted SCPs: cerato-platanin (CP), common in fungal extracellular membrane 1 (CFEM1) and CFEM2. With the exception of more rapid macroconidia germination for one of the transformants (CFEM1OX) compared with the wild-type (WT) strain, no differences were observed in vegetative growth. CFEM1OX also showed a slight increase in disease spread following point inoculation in the moderately susceptible wheat cultivar, Penhold, but not in the resistant or susceptible lines tested. To determine whether any of these SCPs have a role in establishing initial infection, spray inoculation screens are underway. These strains are also being assessed for perithecia development as well as DON production. RNA-sequencing analysis showed altered expression of genes related to, among others, signaling, translation initiation and integral component of membrane.

BASES OF VARIABLE SENSITIVITY TO  
TEBUCONAZOLE IN NEW YORK ISOLATES  
OF *FUSARIUM GRAMINEARUM*

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**ABSTRACT**

Triazole fungicides are an integral component of *Fusarium* head blight management, and their continued efficacy is necessary for sustainable disease control. Properly deploying these chemicals requires accurate monitoring of sensitivity in natural pathogen populations. Current approaches to surveillance are impractical, requiring time consuming growth assays and fungicide amended media. There is a need to understand what causes variation in susceptibility and develop new resistance detection methods that incorporate this understanding. Isolates of *Fusarium graminearum* collected from wheat, barley and corn in New York were assessed for sensitivity to tebuconazole using a discriminating dose derived from a previous study. A subset of these isolates, representing the range of observed sensitivity ( $EC_{50}$  0.22 - 2.85 mg/L), were characterized genetically to find markers for reduced sensitivity. Cytochrome P450 genes (CYP51), involved in triazole resistance of other fungi, were sequenced individually, and whole genome sequences were generated to observe CYP51 promoter regions, ABC transporters and other genes potentially involved in responses to fungicide. While some variation in sensitivity was detected, the majority of isolates were highly susceptible to tebuconazole. A portion of this variation can be attributed to the chemical formulation of tebuconazole used in relative growth assays. No single nucleotide polymorphisms in the three CYP51 paralogs of *F. graminearum* were associated with reduced sensitivity, though genome sequences may contain more useful data. This work highlights the difficulty of efficiently surveilling fungicide sensitivity at large scales and underlines the value of new tools for monitoring resistance.

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OVER-EXPRESSION OF THE *FUSARIUM*  
*GRAMINEARUM* MITOGEN ACTIVATED  
PROTEIN KINASE, MGV1

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**ABSTRACT**

Protein kinases are enzymes that activate/deactivate other proteins (or themselves) by transferring a phosphate group to specific amino acid side chains. The mitogen activated protein kinases (MAPKs) are a family of protein kinases that regulate various cell functions. Three MAPK pathways have been identified in *Fusarium graminearum*, one of the main *Fusarium* species responsible for fusarium head blight (FHB) disease of cereals. It was previously shown that Mgv1 (MAPK for growth and virulence 1) plays a role in mycelia growth, female fertility and the accumulation of trichothecene mycotoxins, such as deoxynivalenol (DON). While it is clear that Mgv1 involves the regulation of various traits, little is known about the biochemical pathway of this MAPK. With the goal of identifying downstream targets of Mgv1 activity, transformants over-expressing the *MGV1* gene *in locus* were obtained by *Agrobacterium*-mediated transformation. The radial growth of the transformant colonies were compared to wild type (WT), showing a slight reduction in growth rate. FHB disease assays were conducted in *Brachypodium distachyon*, a plant model for cereal crops, and no difference in pathogenicity was observed between the WT and transformants. Quantitative RT-PCR analysis confirmed up-regulation of the *MGV1* transcripts, and also revealed downregulation of the *TRI5* gene encoding trichodiene synthase enzyme involved in trichothecene biosynthesis. The results of the immunoblot experiment suggest an increase in a phosphoactivated MAPK in the transformants. The *MGV1* overexpression strains will be used to further characterize the role of Mgv1 in various pathways, including cell wall formation, and to identify the downstream proteomic and genetic elements of the Mgv1 signalling pathway.

AN ARABINOBIOL-HYDROLASE (ARB93B) FROM  
*FUSARIUM GRAMINEARUM* IS ASSOCIATED  
WITH WHEAT HEAD BLIGHT DISEASE

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**ABSTRACT**

Fusarium head blight (FHB), caused by the fungus *Fusarium graminearum*, is one of the most important diseases of wheat and barley worldwide. FHB not only reduces crop yield, but the fungus also contaminates grains with mycotoxins, which are harmful to humans and animals. A previous study demonstrated that *F. graminearum* secreted two  $\alpha$ -L-arabinanases (Arb93A and Arb93B) which belong to the glycoside hydrolase 93(GH93) family. Members of the GH93 family, which include sialidases, share a six-bladed  $\beta$ -propeller structure and play important roles in microbial pathogenesis. In this study, we investigate the role of Arb93B in wheat head blight. Arb93B deletion mutants were similar to the wild-type strain PH-1 in growth, morphology, and production of 15-ADON in liquid culture. FHB pathogenesis assays on wheat heads inoculated with deletion mutants of Arb93B exhibited about 50-60% less FHB disease and reduced DON contamination compared to heads inoculated with wild type PH-1. Gene expression studies revealed that expression of Arb93A was induced at 3 h post inoculation (hpi), reached its highest level at 12 hpi, and then was gradually reduced at 36 hpi and 7 d post inoculation. In contrast, the transcript of Arb93B was first detected at 36 hpi, and was reduced at 7 d post inoculation. Examination of Arb93B protein sequence indicated that it contains a chloroplast localization peptide, however, the fusion protein of GFP and predicted chloroplast peptide did not localize in the chloroplast via *Agrobacterium*-mediated transient expression. Further investigations are underway to determine the localization of the fusion protein.

# A DEOXYNIVALENOL (DON) TRANSPORTER FROM A LIBRARY OF DON-DETOXIFYING MICROORGANISMS

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## ABSTRACT

New strategies are needed to mitigate the mycotoxin deoxynivalenol (DON). Microorganisms were isolated from the environment, and cultured in a mineral salt media using 100 ppm DON as the sole carbon source. Degenerate Oligonucleotide Primed (DOP) –PCR was used to create a DNA library of microbial fragments from these environmental samples. The resulting fragments were cloned into a PCR8/TOPO vector, and were recombined into the yeast vector, pYES-DEST52. Two DON-sensitive yeast strains were transformed with this yeast expression vector, containing a wide array of random microbial fragments from our DOP-PCR microbial DNA library. A fragment was identified that enabled one of the DON-sensitive yeast strains to thrive in the presence of 100 ppm DON. GC/MS analysis of yeast culture extracts showed that the structure of DON appeared unchanged following DON-feeding studies, suggesting that the fragment may be a DON transporter. Here, we describe a series of studies to demonstrate that the microbial fragment (named TER-B) is likely a DON transporter. Ferulic acid, a transport inhibitor for the DON pump TRI12, was used in an attempt to inhibit TER-B. DON transporters could be used in the future to improve the detoxification of mycotoxins in living systems, and could find immediate application in commercial fermentation scenarios such as the production of beer and fuel ethanol.

## ACKNOWLEDGEMENT AND DISCLAIMER

This material is based upon work supported by the U.S. Department of Agriculture, under Agreement Nos. 59-0206-2-082 and 59-0206-6-017. This is a cooperative project with the U.S. Wheat & Barley Scab Initiative. Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the author(s) and do not necessarily reflect the view of the U.S. Department of Agriculture.



# FUSARIUM TRANSPORTERS FOR ENHANCED RESISTANCE TO FHB

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## ABSTRACT

*Fusarium* head blight pathogens produce trichothecene mycotoxins such as deoxynivalenol (DON) that are critical for determining the outcome of plant disease interactions. While much progress has been made in understanding the enzymatic pathways for DON biosynthesis, little is known about how toxins are exported from fungal cells and delivered to the host. The specific goal of our research is to examine several candidate multidrug transporters in *Fusarium graminearum* for their potential role in trichothecene export and fungal virulence. We have identified four co-regulated multidrug resistance transporters that, along with the trichothecene efflux pump Tri12, may be essential for maximum trichothecene export. Each of the five genes have been individually deleted and the mutant alleles are being combined by sexual recombination to create strains with deletions in combinations of two and three per strain. These genotypes are currently being tested for their ability to accumulate DON *in vitro* and *in planta* and for their effect on fungal virulence. Additionally, the genes will be expressed in yeast to determine their ability to allow for DON resistance when expressed in this heterologous host. This information is potentially useful because trichothecene exporters that deliver toxin to the plant when expressed in *Fusarium*, may allow for resistance to DON if expressed in plants. Our ultimate goal is to develop transgenic wheat and barley lines with increased trichothecene tolerance achieved by expression of *Fusarium* proteins conferring resistance to DON. This transgenic approach may represent a novel strategy by which small grain crops may escape the toxic effects of pathogen-produced small molecules.

