Proceedings of the

2019 National Fusarium Head Blight Forum

Hyatt Regency Milwaukee
Milwaukee, Wisconsin
December 8-10, 2019

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Proceedings compiled and edited by: S. Canty, A. Hoffstetter, H. Campbell and R. Dill-Macky

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FHB MANAGEMENT

FUSARIUM HEAD BLIGHT MANAGEMENT IN ALABAMA

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ABSTRACT

As part of the multi-state FHB Management Coordinated Project (MGMT_CP), we evaluated integrated FHB and DON management strategies on soft red winter wheat grown in the Southeastern U.S. states, with emphasis on Miravis Ace® (Adepydin® + propiconazole). An Integrated Management (IM) trial was done at the Plant Breeding Unit (east-central AL, 32.50, -85.89) of the Alabama Agricultural Experiment Station. The factorial set of treatments consisted of four varieties and six fungicide/inoculation regimen, in three blocks arranged in a split-plot with varieties as the main plots. The varieties were Jamestown (moderately resistant), SS 5550 (susceptible), Pioneer 26R94 (moderately susceptible) and Pioneer 26R59 (susceptible). Fungicides were applied using a CO2 backpack sprayer at 32 psi with three TX-12 hollow cone nozzles spaced 20 inches apart and 15 inches above the canopy. Miravis Ace treatment at anthesis was compared with inoculated and non-inoculated treatments with Prosaro® at anthesis and non-treated controls. Inoculation was done late March using *Fusarium graminearum*-infested corn applied at 21 g m^{-1}. FHB intensity and leaf rust severity were rated on 2 May 2019. Seventeen days after fungicide application, forty heads per plot were collected to estimate the FHB index (IND). Field ratings of leaf rust and FHB intensity were low and IND was < 3% in all plots. While FHB intensity was lower in Pioneer 26R59 than in Jamestown or SS 5550, no significant differences in IND were noted among varieties. Pioneer 26R59 had lower yield (bushels/A) and test weight compared to other varieties; no differences in disease levels were noted due to fungicides. At the Tennessee Valley Research and Extension Center (north central AL, 34.69, -86.89), the variety Croplan 9415 (moderately susceptible) was treated with Miravis Ace and Prosaro at full head or anthesis. No significant differences in FHB levels were noted among the fungicide treatments. Yield was numerically greater with any fungicide compared to non-treated controls and test weights were improved with either Miravis Ace treatment compared to the Prosaro treatments and the non-treated control.

ACKNOWLEDGEMENT AND DISCLAIMER

This material is based upon work supported by the U.S. Department of Agriculture, under Agreement No. 59-0206-6-008. 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.

CHARACTERIZATION OF SPECIES COMPOSITION, CHEMOTYPE, AND *IN VIVO* AND *IN VITRO* FUNGICIDE SENSITIVITY OF *FUSARIUM* FROM WHEAT AND CORN IN MICHIGAN, USA

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ABSTRACT

Fusarium species are a major concern due to mycotoxin contamination of wheat grain as well as corn grain in Michigan. To characterize the population of *Fusarium* in Michigan, over 500 isolates were collected and species composition, chemotype (15-ADON, 3-ADON, NIV, NX), and fungicide sensitivity are being determined. Thus far, *F. graminearum* was the major species associated with wheat, but members of the *Fusarium tricinctum* complex, *F. culmorum*, *F. cerealis*, *and F. poae* were found as well. Greater species diversity was found in corn, with a smaller proportion identified as *F. graminearum* and more identified from the *Fusarium fujikuroi* complex. *In vitro* sensitivity to triazole chemistries (metconazole, tebuconazole, and prothioconazole) were assessed with mycelial growth assays. Isolates were most sensitive to metconazole, and less sensitive to prothioconazole and tebuconazole. A small portion of *F. graminearum* isolates had EC_{50} values 10-100 fold greater than the most sensitive isolates. In order to determine if this reduced sensitivity *in vitro* would lead to practical resistance, a field trial was established in 2019. A subset of *F. graminearum* isolates were chosen for investigation, four identified as sensitive *in vitro* (EC_{50} 0.01 -0.1 ppm), and four with reduced sensitivity *in vitro* (atleast 10-fold greater). Plots were inoculated with spore suspensions of each isolate 48 hours prior to fungicide applications in a factorial manner in a randomized complete block design. No differences in the relative fungicide efficacy were found, signaling no practical resistance currently exists in *F. graminearum* despite differences *in vitro* and widespread use in wheat throughout Michigan for more than 10 years.

ACKNOWLEDGEMENT AND DISCLAIMER

This material is based upon work supported by the U.S. Department of Agriculture, under Agreement No. 59-0206-9-189. 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.

COMPARISON OF MIRAVIS ACE, PROSARO AND CARAMBA FOR MANAGEMENT OF DON IN WINTER BARLEY

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ABSTRACT

In a Fusarium head blight (FHB) epidemic, the combination of cultivar resistance and effective fungicide application is expected to provide maximum reduction of DON in winter barley. Our previous work showed that Prosaro® at either 100% spikes fully emerged or 6 days later provided modest DON reduction, and was superior to an application at 50% spikes emerged. In the present experiment, Miravis Ace® was evaluated for two years (2017-18 and 2018-19) in a misted, inoculated FHB nursery at Raleigh, North Carolina. Timing of application was again a factor of interest. The three winter barley cultivars tested had different levels of FHB resistance: Violetta (MR), Thoroughbred (MR/MS), and Flavia (S). Violetta and Flavia were medium-late two-row malting cultivars, while Thoroughbred was a medium-maturing six-row feed type with acceptable malt quality.

Inoculation was provided via *Fusarium*-infected corn spawn, and the experiment was mist-irrigated. Fungicides were Miravis Ace (adepidyn + pydiflumetofen), Prosaro (prothioconazole + tebuconazole), and, in the second year, Caramba® (metconazole). The three timings for fungicide application were: 50% spike emergence, 100% spike emergence, and 100% emergence + 6 days. There were four replicate blocks in each year. Following are the DON results:

DON (ppm)	Fungicide timing			
2018	**None**	**50% headed**	**100% headed**	**6 d later**
Unsprayed	3.2			
Miravis Ace		2.0	1.1	1.1
Prosaro		1.8	1.4	1.1
2019				
Unsprayed	10.7			
Miravis Ace		4.0	2.7	1.4
Prosaro		7.1	4.7	1.7
Caramba		9.3	6.8	3.4
GRAND MEANS	**7.0a**	**4.3b**	**3.0c**	**1.6d**

Overall, the latest timing was the most effective in minimizing DON. Like Prosaro and Caramba, Miravis Ace was less effective when applied at 50% head emergence than at the later timings. Comparing Prosaro and Miravis Ace across years, there was no significant difference in DON levels ($P = 0.06$); each reduced DON significantly relative to the untreated check ($P < 0.0001$). Yields and test weights were improved significantly, across cultivars and fungicides, by spraying at any of the timings as compared to the untreated check ($P \leq 0.01$); no specific timing was better for yield or test weight.

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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 2019, we observed the disease and yield impact of cultivar susceptibility, inoculation with *Fusarium graminearum*, and treatment with organic and conventional 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 30 April 2019. 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^{-1} 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 22 June 2019 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 approximately four days after heading on 29 June 2019 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_2 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 12 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 1 August 2019. 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 2019 growing season were below average for temperature and precipitation. There were 164 less growing degree-days and 2.39 inches less of precipitation compared to the 20-year average. Overall conditions were not highly conducive for FHB infection.

There was no significant cultivar by fungicide treatment interactions for DON or yield. This indicates that under high 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). Overall DON concentrations were extremely low and ranged from 0.10 to 0.23 ppm. The barley yields did respond significantly to the fungicide treatments (Table 2). The mean yield for the trial was 85.4 bushels per acre, above average for the region.

Although small, there were significant differences detected in DON concentrations among varieties (Table 3). Yield did not differ significantly among the varieties.

Even though all of the variety+fungicide+timing treatments resulted in DON concentrations below 1 ppm, it's important to note that Conlon, a moderately resistant variety, still had a significantly lower incidence of DON compared to Rasmussen, a susceptible variety. This indicates the importance of selecting resistant cultivars to manage FHB in our region.

In 2019, the application of fungicides Prosaro®, Caramba® , Mirvais Ace® and ChampION™ did not reduce DON concentrations compared to the inoculated control. Interestingly, yields did not vary significantly between fungicide type, application or variety. A conducive environment is essential to cultivating disease. In 2019, FHB was low and the return on fungicide applications was not observed in this season. However, it was clear that a yearly investment in varieties that are moderately resistant to FHB is important.

ACKNOWLEDGEMENT AND DISCLAIMER

This material is based upon work supported in part by the U.S. Department of Agriculture under agreement No. 59-0206-4-035. 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.

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

Fungicide treatments	Company	Fungicide active ingredient	Application rates
Control			Water
Fusarium graminearum			40,000 spores/ml
Prosaro SC®	Bayer CropScience	Prothioconazole + tebuconazole	6.5 fl oz ac^{-1} + Induce at 0.125% V/V
Caramba®	BASF Ag Products	Metconazole	14 fl oz ac^{-1} + Induce at 0.125% V/V
Champ ION^{++}	NuFarm	Copper hydroxide	1.5 lbs ac^{-1}
Mirvais Ace®	Syngenta	Adepidyn/Pydiflumetofen) + Propiconazole	13.7 fl oz ac^{-1} Induce at 0.125% V/V

Table 2. Main effect treatment on deoxynivalenol (DON) contamination and grain yield at Alburgh, VT 2019.

Fungicide treatment	DON	Yield
	ppm	bu ac^{-1}
Non-sprayed, non-inoculated control	0.10	87.5
Inoculated FGS 10.1	0.24	79.6
Caramba (14 fl oz) at heading	0.21	79.3
Caramba (14 fl oz) 5 days after heading	0.18	90.2
ChampION (1.5 lbs) at heading	0.16	78.3
ChampION (1.5lbs) 5 days after heading	0.21	80.3
ChampION (1.5 lbs) at heading and 5 days after heading	0.15	81.6
Mirvais Ace (13.7 fl oz) at early heading	0.10	87.8
Mirvais Ace (13.7 fl oz) at heading	0.11	93.8
Mirvais Ace (13.7 fl oz) 5 days after heading	0.10	94.7
Prosaro SC (6.5 fl oz) at heading	0.21	85.8
LSD (P=0.05)	NS	NS

Table 3. Main effect of cultivar on deoxynivalenol (DON) contamination and grain yield at Alburgh, VT 2019.

Cultivar treatment	**DON**	**Yield**
	ppm	bu ac^{-1}
Conlon	0.08	88.2
Robust	0.24	82.5
LSD (P=0.05)	0.05	NS

APPLICATION OF MODEL ENSEMBLES TO THE PREDICTION OF FUSARIUM HEAD BLIGHT

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ABSTRACT

Weather-based models for Fusarium head blight (FHB) are the driving force behind the Fusarium Head Blight Prediction Center (FHBPC; http://www.wheatscab.psu.edu/). This resource is used in 30 U.S. states where FHB and deoxynivalenol (DON) contamination consistently reduce the quality and yield of wheat and barley. The first-generation of predictive models were developed with just 50 observations (unique site-year-variety combinations). Cooperation with the Integrated Management Coordinated Project (IM-CP) expanded the data set, with nearly 1,000 cases now available for modeling. Some of the recent additions to the data set are noteworthy, because they represent striking deviations from normal temperature and rainfall patterns. Bringing these observations into the available data set will expand the range of conditions used in model development, and lead to more robust predictions in an era of climate change. We currently have a rich set of logistic regression models representing four generational cycles of development. Summary metrics reveal that the models have gotten better over the generations, reflecting a better understanding of the relationships between weather and FHB epidemics. Despite these improvements, there are limitations in how well any one simple model captures the dynamics of an epidemic. Combining multiple simple models can yield a predictive performance that is superior to that of any one of the individual component models. The technique of combining models is generally known as ensembling and is common in weather forecasting, for example. Hierarchical cluster analysis of the FHB models indicate that there are at least 4 groups of models with respect to Brier score (a statistic comparing model performance). Ensembles representing these groups captured more information and improved prediction accuracy relative to the individual models. This ensemble approach represents a potentially important paradigm shift within plant disease forecasting. In time, such ensemble approaches should improve the predictions of FHB epidemics in the U.S. delivered via the FHBPC.

ACKNOWLEDGEMENTSAND DISCLAIMER

This material is based on work supported by the U.S. Department of Agriculture, under Agreements 59-0206-6-015 and 59-0206-4-018. 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.

IMPACT OF PREDICTION TOOLS FOR FUSARIUM HEAD BLIGHT IN THE US, 2009-2019

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ABSTRACT

A multi-state effort to predict epidemics of Fusarium head blight (FHB) was operational between 2009-2019. 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. During the 2019-growing season (1 March – 15 August), the prediction tools provided over 6,672 sessions (11,247 page views) for 3,415 users in the US. 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 95 commentaries were submitted in 2019. The FHB Alert System sent commentary to 1,145 subscribers in 2019.

Users of the FHB prediction models and the FHB Alert System were surveyed annually in 2010-2014, and then again in 2017-2019. The survey results included input from 1,896 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. 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 90% 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 49% of the respondents, and motivated another 41% to seek advice from others. The 2012-2014 and 2017-2019 surveys asked growers to estimate the monetary value of the information provided to their farm or business. These surveys indicate that the median monetary value of the information provided by the prediction system was $12,158 per user during these time periods. Combining this figure with use statistics suggests that impact of the FHB prediction model exceeds $56 million annually.

ACKNOWLEDGEMENTSAND DISCLAIMER

This material is based on work supported by the U.S. Department of Agriculture, under Agreement Nos. 59-0206-6-015, 59-0206-6-016, 59-0206-8-187, 59-0206-8-215, and 59-0206-6-001. 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.

SENSITIVITY OF *FUSARIUM GRAMINEARUM* ISOLATES CAUSING WHEAT SCAB IN PENNSYLVANIA TO TRIAZOLE FUNGICIDES

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ABSTRACT

Fusarium head blight (FHB or scab) is the major head disease that impacts yield and grain quality of small grains in Pennsylvania, mainly due to accumulation of the mycotoxin deoxynivalenol (DON). Historically, triazole products have been the only fungicide option for effective management of FHB and DON. An *in vitro* assay was conducted to investigate the sensitivity of a recently collected population of *Fusarium graminearum* from Pennsylvania to tebuconazole and metconazole fungicides. During summer 2018, FHB-infected wheat spikes were collected from wheat-producing regions (South, Central, and North) of Pennsylvania using a standardized sampling procedure. In the laboratory, 295 *Fusarium graminearum* strains were isolated from FHB-symptomatic spikelets. Based on this previous collection, ten isolates from each region were selected to conduct this experiment. Isolates were grown on PDA under fluorescent light with a 12-h photoperiod for 7 days. The two triazole-based fungicides tested were aqueous suspensions of commercially formulated metconazole and tebuconazole. The effective concentration for each fungicide to limit growth by 50 percent (EC_{50}) values were determined based on a mycelial growth assay. Tested concentrations for both fungicides included 0, 0.01, 0.1, 0.5, 1, 10 mg/L. One mycelial agar plug (6 mm) from the edge of a culture of each isolate was placed in the center of a petri dish (90 mm). For each combination of isolate–dose–fungicide, two replicates (petri dishes) were used. After 5 days of incubation at 25°C in darkness, average radial growth was measured using two perpendicular directions and the agar plug diameter was subtracted. The experiment was conducted twice. EC_{50} estimates for metconazole ranged from 0.009 to 0.071 mg/L (mean = 0.031 mg/L). EC_{50} estimates for tebuconazole were statistically higher ($\alpha = 0.01$) than metconazole, with values for tebuconazole ranged from 0.0790 to 0.7970 mg/L (mean = 0.2514 mg/L). The isolates did not differ significantly from each other in terms of EC_{50} estimates for both fungicides. Results of this study revealed that *Fusarium graminearum* isolates associated with FHB of wheat in Pennsylvania were sensitive to triazole-based fungicides *in vitro*, regardless of sampling region.

ACKNOWLEDGEMENT AND DISCLAIMER

This material is based upon work supported by the U.S. Department of Agriculture, under Agreement No. 59-0206-9-064. 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.