# INVESTIGATING BARLEY DEFENSE RESPONSES AND INTERACTIONS WITH *FUSARIUM GRAMINEARUM*

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## ABSTRACT

We are studying two aspects of resistance to FHB. Barley harbors a moderate resistance response where *F. graminearum* does not spread from individual infection sites. This resistance response differs in two-row vs. six-row barley, which differ in arrangement of florets and trichome morphology. Our previous work has shown focal accumulation of plant defense compounds (cellulose and lignin) at trichomes on inoculated barley florets, with greater numbers of focal accumulations of these defense compounds on two-row barley varieties with small, domed trichomes. Near isogenic lines (NILS) of barley with loci for two- or six-row trichome characteristics are being studied for focal accumulation. The NILs have allowed us to elucidate the importance of trichome morphology in resistance responses. The *vrs1.c* locus is associated with trichome morphology on the lemma and appears to be important to the cellulose and lignin burst. This six-row allele in a NIL has increased numbers of foci compared to the other NILs and wild-type lines. Cross sectioning of inoculated samples will be done to determine if the observed foci result in the blocking of fungal penetration into the plant cells.

Barley varieties are available with partial (P) or complete (R) resistance to powdery mildew, a biotrophic pathogen, which show increased susceptibility to FHB. We hypothesize that the defense compound accumulation seen in trichomes is decreased in the R lines, allowing *F. graminearum* to penetrate barley florets more readily. Data generated in the R line, P line, and the parent line show foci in samples, with very few observed in any sample. No statistically significant differences have been shown in any lines studied, indicating that any increased susceptibility to *F. graminearum* previously reported are not due to this focal accumulation response.

## ACKNOWLEDGEMENT AND DISCLAIMER

This material is based upon work supported by the U.S. Department of Agriculture, under Agreement Nos. 59-0206-1-120 and 59-0206-6-004. This is a cooperative project with the U.S. Wheat & Barley Scab Initiative. Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the authors and do not necessarily reflect the view of the U.S. Department of Agriculture.

THE RESPONSE OF *FUSARIUM*  
*GRAMINEARUM* TO SILICA  
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**ABSTRACT**

Silica is a common nutrient found in soil and is an important structural component of trichomes xylem, stomate, and silica/cork cells in barley and other grasses. It has been observed by us and by others that important life cycle stages of *F. graminearium* involve silica accumulating host cells. Young hyphae wrap around trichome before penetrating. Perithecial initials form beneath stomates and silica/cork cells and erupt through them to mature. Hyphae move aggressively through the xylem vessels to spread through the host. Our project is designed to investigate the relationship between the FHB fungus and plant silica. We grew barley in low silica conditions to determine whether low silica barley plants would have altered disease phenotypes. Perithecia production by *Fusarium graminearium* increases as the concentration of silica decreases in the medium that the host (*Hordeum vulgare L.*) is grown in. Additionally, the production of the red pigmented polyketide aurofusarin increases when the cultures are exposed to silica amended medium compared the same medium with no silica added. These and other observations are strong indications of a response by the pathogen to reduced silica *in vitro* and in the host grown in low or high silica environments. Recommendations that silica supplementation of fields will enhance host resistance may be affected by these experiments.

**ACKNOWLEDGEMENT AND DISCLAIMER**

This material is based upon work supported by the U.S. Department of Agriculture, under Agreement No. 59-0206-6-004. This is a cooperative project with the U.S. Wheat & Barley Scab Initiative. Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the author(s) and do not necessarily reflect the view of the U.S. Department of Agriculture.

# UPDATING POPULATION STRUCTURE AND GENETIC DIFFERENTIATION OF *FUSARIUM GRAMINEARUM* POPULATIONS FROM THREE MAJOR US REGIONS USING GENOTYPING-BY-SEQUENCING

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## ABSTRACT

Population genomic studies of *Fusarium graminearum* (Fg) isolates provide an important complement to experimental studies that investigate the interaction of this important pathogen with its hosts and functional differences between different pathogen populations. Jointly, these studies can identify the genetic basis of functional differences between populations that can affect pathogen management and strategies for developing host resistance. Our broader aims include characterizing the variability along chromosomes of patterns such as variant density, genetic differentiation between populations, and genetic recombination to gain insight into evolutionary processes in this species. Here, we provide an update of our USWBSI-funded population genomics project that uses genotyping by sequencing (GBS) for the genetic analysis of Fg isolates from the Americas. We have expanded our sample to nearly 600 isolates, focusing on isolates from the main geographical regions: the Upper Midwest, New York state, and Louisiana, but also including isolates from Uruguay. Our sample includes isolates from the 3-ADON, 15-ADON, NX-2, and NIV toxin types, allowing us to measure differentiation between major American populations and genetic exchange between them. We provide an update on our investigation of population structure, adaptive differentiation, and footprints of natural selection. We also describe patterns of linkage disequilibrium between variants in the populations.

## ACKNOWLEDGEMENT AND DISCLAIMER

This material is based upon work supported by the U.S. Department of Agriculture, under Agreement Nos. 59-0206-1-113 and 59-0206-6-002. This is a cooperative project with the U.S. Wheat & Barley Scab Initiative. Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the authors and do not necessarily reflect the view of the U.S. Department of Agriculture.

A CELL-FREE PROTEIN SYNTHESIS SYSTEM  
TO SCREEN ENZYMES THAT MODIFY  
THE MYCOTOXIN DEOXYNIVALENOL

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## ABSTRACT

The trichothecene mycotoxin deoxynivalenol (DON) is an important contaminant of crops such as wheat, barley, and corn. Current strategies to eliminate DON have centered on traditional breeding strategies to generate resistant crop lines. Enzymes may also be used to detoxify DON, but most enzyme screening methods are time-consuming and expensive. We engineered a cell free protein synthesis (CFPS) system to express known *Fusarium* acetyltransferase enzymes (FsTRI101, FgTRI101, and FoTRI201) to modify DON. When FsTRI101, FgTRI101, and FoTRI201 were expressed in the presence of 40 ug/ml DON for 30 min, the enzymes converted nearly all of DON to 3-acetyl-deoxynivalenol (3-ADON). Western blots confirmed the presence of all three acetyltransferase proteins produced in the CFPS system. In a temperature gradient assay, two enzymes (FgTRI101 and FoTRI201) produced in the CFPS system were incubated at five different temperatures (30°C, 40.3°C, 49.5°C, 60.5°C, and 69.5°C) for 30 min and then exposed to 40 ug/ml DON for an additional 30 min at the corresponding temperature. Results indicated that FgTRI101 and FoTRI201 were able to convert DON to 3-ADON at 30°C and 40.3°C, but not at higher temperatures. CFPS systems could be used to easily screen for functional enzymes (e.g., epoxide hydrolases) that can detoxify DON, and resulting enzymes could be used by commercial fermentation industries.

## ACKNOWLEDGEMENT AND DISCLAIMER

This material is based upon work supported by the U.S. Department of Agriculture, under Agreement Nos. 59-0206-2-082 and 59-0206-6-017. This is a cooperative project with the U.S. Wheat & Barley Scab Initiative. Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the author(s) and do not necessarily reflect the view of the U.S. Department of Agriculture.



**VARIETY  
DEVELOPMENT  
AND  
HOST PLANT  
RESISTANCE**





# INFLUENCE OF ENVIRONMENTAL SELECTION ON PREDICTION ACCURACY OF TRAINING POPULATION IN THE UNIFORM SOUTHERN SOFT RED WINTER WHEAT SCAB NURSERY

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## ABSTRACT

The Southern Uniform Winter Wheat Scab Nursery is evaluated annually in up to nine locations to determine the resistance in advanced generation breeding lines relative to the check cultivars Ernie, Jamestown, Bess and Coker 9835 (susceptible). Disease development varies across locations and years so selection of environments to include in training populations for Genomic Selection is necessary. The objective of this research was to compare three methods of choosing environments to maximize prediction accuracy for Scab resistance. The methods were: 1) all environments included, 2) environments selected based on principle component analyses of FHB severity means, and 3) environments selected based on performance of check cultivars for *Fusarium* damaged kernels (FDK) and Deoxynivalenol (DON) concentration in grain. Phenotypic data included 3,818 observations on 334 genotypes evaluated in 68 environments from 2011 to 2017. Three training populations were created including 68, 49 and 39 environments using Methods 1, 2 and 3 respectively. In each training population, least square means were calculated for each line using mixed models in R studio. The three methods were compared by calculating prediction accuracy via cross-validation (5 folds, 50 cycles) in the training populations using the 'rrblup' package in R Studio. A total of 49,441 SNPs went into the analysis after running the GBS pipeline, SNP filtering and missing marker imputation. The lowest prediction accuracies for FHB Severity, FDK and DON were obtained with Method 1, and Method 2 did not provide better prediction than Method 1, perhaps due to the subjective nature of the severity score. In spite of significant missing data for FDK (33%) and DON (58%), the highest prediction accuracies were obtained using Method 3 because FDK and DON likely suffered less judgmental error (FHB Severity (0.68), FDK (0.64) and DON (0.54)). Lower predictions for DON were influenced by the relatively large amount of missing data for this trait. These results suggested that choosing training population environments based on rank and mean of check cultivars should be implemented when disease level is not uniform over all environments. Likewise, more resources should be directed towards obtaining more FDK and DON evaluations. A rise in prediction accuracy is expected after adding significant markers as a covariate in this model.

## ACKNOWLEDGEMENT AND DISCLAIMER

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## SCREENING FOR RESISTANCE TO FUSARIUM HEAD BLIGHT IN SPRING WHEAT AND BARLEY IN IDAHO

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### ABSTRACT

Since 2015, the University of Idaho Cereals Extension program in Aberdeen, ID has been screening for Fusarium head blight (FHB) and deoxynivalenol in spring wheat and barley. It is crucial to continue monitoring FHB and DON levels of both widely grown and new generation varieties. Early May of 2017, 61 spring wheat (hard red, hard white, soft white and durum) and 64 spring barley (six-rowed, two-rowed malt and two-rowed feed) varieties were planted in a randomized complete block design with 2 replications. Approximately 30 g/m<sup>2</sup> of corn inoculum were spread 2-3 weeks before barley head emergence and wheat anthesis. Wheat plots were marked with colored flags according to flowering date and were evaluated for FHB severity 21 days thereafter. Wheat plots were also rated for *Fusarium*-damaged kernels (FDK). Barley plots were marked with colored flagged at head emergence and sprayed with conidial suspension (100,000 macroconidia/L) using a CO<sub>2</sub> sprayer with 8003 VS nozzles at a ground speed of 1 ft/s at 40 psi. Barley plots were supplemented with a second conidial spray and evaluated for FHB severity at 7 and 21 days after the first spray inoculation, respectively. Data were analyzed using the GLIMMIX procedure (SAS 9.4). For wheat, FHB index and FDK were rated up to 64.4% (XA9502) and 13.2% (WB936), respectively. Yield and test weight ranged from 35.4 (Imperial) to 117.2 (Tekoa) bu/A and from 47.0 (WB936) to 62.0 (Rollag and WA8277) lb/bu, respectively. For barley, FHB index values were 21% and below with the exception of the two Yuma highland varieties. Yield and test weight were up to 134.6 (Champion) bu/A and 53.2 (Clearwater) lb/bu, respectively. Wheat and barley subsamples were sent to University of Minnesota for DON analysis. Although FHB has had a lower incidence on barley compared to wheat, threshold levels in malt barley is much lower. Screening for FHB resistance in wheat and barley is necessary to reduce economic impacts and provide appropriate management recommendations to local growers in the area.

### ACKNOWLEDGEMENT AND DISCLAIMER

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MULTIPLEX RESTRICTION AMPLICON SEQUENCING  
(MRASEQ), A NEW NEXT GENERATION  
SEQUENCING-BASED MARKER PLATFORM  
FOR WHEAT AND BARLEY BREEDING

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## ABSTRACT

Marker-assisted breeding enables the indirect selection of traits that are difficult and/or costly to phenotype thereby saving time and money, and increasing selection efficiency. To be useful in breeding programs, markers for genome-wide genotyping must be low cost, randomly distributed throughout the genome, high-throughput, and technically simple. We developed a PCR and NGS-based, low cost, high-throughput genotyping technology for genome-wide marker assays. This technology, designated as Multiplex Restriction Amplicon Sequencing (MRASEq), reduces genome complexity by PCR-amplification of selected portions of genomic regions flanked by restriction sites and is achieved using tailed and semi-degenerate PCR primers with restriction enzyme sequence at the 3'-end. MRASEq is flexible because the restriction enzyme sequence and the adjacent degenerate base sequence in the primers can be altered to suit the species of interest. MRASEq uses restriction sites as primer sites and does not make use of restriction enzymes. The incorporation of unique barcodes during a second PCR allows hundreds of samples to be multiplexed in one sequencing run. Linkage mapping of polymorphic MRASEq SNP markers in an allohexaploid wheat biparental population showed random distribution of SNPs across genomes. MRASEq on wheat and barley natural populations generated thousands of SNPs suitable for genomic selection. Therefore, this marker platform can be used for linkage mapping, background selection, or any other purpose in which large numbers of markers are needed. This simple, flexible and high-throughput genotyping method should be useful for wheat and barley FHB QTL analysis and breeding to improve resistance to FHB.

## ACKNOWLEDGEMENT AND DISCLAIMER

This material is based upon work supported by the U.S. Department of Agriculture. This is a cooperative project with the U.S. Wheat & Barley Scab Initiative. Any opinions, findings, conclusion, 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.

UPDATE ON FUSARIUM HEAD BLIGHT RESEARCH  
AND BREEDING IN SPRING WHEAT AT UNIVERSITY OF  
IDAHO WHEAT BREEDING AND GENETICS PROGRAM

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**ABSTRACT**

Fusarium Head Blight (FHB) has become an important disease for wheat and barley in Idaho and the Pacific Northwest (PNW) due to factors such as climate change and crop rotation. Research and breeding work have been initiated in spring wheat in 2008 under support from University of Idaho Hatch Fund and Idaho Wheat Commission, thereafter by the US Wheat and Barley Scab Initiative and Wheat CAP. We have developed a FHB tolerant cultivar 'UI Stone', a high yielding soft white spring wheat being widely grown in Idaho and PNW. We also conducted various research projects, including bi-parental and genome-wide association mapping and molecular marker assisted selection of FHB resistance. The improved FHB resistant lines and germplasm are in pipe-lines to the market place. Novel selection method, genomic selection is being developed and implemented in selection of improved FHB resistance under collaboration with Washington State University.

**ACKNOWLEDGEMENT AND DISCLAIMER**

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FHB IMPACTS ON SOUTHEASTERN MILLERS,  
FARMERS, SEEDSMEN AND BREEDERS  
Jimmy Clements

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**ABSTRACT**

**Millers** - Must meet demands/requirements of their customers (bakers/flour makers. Must meet the minimum government requirements, local grown by truck vs rail cars.

**Farmers** - Must meet demands/requirements of their customers (miller/feedlots). Must manage their crop for control of FHB follow Land Grant and local buying points advice.

**Seedsmen** - must meet demands/requirements of their customers (farmers). Deliver high yield and high disease resistant planting seed, educate their dealers on how to support their growers, reach out to Land Grants and ensure they are on top of FHB in their area.

**Breeders** - Must meet demands/requirements of their customers (Millers, Farmers, and Seedsmen). Must have yield, and disease package for the area covered.

**Overview** - All the above must meet demands/requirements of their customers.

# DEVELOPMENT OF DIVERSE FUSARIUM HEAD BLIGHT VARIETIES IN SOUTH AFRICA

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## ABSTRACT

Fusarium Head Blight caused predominantly by *Fusarium graminearum*, is a major disease under irrigation on wheat and barley in South Africa. Environmental conditions, corn/wheat crop rotation, tillage practices and the planting of highly susceptible cultivars, cause yield losses of up to 40% under severe disease pressure. Infected grain may contain mycotoxins that are harmful to humans and animals when consumed. No fungicides are registered on wheat in South Africa to control this disease, nor are any resistant cultivars available. For these reasons, it is important to utilize genetic resistance that will provide sustainable and cost effective control for this disease. This paper discusses the evaluation of wheat germplasm to test for resistance against the South African FHB complex to utilize in a back cross programme. Twelve resistant donors were imported and evaluated in the field for *Fusarium* resistance together with Sumai 3, Frontana and Gamenya that were used as resistant and susceptible controls respectively. Entries were planted in a honeycomb design, spray inoculated during flowering with a cocktail of five different *Fusarium* isolates and were evaluated for Type II resistance by using the CIMMYT scale. These entries were evaluated over a three-year period to determine the resistance of each donor. Five of these donors were included in a backcross pre-breeding programme with three local cultivars. Over twenty SSR markers linked to well-characterized FHB resistance genes/QTL from specific donors have been used across the different crossing combinations since 2013. Currently, the developed material is at BC<sub>3</sub>F<sub>1</sub> generation. Selections were made based on the presence of at least one major gene/QTL (*Fhb1*, *Fhb2*, *Fhb4*, *Fhb5*, *3A*, *4A*, *4D* and *7D QTL*) for FHB resistance confirmed by the presence of both flanking markers. Based on initial marker data, most of the families contain different FHB resistance genes/QTL in combination with targeted rust resistance genes. These promising lines will be top-crossed with one another to stack several different FHB resistance genes/QTL and rust resistance genes into single lines, to certainly establish diverse FHB resistant varieties in South Africa.