INTEGRATED MANAGEMENT OF SOFT RED WINTER WHEAT IN ILLINOIS - 2019

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ABSTRACT

Wheat is an economically important field crop in Illinois, resulting in the production of over $150 million USD in 2018. Recent epidemics of Fusarium head blight (FHB) continue to result in production losses due to yield and quality reductions. The use of moderately resistant varieties combined with a recommended fungicide applied within five days of flowering (Feeke's growth stage (FGS) 10.5.1) has been shown to provide the greatest suppression of disease. However, recommended FHB management strategies only use triazole-based fungicides such as Caramba®, Prosaro®, and Proline®. Miravis Ace® was released early this year by Syngenta for use in managing FHB. This fungicide incorporates adepidyn, a succinate dehydrogenase inhibitor, as the active ingredient, and is marketed as controlling FHB when applied as early as FGS 10.3. We assessed the utility of Miravis Ace for suppressing FHB as part of an integrated management program at field sites in Marion and Urbana, IL. A split plot design was used, with variety as the main plots and fungicide treatment (Caramba, Prosaro, Miravis Ace) and timing (FGS 10.3, FGS 10.5.1, FGS 10.5.1 + 5) as subplots. FHB infection of the spike, *Fusarium* damaged kernels (FDK), test weights, vomitoxin (DON), and yields were recorded and statistically analyzed. Levels of FHB, FDK, and DON were lower in the MR compared to the MS variety, and yields equivalent. Regardless of product, fungicides applied at FGS 10.5.1 -10.5.1+5d resulted in greater test weights and yields compared to the FGS 10.3 timing, whereas DON levels and FDK were the lowest in FGS 10.5.1 + 5 treatments. Across timings, Miravis Ace treatments provided the greatest test weights, and suppression of FHB, FDK, and DON equivalent to Prosaro and Caramba. Based on our data, Miravis Ace should be included as a recommended fungicide for managing FHB and DON as part of an integrated management plan. However, application of fungicides at 10.5.1-10.5.1+5 d still provides the greatest disease control.

ACKNOWLEDGEMENT AND DISCLAIMER

This material is based upon work supported by the U.S. Department of Agriculture, under Agreement No. 59-0206-8-211. 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.

EVALUATING ADEPIDYN AND HOST RESISTANCE AT TWO YEARS AND LOCATIONS TO REDUCE FUSARIUM HEAD BLIGHT AND DEOXYNIVALENOL IN SPRING BARLEY

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ABSTRACT

Fusarium head blight (FHB) is a serious problem for malting barley companies and production areas in the Midwest United States. Strategies involving fungicide timing are being tested to help control this devastating disease. Two barley FHB integrated management trials were established in 2018 and 2019 at Fargo and Langdon, North Dakota. The trials evaluated the effect of adepidyn plus propiconazole (Miravis Ace®, Syngenta) along with standard FHB treatments with varietal resistance on reducing deoxynivalenol (DON) and FHB. Trials were designed in a randomized complete block with a split-plot arrangement with four replications at the two 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 3 to 7 days after heading, metconazole at heading, adepidyn + propiconazole at 50% heading, adepidyn + propiconazole at heading and adepidyn + propiconazole 3 to 7 days after heading. Corn spawn served as the inoculum source at Langdon and Fargo in addition to *Fusarium* spores in Fargo (2018 only). Inoculum was applied to all treatments except for the non-treated, non-inoculated check. The level of FHB severity and incidence was evaluated around the Feekes 11.2 growth stage (mid to hard dough). Yield and DON were obtained after harvest. Data were analyzed using Proc GLM and means were separated with LSD (P=0.05). Results indicated that applying fungicides generally lowered DON significantly compared to non-treated controls. Both FHB and DON levels were higher in susceptible varieties. Regardless of fungicide, applications made 3 to 7 days after heading had the lowest levels of DON, FHB incidence and highest yield. Adepidyn + propiconazole applied 3 to 7 days after heading had the lowest DON levels and the highest yield compared to all other treatments. Results from this study will help update FHB fungicide recommendations for spring barley production.

ACKNOWLEDGEMENT AND DISCLAIMER

This material is based upon work supported by the U.S. Department of Agriculture, under Agreement Nos. 59-0206-8-199. This is cooperative project with the U.S. Wheat and Barley Scab Initiative. Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the authors and do not necessarily reflect the view of the U.S. Department of Agriculture.

IMPACT OF ENVIRONMENTAL CONDITIONS ON FUNGICIDE ABILITY TO CONTROL FUSARIUM HEAD BLIGHT UNDER FIELD NURSERY CONDITIONS

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ABSTRACT

Large variations in environmental conditions, including precipitation, are characteristic of changing weather patterns. More years with more extreme conditions are becoming frequent in agriculture. In the 2017-2018 Kansas winter wheat growing season, rainfall totaled 8.87", compared to the 2018-2019 season rainfall total of 26.87". These two extreme seasons in consecutive years presented the opportunity to examine a sample of fungicides' ability to control Fusarium head blight in a disease nursery under two extreme environmental conditions.

Comparisons were made using a scab susceptible cultivar, 'Larry', planted in a randomized complete block design with five (2019) or seven (2018) replications. Treatments included commercially available fungicides and an untreated control. Applications were made at the rate of 20 gal/A using a backpack CO2 sprayer equipped with flat-fan nozzles (8002 VS) with 20-in. spacing at 30 psi.

While in near drought conditions, we observed that fungicides are able to maintain control over the pathogen. However, under extreme precipitation conditions, losses to abiotic stress outweighed the benefits of fungicide applications.

This research suggests that under high disease pressure and extreme weather conditions, fungicides fail to perform better than non-treated checks.

FUNGICIDE EFFICACY AND TIMING FOR MANAGEMENT OF FUSARIUM HEAD BLIGHT IN MALTING BARLEY IN VIRGINIA

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ABSTRACT

Barley is a minor crop in Virginia, but there is an emerging specialty market for malting barley in the region. However, quality standards require low Fusarium head blight (FHB) and deoxynivalenol (DON) contamination in malting barley, so effective disease management approaches are needed. Optimum application timings of fungicides for FHB and DON control have been evaluated extensively for wheat, and the current recommendation is to apply the fungicide at early flowering. In contrast to wheat for which flowering occurs after heading, in barley flowering coincides with head emergence, and the efficacy of FHB fungicide applications prior to and after heading of the barley crop has not been evaluated in Virginia. In addition, though barley varieties vary in susceptibility to FHB and DON contamination, we do not have data on the efficacy of integrated disease management that incorporates both host resistance and optimum application of an effective fungicide. Thus, the objective of our study was to evaluate the efficacy of fungicide chemistries and timings for FHB and DON control in two malting barley varieties at multiple locations in Virginia during the 2019 growing season. Experiments were conducted at locations in Blackstone, New Kent, Mount Holly, and Suffolk, VA. Prothioconazole + tebuconazole (Prosaro®, Bayer CropScience), metconazole (Caramba,® BASF), and pydiflumetofen + propiconazole (Miravis Ace®, Syngenta) were applied at either the boot stage, anthesis (heading/early flowering, Feekes stage 10.5), or 4-6 days after anthesis. Two malting barley varieties, Calypso and Flavia, were compared. Experiments at Blackstone and New Kent were subjected to natural sources of inoculum whereas trials at Mount Holly and Suffolk were inoculated with *Fusarium graminearum* to promote development of Fusarium head blight (FHB). Disease pressure varied among sites with relatively high severity of net blotch in Blackstone and Suffolk and high severity of FHB in Mount Holly. Overall, Calypso had greater severity of net blotch compared to Flavia, and pydiflumetofen + propiconazole provided the greatest control of foliar disease. When FHB severity was high, Flavia had higher levels of FHB compared to Calypso and all fungicides and application timings reduced disease. Significant differences in yield were not detected among fungicide treatments, but fungicide treated plots generally had greater yields compared to plots that did not receive a fungicide application. Results indicate both variety selection and judicious use of fungicides are needed to maximize malting barley yield and quality in Virginia.

ACKNOWLEDGEMENT AND DISCLAIMER

This material is based upon work supported in part by the U.S. Department of Agriculture, under Agreement No. 59-0206-6-010. 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.

PRE-HARVEST RAINFALL AND HARVESTING STRATEGY EFFECTS ON THE QUALITY OF FHB AFFECTED GRAIN

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ABSTRACT

Although measures of Fusarium head blight (FHB) such as disease incidence, index, and *Fusarium* damaged kernels are good indicators of deoxynivalenol (DON) contamination, DON levels may be lower or higher than one would expect based on visual symptoms under certain conditions. One possible explanation for relationships between visual estimates of FHB and DON breaking down could be the conversion of DON to DON-3-Glucoside (D3G), a masked form of the toxin that is missed by common DON testing methods. Failure to detect masked forms of DON and other toxins is a major food safety concern. DON-to-D3G conversion could be affected by several pathogen, host, and environmental factors. Here we investigated the effects of pre-harvest rainfall and harvesting strategies to mitigate toxin contamination on wheat grain quality. The experimental design was a randomized complete block, with a split-split-plot arrangement of harvest time (early harvest – 36 days after anthesis [DAA] and late harvest - 46 DAA) as whole-plot, number of successive days with rain immediately prior to harvest (0, 5 and 10 days) as sub-plot, and *Fusarium graminearum* inoculum density (0, 5 x 10^4, and 1 x 10^5 spores.mL^{-1}) as sub-sub-plot. Separate plots of a susceptible cultivar were spray inoculated at anthesis with the desired spore concentration, resulting in a range of baseline FHB index levels under which the rainfall and harvest treatment effects were evaluated. Mean FHB index was 30.0 (23.6-32.4%), 36.7 (30.8-41.8%), and 40.9% (35.3-46.4%) in plots with the natural infection, low, and high inoculum densities, respectively. Plots that received 5 or 10 consecutive days of simulated rain immediately before harvest had lower mean DON, higher mean D3G and zearalenone (ZEA), and lower mean test weight than plots not subjected to simulated rainfall. There was a significant positive relationship between DON and D3G in plots that received simulated rain, but not in plots without simulated rainfall. DON conversion to D3G (based on the D3G/DON ratio) increased and test weight decreased as the number of pre-harvest rainy days increased from 5 to 10 days. Early harvest did not effectively reduce mean DON. However, compared to plots harvest late and subjected to pre-harvest rainfall, plots harvested early and not exposed to simulated rainfall consistently had higher mean test weight and lower mean D3G and ZEA. Analyses will be conducted to formally quantify the observed effects and associations.

ACKNOWLEDGEMENTS AND DISCLAIMER

This material is based upon work supported by the U.S. Department of Agriculture, under Agreement No. 59-0206-8-187. 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.

ASSESSMENT OF FUSARIUM HEAD BLIGHT IN SMALL GRAINS USING AERIAL METHODS

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ABSTRACT

Fusarium head blight (FHB, or scab) is a disease of small grains caused by *Fusarium graminearum* that lowers yield and contaminates grain with mycotoxins called deoxynivalenol (DON). FHB epidemics have become more common in the mid-Atlantic in recent years, where environmental conditions and production practices favor disease development. By developing resistant cultivars, we are able to more readily ensure a reliable crop and safe food supply. However, current field assessment methodologies for evaluating FHB and time consuming, and breeders are limited to a single date to assess the incidence and severity of the disease. Therefore, the objectives of this study are to 1.) Explore the ability to optically assess FHB incidence and severity in the field in comparison to currently adopted methods of visual assessment; and 2.) Characterize the practicality of aerial imagery as a means to more efficiently and effectively quantify disease progress throughout the growing season in both a breeding and fungicide application program. FHB incidence and severity was collected to determine FHB index beginning twenty-one days after flowering, and continuing three times weekly until maturity. Aerial images were collected simultaneously with a MicaSense RedEdge sensor. Images were used to extract normalized difference red edge (NDRE) and normalized difference vegetative index (NDVI). NDRE and NDVI was ground-truthed with visual incidence and severity date to determine the effectiveness of aerially collected data. With the exception of two varieties, NDVI ranked the varieties the same as FHB index twenty-one days after flowering. Varieties that had the highest FHB index had the lowest NDVI and NDRE; while varieties with the lowest FHB index had the highest NDVI or NDRE.

FUSARIUM HEAD BLIGHT MANAGEMENT COORDINATED PROJECT: INTEGRATED MANAGEMENT TRIALS 2018-2019

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INTRODUCTION

Efforts to evaluate integrated management strategies for Fusarium head blight (FHB) and deoxynivalenol (DON) management in wheat and barley continued in 2018 and 2019. The focus of this round of integrated management coordinated project (IM_CP) was Miravis Ace®, a new Succinate Dehydrogenase Inhibitor (SDHI; Adepidyn - Pydiflumetofen) + Demethylation Inhibitor (DMI; Propiconazole) premix fungicide that was recently labeled for managing diseases of wheat, barley, and other small grain crops. Preliminary results from a limited number of trials showed that when applied at early anthesis (Feekes 10.5.1) or within the first 6 days after early anthesis, Miravis Ace was just as effective as Prosaro® and Caramba® (3,4,5). This suggested that like the latter two fungicides, this new fungicide alone will not be sufficient to manage FHB and DON. Based on results from previous IM_CP, we hypothesized that Miravis Ace will be most valuable for FHB management when combined with other management strategies such as genetic resistance, tillage, and crop rotation as part of an integrated management program (1,4,7). **The objective of this study was to evaluate the integrated effects of fungicide programs (products and timings) and genetic resistance on FHB and DON in all major grain classes, with emphasis on the new fungicide, Miravis Ace**.

MATERIALS AND METHODS

To accomplish the aforementioned objective, field experiments were conducted in 22 US wheat-growing states in 2018 and 2019. The standard protocol consisted of the application of fungicide treatment programs (sub-plot; **Table 1**) to plots of cultivars (whole-plot) with different level of resistance to FHB - susceptible (S), moderately susceptible (MS), and moderately resistant (MR). The experimental design was a randomized complete block, with at least 4 replicate blocks. In most experiments, plots were spray inoculated with a spore suspension of the fungus approximately 24-36 h after the anthesis treatments were applied, with or without mist-irrigation. Trials were naturally infected at some locations. FHB index (IND) was rated or calculated as previously described (2,6) on 60-100 spikes per plot at approximately Feekes 11.2. Plots were harvested and a sample of grain from each experimental unit was sent to a USWBSI-supported laboratory for mycotoxin analysis. Linear mixed models (multi-location) were fitted to the pooled arcsine square root-transformed IND and log-transformed DON data to evaluate the main and interaction effects of fungicide treatment and genetic resistance on IND and DON. Overall percent control/reduction relative to the nontreated susceptible check was also estimated for each management program as a measure of efficacy.

Table 1. The following core treatments were randomly assigned to experimental units. All fungicide treatments were applied along with a nonionic surfactant

Treatment[a]	Product/inoculation	Rate	Timing
1 (CK)	Untreated check, inoculated	…	…
2 (I)	Prosaro, inoculated	6.5 fl oz/A	Anthesis
3 (II)	Miravis Ace, inoculated	13.7 fl oz/A	Anthesis
4 (III)	Miravis Ace, inoculated	13.7 fl oz/A	Feekes 10.3
5 (PRO_A)	Prosaro, non-inoculated	6.5 fl oz/A	Anthesis
6 (CK0)	Untreated, non-inoculated	…	…

RESULTS AND DISCUSSION

Mean Fusarium head blight index (IND) and deoxynivalenol (DON) grain contamination data from 31 environments (trial x state x year combinations), representing different wheat market classes, are summarized for different cultivar resistance x fungicide program combinations in Figure 1 and 2. Averaged across management combinations, mean IND ranged from 0 to 74% and DON from 0 to 57 ppm.

FHB index

Mean IND was more variable across environments on S (interquartile range [IQR] 9 to 25%) and MS (IQR 4 to 12%) cultivars, than on MR (2 to 10%) cultivars. This in part reflects the fact that there were fewer environments with S and MS cultivars than with MR cultivars (**Fig. 1A**). The susceptible, nontreated check (S_CK) had the higher mean IND (22.6 %), whereas the application of Prosaro or Miravis Ace at anthesis to a moderately resistant cultivar resulted in the lowest means, 2.9 and 2.5% for MR_I and MR_II, respectively (**Fig. 2A**). For all tested resistance classes, all fungicide programs resulted in significantly lower mean IND (on the arcsine square root-transformed scale) than the nontreated check, and differences between pairs of fungicide programs were statistically significant. The only exceptions were for comparisons between Prosaro and Miravis Ace at anthesis on

MR cultivars and between Prosaro at anthesis and Miravis Ace at Feekes 10.3-5 on MS and S cultivars.

Deoxynivalenol

DON contamination results were somewhat different from those observed for IND. For instance, MS_III (application of Miravis Ace to an MS cultivar at Feekes 10.3-5) had the highest mean DON across trials, whereas management combinations consisting of a Prosaro or Miravis Ace application at anthesis to an MR (MR_I and MR_II) or S (S_I and S_II) cultivar had the lowest overall mean levels of the toxin (**Fig. 1B and 2B**). For all tested resistance classes, treatments applied at anthesis resulted in significantly lower mean DON (on the log-transformed scale) than the nontreated check and the Feekes 10.3-5 application of Miravis Ace.

As additional data become available, a more complete set of analyses will be performed. However, the results summarized herein suggest that while a Feekes 10.3-5 application of Miravis Ace may suppress FHB IND to levels comparable to those achieved with an anthesis application of Miravis Ace or Prosaro, such an early application is considerably less effective than the anthesis applications in terms of DON suppression.