## ACKNOWLEDGEMENT AND DISCLAIMER

This material is based upon work supported by the Winter Cereal Trust (WCT) and the Agricultural Research Council (ARC). Any opinions, findings, conclusions or recommendations expressed in this publication are those of the authors and do not necessarily reflect the view of the WCT or ARC. A full copy of this disclaimer can be accessed on <http://www.arc.agric.za/Pages/Terms-and-Conditions.aspx>



ASSESSMENT OF FUSARIUM HEAD BLIGHT  
RESISTANCE IN REGISTERED SPRING WHEAT  
VARIETIES UNDER NATURAL CONDITIONS  
IN MANITOBA, CANADA

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**ABSTRACT**

Fusarium head blight (FHB) has been a major issue facing spring wheat growers in Manitoba since the first major outbreak in the province in 1993. Varietal resistance and foliar fungicides are the two main methods for managing this disease. FHB resistance ratings are assigned to varieties based on three years of testing in the Variety Registration Trials located across the Canadian prairies. The list of registered varieties available to Manitoba growers continues to increase each year with limited disease data available comparing older varieties to newly registered varieties. In 2009, this study was initiated to compare FHB levels in Manitoba Crop Variety Evaluation Team (MCVET) trials in various locations across the province under natural conditions. Harvested samples are collected and analyzed for *Fusarium* damaged kernels (FDK) and levels of deoxynivalenol (DON). Using a mixed model analysis, mean levels of FDK and DON are determined and used to compare varieties adjusting for factors such as year, location, and interactions. The levels of FHB infection vary from year to year, but most of the varieties perform as expected based on their FHB resistance ratings given at registration. However, through this study a limited number of varieties show FDK and DON levels inconsistent with their FHB resistance ratings. The purpose of this post-registration assessment is to assist producers and agronomists in their selection of spring varieties to help manage this disease.

## EVALUATION OF WINTER BARLEY CULTIVAR NOMINI FOR QUANTITATIVE RESISTANCE TO FUSARIUM HEAD BLIGHT

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### ABSTRACT

Fusarium head blight (FHB), caused by the pathogen *Fusarium graminearum* Schwabe, can result in severe yield and quality losses for barley (*Hordeum vulgare*) producers in the Mid-Atlantic region via kernel damage and production of mycotoxins. The demand for cultivars with enhanced resistance to FHB and lower Deoxynivalenol (DON) accumulation is essential to barley producers to meet the current and future market demands for winter barley in the production of health foods, livestock feed, and malt products. The objectives of this study are to identify, characterize and map the FHB resistance QTL in the hulled winter barley cultivar Nomini and to develop diagnostic markers for use in marker-assisted selection to help pyramid and enhance overall scab resistance in barley. First year FHB phenotypic field data was collected in a Thoroughbred/Nomini population in scab nurseries in KY and VA during 2017, where mean FHB index values for the parents Nomini and Thoroughbred were 5.5 and 11.3, respectively (Table 1). Nomini mapping populations (Thoroughbred/Nomini and Violetta/Nomini) will be evaluated in scab nurseries during 2017-18. A total of 300 mapping population lines (180 RILs from Thoroughbred/Nomini and 120 DH lines from Violetta/Nomini) will be sent for 9K SNP genotyping to Fargo, ND during FY 2017-18 and FY 2018-19, respectively. We will identify, validate, and develop diagnostic markers for major scab resistance QTL derived from Nomini and Violetta, which has also expressed resistance to FHB, and compare these with the QTL previously identified in Eve and other reported sources.

### ACKNOWLEDGEMENT AND DISCLAIMER

This material is based upon work supported by the U.S. Department of Agriculture, under Agreement No. 59-0206-4-032. This is a cooperative project with the U.S. Wheat & Barley Scab Initiative. Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the authors and do not necessarily reflect the view of the U.S. Department of Agriculture.

**Table 1.** Mean FHB Incidence, Severity and Index values for Thoroughbred/Nomini population in Mt. Holly, VA and Lexington, KY in 2017.

Parent Variety	Incidence (%)			Severity (%)			FHB Index (0-100)		
	VA	KY	Over Locations	VA	KY	Over Locations	VA	KY	Over Locations
Thoroughbred	77.5	95.0	<b>86.3</b>	16.7	10.0	<b>13.4</b>	12.9	9.7	<b>11.3</b>
Nomini	66.3	60.0	<b>63.1</b>	8.3	8.0	<b>8.2</b>	5.6	5.3	<b>5.5</b>
Population (N=180)	95.8	78.1	<b>82.0</b>	21.0	12.4	<b>16.7</b>	18.5	10.3	<b>14.4</b>
Mean	76.5	77.7	<b>77.1</b>	15.3	10.1	<b>12.7</b>	12.3	8.4	<b>10.4</b>

# MAPPING QUANTITATIVE TRAIT LOCI FOR FUSARIUM HEAD BLIGHT RESISTANCE IN WINTER WHEAT

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## ABSTRACT

Fusarium head blight (FHB) is one of the devastating wheat diseases in the world. It not only causes great yield losses, but also lowers grain quality due to mycotoxins produced by the pathogen *Fusarium graminearum*. Wheat FHB resistance is a quantitative trait and controlled by multiple quantitative trait loci (QTLs). To identify consistent QTLs for FHB resistance in a US winter wheat CI13227 and markers tightly linked to these QTLs for marker-assisted breeding, we developed a population of 177 double haploid (DH) lines from the cross between Lakin and CI13227. We used Illumina wheat 90K single nucleotide polymorphism (SNP) chips to genotype the population for construction of a SNP map and evaluated the DH population for FHB resistance in three greenhouse experiments from 2016-2017 by using point inoculation and one field experiment (2017) using grain pawn inoculation. QTL mapping found four QTLs on chromosomes 4B, 5AL, 7A, and 2D, which explained 8-17% of the phenotypic variation for FHB resistance in different experiments. The 4B QTL showed the largest effect and were consistently detected in three experiments. QTL on 5A explained 11-12% of the phenotypic variation and detected in two greenhouse experiments. Other two QTLs were detected in only one experiment. The QTLs on the chromosome 4B and 2D showed a high correlation with plant height, suggesting a polytropic effect of these QTLs. These QTLs can be pyramided with *Fhb1* or QTLs from other sources to improve wheat FHB resistance.

# COLLABORATIVE DOUBLED HAPLOID BREEDING FOR FUSARIUM HEAD BLIGHT RESISTANCE IN BARLEY

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## **ABSTRACT**

Breeding barley is a long-term process that requires multiple cycles of self-pollination to achieve complete homozygosity. Doubled haploid (DH) production leads to complete homozygosity in a single generation, thus bypassing the complications of field, greenhouse, or off-season generation advance. Completely homozygous material facilitates the phenotyping of complex traits and simplifies integration of phenotype with genotype for gene discovery and characterization. A collaborative network - in which multiple investigators provide germplasm of interest and a central facility produces doubled haploids - can generate synergies and efficiencies. The USWBSI is supporting collaborative DH production at OSU, starting fall, 2017. F1s were solicited from barley researchers and received from Cornell (Sorrells) and Virginia Tech (Brooks & Griffey). In order to show the projected flow of the USWBSI project, the path of an ongoing project (supported by the American Malting Barley Association (AMBA)) is presented. The AMBA project is designed to generate facultative/winter 2-row malting barley germplasm with superior low temperature tolerance.

## **ACKNOWLEDGEMENT AND DISCLAIMER**

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# FHB RESISTANCE AND AGRONOMIC PERFORMANCE IN GEORGIA SOFT RED WINTER WHEAT GERMPLASM

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## ABSTRACT

The most efficient approach to control Fusarium Head Blight (FHB) is the development of resistant wheat cultivars. Local broadly adapted cultivars of Soft Red Winter Wheat (SRWW) have been crossed with *Fhb1* derived lines, Truman, Neuse, and Jamestown to introduce FHB resistant QTL into GA adapted SRWW genetic backgrounds. Two wheat lines, GA 051207-14E53 and GA JT141-45 with moderate levels of FHB resistance, were released in 2017. GA 051207-14E53 has the QTL 1A and 6A from Neuse. GA 051207-14E53 is from the cross of AGS 2000/SC996284/IN981359C1. IN981359C1 was used due to its scab resistance. GA 051207-E53 is a high grain yielding, medium maturing, awnless, good test weight, medium height line and has the *H9* gene for Hessian fly resistance. GA JT141-14E45 is from the cross of AGS 2026 / Jamestown. AGS 2026 was crossed due to its Hessian fly resistance (*H13*). Jamestown was used due to its scab resistance (1B and 6A). GA JT141-14E45 is a high grain yielding, medium maturing, awnless, good test weight, medium height line. It has the QTL 1B from Jamestown and has the *H13* gene for Hessian fly resistance. In 2017, other elite lines with FHB resistance derived from Truman/Bess, Neuse, MD08-27-E9 and Jamestown, were evaluated under Georgia's field conditions for FHB resistance and agronomic performances. Among these, an elite line, GA 09129-16EL56, was identified with good FHB resistance. GA0912916EL56 derived from the 991109-6E8 \*2/ IL00-8530 cross, has the scab QTL 1A and 4A from Neuse and 1B from Jamestown. It has similar FHB ratings as Jamestown for incidence, index and ISK. Similarly, GA 141077-G5 derived from the cross AGS 2033 \*2 / MD 08-26-H2 was identified as a high yielding line with the QTL *Fhb1* and 5AS. Several double haploid lines with moderate levels of FHB resistance derived from Jamestown, Truman, Neuse or with *Fhb1* markers were also identified with high grain yield potential and will be further evaluated. Marker-assisted selection, double haploid development and field phenotyping will enhance further breeding for FHB resistance in our program.