ACKNOWLEDGEMENTS AND DISCLAIMER

This material is based upon work supported by the U.S. Department of Agriculture, under Agreement Nos. 59-0206-8-187, 59-0206-8-195, 59-0206-9-120, 59-0206-6-008, 59-0206-5-007 & 58-6070-8-019, 59-0206-8-192, 59-0206-8-189, 59-0206-9-112, 59-0206-8-190, 59-0206-6-015, 59-0206-4-016 & 59-0206-9-117, 59-0206-8-210, 59-0206-4-012 & 59-0206-8-199, 59-0206-8-211, 58-2050-8-013, 59-0206-6-010, 59-0206-6-012, 59-0206-9-123, 59-0206-6-014, and 59-0206-6-009. 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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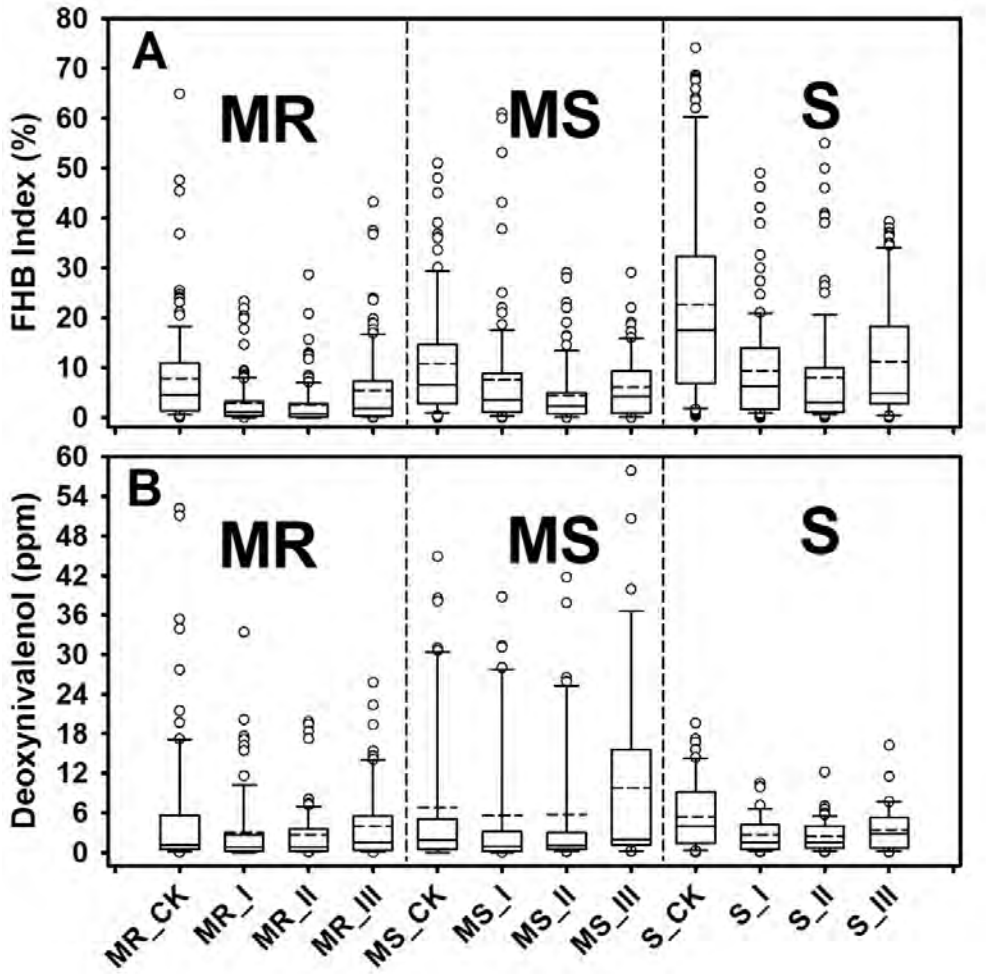

Fig. 1. Boxplots showing the distribution of **A**, mean Fusarium head blight index and **B**, deoxynivalenol grain contamination for different fungicide program x cultivar resistance management combinations. **S**, **MS**, and **MR** represent susceptible, moderately susceptible, and moderately resistant, respectively, whereas **CK** = nontreated, **I** = treated with Prosaro (6.5 fl. oz.) at Anthesis, **II** = treated with Miravis Ace (13.7 fl. oz.) at anthesis and **III** = treated with Miravis Ace (13.7 fl. oz.) between Feekes 10.3 (early head emergence) and 10.5 (complete head emergence).

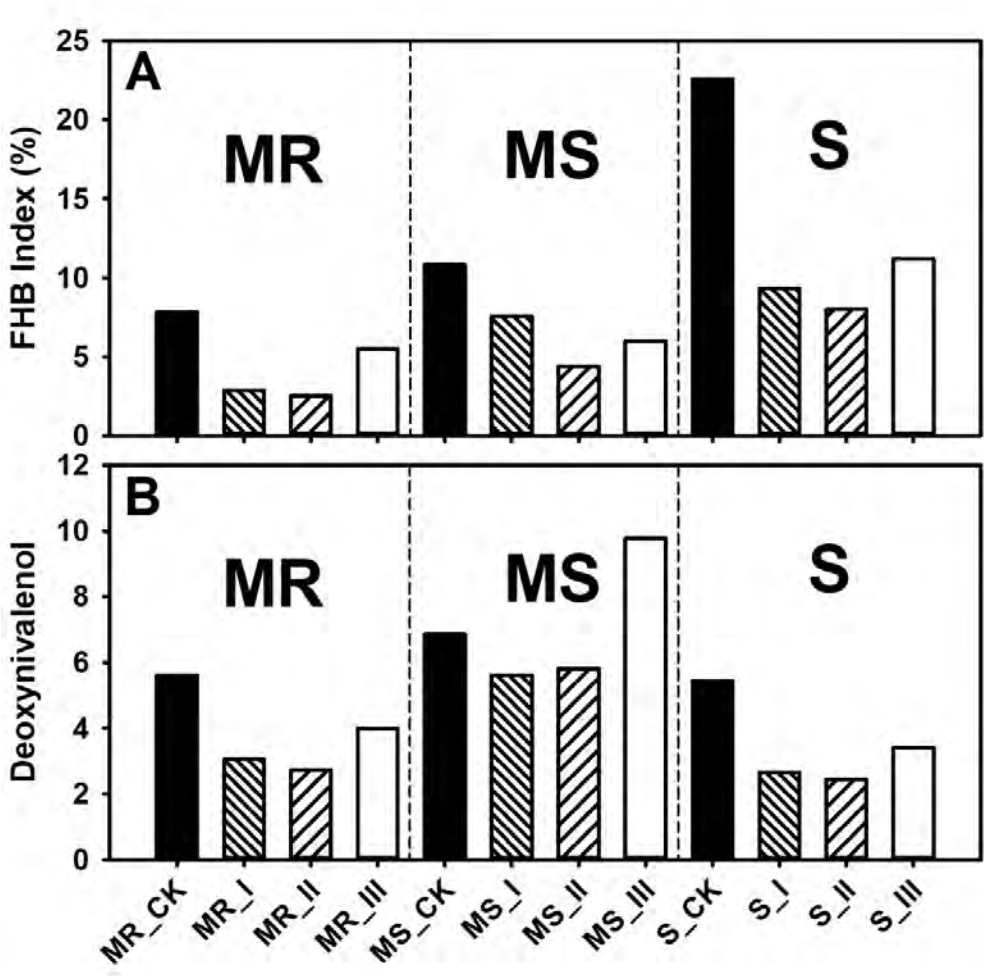

Fig. 2. Mean **A**, Fusarium head blight index and **B**, deoxynivalenol grain contamination for different fungicide program x cultivar resistance management combinations. **S**, **MS**, and **MR** represent susceptible, moderately susceptible, and moderately resistant, respectively, whereas **CK** = nontreated, **I** = treated with Prosaro (6.5 fl. oz.) at Anthesis, **II** = treated with Miravis Ace (13.7 fl. oz.) at anthesis and **III** = treated with Miravis Ace (13.7 fl. oz.) between Feekes 10.3 (early head emergence) and 10.5 (complete head emergence).

FUSARIUM HEAD BLIGHT MANAGEMENT COORDINATED PROJECT: UNIFORM FUNGICIDE TRIALS 2018-2019

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INTRODUCTION

Miravis Ace®, a new Succinate Dehydrogenase Inhibitor (SDHI; Adepidyn/Pydiflumetofen) + Demethylation Inhibitor (DMI; Propiconazole) premix fungicide, was recently labeled for management of diseases of wheat, barley, and other small grain crops. Preliminary results from a limited number of trials showed that when applied at early anthesis (Feekes 10.5.1) or within the first 6 days after anthesis, Miravis Ace was just as effective as Prosaro® and Caramba® (2,3,4). However, one of the primary questions being asked about Miravis Ace is whether it is just as effective when applied at Feekes 10.3 (early heading). If it is, this will extend the application window to as many as 10 days, allowing greater flexibility in terms of application timing. In addition, having a new, effective fungicide, particularly one of a different chemistry, and a wider application window creates opportunities for evaluating two-treatment fungicide programs for FHB and DON management. **The objective of this study was to compare the efficacy of Miravis Ace when applied at, before, or after anthesis, or sequentially with a DMI fungicide to that of a standard anthesis-only application of Prosaro or Caramba**.

MATERIALS AND METHODS

To accomplish the aforementioned objective, field experiments were conducted in 10 US wheat-growing states in 2018 and 2019. The standard

protocol consisted of the application of fungicide treatments (**Table 1**) to plots of a susceptible cultivar. The experimental design was a randomized complete block, with at least 4 replicate blocks. In all experiments, plots were artificially inoculated with either *F. graminearum*-colonized grain spawn (5) or a spore suspension of the fungus applied approximately 24-36 h after anthesis. Plots were mist-irrigated during and shortly after anthesis in some experiments to enhance inoculum production and infection. FHB index (IND) was rated or calculated as previously described (1,6) on 60-100 spikes per plot at approximately Feekes growth stage 11.2. Grain was harvested and samples were sent to a USWBSI-supported laboratory for mycotoxin analysis. Linear mixed models (multi-location) were fitted to the pooled arcsine square root-transformed IND and log-transformed DON data to evaluate treatment effects. Overall percent IND and DON control/reduction relative to the check was also estimated as a measure of efficacy.

Table 1. The following treatments were randomly assigned to experimental units. All fungicide treatments were applied along with a nonionic surfactant.

	Treatment - product, rate and timing
Core	
1	Untreated check
2	Prosaro at 6.5 fl oz/A at anthesis
3	Caramba at 13.5 fl oz/A at anthesis
4	Miravis Ace at 13.7 fl oz/A at Feekes 10.3
5	Miravis Ace 13.7 fl oz/A at Anthesis
6	Miravis Ace at 13.7 fl. oz. at anthesis followed by Prosaro at 6.5 fl. oz. at 4-6 days after
7	Miravis Ace at 13.7 fl. oz. at anthesis followed by Caramba at 13.5 fl. oz. at 4-6 days after
Optional	
8	Miravis Ace at 13.7 fl. oz. at anthesis followed by tebuconazole at 4 fl. oz. 4-6 days after
9	Miravis Ace at 13.7 fl. oz. at 4-6 days after anthesis
10	Prosaro at 6.5 fl oz at 4-6 days after anthesis

RESULTS AND DISCUSSION

Mean Fusarium head blight index (IND) and deoxynivalenol (DON) contamination data from 26 environments (trial x state x year combinations), representing different wheat market classes, are summarized for different fungicide treatments in Figure 1 and 2. Plot-level mean index ranged from 0 to 68% and DON from 0.16 to 39 ppm. For both responses, the nontreated check has the highest over means, whereas treatments that consisted of an early anthesis (Feekes 10.5.1) application of Miravis Ace followed by an application of Prosaro, Caramba, or tebuconazole 4-6 days later had the lowest means (**Fig. 1** and **2**).

FHB index

All treatments resulted in significantly lower mean FHB IND (on the arcsine square root-transformed scale) than the nontreated check. Treatments applied at anthesis reduced mean IND by 51 (Caramba) to 66% (Miravis Ace) relative to the check, whereas those consisting of sequential applications of Miravis Ace and a DMI reduced the mean by 73 (Miravis Ace followed by Prosaro) to 88% (Miravis Ace followed by tebuconazole). Differences between pairs of anthesis-applied (Feekes 10.5.1) treatments were not statistically significant. Similarly, differences between treatments applied at Feekes 10.5.1 and Miravis Ace applied at early

heading were not statistically significant. On the other hand, treatments with sequential applications (Miravis Ace followed by a DMI) resulted in significantly lower mean IND than treatments with a single application.

Deoxynivalenol

All treatments resulted in significantly lower mean DON contamination of grain (on the transformed scale) than the nontreated check. All treatments that included an application at or within the first 6 days after early anthesis resulted in significantly lower mean DON than the early application of Miravis Ace. Among the treatments applied at early anthesis alone, Miravis Ace resulted in the lowest mean DON; differences between pairs of log-transferred means were statistically significant for Miravis Ace vs Prosaro and Miravis Ace vs Caramba. As was the case with IND, treatments with sequential applications (Miravis Ace followed by a DMI) resulted in significantly lower mean DON than treatments with a single application. Anthesis-only treatments reduced DON by 31 to 44% and sequentially applied treatments reduced the toxin by 56%, compared to only 9% with the Feekes 10.3-5 Miravis Ace treatment.

As additional data become available, a more complete set of analyses will be performed. However, the results summarized herein suggest that while a Feekes 10.3-5 application of Miravis Ace may suppress FHB IND to levels comparable to, or even better than, those achieved with an anthesis application, such an early application is considerably less effective than a single anthesis application in terms of DON suppression.

ACKNOWLEDGEMENTS AND DISCLAIMER

This material is based upon work supported by the U.S. Department of Agriculture, under Agreement Nos. 59-0206-8-187, 59-0206-8-195, 59-0206-9-120, 59-0206-6-008, 59-0206-5-007 & 58-6070-8-019, 59-0206-8-192, 59-0206-8-189, 59-0206-9-112, 59-0206-8-190, 59-0206-6-015, 59-0206-4-016 & 59-0206-9-117, 59-0206-8-210, 59-0206-4-012 & 59-0206-8-199, 59-0206-8-211, 58-2050-8-013, 59-0206-6-010, 59-0206-6-012, 59-0206-9-123, 59-0206-6-014, and 59-0206-6-009. 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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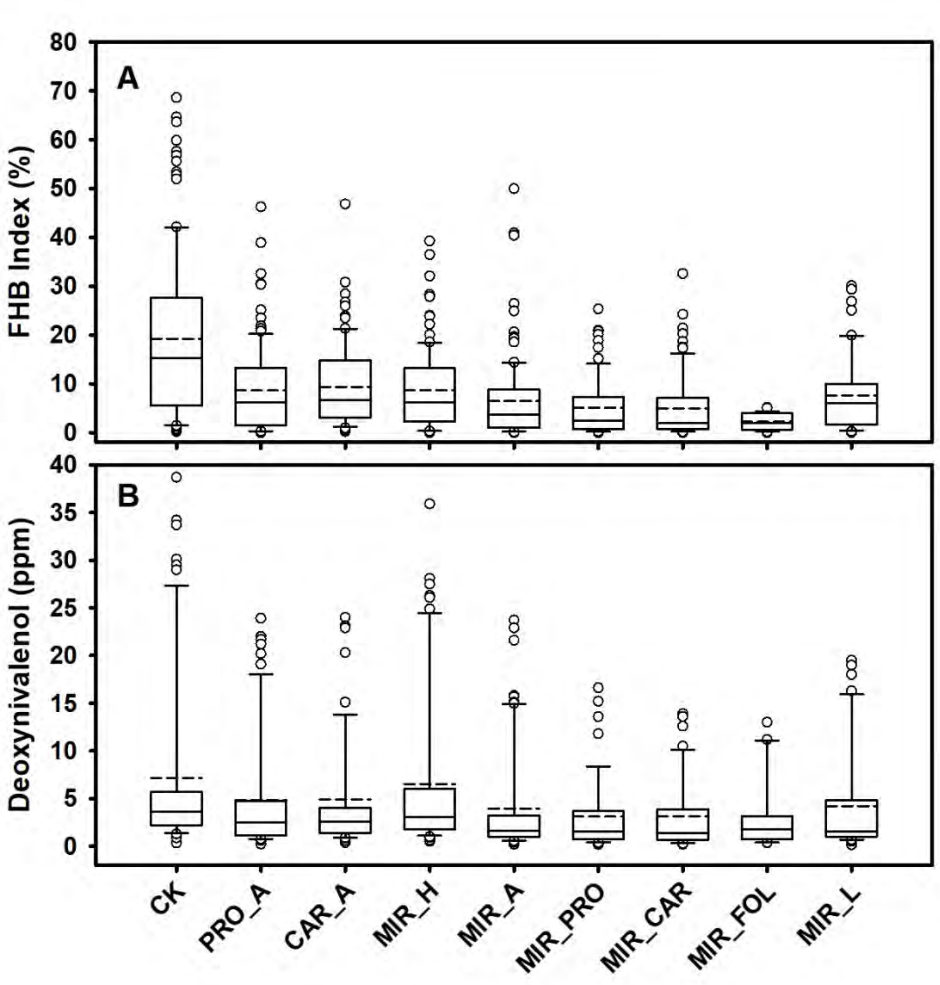

Fig. 1. Boxplots showing the distribution of **A**, mean Fusarium head blight index and **B**, deoxynivalenol grain contamination for different fungicide treatments. **PRO_A** = Prosaro at 6.5 fl. oz applied at anthesis, **CAR_A** = Caramba at 13.5 fl. oz applied at anthesis, **MIR_H** = Miravis Ace at 13.7 fl. oz applied at Feekes 10.3-5, **MIR_A** = Miravis Ace at 13.7 fl. oz applied at anthesis, **MIR_PRO** = Miravis Ace at anthesis followed by Prosaro 4-6 days later, **MIR_CAR** = Miravis Ace at anthesis followed by Caramba 4-6 days later, **MIR_FOL** = Miravis Ace at anthesis followed by Tebuconazole (4 fl. oz) 4-6 days later, and **MIR_L** = Miravis Ace applied at 4-6 days after anthesis.

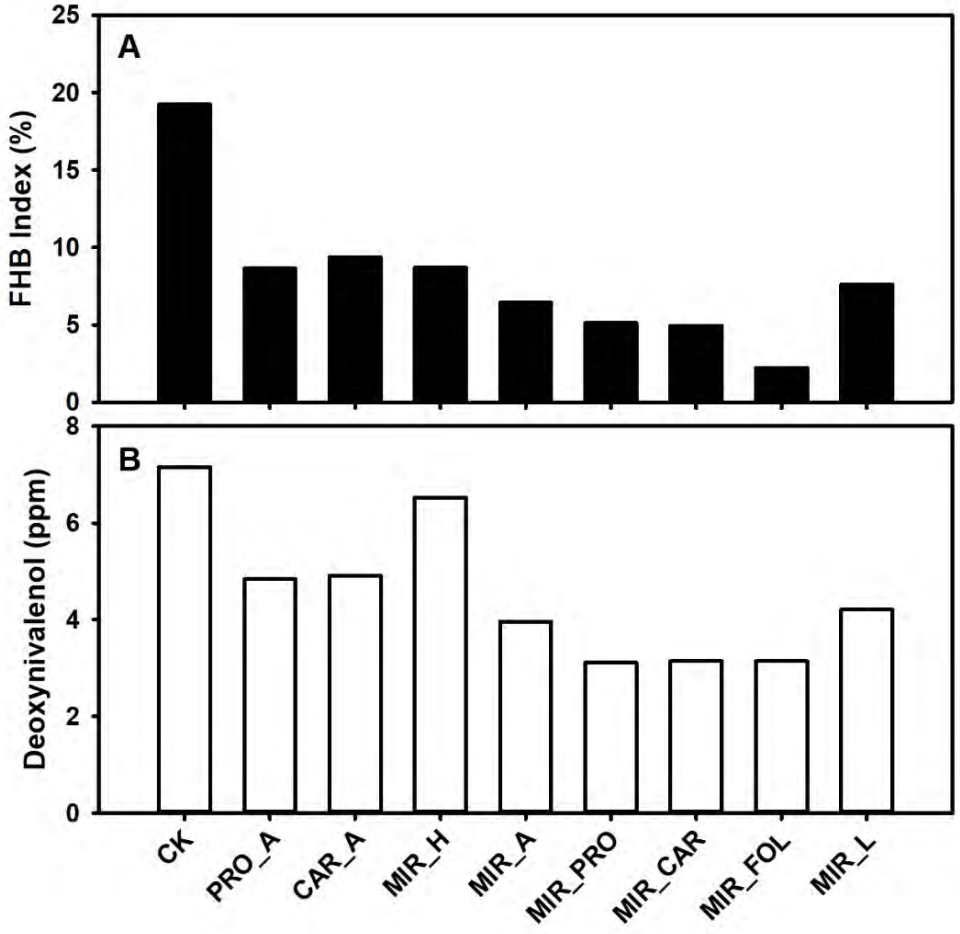

Fig 2. Mean **A**, Fusarium head blight index and **B**, deoxynivalenol grain contamination for different fungicide treatments. **PRO_A** = Prosaro at 6.5 fl. oz applied at anthesis, **CAR_A** = Caramba at 13.5 fl. oz applied at anthesis, **MIR_H** = Miravis Ace at 13.7 fl. oz applied at Feekes 10.3-5, **MIR_A** = Miravis Ace at 13.7 fl. oz applied at anthesis, **MIR_PRO** = Miravis Ace at anthesis followed by Prosaro 4-6 days later, **MIR_CAR** = Miravis Ace at anthesis followed by Caramba 4-6 days later, **MIR_FOL** = Miravis Ace at anthesis followed by Tebuconazole (4 fl. oz) 4-6 days later, and **MIR_L** = Miravis Ace applied at 4-6 days after anthesis.

CAN AGRONOMIC PRACTICES REDUCE DON?

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ABSTRACT

The most common agronomic practices to reduce the risk of deoxynivalenol (DON) contamination in wheat are to utilize moderately resistant wheat cultivars and to apply a fungicide at beginning flowering (Feekes 10.5.1; Zadoks 60). However, there are certain situations in which the wheat crop does not flower uniformly. This results in questions of when to apply the fungicide and what level of efficacy is achieved. To determine whether there are additional agronomic practices that can implemented to increase the uniformity of wheat heading and flowering and reduce DON contamination, seeding rate and in-furrow phosphorus fertilizer were examined. Field trials were established in the fall of 2016, 2017, and 2018 at the University of Kentucky Research and Education Center on two soil types: Crider silt loam (Typic Paledalf) and a Zanesville silt loam (Oxyaquic Fraguidalf). The experimental design was a randomized complete block with five replications and two environments. One environment was a mist-irrigated Fusarium head blight nursery that was inoculated with *Fusarium graminearum*-infested corn; the second environment was a non-inoculated control. The treatments included two seeding rates (377 and 603 pure live seed m^{-2}); two cultivars (Pembroke 2016 with moderate resistance to *F. graminearum* and Pioneer 26R53 with moderate susceptibility to *F. graminearum*); and two in-furrow phosphorus treatments (0 kg P_2O_5 $hectare^{-1}$ and 47 kg P_2O_5 $hectare^{-1}$). In the spring of 2018 and 2019, flowering uniformity was determined by flagging a one meter length of a single row and measuring the date of the following growth stages for all main stems and primary tillers within that area: heading (Feekes 10.5; Zadoks 58), beginning flowering (Feekes 10.5.1; Zadoks 60), one-half flowering complete (Feekes 10.5.2; Zadoks 64) and full flowering (Feekes 10.5.3; Zadoks 68). For all three years, *F. graminearum* incidence, severity, and index, *Fusarium* damaged kernels (FDK), DON contamination, grain yield, and test weight were measured. Data analyses, which include all three years, are on-going. Preliminary analyses of the 2017 and 2018 data suggest that the in-furrow phosphorus application did not impact ($P \geq 0.05$) FDK, DON contamination, or test weight. However, it appears that differences ($P < 0.05$) for grain yield were found between 2017 and 2018. In 2017, which had a warm, mild winter, in-furrow phosphorus application did not affect ($P \geq 0.05$) grain yield. In 2018 and 2019, which was a more typical cool winters, in-furrow phosphorus application increased ($P < 0.05$) grain yield. More detailed analyses of all three years will provide a better understanding of whether additional agronomic practices can reduce DON contamination of wheat.

ACKNOWLEDGEMENT

Funding provided by the Kentucky Small Grains Promotion Council. This work could not have been completed without the assistance of Conner Raymond and the following undergraduate interns:Kelly Eicher, Bradley James, Bailey Webster, Carrie Ann Followell, Mary Grace Jackson, Hunter Adams, Curtis Bradley, Jacob Foote, and Gracie Harper.

DEOXYNIVALENOL CONTAMINATION AND *FUSARIUM GRAMINEARUM* INFECTED WHEAT KERNELS FROM VARIOUS PRODUCTION PRACTICES

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ABSTRACT

Deoxynivalenol (DON) contamination caused by *Fusarium graminearum* is often a great concern to soft red winter wheat millers and producers. A field study was established to investigate intensive agronomic practices to decrease DON levels in harvested grain, in addition to current practices of planting cultivars with moderate resistance to *F. graminearum* infection and applying efficacious fungicides at beginning anthesis (Feekes 10.5.1). A kernel plating study was also conducted to evaluate the effect of harvest timing and phosphorus fertilization on the percentage of healthy looking kernels infected with *F. graminearum*. The objectives of this study were to determine the effect of DON contamination and percentage of healthy looking kernels infected with *F. graminearum* from i) harvesting grain at different moisture concentrations and ii) in-furrow application of phosphorus at planting. Field trials were established in Princeton KY in the fall of 2016 and 2017. Treatments included two planting dates (mid-October and mid-November), two harvest timings (20 to 22% grain moisture or 13 to 15% grain moisture), two soft red winter wheat cultivars (moderately resistant to FHB and moderately susceptible to FHB) and two phosphorus applications applied in-furrow at planting (0 kg ha^{-1} or 47 kg ha^{-1} P_2O_5). The plating study was conducted as a randomized complete block design. Twenty healthy looking kernels from each plot were surface sterilized with a 90% ethanol solution, followed by a 10% bleach solution, and finally a sterile water wash before being plated on pentachloronitrobenzene (PCNB) agar plates used to select for *F. graminearum*. This process was replicated six times to total 120 seeds evaluated from each plot. Seed germination was measured five days after plating while percentage of *F. graminearum* infected seeds were measured five and six days after plating. Deoxynivalenol contamination was measured using Environlogix Mycotoxin Test Strips (Portland, ME) on the harvested wheat grain. Results from this study will help understand if additional management practices could reduce DON contamination.

ACKNOWLEDGEMENT

Funding provided by the Kentucky Small Grain Promotion Council. This work could not have been complete without the help of Bella Usenza, Curtis Bradley, Conner Raymond, Hunter Adams, Jacob Foote, Gracie Harper, Bradley James, Carrie Ann Followell, Mary Grace Jackson, Kelly Eicher, and Bailey Webster.

EVALUATION OF DIFFERENT SPRAY NOZZLE SYSTEMS AND GROUND SPEEDS FOR COVERAGE OF SIMULATED WHEAT HEADS AND EFFICACY AGAINST FUSARIUM HEAD BLIGHT AND DEOXYNIVALENOL IN WINTER WHEAT

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ABSTRACT

One of the primary practices used to manage Fusarium head blight (FHB) and deoxynivalenol (DON) contamination in harvested grain is a fungicide application to wheat heads. Because of the vertical orientation of wheat heads, achieving a high level of coverage with a ground rig sprayer can be difficult. Fungicide movement within wheat heads generally is considered to be minimal; therefore, achieving a greater level of wheat head coverage with a fungicide could result into improved efficacy. A field research trial was conducted near Princeton, KY in 2017, 2018, and 2019 with an objective to compare different spray nozzle systems and different speeds of a ground rig sprayer for coverage of a simulated wheat head. Nozzles evaluated were AI 3070, AI TTJ60, and TTJ60, which were all manufactured by TeeJet Technologies (Wheaton, IL). The AI TTJ60 and TTJ60 nozzles were evaluated at different angles relative to the wheat heads, and all nozzles were evaluated at ground rig speeds of 8, 12, and 15 mph. Simulated wheat heads that were the approximate same circumference and height as wheat heads were constructed from water-sensitive cards wrapped around tubes and mounted on rods in a wheat field. Water was applied through the sprayer, and cards were collected, digitally scanned, and evaluated using image analysis software. Prior to the 2018 growing season, the ground rig sprayer was modified to spray water out of one side of the spray boom and Prosaro® fungicide (Bayer CropScience, St. Louis, MO) out the other side. This allowed for evaluation of the different nozzles and ground speeds for management of FHB and DON in the subsequent two growing seasons. The main effect of nozzle was significant for head coverage and DON reduction, but not for FHB reduction. The main effect of ground rig speed and the interactive treatment of nozzle*speed were not significant for coverage, FHB, or DON reduction. The AI 3070 nozzle and the TTJ60 nozzle oriented as recommended by the manufacturer provided the greatest head coverage (12.3% and 12.2%, respectively), which were significantly ($P \leq 0.05$) better than the other nozzle treatments. Similarly, the TTJ60 nozzle oriented as recommended by the manufacturer provided the greatest reduction in DON (77%), which was significantly better than all other treatments except the AI 3070 nozzle (66%). Although more analyses are needed to better understand the relationship between coverage and efficacy, the results presented herein provide evidence that variables associated with spray nozzles and ground rig sprayers can be adjusted to optimize fungicide efficacy.

ACKNOWLEDGEMENT

This material is based upon work supported by the Kentucky Small Grain Growers Association.

FIELD EVALUATION OF PYDIFLUMETOFEN + PROPICONAZOLE AND PROTHIOCONAZOLE + TEBUCONAZOLE EFFICACY ON *FUSARIUM GRAMINEARUM* IN SOUTH DAKOTA

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ABSTRACT

A triozole based fungicide; Prosaro® (Bayer Crops Science) has been one of the major fungicides used for managing Fusarium head blight (FHB), mainly caused by *Fusarium graminearum*. Prosaro is a combination of prothioconazole + tebuconazole both of which are involved in inhibition of sterol biosynthesis, which compromises fungal cell membrane composition and functionality leading to suppressed hyphal growth and eventual cell death. Recently, Miravis Ace (SYNGENTA) was introduced for FHB management. Miravis Ace® combines the sterol biosynthesis inhibition capability of propiconazole and the ability of pydiflumetofen to disrupt fungal cell respiration through succinate dehydrogenase inhibition. The two fungicides were applied to three cultivars namely, Brick (moderate resistance), Prevail (moderate resistance) and Samson (susceptible) in an FHB management field trial conducted in South Dakota for two seasons. The objective was to assess the efficacy of Prosaro and Miravis Ace in FHB management and impact of time of application for Miravis Ace. All plots were inoculated *F. graminearum* infested corn spawn at Feekes 9 and misted to maintain wet conditions. Both fungicides were applied at anthesis; Miravis Ace was also applied at heading based on product label recommended. Disease incidence, severity, index, *Fusarium* damaged kernels (FDK) and deoxynivalenol (DON) were collected and analyzed using the generalized linear model applicable link functions. No significant differences were observed between Prosaro @ 6.7fl oz/ac and Miravis Ace @ 13.7fl oz/ac applied at anthesis across cultivars and FHB metrics except for DON concentration in the susceptible cultivar Samson (p=0.0008). In terms of application timing for Miravis Ace, spraying at heading generally resulted in higher FHB incidence, severity, index, FDK and DON compared to anthesis timing although the differences were mostly statistically insignificant. However, FHB severity, index and DON which were significant ($p \leq 0.05$) for Samson. These preliminary observations suggest that Prosaro and Miravis Ace are efficient for FHB management, particularly when applied at anthesis.

ACKNOWLEDGEMENT AND DISCLAIMER

This material is based upon work supported by the U.S. Department of Agriculture, under Agreement No 59-0206-8-192. 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.