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THE 2017 UNIFORM SOUTHERN SOFT RED  
WINTER WHEAT SCAB NURSERY

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**ABSTRACT**

The Uniform Southern Soft Red Winter Wheat Scab Nursery provides breeders in the public and private sectors the opportunity for multi-environment, independent evaluations of FHB resistance in advanced generation breeding lines compared with the current most resistant check varieties ‘Ernie’, ‘Bess’ and ‘Jamestown’. Valuable data are provided on resistance to other important fungal and viral diseases, milling and baking quality and agronomic characteristics. In addition, genotypic analyses identify alleles present at numerous important loci. In addition, we provide Genomic Estimated Breeding Values (GEBV) for nursery entries. These were estimated from a training population of nursery entries from 2011 to 2016. A combined mixed model analysis of the phenotypic data from 2011 to 2016 was performed using SAS 9.3 and BLUEs for each genotype were recorded. The number of SNP markers utilized was 35,706. The Genotypic Selection model utilized Ridge Regression BLUP through the R-package rr-BLUP to predict GEBVs for individuals in the 2017 nursery. GS model accuracy was evaluated by Pearson correlations between GEBVs and best linear unbiased estimate (BLUE) for the 2017 lines. Correlation coefficients varied between 0.63 for FHB Incidence to 0.29 for Heading Date. The 2016-17 nursery comprised 42 advanced generation breeding lines and four check cultivars. Five U.S. public programs (Arkansas, Georgia, Louisiana, North Carolina, and Virginia), and two private companies (KWS and Limagrain) submitted entries. Data were returned from up to eight locations in the US. In addition, three USDA-ARS laboratories conducted evaluations for Hessian fly resistance, milling and baking quality and marker genotypes.

Copies of the full report will be available at the 2017 National Fusarium Head Blight Forum and subsequently on line at the USWBSI web site: <https://www.scabusa.org/>.

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**Table 1.** Phenotypic means across locations, correlations between GEBV and phenotypic means and genotypic content of regions associated with FHB resistance.

Cultivar/ Designation	FHB Incidence		FHB Severity		FHB Index		FDK		ISK		DON	
	RANK	RANK	RANK	RANK	RANK	RANK	RANK	RANK	RANK	RANK	RANK	RANK
1 ERNIE	42	16	23	12	14	21	28	13	28	7	9	9
2 COKER9835	74	46	54	46	45	46	71	46	61	46	13	25
3 BESS	36	5	17	3	7	1	12	1	23	1	8	5
4 JAMESTOWN	42	16	16	2	10	6	21	3	27	4	7	1
5 NC13-20076	39	9	21	8	11	11	22	5	29	10	7	1
6 VA13W-38	43	19	23	12	11	11	41	37	31	16	10	15
7 ARLA07133C-19-4	34	2	20	7	10	6	24	7	27	4	14	28
8 ARLA07133C-3-4	46	29	26	22	19	31	35	26	36	29	11	17
9 ARLA06146E-1-4	44	24	19	5	10	6	27	11	31	16	9	9
10 AR08109-17-2	43	19	26	22	10	6	23	6	29	10	12	22
11 AR08015-17-4	43	19	33	37	21	37	37	29	40	36	16	36
12 AR08057-5-1	43	19	34	42	21	37	43	39	40	36	11	17
13 LES15-5369	38	8	21	8	9	4	31	19	29	10	7	1
14 LES15-5499	37	7	26	24	11	11	29	16	28	7	10	15
15 LES15-5605	41	13	24	17	12	16	29	16	30	14	15	31
16 GA09343-16ES3	52	40	33	37	21	37	57	45	47	45	18	40
17 GA09410-16ES22	55	43	34	42	20	32	40	34	40	36	19	42
18 GA09129-16EL56	41	13	24	17	13	18	32	23	31	16	12	22
19 GA121176-16JS49	50	37	27	27	21	37	45	42	43	43	13	25
20 GA09163-16ES19	40	12	23	12	12	16	28	13	30	14	21	44
21 GA09144-16ES23	47	32	27	27	15	24	31	19	34	23	15	31
22 GA05450-16ES8	52	40	30	32	20	32	45	42	42	42	20	43
23 GA09054-16ES25	48	34	33	37	22	42	38	30	41	40	14	28
24 KWS095	41	13	25	20	13	18	33	24	34	23	9	9
25 KWS103	35	3	23	12	10	6	24	7	27	4	8	5
26 KWS114	43	19	37	45	16	25	35	26	37	30	15	31
27 KWS122	33	1	23	12	9	4	18	2	25	2	9	9
28 KWS133	45	25	27	27	14	21	34	25	34	23	15	31
29 KWS141	36	5	24	17	11	11	21	3	28	7	12	22
30 LA08265C-50	47	32	30	32	20	32	31	19	38	33	9	9
31 LA09101UB-48-3-5	45	25	21	8	13	18	31	19	31	16	8	5
32 LW08049C-74-2-5	42	16	29	30	17	29	25	9	33	21	11	17
33 LA10081C-18	48	34	25	20	16	25	40	34	37	30	17	38
34 LA11309GS-16	46	29	29	30	18	30	38	30	35	28	8	5
35 NC13-23443	55	43	33	37	23	43	36	28	34	23	7	1
36 NC13-21213	45	25	32	36	20	32	48	44	41	40	9	9
37 NC13-20332	55	43	26	24	16	25	38	30	37	30	14	28
38 NC14-23372	50	37	19	5	11	11	28	13	32	20	11	17
39 NC14-23373	39	9	15	1	7	1	26	10	25	2	17	38
40 VA13W-174	46	29	21	8	14	21	44	41	34	23	11	17
41 VA09MAS2-131-6-2	49	36	31	34	21	37	41	37	38	33	15	31
42 VA14W-32	51	39	36	44	27	45	43	39	46	44	22	45
43 VA07MAS1-7047-1-1-4-2	35	3	17	3	8	3	38	30	29	10	18	40
44 DH12SRW056-058	39	9	26	24	16	25	27	11	33	21	16	36
45 DH11SRW061-16	45	25	33	37	23	43	30	18	38	35	13	25
46 VA09MAS2-131-6-2-4	52	40	31	34	20	32	40	34	40	36	23	46
Mean	45		27		16		34		34		13	
LSD (0.05)	26		24		24		28		19		10	
CV%	29.2		46.4		77.9		42.2		28.6		38.6	
Mean v GEBV Correlation	0.63		0.53		0.54		0.61		0.57		0.39	

Table 1. Continued

Cultivar/ Designation	Heading Date		Plant Height		Flour Yield %		Softness Equiv. %		Hessian Fly		Fhb1	Fhb Massey 3BL	Fhb 5A_Ning	Bess 2B	Bess 3B	Jamestown 1B	Jamestown 6A	NC-Neuse 1A	NC-Neuse 6A
	RANK	RANK	RANK	RANK	RANK	RANK	Bio. L	H13											
1 ERNIE	109	11	33	9	65.6	38	54.1	19	0-16	no	no	YES	YES	no	no	no	no	YES	YES
2 COKER9835	110	17	32	4	66.8	18	61.0	1	0-20	no	no	no	no	no	no	no	no	no	no
3 BESS	113	34	37	41	66.9	17	55.4	13	0-22	no	no	no	no	YES	YES	YES	no	YES	no
4 JAMESTOWN	106	2	32	4	64.3	44	54.1	19	0-16	no	no	no	no	no	no	YES	YES	YES	no
5 NC13-20076	109	11	37	41	65.6	38	55.4	13	0-17	no	no	no	no	no	no	no	no	het	ND
6 VA13W-38	109	11	34	16	66.3	26	50.5	35	0-17	no	no	no	no	no	no	YES	YES	no	no
7 ARLA07133C-19-4	111	24	35	30	67.5	13	50.3	38	0-18	no	no	no	no	no	no	YES	no	no	no
8 ARLA07133C-3-4	109	11	36	35	66.3	26	57.1	6	0-19	no	no	no	no	no	no	no	no	het	no
9 ARLA06146E-1-4	110	17	36	35	65.4	41	53.6	23	0-15	no	no	no	no	no	no	YES	YES	YES	no
10 AR08109-17-2	112	30	34	16	67.6	12	45.9	45	0-16	no	no	no	no	no	no	no	no	no	het
11 AR08015-17-4	112	30	38	46	68.7	4	37.9	46	0-15	no	no	no	no	no	no	no	no	no	no
12 AR08057-5-1	113	34	37	41	68.8	3	55.7	10	0-15	no	no	no	no	no	no	no	Het	no	no
13 LES15-5369	113	34	34	16	66.4	24	57.6	5	0-17	no	no	YES	no	no	no	no	no	YES	no
14 LES15-5499	115	45	34	16	65.8	33	53.6	23	0-23	no	no	no	no	no	no	YES	no	YES	no
15 LES15-5605	114	42	33	9	69.0	2	59.2	3	0-17	no	no	no	no	no	no	YES	no	YES	no
16 GA09343-16ES3	111	24	34	16	68.3	6	50.1	40	0-18	no	no	no	no	no	no	no	no	no	no
17 GA09410-16ES22	110	17	31	2	65.8	33	51.1	32	0-16	Het	no	no	no	no	no	no	no	no	no
18 GA09129-16EL56	108	8	36	35	66.5	21	49.1	42	0-17	no	no	no	no	no	no	YES	Het	YES	no
19 GA121176-16JS49	105	1	33	9	67.1	15	55.1	15	0-16	no	no	no	no	no	no	YES	YES	no	no
20 GA09163-16ES19	110	17	34	16	66.4	24	47.9	44	0-11	no	no	no	no	no	no	no	YES	no	no
21 GA09144-16ES23	108	8	34	16	63.4	46	55.6	12	0-17	no	no	no	**	no	no	no	Het	het	no
22 GA05450-16ES8	111	24	34	16	65.2	42	52.1	30	0-21	no	no	no	no	no	no	no	no	no	no
23 GA09054-16ES25	106	2	33	9	63.7	45	52.2	28	21-0	Yes	no	no	no	no	no	no	Het	het	no
24 KWS095	113	34	36	35	69.6	1	58.7	4	0-23	no	Fhb1	no	no	no	no	no	no	no	no
25 KWS103	114	42	36	35	68.6	5	55.8	9	0-19	no	no	Het	no	no	no	no	no	YES	YES
26 KWS114	115	45	37	41	68.1	8	49.0	43	0-15	no	no	no	no	no	no	no	no	YES	YES
27 KWS122	114	42	37	41	66.5	21	50.4	36	0-17	no	no	no	no	no	Het	no	no	YES	YES
28 KWS133	113	34	35	30	64.5	43	55.1	15	0-17	no	no	no	no	no	no	Het	no	YES	YES
29 KWS141	112	30	34	16	66.6	20	55.7	10	0-19	no	no	YES	no	no	no	YES	no	YES	no
30 LA08265C-50	111	24	35	30	65.7	37	52.2	28	0-16	no	no	no	no	no	no	YES	no	YES	no
31 LA09101UB-48-3-5	110	17	34	16	66.0	31	60.3	2	0-13	no	ND	no	no	no	no	YES	no	YES	no
32 LW08049C-74-2-5	110	17	35	30	68.3	6	56.3	7	0-18	no	no	no	no	no	no	no	no	no	no
33 LA10081C-18	108	8	34	16	65.8	33	52.6	26	0-14	no	no	no	no	no	no	no	no	YES	no
34 LA11309GS-16	107	5	33	9	67.1	15	54.0	21	0-18	no	no	no	no	no	no	no	no	YES	YES
35 NC13-23443	113	34	36	35	67.8	11	56.1	8	0-14	no	no	no	no	no	no	YES	no	no	no
36 NC13-21213	111	24	34	16	65.6	38	54.4	18	18-0	Yes	no	no	no	no	no	YES?	no	YES	Het
37 NC13-20332	112	30	32	4	66.3	26	52.4	27	0-13	no	no	no	no	no	no	YES	Het	YES	no
38 NC14-23372	113	34	34	16	67.9	9	51.0	33	0-18	no	Fhb1	no	YES	no	no	YES	no	YES	YES
39 NC14-23373	113	34	34	16	67.2	14	51.8	31	0-17	no	Fhb1	no	no	no	no	YES	no	YES	YES
40 VA13W-174	107	5	33	9	66.0	31	53.8	22	0-23	no	no	no	no	no	no	no	no	YES	Het
41 VA09MAS2-131-6-2	109	11	30	1	65.8	33	50.9	34	0-15	no	no	no	no	no	no	no	no	YES	no
42 VA14W-32	106	2	31	2	66.3	26	53.4	25	0-14	no	no	no	no	no	no	no	no	no	no
43 VA07MAS1-7047-1-1-4-2	107	5	32	4	66.8	18	50.3	38	0-16	no	no	Het	no	no	no	YES	YES	no	no
44 DH12SRW056-058	111	24	35	30	67.9	9	54.5	17	0-19	no	no	YES	no	no	no	YES	no	no	no
45 DH11SRW061-16	109	11	33	9	66.2	30	49.3	41	0-17	no	no	no	no	no	no	no	no	no	no
46 VA09MAS2-131-6-2-4	110	17	32	4	66.5	21	50.4	36	0-18	no	no	no	no	no	no	no	no	YES	no
Mean	110		34		66.6		53.1												
LSD (0.05)	7		4		.		.												
CV%	3.3		5.2		.		.												
Mean v GEBV Correlatio	0.29		0.35		.		.												

# PREDICTING GENETIC VARIANCE AND CORRELATION IN BARLEY USING GENOME-WIDE MARKERS

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## ABSTRACT

When breeding for a quantitative trait, such as *Fusarium* head blight (FHB) severity, the mean of the selected individuals ( $\mu_{sp}$ ) is expected to be greater in a population with greater genetic variance ( $V_G$ ), assuming constant mean ( $\mu$ ). Furthermore, selection on one quantitative trait can impact breeding progress of another trait if they are genetically correlated. If such correlations are due to linked causal loci, certain parent combinations may be more favorable for shifting the genetic correlation ( $r_G$ ). Taken together, the ability to predict  $V_G$  for a single trait and  $r_G$  between traits within a bi-parental population – prior to developing that population – would be very useful. One method to make such predictions uses genomewide marker effects and simulated recombinant inbred families. To test the effectiveness of this method in a new two-row barley (*Hordeum vulgare* L.) breeding program, we predicted among 330,000 simulated families the values of  $\mu$ ,  $V_G$ ,  $\mu_{sp}$ , and  $r_G$  for important agronomic and disease resistance traits. We selected and created 27 families based on these predictions, and we evaluated families for FHB severity, heading date, and plant height across two locations in the first year of a multi-year project. Among families, the fold range in estimated  $V_G$  was 5 for FHB severity, 26 for heading date, and 95 for plant height. The prediction accuracies of  $V_G$  and  $\mu_{sp}$  were highest for heading date and lowest for FHB severity, mirroring the trait heritabilities. Though we observed a large range in estimated  $r_G$ , prediction accuracy of  $r_G$  was poor. Additional data is need to validated predictions in an applied breeding program.

## ACKNOWLEDGEMENT AND DISCLAIMER

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DEVELOPMENT OF FHB SOLUTIONS:  
A SEED INDUSTRY PERSPECTIVE  
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**ABSTRACT**

The development of Fusarium head blight (FHB) solutions can be accomplished using the tools available at a large agricultural company. The approaches considered for a multinational agricultural company will differ from those of a university wheat breeding effort focused on a smaller region. These decisions include the value of FHB tolerance relative to other regionally important wheat traits such as quality, agronomic traits, and ultimately, yield. Considerations also include the cost/value to develop FHB solutions in wheat vs. the value of the development of agronomic solutions in more profitable crops. The benefits of working on wheat in a large company includes the availability of technologies developed for crops such as corn and soy. These tools include marker platforms, genomic resources and biotechnology.

## A REGIONAL APPROACH TO GENOMIC SELECTION FOR SCAB RESISTANCE

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### ABSTRACT

Prediction of Genomic Estimated Breeding Values (GEBVs) for genotypes with no available phenotypic data is central to Genomic Selection (GS) approaches. Public winter wheat breeding programs in the United States have a long tradition of germplasm exchange and collaborative testing of advanced experimental lines prior to cultivar release. Historical unbalanced cooperatives nurseries phenotyped in multiple environments and connected by performance checks allow for use of these data sets as a training population (TP) to integrate genomic predictions in the pipeline of ongoing breeding programs without extra phenotyping efforts. We utilized historical genotypic and phenotypic information from SUWWSN, NUWWSN, and PNWWSN to construct GS models for different traits related with Fusarium Head Blight (FHB, *Fusarium graminearum*) resistance that yield predictive abilities above 0.5 in cross validation studies. GS model predictive ability can also be improved by fine tuning the TP design and by incorporating markers linked to major QTLs associated with FHB as fixed covariates.