MANAGEMENT OF FHB AND DON USING FUNGICIDES AND HOST RESISTANCE IN HARD SPRING WHEAT IN IDAHO

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ABSTRACT

FHB Management Coordinated Project (MGMT_CP) comprised of the Integrated Management (IM) and Uniform Regional Trial (UFT) projects were conducted at the University of Idaho Aberdeen Research and Extension Center for two consecutive years (2018 and 2019) to evaluate FHB and DON management strategies with emphasis on Miravis® Ace (pydiflumetofen + propiconazole). Here we report the results of the 2019 trial. IM and UFT experimental plots (5 × 9.3 ft) were arranged in a randomized complete block design with 4 replicates. Plots were inoculated with Fusarium graminearum macroconidial suspensions (100,000 spores/ml) 24-36 hours following the anthesis application of fungicides. For the IM trial, we evaluated the integrated effects of fungicides and genetic resistance in hard spring wheat. The IM trial had a split-plot arrangement, with varieties (Kelse, IDO1602S, LCS Star and Rollag) as main plots and fungicide treatments as sub-plots. Miravis Ace treatments applied at heading or at anthesis were compared with both inoculated and non-inoculated Prosaro® treatments and untreated controls. For the UFT trial, a single susceptible variety (Jefferson) was used to compare the efficacy of single application of Miravis Ace at heading or anthesis, to the standard applications of Prosaro or Caramba® or in combination 7 days post-anthesis. FHB incidence and index (plot severity) were determined from 100 randomly chosen heads per plot at soft dough. Plots were harvested with a small plot combine and yield (bu/A) was determined with the HarvestMaster system. Subsamples were measured for test weight and *Fusarium*-damaged kernels (FDK, %) post-harvest. Data were analyzed using the GLIMMIX procedure in SAS 9.4. FHB establishment in the two trial fields was moderate with the highest mean disease incidence of 28 and 26.5% in the IM and UFT trials, respectively. In the IM trial, there were significant differences (α=0.05) in FHB incidence and index (P<0.0001) among varieties and fungicides, respectively. Interaction effects between varieties and fungicides were significant for FHB incidence (P=0.0009) and index (P<0.0001). The mean highest and lowest FHB incidence (20.6 and 0.6%) and index (13.6 and 0.2%) were observed in Kelse and Rollag, respectively. Among fungicide treatments, Miravis Ace applied at anthesis was the most effective with the lowest FHB incidence and index of 5.9 and 2.6%, respectively. The untreated plots had the highest incidence (18.4%) and index (12.6%). Both varieties and fungicides had significant effects on DON content (P=0.0005 for varieties and P<0.0001 for fungicides), test weight (P<0.0001), FDK (P<0.0001), and yield (P=0.039 for varieties and P=0.0303 for fungicides). In the UFT trial, fungicide treatments had significant effects on FHB incidence (P<0.0001), index (P<0.0001), DON content (P<0.0001), test weight (P=0.0118) and FDK (P=0.0026). Combined applications of Miravis Ace at anthesis and Caramba at post-anthesis resulted in the lowest FHB incidence (1.5%), index (0.75%) and DON (0.1 ppm). The untreated check had the highest FHB incidence (26.5%), index (22.4%) and DON (1.0 ppm).

ACKNOWLEDGEMENT AND DISCLAIMER

This material is based upon work supported by the U.S. Department of Agriculture, under Agreement No. 58-2050-8-013. 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.

FOOD SAFETY AND TOXICOLOGY

STEPS FOR APPROVING AND VALIDATING COMMERCIAL MYCOTOXIN TEST KITS

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ABSTRACT

Since the early 1990's the United States Department of Agriculture's Federal Grain Inspection Service (FGIS) has provided official mycotoxin testing services throughout the United States for domestic and export grains, oilseeds, and processed-grain commodities. Official testing services are available for aflatoxins, deoxynivalenol, fumonisins, ochratoxin A, and zearalenone. Testing at field locations requires rapid, simple, inexpensive, and accurate methods to effectively assess the quality of U.S. grain. An important part of quality assurance for official mycotoxin testing is the Mycotoxin Test Kit Evaluation (MTKE) Program, through which FGIS evaluates and certifies the conformance of quantitative rapid mycotoxin test methods to specific criteria. Rapid test kits, which are certified by FGIS, can only be used for official mycotoxin testing. FGIS establishes design criteria and performance specifications that mycotoxin test kits must meet to be considered for use in official inspection. These criteria are updated time to time to accommodate the recommendations of industry leaders and the market needs. The latest update has been effective from June 01, 2018. Test kit manufacturers may submit a test kit for evaluation by FGIS staff. Submission packets are reviewed by FGIS staff in order of receipt. The submission packet must include all documentation needed to demonstrate that the test kit meets the established FGIS design criteria and performance specifications. Incomplete submissions, submission not conforming to FGIS requirements, and submissions containing excessive errors can be rejected. If the submission is accepted, arrangements for analyst training and FGIS performance verification will be made with the applicant. The FGIS analysts that conduct performance verification testing will be trained in the operation of the test kit by the applicant. After evaluation of the test kit in the FGIS laboratory, if the test kit meets all design and performance requirements, FGIS issues a Certificate of Conformance (COC) stating that the test kit has met the criteria. Upon issuance of the official test kit instructions, the test kit may be used for official inspection. The COC will be valid for three years from its date of issuance. Renewal of a COC requires a full submission and evaluation. If the test kit fails to meet all of the criteria specified herein, the test kit can be resubmitted. When the test kit is resubmitted, the applicant must state the corrective action that was taken to bring the test kit into conformance with FGIS requirements.

ACKNOWLEDGEMENT AND DISCLAIMER

This material is based upon work supported by the U.S. Department of Agriculture.

FUNGAL LOCALIZATION AND MYCOTOXIN PRODUCTION IN *FUSARIUM* INFECTED GRAIN AND MALT KERNELS

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ABSTRACT

Some FHB infected grain samples, including barley, wheat, rye, and triticale had been identified as producing large amounts of DON during malting. Objectives of the current study were to investigate the distribution of DON levels between single kernels of each grain and malt, and to associate mycotoxin production with the location of *Fusarium* hyphae. Average DON levels were found to increase during malting of the selected samples: Barley 0.6 to 5.4 μg/g, wheat <0.2 to 2.1 μg/g, rye <0.5 to 9.3 μg/g, and triticale 0.9 to 23.8 μg/g. However, there was considerable variation when single kernels were examined, and in general around 80% of all unmalted grains had no detectable DON or <0.2 μg/g. A smaller number of grains had high levels: up to 12.1 μg/g (barley), 35.3 μg/g (wheat), 20.5 μg/g (rye), and 220.0 μg/g (triticale). Following malting, over 70% of the kernels had DON levels above 1.0 μg/g, which suggests cross-contamination or growth of existing *Fusarium.* The DON levels on malt kernels ranged from <0.2 μg/g up to 219.2 μg/g in malted barley, 365.6 μg/g in malted wheat, 437.0 μg/g in malted rye, and 343.7 μg/g in malted triticale. In the final phase of this work single kernels of each grain and malted were selected, and sectioned into 3 cross-sections. The basal portion was used for mycotoxin analysis, and the others for hyphal localization by scanning electron microscopy and confocal laser scanning microscopy. With barley kernels (DON levels <10 μg/g) only small amounts of hyphae were observed in the husk, spongy parenchyma, furrow vascular bundle, and/or cavity tissues. However, with malted barley kernels (DON > 100 μg/g), heavy infection of hyphae was found in internal tissues, including the aleurone layer, furrow crease and cavity, endosperm transfer layer, dorsal vein, embryo, and even starchy endosperm. With wheat, rye, and triticale grains, it was relatively common to find the internal infection, but it was thinner in the kernels with lower DON levels (<10 μg/g). However, when grain and malt kernels from these grains with higher DON (>100 μg/g) were examines, the hyphal growth was extremely thick in the internal tissues. Metagenomic analysis of dehusked samples showed that *Fusarium graminearum* was the predominant fungus associated with the interior of these grain and malt kernels.

ACKNOWLEDGEMENT AND DISCLAIMER

This material is based upon work supported by the U.S. Department of Agriculture under Agreement No. 59-0206-9-064, and also by the American Society of Brewing Chemists (ASBC). The USDA support 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 and ASBC. The authors acknowledge NDSU Advanced Imaging & Microscopy Laboratory and Electron Microscopy Center for assistance with this work.

GENE DISCOVERY AND ENGINEERING RESISTANCE

HIGH VS. LOW DON ACCUMULATING LINES OF BARLEY EVALUATED BY DIP INOCULATION OF FUSARIUM HEAD BLIGHT

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ABSTRACT

Fusarium head blight (FHB) is an important disease of barley and the contamination of grain with the mycotoxin deoxynivalenol (DON) causes adverse health and severe economic consequences. Evaluating resistance in the field is complicated by environmental factors. As a result, determination of host resistance factors has been difficult. Green house and growth chamber experiments could ameliorate these confounding factors given the right strategy for inoculation. Here we developed a simple dip inoculation method for high-throughput evaluation of FHB resistance in barley using spore concentrations to separate the effects of type I resistance against initial infection from type V resistance to DON accumulation. Fifteen barley lines of genetically diverse backgrounds were evaluated for visual severity and accumulation of DON when inoculated with *F. graminearum*. Higher concentrations of conidia equated to better differentiation of lines based on accumulation of DON. This robust protocol for dip inoculation will enhance breeding and screening for DON resistance to FHB in barley. From the fifteen barley lines tested, two high-DON and two low-DON-accumulating barley lines were identified that are parents of extant bi-parental mapping populations. Transcriptomic responses to wild type *F. graminearum* and Δtri5, at 1 and 3 days post-inoculation, were assessed for these parent lines. Low DON lines had more genes expressing in response to pathogen infections than the high DON lines. All lines had more genes responding to the DON-deficient Δtri5 mutant than to wild type, suggesting that DON suppresses pathogen response. Responsive genes in the low DON barley lines include UDP-glycosyltransferases, pathogenesis-related proteins, and chitinases. These genes are potential breeding targets for future work.

ACKNOWLEDGMENTS AND DISCLAIMERS

This material is based upon work supported by the U.S. Department of Agriculture, Improvement of Barley and Oat for Enhanced Productivity, Quality, and Stress Resistance, Project Number 2050-21000-034-00-D. This is a cooperative project with the U.S. Wheat & Barley Scab Initiative and is supported by projects FY16-BR-013 and FY16-BA-005.

THE UDP-GLUCOSYL TRANSFERASE UGT13248 CATALYZES DON TO DON-3-GLUCOSE CONVERSION IN BARLEY AND AFFECTS DON ACCUMULATION IN SPIKES

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ABSTRACT

Fusarium head blight (FHB) of *Hordeum vulgare* (barley), is primarily caused by the fungal pathogen *Fusarium graminearum*. FHB leads to yield losses and reduction in grain quality mainly by accumulation of trichothecene mycotoxins, e.g. deoxynivalenol (DON). Glycosylation of DON to DON-3-glucose (D3G) is thought to be catalyzed by UDP-glucosyl transferases (UGTs). Barley UGT13248, was previously shown to convert DON to D3G in yeast, *Arabidopsis* and wheat. In wheat, expression of UGT13248 decreased disease severity of FHB. We identified two TILLING lines with amino acid changes close to the UDP-sugar binding site of UGT13248 in the Morex background, UGT13248 (T368I) and UGT13248 (H369Y). Roots of these plants showed increased sensitivity to DON-containing media. Additionally, DON to D3G conversion was strongly, reduced in UGT13248 (T368I) and UGT13248 (H369Y) spikes compared to controls. Further, field experiments showed increased FHB disease severity in some environments and reduced D3G production. Our data suggest that barley UGT13248 catalyzes the majority of DON 3-glycosylation in barley.

ACKNOWLEDGEMENT AND DISCLAIMER

This material is based upon work supported by the U.S. Department of Agriculture, under Agreement No. 59-0206-8-203. 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.

IMPROVEMENT OF FHB RESISTANCE IN HARD WINTER WHEAT USING BSMV-MEDIATED CRISPR/CAS9 GENE EDITING SYSTEM

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ABSTRACT

Fusarium head blight (FHB) is one of the most destructive wheat diseases worldwide. In the past several decades, severe and frequent FHB epidemics have caused significantly reduction in wheat grain yield and quality, which threatens world food security. Improving FHB resistance in wheat cultivars can minimize FHB damage. To create new sources of resistance, disabling susceptibility genes (S-genes) is a promising strategy. Recently developed CRISPR/Cas9 gene-editing technology can precisely knock out the targeted S-genes to validate gene functions and create new sources of resistance for breeding. We have recently cloned *Fhb1*, an FHB resistance gene with a major effect on type II resistance from Chinese cultivar sources, named as a histidine-rich calcium binding protein (*TaHRC*). The wide type allele of *TaHRC* conditions FHB susceptibility (*TaHRC_S*), whereas the mutant allele with a deletion in the start codon region of the gene conditions FHB resistance. Previously, we developed a novel *Barley stripe mosaic virus* (BSMV)-mediated CRISPR/Cas9 gene-editing system and successfully used the system to knock out *TaHRC-S* allele in a spring wheat variety 'Bobwhite', and the results validated that *TaHRC* regulated *Fhb1* resistance and the BSMV-mediated CRISPR/Cas9 system was applicable for gene editing in hexaploidy wheat. In this study, we selected a US hard winter wheat variety 'Everest' that carries *TaHRC_S* allele but with very low transformation efficiency for BSMV-mediated gene editing. We made a cross using the Cas9-overexpressed (Cas9-OE) 'Bobwhite' that developed from a previous project to transfer the *Cas9* gene into 'Everest' background. Using the BSMV-mediated gene-editing system, we knocked out *TaHRC_S* in the Cas9-OE 'Everest' and found that the edited 'Everest' plants showed a significant increase in FHB resistance. The results confirmed that loss-of-function mutation in *TaHRC_S* can improve FHB resistance in a hard winter wheat genetic background, and the BSMV-mediated CRISPR/Cas9 gene editing system is effective for creating new sources of disease resistance and for validating gene functions in gene cloning studies.

ACKNOWLEDGEMENT AND DISCLAIMER

This material is based upon work supported by the U.S. Department of Agriculture, under ARS Agreement No. 59-0206-6-007. This is a cooperative project with the U.S. Wheat & Barley Scab Initiative (Project ID Nos. FY18-BA-045 and FY18-TR-007). 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.

TESTING TRANSGENIC SPRING WHEAT AND BARLEY LINES FOR REACTION TO FUSARIUM HEAD BLIGHT: 2019 FIELD NURSERY REPORT

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ABSTRACT

The 2019 field screening nursery consisted of 67 wheat and 8 barley entries evaluated in adjacent experiments. Entries within each experiment were arranged in a randomized complete block design with four replications in a field located at UMore Park, Rosemount MN. Trial entries and untransformed parental controls* were submitted by the University of Minnesota (20 wheat lines + Linkert* and Rollag* and 8 barley lines + Rasmusson*), Rutgers University (4 wheat lines + RB07*), University of North Texas (9 wheat lines + Bobwhite*) and the USDA-ARS (8 wheat lines + CB037*). Lines with known reactions to Fusarium head blight (FHB) were also included as checks. The wheat checks included were the moderately resistant cultivars Alsen, Linkert, Rollag, and Sumai 3 and the susceptible cultivar Wheaton. The barley checks were the moderately resistant cultivar Quest and the susceptible cultivar Stander. Individual plots were 2.43 m long single rows. The trial was planted on May 17, 2019. All plots were inoculated twice. The first inoculations were applied between July 1 and 10 to coincide with anthesis for wheat and head emergence for barley. The second inoculation was applied three days after the initial inoculation (d.a.i.) for each plot with the last inoculations conducted on July 15. The inoculum was a composite of 26 *F. graminearum* isolates, applied at a concentration of 100,000 macroconidia. ml^{-1} with Tween 20 (polysorbate) added at 2.5 $ml.L^{-1}$ as a wetting agent. The inoculum was applied using a CO_2-powered backpack sprayer fitted with a SS8003 TeeJet spray nozzle with an output of 10 $ml.sec^{-1}$ at a working pressure of 275 kPa. Mist-irrigation was applied from the first inoculation on June 29 through July 22 to facilitate FHB development. FHB incidence and severity were assessed visually 17-19 d.a.i. on 20 arbitrarily selected heads per plot. FHB incidence was determined by the percentage of spikes with visually symptomatic spikelets of the 20 heads observed. FHB severity was determined as the percentage symptomatic spikelets of the total of all spikelets observed. Plots were hand harvested at maturity; between August 2 and 23, 2019. Approximately forty heads where harvested from each plot, threshed and the seed cleaned by hand. The wheat grain was used to determine the percentage of visually scabby kernels (VSK) and then all samples (wheat and barley) were ground and submitted for deoxynivalenol (DON) analysis. Mean FHB severities for the wheat untransformed parental and/or checks Alsen, Bobwhite, CB037, Linkert, RB07, Rollag, and Sumai 3 were 41%, 61%, 52%, 35%, 39%, 37%, and 34%, respectively. The mean FHB severity for the susceptible wheat check Wheaton was 53%. The mean FHB severity for the untransformed parent barley check Rasmusson was 43% while the barley checks Quest and Stander had a mean FHB severities of 23% and 62%, respectively.

The FHB severity data indicated that resistance was improved in some transformed lines compared to the untransformed checks. The DON data are not yet available, although we expect this data will be included in the poster presented at the forum.

ACKNOWLEDGEMENTS AND DISCLAIMER

We would like to acknowledge Dr. Yanhong Dong for conducting the mycotoxin analyses.

This material is based upon work supported by the U.S. Department of Agriculture, under Agreements No. 59-0206-4-016. 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.

RESPONSE OF WHEAT CONSTITUTIVELY EXPRESSING MONOLIGNOL BIOSYNTHESIS GENES TO FUSARIUM HEAD BLIGHT

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ABSTRACT

The overall goal of this research is to utilize wheat lines constitutively expressing sorghum genes involved in the monolignol biosynthesis pathway, to identify unique sources of resistance to Fusarium head blight (FHB). Toward this goal, we have identified lines with enhanced resistance. We transformed the spring wheat cultivar CB037 to constitutively express one of four sorghum genes: one encoding for the transcription factor, *SbMyb60*, involved in regulation of genes in sorghum monolignol biosynthesis, and the monolignol biosynthetic enzymes coumaroyl shikimate 3-hydroxylase (*SbC3H*), caffeoyl coenzyme A 3-*O*-methyl transferase (*SbCCoAOMT*) and 4-coumarate:CoA ligase (*SbBmr2*). The transgenic lines were assessed for responses to the FHB pathogen, *Fusarium graminearum*, in greenhouse and field trials. For both greenhouse and field artificial inoculations, lines constitutively expressing *SbC3H* or *SbCCoAOMT* exhibited increased resistance to FHB. Lead events (two to four) were grown in a greenhouse and inoculated with *F. graminearum* by spray inoculation, to test for Type I resistance to FHB infection, and single-floret inoculation, to test for Type II resistance to pathogen spread. Area under the disease progress curve (AUDPC) was assessed for each, then grain was allowed to mature and the proportion of *Fusarium* damaged kernels (FDK) was determined. For spray inoculations, the AUDPC for one lead event constitutively expressing *SbC3H* (C3H_885) was significantly less than that for the moderately-susceptible parental genotype, CB037, although FDK was not significantly different. For all other constitutive expression lines, AUDPC and FDK were not significantly different from those of CB037 except for one *SbBmr2* line in which FDK was higher. For single-floret inoculations, lines C3H_885 and CCoAOMT_894_A (constitutively expressing *SbCCoAOMT*) each had an AUDPC significantly less than that for CB037. FDK for C3H_885 was again similar to that of CB037 while FDK for CCoAOMT_894_A was not significantly different from that of the moderately-resistant check Sumai 3. In general, *SbBmr2* and *SbMyb60* constitutive expression lines were susceptible to FHB in greenhouse assays. Field spray inoculations also were performed (UMore Park, Rosemount, MN) during the 2018 season and FHB Incidence and Index (severity), proportion of FDK and DON levels were determined. All lines had similar levels of FHB Incidence with C3H_885 having the lowest. The constitutive expression lines C3H_885 and CCoAOMT_894 (from a different event than CCoAOMT_894_A) had FHB Indices not significantly different from that of Sumai 3. A line with constitutive expression of the SbMyb60 transcription factor, Myb_860, had significantly reduced proportion of FDK as compared with CB037. The DON level of C3H_885 was significantly reduced as compared with that of CB037. All other overexpression lines had FHB Indices, FDK or DON levels similar to or significantly greater than those of CB037. Because constitutive expression of *SbC3H* or *SbCCoAOMT* resulted in wheat lines with increased resistance in both greenhouse and field assessments, these lines provide valuable

materials for future research to identify genes and pathways with altered activities in response to FHB associated with the observed increased resistance.

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.

ENGINEERING GENE-FOR-GENE RESISTANCE TO FUSARIUM HEAD BLIGHT IN WHEAT AND BARLEY

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ABSTRACT

This project focuses on generating novel, new-to-nature disease resistance traits to Fusarium head blight (FHB) (*Fusarium graminearum*). Our approach, known as 'decoy engineering', represents an extension of our work on the *Arabidopsis* resistance protein, RPS5, which mediates recognition of the effector protease AvrPphB by detecting cleavage of one of AvrPphB's targets, PBS1 (Kim et al., 2016). We have recently shown that we can expand the recognition specificity of RPS5 by adding PBS1 'decoys' that function as substrates for other pathogen proteases. Cleavage of these modified PBS1 proteins by pathogen proteases activates RPS5, thereby conferring resistance to multiple pathogens (Kim et al., 2016). Here, we show that, like *Arabidopsis*, barley and wheat each contain a resistance protein that recognizes AvrPphB and contain PBS1 proteins that are cleaved by AvrPphB (Carter et al., 2019). We, therefore, predict that given the functional conservation of AvrPphB recognition in both barley and wheat, it is likely the decoy engineering technology can be extended to these crop plants with the goal of introducing novel disease resistance traits to FHB. Specifically, we propose to modify a *PBS1* orthologous gene in wheat and barley to enable recognition of proteases secreted by *F. graminearum* during infection, as was done in *Arabidopsis* and soybean (Kim et al., 2016; Helm et al., 2019). Using transcriptome data sets generated by Brown et al. (2017) and Dilks et al. (2019), we identified ten candidate proteases from *F. graminearum* whose expression is highly to moderately induced *in planta* compared to when growing *in vitro*. Additionally, these proteases are highly conserved among *F. graminearum* isolates from USA, Brazil, and Australia and each shows a low level of polymorphisms, suggesting these proteases have a functional role during the infection process. Future work will involve identifying the peptide sequences recognized by each of the candidate proteases and subsequently generating the appropriate wheat and barley PBS1 decoy proteins.

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GENETIC ANALYSIS OF FUSARIUM HEAD BLIGHT SEVERITY, MALTING QUALITY AND AGRONOMIC TRAITS IN THE CENTROMERIC REGION OF CHROMOSOME 6H IN BARLEY

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ABSTRACT

Fusarium head blight (FHB) caused by *Fusarium* species has been a serious problem for wheat and barley growers and industries. Genetic studies have identified many quantitative trait loci (QTL) contributing to host resistance to FHB. Mapping studies utilizing Chevron, a six-rowed resistant landrace originated from Switzerland, have consistently detected FHB resistance and DON accumulation QTL in the centromeric region of chromosome 6H. This region is also associated with QTL for grain protein content (GPC), kernel discoloration (KD), net blotch (NB), plant height, heading date and yield. To elucidate the relationship among these correlated trait QTL, two mapping populations were developed. Population Gen10a was derived from Gen2-129, a NIL in the Lacey background carrying Chevron allele for the 6H QTL region (~ 25.0 cM), crossed with Lacey and consisted of 1941 F_2 plants. Genotyping of F_2 with SSR markers resulted in 249 F_4 Gen10 recombinant (r)NILs of which 101 rNILs were genotyped with a 50K SNP chip and selected for phenotyping. The rNILs represented 22 recombinant classes (RCs) with between 1 and 13 individual lines nested within each class. DON accumulation, FHB severity, GPC, physiological maturity, NDVI, stem breakage, KD, heading date, height, adult NB, seedling NB, bacterial leaf streak (BLS) and yield were evaluated in field or greenhouse trials in 2015 and 2016. Significant variation among RCs for various traits were identified and additive allelic effects were most significant near the *HvNAM-1*/*Gpc-1* locus. The second population Gen10b was developed using Lacey crossed with two Gen10 lines derived from Gne10a population which shared an overlapped region of ~ 3.0 cM containing the *Gpc-1* locus. This population of 2,082 F_2 plants was used to further dissect the relationships among DON, GPC, maturity and FHB severity. Recombinants identified from the Gen10b population were genotyped with 34 SNP markers covering the target region (~ 3.0 cM), which resulted in the identification of 37 rNILs representing 12 recombinant classes. Selected homozygous $F_{2:4}$ rNILs were tested for FHB severity, GPC and DON accumulation in field and greenhouse conditions from 2016-2019. Multiple lines showed lower FHB severities than the resistant parents in some environments. Preliminary results suggest that reduced DON accumulation and FHB severity are associated with the *Gpc-1* locus. Lines with lower FHB severity and lower GPC could be used in breeding for malting barley cultivars.

CRISPR-EDITING HOST SUSCEPTIBILITY GENES TO IMPROVE FUSARIUM HEAD BLIGHT DISEASE RESISTANCE

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ABSTRACT

Fusarium head blight (FHB) caused by *Fusarium graminearum* is a devastating disease for wheat and barley, resulting in significant yield loss and reduced grain quality due to mycotoxin contamination. CRISPR/Cas9 (Clustered Regularly Interspaced Short Palindromic Repeats/CRISPR-associated 9 nuclease) gene editing technology was used in this study to knock-out (KO) the host genes involved in *F. graminearum* susceptibility. Previous studies have shown that the Arabidopsis *DMR6* gene, encoding a putative 2-oxoglutarate Fe(II)-dependent oxygenase (2OGO), is an immunity suppressor whose mutation results in enhanced expression of plant defense genes. Published results have also shown that *F. graminearum* exploits the host ethylene signaling pathway to establish infection and that the attenuation of the core *ethylene insensitive 2* (*EIN2*) gene improved *F. graminearum* resistance in both Arabidopsis and wheat plants. In this study, both the *At2OGO* and *AtEIN2* genes were knocked out in Arabidopsis (Col-0) plants by our CRISPR-gene editing platform. Our results showed that the *At2OGO*-KO and *AtEIN2*-KO mutants were more resistant to *F. graminearum* infection and proliferation than wild type (WT) plants. Real-time PCR assay was used to analyze the molecular mechanisms for improved FHB resistance in these KO plants. This analysis showed that some of the salicylic acid (SA) and jasmonic acid (JA) signaling pathways-related genes were highly upregulated in the *At2OGO*-KO plants and were differentially expressed in the *AtEIN2*-KO plants. We have cloned the barley orthologous *Hv2OGO* and *HvEIN2* cDNAs from Conlon cultivar and used these to complement *At2OGO*-KO and *AtEIN2*-KO mutant Arabidopsis plants. FHB susceptibility was recovered in both the *At2OGO*-KO/*Hv2OGO* and *AtEIN2*-KO/*HvEIN2* Arabidopsis plants, indicating that these two genes are involved in conditioning barley to *F. graminearum* infection. We have used a CRISPR-editing vector tailored to monocot plants to similarly edit the *Hv2OGO* gene in barley plants (cv. Conlon), which we are currently testing for enhanced FHB resistance.

ACKNOWLEDGEMENT AND DISCLAIMER

This material is based upon work partially supported by the U.S. Department of Agriculture, under Agreement No. 58-2050-8-012. 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.

GREEN LEAF VOLATILES (GLVS) EFFECTIVELY INHIBIT *FUSARIUM GRAMINEARIUM* BUT THE IMPACT ON INFECTION IN WHEAT EXPOSED TO EXOGENOUS SUPPLIED GLVS IS COMPLICATED

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ABSTRACT

Plant-derived volatile organic compounds (VOCs) are produced when plants are under both abiotic and biotic stress. They play important roles in plant growth regulation, plant communications, plant-microbe interactions, and defense. However, it is not clear how specific VOCs impact plant pathogenesis and if GLV may enhance resistance to Fusarium head blight (FHB). We found that a green leaf volatile (GLV), (E)-2-hexenal, completely inhibits the growth of *F. graminearum* at concentrations less than 15 ppm. However, we found that exposure of wheat seedlings grown in enclosed Sigma plant boxes (1 liter) to GLVs can lead to enhanced fungal growth relative to mock controls. The main objective of this study is to determine if VOCs affect susceptibility of wheat to FHB. Using time-course studies with a range of GLV concentrations, we present the impact of exogenous application of GLV on plant defense gene response and impact on resistance to *F. graminearum*. In addition, previous research in the lab has found that the retromer is a key factor for fungal susceptibility to VOCs and that disruption of retromer function by GLVs treatment is hypothesized to impact the plant response to the fungus.

ACKNOWLEDGEMENT AND DISCLAIMER

This material is based upon work supported by the U.S. Department of Agriculture, under Agreement No. 59-0206-8-213 (McLaughlin). 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.

NON-SPECIFIC LIPID TRANSFER PROTEINS (NSLTPS) HAVE ANTIFUNGAL AND ANTI-ROS PROPERTIES THAT ENHANCE RESISTANCE OF WHEAT TO *FUSARIUM GRAMINEARUM* INFECTION AND DEOXYNIVALENOL EXPOSURE

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ABSTRACT

Plant non-specific lipid transfer proteins (nsLTPs) are involved in abiotic and biotic stress responses including the response to fungal pathogens. Plant nsLTPs have been shown to be induced by *Fusarium graminearum* and exposure to trichothecenes, in addition to other oxidants. Earlier work identified an Arabidopsis nsLTP (AtLTP4.4) as protective against trichothecene exposure when overexpressed in the plant. In addition, numerous reports in the literature have found that nsLTPs have antimicrobial properties. To test if AtLTP4.4 has antifungal properties, we purified AtLTP4.4 expressed in *Pichia pastoris* and show that AtLTP4.4 exhibits potent antifungal activity against *F. graminearum*. Transgenic wheat lines overexpressing nsLTPs (AtLTP (1126:AtLTP4.4 and 1088:AtLTP in the RB07 genetic background) were tested and we found that overexpressing nsLTPs reduced fungal growth in both leaf sections and floral tissues inoculated with *F. graminearum*. In addition, we found that DON induces ROS in wildtype RB07 leaf sections and that overexpression of AtLTP4.4 substantially and significantly ($p<0.001$) reduces ROS induction upon exposure to the toxin over a ten-hour time period. We also see ROS protective effects when protoplasts from RB07 and the transgenic lines are tested. We found that DON induces H_20_2 in wildtype RB07 protoplasts and overexpression of AtLTP significantly ($p<0.001$) reduces ROS induction upon exposure of the protoplasts to the toxin. To determine if expression of AtLTP4.4 protects wheat from *F. graminearum* infection we tested transgenic lines in both the greenhouse (Rutgers University) and field (Rosemount, MN). Results from the greenhouse show that overexpression of AtLTP4.4 increase resistance to *F. graminearum* in the early stages of floral growth (7 and 14 DPI). Data from the field tests for FHB severity and DON (ppm) content will be presented.

ACKNOWLEDGEMENT AND DISCLAIMER

This material is based upon work supported by the U.S. Department of Agriculture, under Agreement No. 59-0206-6-005 (Tumer), Agreement No. 59-0206-6-007 (Trick), and Agreement No. 59-0206-4-016 (Dill-Macky). 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.

TARGETING FUNGAL VIRULENCE GENES VIA HOST-INDUCED GENE SILENCING (HIGS) FOR ENHANCING PLANT RESISTANCE TO *FUSARIUM GRAMINEARUM*

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ABSTRACT

Host-induced gene silencing (HIGS) provides a mechanism to target the transcripts of pathogen genes for silencing. The goal of this project is to utilize HIGS to target the transcripts of *Fusarium graminearum* (*Fg*) pathogenicity genes to promote wheat resistance to Fusarium head blight and Fusarium seedling blight. In HIGS, double stranded RNA (dsRNA) corresponding to a fungal gene is expressed in the host plant. The resultant dsRNA is processed into small RNAs in the plant. These small RNA once taken up by the fungus are expected to destabilize transcripts of the targeted fungal gene. HIGS has been utilized to target two virulence factors in *Fg*, a secretory lipase FGL1, and a secretory salicylate hydroxylase *FgNahG* that limits accumulation of the defense signaling metabolite salicylic acid. dsRNA derived from these genes when expressed in Arabidopsis and wheat enhanced resistance against *Fg* infection, thus confirming the effectiveness of HIGS as a strategy to control *Fg* infection.

ACKNOWLEDGEMENT AND DISCLAIMER

This material is based upon work supported by the U.S. Department of Agriculture, under Agreement No. 59-0206-7-006 and 59-0206-6-007. 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.

TARGETING PATHOGENICITY MECHANISMS TO PROMOTE FHB-RESISTANCE IN WHEAT

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ABSTRACT

Pathogenicity, which is the ability of a pathogen to cause disease, is determined by fungal virulence mechanisms and host mechanisms that contribute to susceptibility. Pathogenicity mechanisms provide excellent targets for controlling disease caused by *Fusarium graminearum*. I will discuss two approaches underway in our labs to enhance plant resistance to *F. graminearum* by targeting knock-down of genes associated with mechanisms that contribute to pathogenicity. The first involves enhancing resistance against *F. graminearum* by knock-down of a class of plant lipoxygenases, which are involved in oxylipin (oxidized lipid) metabolism that contribute to susceptibility. The second approach involves the utilization of host-induced gene silencing (HIGS) to knock-down expression of fungal virulence genes. HIGS comprises the expression of double stranded RNA (dsRNA) corresponding to fungal genes in the host plant. Small RNA, resulting from the processing of the dsRNA, when taken up by the fungus are expected to destabilize accumulation of the corresponding fungal gene transcript to attenuate fungal pathogenicity. We have successfully utilized HIGS in Arabidopsis and wheat to target two secretory protein-encoding *F. graminearum* virulence genes to enhance resistance against *F. graminearum*.

ACKNOWLEDGEMENT AND DISCLAIMER

This material is based upon work supported by the U.S. Department of Agriculture, under Agreement No. 59-0206-7-006, 59-0200-6-018, and 59-0206-6-007. 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.