### ACKNOWLEDGEMENT AND DISCLAIMER

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# COMBINING CHROMOSOME 5A FHB RESISTANCE QTL WITH *FHB1* IN HARD RED WINTER WHEAT LINES

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## ABSTRACT

The NDSU HRWW breeding germplasm is of recent origin and during its assembly very little evidence of 'native' FHB resistance occurred. Known FHB quantitative trait resistance loci (QTL) are therefore being transferred from spring wheat, including *Fhb1* and *Qfhs.ifa-5A* (both from CM82036), and *QTL5A-1* and *QTL5A-2* (both from PI277012). Individually, these QTL confer only partial resistance to FHB, and ideally, they must be deployed in more effective, complementing combinations. This study attempts to develop winter wheat breeding lines with pyramids of *Fhb1* and one or more of the chromosome 5A QTL and to evaluate their performance in the field. Initially, resistance QTL were introgressed from the spring wheat accessions CM82036 and PI277012. Following two seasons of field selection under artificial and natural infection, resistant winter lines were derived. These were characterized with molecular markers and finally the resistance in each was corroborated by also evaluating for FHB type II resistance in a greenhouse trial. A cross was then made employing the most resistant line from each of the two transfer attempts (14K456-K-1 and Novus-4). 400 F<sub>2</sub> plants were screened with *Fhb1* and *Qfhs.ifa-5A* markers. This identified 17 families homozygous for *Fhb1* only, and 19 families that were homozygous for *Fhb1* and *Qfhs.ifa-5A*. Four F<sub>3</sub> plants were sampled per family (total = 144) for 9K SNP analyses and F<sub>4</sub> sub-families of each were included in a greenhouse FHB trial with four replicates. The results showed very similar distributions of resistance in the two groups (*Fhb1* homozygotes versus *Fhb1* plus *Qfhs.ifa-5A* homozygotes); however, not surpassing the 14K456-K-1 resistance. This would suggest the presence of an additional PI277012-derived QTL in the *Fhb1* only group, which could occur in the same chromosome region as *Qfhs.ifa-5A*. The SNP data are currently being analyzed in an attempt to confirm this. F<sub>5</sub> families were planted in field rows in the fall of 2017 for evaluation of their winter survival, agronomic performance and FHB resistance.

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# GWAS OF SCAB TRAITS IN THE ELITE EASTERN WHEAT MAPPING PANEL IN AN ARTIFICIALLY WARMED ENVIRONMENT

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## ABSTRACT

Fusarium head blight (FHB) epidemics are strongly driven by meteorological factors. Due to the ongoing changes in climate there is a risk for an increased disease pressure, mainly due to temperature and rainfall variation. For this reason, we evaluated the effect of climate variation on crop resistance to FHB. Our goals for this study were: 1) to evaluate scab traits in the TCAP elite eastern mapping panel under warmed and control conditions and 2) to conduct a genome wide association study (GWAS) to identify potential QTL associated with resistance to FHB. We evaluated two hundred and forty wheat cultivars and breeding lines from the mapping panel over two years (2016 – 2017) at Lexington, KY. The experiment was conducted as a nested factorial design. Cables were buried to increase soil temperature by  $\pm 3^{\circ}\text{C}$  in the warmed treatment. The experimental unit was a hill plot. Two replications per genotype per treatment were planted in 2016, and four replications in 2017. The traits measured were: heading date (Julian), plant height (cm), FHB rating (0 – 9), and FDK (0 – 90%; only measured in 2017). There were significant ( $p \leq 0.05$ ) differences among the mapping panel entries for all traits evaluated. Significant treatment x genotype interaction for FHB rating and *Fusarium*-damaged Kernels (FDK) were observed. Broad sense heritabilities ranged from 0.30 to 0.90. Correlations between traits varied between 2016 and 2017. FHB rating was negatively correlated with height in warmed and controlled environments in 2016. Moreover, in the warmed treatment, FHB rating and heading date were highly positively correlated in 2017. FDK was positively correlated with rating in both treatments. The association study was conducted each year. The analysis indicated possible QTL for FHB rating located on chromosomes 3A and 2B in the warmed treatment for 2016 and 2017, respectively. Potential QTL associated with FDK were located on chromosome 7B in the warmed environment. Implications for breeding programs will be discussed.

## ACKNOWLEDGEMENT AND DISCLAIMER

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IDENTIFICATION AND MOLECULAR MAPPING OF  
QUANTITATIVE TRAIT LOCI FOR RESISTANCE  
TO FUSARIUM HEAD BLIGHT IN CULTIVATED  
EMMER PI 272527

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## ABSTRACT

Improvement of modern durum wheat (*Triticum turgidum* subsp. *durum*) for resistance to Fusarium head blight (FHB) has been seriously hampered worldwide due to lack of sources of high levels of resistance to this disease in durum germplasm. In searching for sources of FHB resistance that can be used for durum improvement, we identified a cultivated emmer wheat (*T. turgidum* subsp. *dicoccum*) accession, PI 272527, with a high level of resistance to FHB. To identify quantitative trait loci (QTL) associated with FHB resistance, a mapping population consisting of 219 recombinant inbred lines was developed from a cross between the durum variety 'Divide' and PI 272527. This population was genotyped using Illumina iSelect 90K array and a high density SNP linkage map was constructed with 10,486 SNP markers covering all 14 chromosomes. The population was also phenotyped for FHB resistance in two greenhouse and five field experiments during 2015 and 2016 growing seasons. Through homogeneity tests, we were able to combine disease severity data from the first greenhouse experiment and two field experiments in 2015 into one data set and combine the second greenhouse experiment and two of three field experiments in 2016 into another data set. However, the disease severity data collected from the third field experiment in 2016 could not be combined with either of the two combined data sets. Therefore, the SNP marker data and three sets of FHB data were used for subsequent QTL analysis. The results indicated that two QTL from Divide were detected on chromosomes 2A and 4A, and four QTL from PI 272527 were identified on chromosomes 1A, 3A, 5A, and 7B, respectively. The QTL on chromosome 5A, which conferred a major effect and was the only QTL detected in all greenhouse and field experiments, is likely the same as the one we previously identified in the hexaploid wheat line PI 277012. Based on the chromosomal location and the parentage information of Divide, the 2A QTL is likely the same as the one previously identified in durum variety 'Ben', suggesting that this QTL may be commonly present in the durum varieties developed by the North Dakota State University durum breeding program. Some of the QTL with minor effects derived from PI 272527 likely represent novel loci. Since PI 272527 is the most resistant genotype we have identified so far in tetraploid wheat germplasm collections, its high level of FHB resistance may be due to the additive effects of the QTL detected in this study.

## ACKNOWLEDGEMENT AND DISCLAIMER

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

# FINE MAPPING OF A NOVEL MAJOR QTL FOR FUSARIUM HEAD BLIGHT RESISTANCE IN THE WHEAT LINE PI 277012

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## ABSTRACT

The hexaploid wheat line PI 277012 exhibited a high level of Fusarium head blight (FHB) resistance in both greenhouse and field experiments. Previous QTL analysis of a population consisting of 130 doubled haploid (DH) lines from a cross between PI 277012 and ‘Grandin’ identified two major FHB resistance QTLs located on chromosome arms 5AS and 5AL, respectively. The 5AS QTL (*Qfhb.rwg-5A.1*) peaked at marker *Xbarc40* between markers *Xcfa2104* and *Xgwm617*, while the 5AL QTL (*Qfhb.rwg-5A.2*) peaked at marker *Xcfd39* between markers *Xwmc470* and *Xbarc48*. *Qfhb.rwg-5A.2* is different from those found in other known sources of FHB resistance. To fine map *Qfhb.rwg-5A.2* with more DNA markers, the DH population (GP-DH) was first genotyped using the wheat 9K SNP iSelect assay, and a total of 4317 polymorphic SNPs between PI 277012 and Grandin were mapped to the genetic linkage map previously constructed for chromosome 5A with SSR markers. Sequences of SNP markers within the peak of *Qfhb.rwg-5A.2* were used to search for syntenic region in *Brachypodium distachyon* (Bd) genome and a Bd region containing 2,500 genes were identified. The Bd genes were used as queries to search for the wheat genome survey sequences and 139 contigs were identified for primer design. Ten Cleaved Amplified Polymorphic Sequences (CAPS) markers were developed from the wheat contig sequences and mapped to the *Qfhb.rwg-5A.2* region in the GP-DH population. Additional DNA markers were developed for the *Qfhb.rwg-5A.2* region from the wheat 90K SNP iSelect assay and reference genome sequences of Chinese Spring. By using these DNA markers to genotype 947 recombinant inbred lines from the cross between PI 277012 and Grandin, we delimited *Qfhb.rwg-5A.2* in a 1.09-Mbp genomic region according to the reference genome sequence of Chinese Spring. This study provides DNA markers tightly linked to *Qfhb.rwg-5A.2*, which can be used for marker-assisted selection and facilitate the isolation and functional characterization of the underlying resistance gene.