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

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ABSTRACT

Oxylipins are a class of oxidized lipids, which have variety of biological functions. Lipoxygenases catalyze the first step in the synthesis of oxylipins (oxidized lipids). Previous studies have shown that 9-lipoxygenases (9-LOXs) function as susceptibility factors in Arabidopsis and wheat interaction with *Fusarium graminearum* (*Fg*) (Nalam et al. 2015). Knock-down of 9-LOX encoding genes in the hexaploid wheat cv Bobwhite and in Arabidopsis confer enhanced resistance against *Fg*. Fungal infection was largely confined to the inoculated spikelet on 9-*LOX* silenced wheat lines. *Lpx3* on wheat chromosome 4 is one of the wheat 9-LOX's that contribute towards susceptibility to *Fg*. As a non-GMO approach, TILLING lines with nonsense and/or missense *Lpx3* variants in the hexaploid wheat variety Cadenza and tetraploid wheat variety Kronos have been characterized for their response to *Fg*. FHB incidence was reduced in some of these lines. Also identified during the screening of these TILLING lines, was a hexaploid wheat line that exhibited very high susceptibility to *Fg*. The *Fg* resistance and susceptible TILLING lines are being further characterized and the *Fg*-resistant TILLING lines are being crossed with each other to generate lines with non-sense mutations at multiple *Lpx3* homeologs. The non-GMO strategy provided by these TILLING lines will facilitate the integration of FHB-resistant 9-LOX alleles into FHB resistance breeding programs.

ACKNOWLEDGEMENT AND DISCLAIMER

This material is based upon work supported by the U.S. Department of Agriculture, under Agreement No. 59-0206-7-006 and 59-0200-6-018. 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.

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TOWARDS UNDERSTANDING THE MOLECULAR MECHANISM OF PORE-FORMING TOXIN (PFT)-MEDIATED RESISTANCE AGAINST *FUSARIUM GRAMINEARUM*

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ABSTRACT

Fusarium head Blight (FHB), caused by *Fusarium graminearum*, is one of the most devastating diseases of wheat. Pore-forming toxin-like (*PFT*) gene was shown to be one of the major determinants of *Fhb1* mediated resistance in wheat (Rawat et al. 2016). PFT encodes a chimeric lectin protein with two agglutinin domains and a bacterial pore forming domain. The objective of this study is to understand the molecular mechanism by which PFT confers resistance to *F. graminearum*. PFT is a novel resistance protein and lacks any secretory peptide or transmembrane domain. Using Nicotiana, Arabidopsis and wheat as hosts, we are investigating the molecular mechanism of action of PFT against *F. graminearum.* Stable transgenic lines with GFP-tagged PFT were developed in Arabidopsis, in addition to transiently expressing GFP/ RFP-tagged PFT in Nicotiana. Confocal microscopy and disease assay results demonstrate that PFT is involved in resistance against *F. graminearum* and adopts an unconventional trafficking pathway. Results from the studies will be presented in the meeting.

ACKNOWLEDGEMENT AND DISLAIMER

This material is based upon work supported by the U.S. Department of Agriculture, under Agreement No. 59-0200-6-018. 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.

PATHOGEN BIOLOGY AND GENETICS

HIGHLY AGGRESSIVE AND TOXIGENIC TRANSGRESSIVE PROGENY FROM A CROSS OF MODEL *FUSARIUM GRAMINEARUM* STRAINS PH-1 AND GZ3639 ARE ASSOCIATED WITH A RECOMBINATION HOTSPOT ON CHROMOSOME 2

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ABSTRACT

Fusarium graminearum is homothallic, but it retains an ability to outcross (Bowden and Leslie, 1999). Aggressiveness and mycotoxin production are both quantitative traits influenced by many loci (Talas et al., 2016), and crosses can produce transgressive progeny that are more aggressive and toxigenic than either parent (Cumagun and Miedaner, 2004; Vos et al., 2010). Although rates of outcrossing in the field are unknown, the frequent co-occurrence of strains in wheat heads and the high levels of genetic diversity among isolates suggest that it is probably common (e.g. Bec et al., 2015; Gale et al., 2002; Kelly et al., 2015; Talas and McDonald, 2015a; Zeller et al. 2003, 2004). Recent studies have uncovered direct evidence of recombination within pathogen populations causing Fusarium Head Blight of wheat, e.g. in Europe (Talas and McDonald, 2015b) and North America (Kelly and Ward, 2018). Recombination rates vary across *F. graminearum* chromosomes, with recombination "hotspots" interspersed with areas where recombination is less frequent (Laurent et al., 2018; Talas and McDonald, 2015b; Wang et al. 2017). It has been suggested that recombination at these hotspots produces variants with adaptive advantages, contributing to rapid evolution of more aggressive populations (Cuomo et al. 2007; Laurent et al., 2017; Talas and McDonald, 2015b; Wang et al. 2017). However, this has not been demonstrated directly.

Two *Fusarium graminearum* strains belonging to the dominant North American 15-ADON NA1 population have been widely used as genetic models for sexual development and toxigenicity. PH-1 (NRRL 31084) was isolated from maize in Michigan, while GZ3639 (NRRL 38155) was recovered from wheat in Kansas. Alignment of the original genome assemblies of these two strains generated a list of 10,500 single-nucleotide polymorphism (SNP) markers (Cuomo et al., 2007). The polymorphisms were clustered at telomeres and at several interchromosomal regions which were correlated with recombination hotspots (Cuomo et al., 2007). For the current study, we measured various traits relevant

to fitness and pathogenicity in both strains, and then characterized patterns of inheritance among the progeny of crosses between them.

Both strains were equally aggressive on the susceptible soft red winter wheat (SRWW) variety Pioneer 2555, but GZ3639 was more aggressive on the more resistant SRWW varieties 25R18 and Truman. GZ3639 was also more aggressive in maize stalk assays on Golden Jubilee sweet corn. GZ3639 produced more DON mycotoxin than PH-1 *in planta* on Pioneer 2555, and *in vitro* on rice and in liquid media. Segregation and recombination of unlinked molecular markers among 95 single-ascospore progeny occurred in the expected ratios. The progeny strains were skewed toward increased aggressiveness on Pioneer 2555. Three strains were >3-fold more aggressive than their parents and were also significantly more aggressive than any of their siblings. These highly aggressive strains produced up to 50-fold more DON than either parent. Two progeny pools, one consisting of the ten most aggressive strains, and one of the ten least aggressive, were sequenced by using Illumina paired-end sequencing, and a bulk segregant analysis of marker association with aggressiveness was performed. One region (~300 kb) on chromosome 2 tightly associated with a recombination hotspot was linked to the highly aggressive pool. This region does not include the trichothecene metabolite cluster, but it does include other genes that are potentially important in pathogenicity. This work provides direct evidence for the association of a recombination hotspot with increased aggressiveness in recombinant progeny.

ACKNOWLEDGEMENT AND DISCLAIMER

This material is based upon work supported by the U.S. Department of Agriculture, under Agreement No 58-50208-016. 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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INFECTION CUSHIONS MAKE *FUSARIUM GRAMINEARUM* A TOXIC CEREAL KILLER

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ABSTRACT

Fusarium head blight (FHB) disease of wheat and barley caused by *Fusarium graminearum* decreases grain yield and contaminates grains with trichothecene mycotoxins, such as deoxynivalenol (DON). *F. graminearum* forms multicellular infection structures, including infection cushions (IC), which exhibit elevated TRI5 gene expression on wheat floret tissues (Boenisch and Schäfer, 2011, *BMC Plant Biology*). How those infection structures of *F. graminearum* penetrate the host tissues has not been shown in detail. In this study, we applied live cell imaging, histology, and electron microscopy to elucidate the anatomy and function of IC formed on wheat paleae and glumes. The microscopic results revealed that IC penetrated outer epidermal cell walls of paleae and glumes with numerous penetration pegs, but also colonized the subcuticular space. Epidermal and subepidermal cells underneath IC were often filled with intracellular hyphae, leading to necrotic lesions. To identify genes involved in IC formation and/or function, we compared transcriptome profiles of IC with those from epiphytic runner hyphae (RH) (Mentges et al., 2019, *under review Molecular Plant Pathology*). Hundreds of IC and RH grown on wheat paleae were isolated by laser capture microdissection and used for mRNA isolation and RNASeq. In transcriptomes of RH and IC 85% and 90% of the all genes in the genome were expressed respectively. A total of 573 differentially expressed genes (DEGs (log2FC± 2)) were identified in IC compared to RH, while 238 were down- and 335 upregulated genes. Among the top 26 highest upregulated DEGs in IC are trichothecene metabolite cluster genes, including TRI4, 5, 6, and 14, confirming elevated DON biosynthesis in IC. Other abundant functional categories of regulated genes in IC vs. RH correspond to CAZys, ROS related enzymes, and putative effector proteins. In summary, hundreds of candidates for virulence factors were identified, which are potential targets to control initial stages of FHB and DON contamination in cereals.

ACKNOWLEDGEMENTS

This project was partially funded by the Deutsche Forschungsgemeinschaft (BO 4098/2-1) and U.G. acknowledges the support by the Austrian Science Fund FWF, Special Research Project “Fusarium” F3705/DFGME1682/6-1 (UG).

COMPARATIVE AGGRESSIVENESS OF *FUSARIUM GRAMINEARUM* ISOLATES CAUSING FUSARIUM HEAD BLIGHT IN PENNSYLVANIA

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ABSTRACT

Pennsylvania (PA) small grain production is divided geographically into three environmentally distinct zones where Fusarium head blight (FHB) epidemics are common. Experiments were performed to compare the aggressiveness of *Fusarium graminearum* isolates collected in 2018 from the three wheat-producing zones of PA. First, a field experiment was conducted at two locations (Manheim and Rock Springs) in PA in 2019 using two winter wheat varieties ('Agrimaxx 446' = Rock Springs; 'MAS#67' = Manheim). Treatments included a single representative isolate from each management zone (North, Central, and South) and an untreated check. Four replications were used at each location. The spikes were spray-inoculated at anthesis (Manheim = 05/23/19; Rock Springs = 05/29/19) with a conidial suspension of $4x10^4$ spore/ml. Disease incidence and severity (%) were evaluated 18 days after inoculation. Yield (bu/ac) was measured at harvest (Manheim = 07/03/19; Rock Springs = 07/15/19) and adjusted to 13% moisture. Test weight was also recorded. A rolled towel assay was conducted to determine the ratio of germinated/non-germinated seeds (G:NG). Seed samples were submitted to the University of Minnesota for DON analyses. Additionally, 100-kernel weight (100-KW) and *Fusarium* damaged kernels (FDK, %) were assessed. In a second experiment, the mean radial growth rate (millimeters per day) for 295 isolates was determined through an *in vitro* assay. Lastly, the asexual fecundity of the strains was evaluated based on the macroconidia production in culture medium. For the field experiment, no significant treatment effects ($\alpha = 0.05$) were observed for FHB incidence and severity at either location, wherein FHB severity ranged from 64.6 to 74.8% and from 51.7 to 62.6% in Manheim and Rock Springs, respectively. At both locations, yield was also not significantly influenced by treatments. However, higher yields were observed at Rock Springs (mean = 71.9 bu/ac) compared to Manheim (mean = 46.1 bu/ac). All grain quality traits, 100-KW, FDK, and test weight, were significantly affected by treatments. However, this significance was not consistent across locations. The mycelial growth rates were not significantly influenced by isolates sampling zone, with means of 11.0, 10.9, and 10.8 mm/day for North, Central and South, respectively. In general, the population of *F. graminearum* isolates used in this study could not be differentiated based upon the measured traits. This new knowledge will help us increase understanding of the influence of geographic region on the aggressiveness of *F. graminearum* isolates in small grains and the potential for FHB development.

ACKNOWLEDGEMENT AND DISCLAIMER

This material is based upon work supported by the U.S. Department of Agriculture, under Agreement No. 59-0206-9-064. 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.

POTENTIAL OF WILD GRASS HOSTS TO SERVE AS RESERVOIRS OF PATHOGEN GENETIC DIVERSITY AND SOURCES OF INOCULUM

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INTRODUCTION

Primarily understood as an agricultural pathogen, *Fusarium graminearum* is also associated with dozens of non-cultivated or wild hosts. The pathogen's relationship with wild hosts is poorly understood and may have an underestimated influence on epidemiology and evolution. A survey of New York wild grasses, analysis of pathogen population structure, and experimental comparison of host tissues suggest wild grasses impact the epidemiology and evolution of *F. graminearum.*

OBJECTIVES

1. Document incidence of *Fusarium graminearum* in wild grass debris and spikes
2. Characterize pathogen genetic structure and diversity found in non-cultivated grass hosts
3. Compare inoculum production on wheat and wild grass stems

MATERIALS AND METHODS

Field Survey

Wild grass debris and inflorescences were collected from the borders of small grains fields and natural preserves in two regions of New York during the 2015-2017 growing seasons. Twenty unique locations were sampled, and one, Montezuma National Wildlife Refuge, was visited in all three years. In 2017, research locations were divided between central and northeastern NY to include environments from regions with different levels of agricultural production. Sampling focused on grass species that flower simultaneously with winter wheat and was timed to coincide with the 'early grain filling' growth stage in nearby wheat crops. Stem debris that had overwintered naturally was collected in early spring. Pathogen incidence in these tissues was modelled using host density[2] and rainfall[3] as predictors.

Population Genetics Analysis

Isolates from winter wheat, wild grass spikes, and overwintered grass debris were used to understand population structure at the intersection of host communities and land uses. Trichothecene genotypes (n = 900) and microsatellite genotypes from eight loci (n = 700) were analyzed with respect to region, land use, and host source. Fine scale population structure was characterized by counting putatively clonal isolates collected from within a series of 1 m^2 quadrats.

Comparison of inoculum production

Five common grasses [*Bromus inermis* (smooth brome grass), *Dactylis glomerata* (orchardgrass), *Elymus repens* (quackgrass), *Lolium perenne* (perennial ryegrass), and *Phalaris arundinacea* (reed canarygrass)] and one spring wheat cultivar were contrasted in controlled experiments to measure potential inoculum production on stem tissue and host suitability as overwintering sites. Sterile stem tissue segments, each segment

including a single node, were weighed into 0.3 g piles and inoculated with one of ten pathogen isolates. Petri dishes containing inoculated stem tissue and moist filter papers were incubated under laboratory conditions to determine ascospore and conidium production per dry gram of tissue. In a parallel experiment, host tissues were inoculated with a mixture of all ten isolates and placed outdoors under field conditions for an overwintering assay. Pathogen infestation of overwintered stems was recorded in two consecutive springs.

RESULTS AND DISCUSSION

Field Survey

Wild grass stems were infested with *F. graminearum* at an overall incidence of 13.4 % (n = 3671). Pathogen incidence in grass spikes over three years was 15.4 % (n = 3435). Land use and host species were not significant predictors of incidence. Variation was found between regions and years that was linked to host density within 1 km of sampling sites and rainfall in the two months preceding sample collection (Figure 1). Cumulative host density was a marginally better predictor (Δ AIC $\geq$ 2) of incidence than wild grass, small grain, or corn acreage alone. The proportion of wild grasses colonized and the importance of all hosts to describing incidence suggest non-cultivated hosts have significant potential to support local and potentially regional inoculum production.

Population Genetics Analysis

Non-agricultural environments contained a greater frequency of 3ADON genotype isolates than wheat fields (Figure 2) ($P < 0.001$), though no difference was detected between host sources within field sites. This finding supports a hypothesis that one or more agricultural management practices select for a different pathogen population than is found in natural host communities. Uniformity of flowering times and host genetics, fungicide applications, and disturbance of soil and plant residues are some examples of the selective pressures found in agricultural sites but not present in natural spaces. Based on microsatellite markers, all hosts, land uses and regions contained genotypically diverse populations (mean Simpson's $\lambda = 0.92$). The chance of retrieving isolates with shared microsatellite genotypes from within 1 m^2 sampling quadrats was just 4 % in the case of both grass stems and spikes, suggesting even small wild host communities can support numerous individuals.

Comparison of inoculum production

Ascospore and conidia production under laboratory conditions varied significantly by host species, but on average, all hosts supported production of $>10^5$ conidia or ascospores per dry gram of tissue. Pathogen survival in four of the five wild grass hosts tested was comparable to that in wheat, while significantly lower pathogen survival was observed in perennial ryegrass (Figure 3) ($P < 0.001$). Under experimental conditions, wild grass hosts appear comparable to wheat in their ability to harbor *F. graminearum* between years and serve as a substrate for spore production.

ACKNOWLEDGEMENTS AND DISCLAIMER

This material is based upon work supported by the U.S. Department of Agriculture, under Agreement No. 59-0206-4-006. This is a cooperative project with the U.S. Wheat & Barley Scab Initiative. Additional support was provided through National Institute of Food and Agriculture Hatch Project NYC153437. 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. The authors thank Extension Specialists K. Severson, M. Stanyard, and K. O'Neil; C. Nobles and the Uihlein Seed Potato Farm; M. Davis and Willsboro Research Farm; L. Ziemba and the Montezuma National Wildlife Refuge; and the small grains producers who assisted with sampling.