## ACKNOWLEDGEMENT AND DISCLAIMER

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**OTHER**



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# DIRECT AND SECONDARY ECONOMIC IMPACT OF THE U.S. WHEAT & BARLEY SCAB INITIATIVE

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## OBJECTIVE

Estimate the direct and secondary economic impacts of the USWBSI for all Crop Reporting Districts (CRD) and states since its insertion in 1997. This involves estimates of savings from reduction of scab losses to producers and returns on investments from research provided by the Initiative.

## INTRODUCTION

Fusarium Head Blight (FHB) has led to major economic losses for wheat and barley producers. Varietal research has led to the development of varieties that are moderately resistant to FHB. Also, studies indicate combinations of genetic resistance, fungicides, and some management practices (combine settings, tillage practices, etc.) can be used to decrease losses due to FHB. These approaches were developed beginning in 1997, with the introduction of the United States Wheat and Barley Scab Initiative (USWBSI). Prior studies by Johnson, et al. (1998) and Njanje et al. (2004) focused on estimating losses due to scab. Loss estimates were available from 1993 to 2004. No economic study has estimate loss trends and returns on research facilitated by the USWBSI. This study is the comprehensive to date, in scope and timeframe covered. States included for the analysis were all wheat and barley producing states, by crop reporting district, in the US that had experienced significant scab losses.

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## METHODOLOGY

A five-step process was used to analyze the impacts of USWBSI (Wilson et al. 2017). First, we estimated production loss due to FHB from 1993 to 2014 using methods developed by Johnson, et al. (1998) and Njanje et al. (2004). Second, we segregate average losses into two periods; a base period prior to the Initiative (1993 – 1996) and after the Initiative (1997 – 2014). Differences between the base period and subsequent years provided preliminary observations suggesting average losses and the variability of losses have been declining after the insertion of the Initiative in 1997, for most crops and regions. Third, we estimate average revenue loss by multiplying production losses with prices. Savings by the USWBSI accrued when revenue loss declines after the base period (1993-1996), due to reduction in FHB occurrences. The effect of savings from resistant varieties and management practices represent savings to small grain producers and also represent direct and secondary economic benefits to regional economies. Annual savings and expenses (direct federal funding, other farm expenses on fungicides and in-kind contributions from faculty unpaid time) were used to estimate returns on investments; using net present value (NPV), internal rate of return (IRR) and aggregate rate of return to account for risk or variability of savings. As a benchmark, social returns to public expenditures are often compared with the return to U.S. Treasury Bonds as a measure of the opportunity cost of public funds (Magni 2015).

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## RESULTS AND DISCUSSIONS

The value of the USWBSI goes beyond production to other sectors in the economy (agribusiness industry, input supplies, trade, etc.). Total direct and secondary economic gains from FHB reduction in HRS wheat, barley, durum, and SRW wheat from 1997 to 2014 were estimated at \$21.6 billion. This will enable policy makers, industry representatives, and those in academia to evaluate the comprehensive economic value of the USWBSI for HRS only.

Total direct and secondary economic gains from FHB reduction in HRS wheat, barley, durum, and SRW wheat from 1997 to 2014 were estimated at \$21.6 billion (Table 1). The Initiative yields important societal returns. A large body of economic literature, including 35 studies published over the time period of 1965-2005, indicates that the median estimate of the social rate of return was 45 percent per year and that for every \$1 spent on agricultural research, approximately \$10 worth of benefits were returned to the economy (Fuglie and Heisey 2007). Investments with the Scab Initiative has triggered significant investments from producers and other research and outreach efforts. The \$76 million triggered other investments in fungicide use (\$110 million per year), extension and other research (\$12.76 million). These aggregate investments suggest the Initiative has resulted in significant net savings for the period 1997 through 2014, totaling nearly \$5.4 billion (Table 2). The internal rate of returns was 34% (Table 3). The USWBSI has provided significant reduction of FHB losses in most crops and CRD.

The Initiative has also resulted in reduction in variability of losses, providing more stable revenue for growers.

## ACKNOWLEDGEMENT AND DISCLAIMER

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**Table 1.** Gains in Total Economic Output by the Initiative in Barley, Durum, Hard Wheats and Soft Red Winter, by State, 1997 through 2014.

<b>State and Crop</b>	<b>Low Year</b>	<b>High Year</b>	<b>Total</b>	<b>Annual Average</b>
	----- millions \$ -----			
ND				
Barley	0	1.86	1.85	0.1
Durum	0	49.96	656.05	36.4
Hard Wheats	0	210.22	1,397.94	77.66
SD				
Hard Wheats	10.05	20.52	278.15	15.5
MT				
Hard Wheats	0	49.67	189.46	10.5
MN				
Barley	0	5.82	11.53	0.6
Durum	0.88	1.10	17.94	1.0
Hard Wheats	0	178.88	1,261.31	70.1
NE				
Hard Wheats	0	87.13	587.18	32.6
KS				
Hard Wheats	0	282.78	1,871.64	104.0
MD				
Barley	0	1.92	10.42	0.6
SRW	0	5.04	22.80	1.3
VA				
SRW	0	7.02	46.24	2.6
IL				
SRW	20.25	29.55	451.04	25.1
IN				
SRW	254.68	456.99	7,878.25	437.7
MI				
SRW	-3.54	5.49	11.98	0.7
MO				
SRW	5.79	11.56	178.97	9.9
OH				
SRW	4.99	23.05	319.32	17.7
KY				
SRW	0	0.08	0.08	0.004
AR				
SRW	0	8.12	26.66	1.5
GA				
SRW	0	11.32	85.87	4.8
NC				
SRW	0	13.03	77.80	4.3
PA				
SRW	0	4.59	21.33	1.2
ID				
SRW	0	56.13	338.01	18.8
OR				
SRW	0	42.05	270.82	15.0
WA				
SRW	0	156.56	1,120.48	62.25
All States and Crops	670.43	1,949.62	21,605.15	1,200.3



**Table 2.** Savings and NPV Due to USWBSI for Direct Savings

<b>Year</b>	<b>Savings (All Grains)</b>	<b>Funds Provided</b>	<b>Net Savings</b>
Fungicide Investment (Annually)	-	-\$110,000,000	-\$110,000,000
Other Research and Outreach	-	-\$12,764,016	-\$12,764,016
1997	\$444,633,690.17	-\$200,000.00	\$444,433,690.17
1998	\$635,396,348.02	-\$200,000.00	\$635,196,348.02
1999	\$760,191,837.36	-\$3,050,192.00	\$757,141,645.36
2000	\$759,156,194.72	-\$4,228,846.00	\$754,927,348.72
2001	\$675,176,159.09	-\$4,916,501.00	\$670,259,658.09
2002	\$554,084,960.58	-\$4,923,885.00	\$549,161,075.58
2003	\$532,098,360.60	-\$4,922,301.00	\$527,176,059.60
2004	\$585,082,491.22	-\$5,125,318.00	\$579,957,173.22
2005	\$581,338,447.35	-\$5,054,864.00	\$576,283,583.35
2006	\$474,580,971.44	-\$4,993,646.00	\$469,587,325.44
2007	\$313,823,140.04	-\$4,991,809.00	\$308,831,331.04
2008	\$297,170,976.14	-\$4,956,802.00	\$292,214,174.14
2009	\$447,311,657.44	-\$4,927,432.00	\$442,384,225.44
2010	\$387,610,779.80	-\$4,928,531.00	\$382,682,248.80
2011	\$312,392,773.08	-\$4,862,721.00	\$307,530,052.08
2012	\$344,617,558.05	-\$4,423,440.00	\$340,194,118.05
2013	\$371,667,378.73	-\$4,536,580.00	\$367,130,798.73
2014	\$386,931,312.21	-\$4,911,835.00	\$382,019,477.21
Mean	\$492,403,613.11	-\$4,230,816.83	\$488,172,796.28
NPV (billion \$)			\$5.29 -\$5.37*

\*Low range estimate includes in-kind research and outreach expenses

**Table 3.** IRR and Discount Rate

<b>Year</b>	<b>IRR</b>	<b>Discount Rate</b>	<b>ARO I</b>
1997	0%	9.94%	0%
1998	0%	14.92%	0%
1999	15%	-8.25%	16%
2000	23%	16.66%	24%
2001	28%	5.57%	28%
2002	30%	15.12%	30%
2003	32%	0.38%	32%
2004	33%	4.49%	33%
2005	33%	2.87%	33%
2006	34%	1.96%	34%
2007	34%	10.21%	34%
2008	34%	20.10%	34%
2009	34%	-11.12%	34%
2010	34%	8.46%	34%
2011	34%	16.04%	34%
2012	34%	2.97%	34%
2013	35%	-9.10%	34%
<u>2014</u>	<u>35%</u>	<u>10.75%</u>	<u>34%</u>



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