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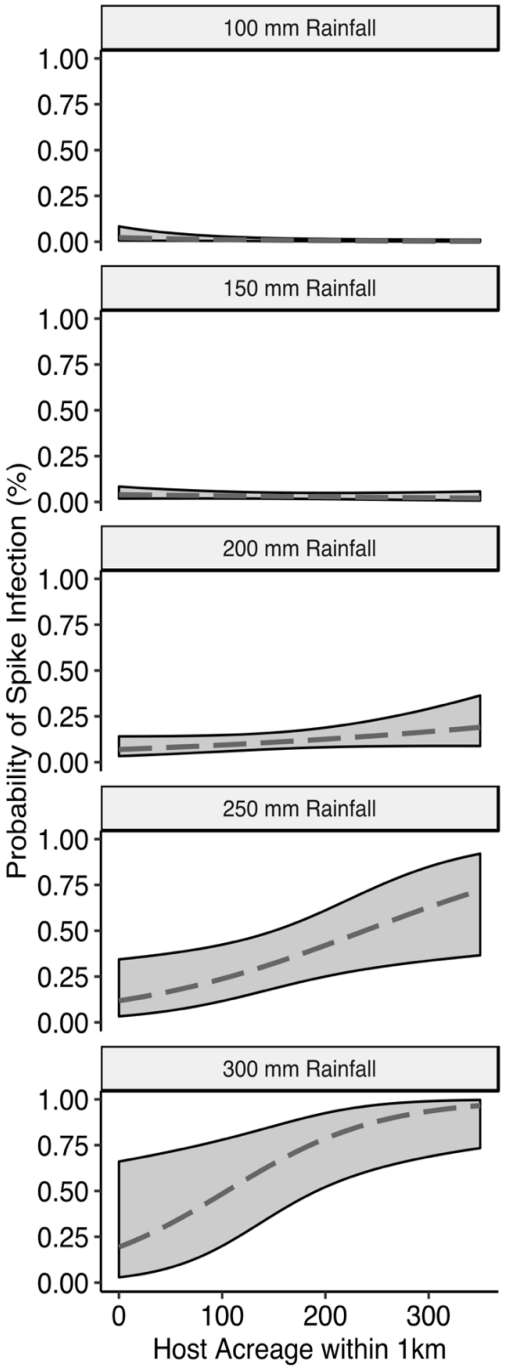

Figure 1. The probability of spike infection by *F. graminearum* was predicted by the interaction of host density and rainfall in the two months prior to sampling. Confidence intervals (95%) around least-squares means are displayed for increasing rainfall and host density. The range of host density and rainfall values displayed represents field conditions observed during the course of this study.

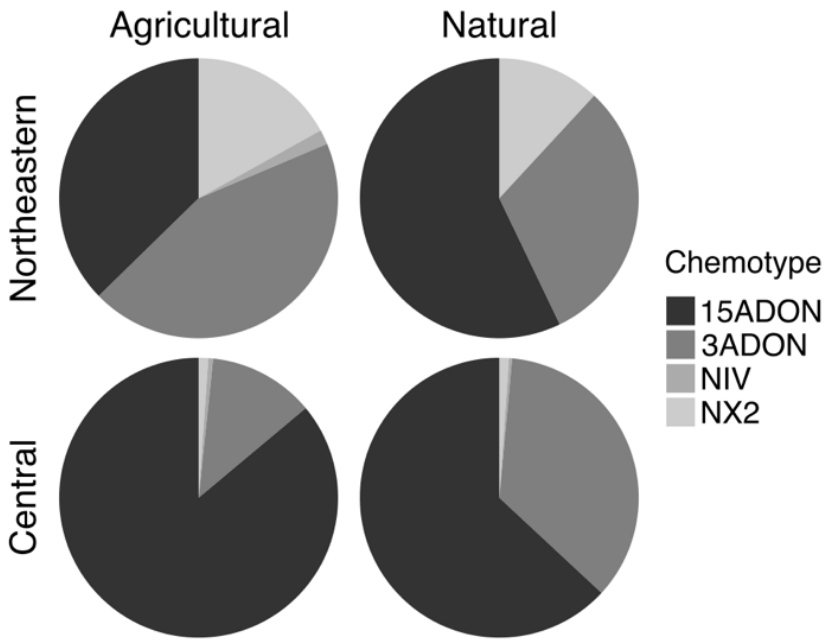

Figure 2. Trichothecene genotype frequencies varied by land use and region.

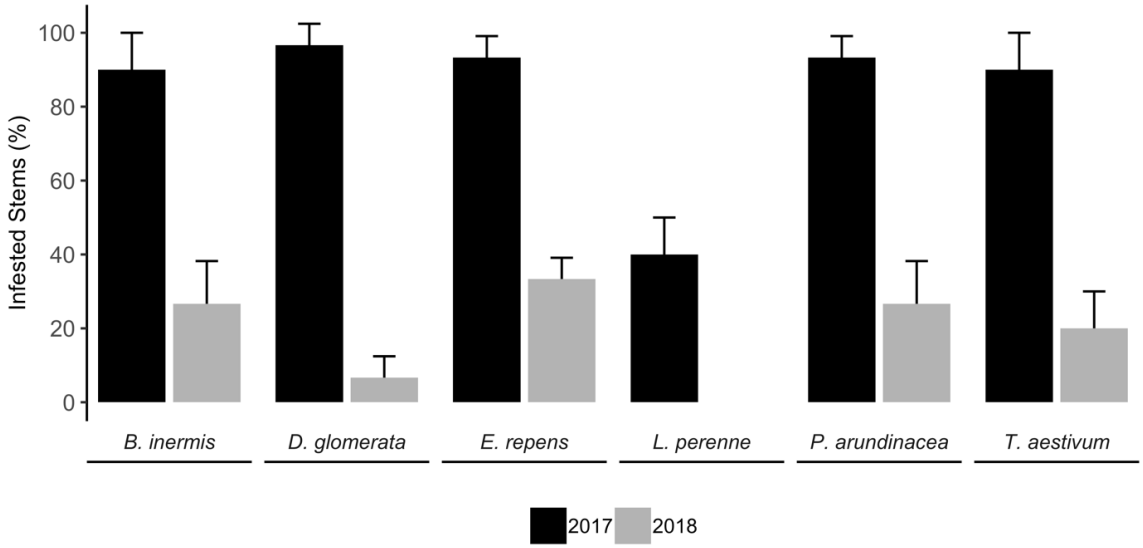

Figure 3. Survival of *Fusarium graminearum* in inoculated grass stems over two winters was comparable between wheat (*Triticum aestivum*) and most grasses. Bars show percent pathogen recovery from stems after one and two years of overwintering. Whiskers represent standard deviation of three replicates, each containing 10 stem segments.

UNDERSTANDING THE GENETIC DIVERSITY OF *FUSARIUM* SPECIES CAUSING FUSARIUM HEAD BLIGHT (FHB) OF WHEAT IN GEORGIA

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ABSTRACT

Fusarium graminearum is a cereal fungal pathogen causing Fusarium head blight (FHB) in wheat (*Triticum aestivum* L.), a major yield-limiting disease with serious food safety issues associated with mycotoxin Deoxynivalenol (DON) contamination. FHB has become an increasing problem in the southeast United States mainly due to increased corn (*Zea mays* L.) acreage. *F. graminearum sensu stricto (s.s.)* with 15ADON chemotype within the 16 biogeographically divergent species complex (FGSC) is the dominant pathogen in U.S. and Canada but shifts in chemotype along with different species have been reported. To elucidate the pathogen diversity in Georgia (GA), USA, we collected nearly 320 isolates from symptomatic wheat heads and corn debris from 47 counties in 2017/18 and 2018/2019. PCR-based identification with translocation elongation factor 1 alpha (*EF-1α*) primers of nearly 50 isolates indicated most were *F. graminearum s.s.* with a few isolates being *F. poae* that are clustered in southwest GA. Chemotyping using multiplex PCR targeting the *Tri3* genes revealed that isolates so far are 15ADON type. Phylogenetic relationship of representative isolates and reference strains of all known FGSC species based on the *EF-1α* gene showed the majority of isolates were clustered within the *F. graminearum* clade. To our knowledge, this is the first report of FGSC and their chemotype in GA. A further study on the population structure, chemotype, and virulence of the additional isolates in this collection are ongoing to better understand the pathogen and assist future disease management strategies.

THE EFFECTOR FGNLS1 IS REQUIRED FOR FULL VIRULENCE OF *FUSARIUM GRAMINEARUM*

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ABSTRACT

Effectors produced by plant pathogenic fungi play important roles in suppression of immunity and promotion of disease in plants. Fusarium head blight (FHB), caused by the fungus *Fusarium graminearum*, is one of the most devastating diseases of wheat and barley worldwide. In addition to reducing crop yield and quality, *F. graminearum* can contaminate grain with trichothecene mycotoxins such as deoxynivalenol (DON). Based on genome sequence data, *F. graminearum* is predicted to produce hundreds of effectors. However, the functions of most of these effectors remain unknown. In this study, we characterized an effector FgNls1 (FGSG_04563), which is predicted to contain multiple eukaryotic nucleus localization signals (NLS). A fusion protein of GFP and FgNls1 accumulated in nucleus of *Nicotiana benthamiana* via *Agrobacterium*-mediated transient expression. Co-immunoprecipitation assays using GFP-FgNls1 fusion protein confirmed its nuclear localization in *N. benthamiana* but did not identify its plant target in *N. benthamiana*. FgNls1 also suppressed cell death induced by Bax when co-expressed with Bax in *N. benthamiana*. Expression of *FgNLS1* was induced during development of FHB in wheat, suggesting a role in pathogenesis. Deletion mutants of *FgNLS1* displayed similar growth and DON production as wild-type parent strain PH-1. Compared to PH-1, Fgnls1 mutants significantly reduced their ability to cause FHB and DON contamination in wheat head. These results indicate that FgNls1 contributes to pathogenesis of *F. graminearum* on wheat by suppressing plant immune responses. Further experiments are underway to identify FgNls1 plant target and elucidate the mode of action of FgNls1 during FHB.

STENOTROPHOMONAS BACTERIA READILY COLONIZES *FUSARIUM GRAMINEARUM* PERITHECIA AND REDUCES PERITHECIA FORMATION

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ABSTRACT

Fusarium graminearum (*Fg*) is the primary fungal pathogen responsible for Fusarium head blight (FHB), a devastating disease of wheat and barley worldwide. In regions where *Fusarium* inoculum is limited, the severity of FHB epidemics and accumulation of trichothecene mycotoxins in grain is strongly driven by the coincidence of inoculum formation during flowering when the plant is most susceptible. An unexplored route to reducing inoculum availability is to recruit parasites of perithecia to reduce the success of spore production or active discharge. A microbiome analysis of *Fg* perithecia identified a bacterial strain of *Stenotrophomonas* from soil that readily colonized *Fg* perithecia, and reduced perithecia formation by approximately half. Additionally, genome sequencing analysis revealed that a *Fg* strain, F131, was heavily contaminated with a bacterial symbiont also identified as *Stenotrophomonas*. This bacterial association between F131 and *Stenotrophomonas* inhibits perithecia formation to an even greater extent. In comparison to F131 that had been cured of the bacterium via antibiotics, the F131 that was associated with *Stenotrophomonas* produced 4-fold fewer perithecia. Further exploration is warranted to determine whether bacterial associates that reduce the success of *Fg* spore production may be useful in managing 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, 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 FLORA AND MYCOTOXIN PROFILES OF COMMERCIAL MALTING BARLEY GRAIN LOTS IN NEW YORK

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ABSTRACT

Production of malting barley for the craft brewing supply chain is a new priority in New York State since 2012. The number of growers and total acreage of this new crop is still low but is expanding each year. Fusarium head blight and grain contamination with trichothecene mycotoxins are already established as major constraints in the production of malting barley in this state. Little is known about the mycoflora of malting barley grain in New York and especially about the spectrum of *Fusarium* species associated with grain and mycotoxin contamination, so research was conducted to establish baseline data on grain mycoflora. A total of 45 malting barley grain samples (24 samples in 2018 and 20 samples in 2019) from commercial producers in New York was collected and analyzed for their fungal flora with a focus on *Fusarium* species profiles. One hundred seeds per sample were surface-disinfected and plated on PDA++ medium under a 12-hour photoperiod of near-UV light for four days. *Fusarium*-like colonies were identified initially based on morphological features and confirmed by TEF-1a gene sequencing and comparison to GenBank sequences. The incidence of recovery of *Fusarium* from grain samples ranged 0% to 45%. *Fusarium* infection incidence and species diversity were higher in 2018, a wetter production season, than in 2019, a drier production season. *Fusarium graminearum* sensu stricto accounted for most isolations from grain in both years. Other *Fusarium* species isolated included *F. avenaceum*, *F. culmorum*, *F. equiseti*, *F. oxysporum*, *F. poae*, and *F. solani*. Quantitative mycotoxin results will also be presented for commercial barley grain lots in association with their *Fusarium* profiles.

ACKNOWLEDGEMENTS

We acknowledge Jennifer Starr and Kevin Myers for expert technical assistance and undergraduate Annie Xu for general lab assistance. This research was made possible by grants for malting barley from the New York State Department of Agriculture and Markets and the Genesee Valley Regional Market Authority and from the U.S. Department of Agriculture, National Institute of Food and Agriculture through Cornell University Hatch Project NYC153437.

THE *FUSARIUM GRAMINEARUM* T-SNARE *SSO1* IS INVOLVED IN GROWTH, DEFENSE, AND DON ACCUMULATION AND VIRULENCE

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ABSTRACT

The plant pathogenic fungus *Fusarium graminearum*, causal agent of Fusarium Head Blight (FHB) disease on small grain cereals, produces toxic trichothecenes which require proper export for full virulence. Two potential modes of mycotoxin transport are membrane-bound transporters, which move toxins across cellular membranes, and SNARE mediated vesicular transport, by which toxins may be packaged as cargo in vesicles bound for organelles or the plasma membrane. In this study we show that deletion of a gene (*Sso1*) for a sub-apically localized t-SNARE protein results in growth alteration, increased sensitivity to xenobiotics, altered gene expression profiles, and reduced DON accumulation *in vitro* and *in planta* as well as reduced FHB symptoms on wheat. Crossing a *Sso1* mutant with an ABC transporter mutant (Δ*abc1*) results in an additive reduction in DON accumulation and almost complete loss of FHB symptoms *in planta*. These results suggest an important role of *Sso1*-mediated sub-apical exocytosis in FHB progression and xenobiotic defense and are the first report of an additive reduction in *F. graminearum* DON accumulation upon deletion of two distinct modes of cellular export. This research provides useful information which may aid in formulating novel management plans of FHB or other destructive plant diseases.

ACKNOWLEDGEMENTS AND DISCLAIMER

The authors acknowledge the Minnesota Supercomputing Institute (MSI) at the University of Minnesota for providing resources that contributed to the research results reported within this paper. Funding was provided by United States Department of Agriculture, United States Wheat and Barley Scab Initiative, award FY18-KI-021. Mention of trade names or commercial products in this article solely for the purpose of providing specific information and does not imply recommendation or endorsement by the United States Department of Agriculture (USDA). USDA is an equal opportunity provider and employer.

DETECTION OF FUSARIUM HEAD BLIGHT IN WHEAT USING A DEEP NEURAL NETWORK AND COLOR IMAGING

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ABSTRACT

Fusarium head blight (FHB) is a devastating disease of wheat worldwide. In addition to reducing the yield of the crop, the causal pathogens also produce mycotoxins that can contaminate the grain. The development of resistant wheat varieties is one of the best ways to reduce the impact of FHB. To develop such varieties, breeders must expose germplasm lines to the pathogen in the field and assess the disease reaction. Phenotyping breeding materials for resistance to FHB is time-consuming, labor-intensive, and expensive using conventional protocols. To develop a reliable and cost-effective high throughput phenotyping system for assessing FHB in the field, we focused on developing a method for processing color images of wheat spikes to accurately detect diseased areas using deep learning and image processing techniques. Color images of wheat spikes at the milk stage were collected in shadow condition and processed to construct datasets, which were used to retrain a deep convolutional neural network model using transfer learning. Testing results showed that the model detected spikes quite accurately in the images as the coefficient of determination for the number of spikes tallied by manual count and the model was 0.80. The model was assessed, and the mean average precision for the testing dataset was 0.9201. On the basis of the results for spike detection, a new color feature was applied to obtain the gray image of each spike and a modified region growing algorithm was implemented to segment and detect the diseased areas of each spike. Results showed that the region growing algorithm performed better than K-means and Otsu's method in segmenting diseased areas. We demonstrates that deep learning techniques enable accurate detection of FHB in wheat based on color image analysis, and the proposed method can effectively detect spikes and diseased areas, thereby improving the efficiency of FHB assessment in the field.

ACKNOWLEDGEMENT AND DISCLAIMER

This research was supported by the USDA-ARS U.S. Wheat and Barley Scab Initiative (Funding No. 58-5062-8-018). 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 project is also partially funded by the National Natural Science Foundation of China (Grant No. 31971786) and the Industrial Partnership Grants from MnDRIVE Robotics, Sensors and Advanced Manufacturing Initiative.

A SIMPLE PCR-BASED METHOD FOR IDENTIFICATION OF FUSARIUM HEAD BLIGHT PATHOGENS

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ABSTRACT

Members of the *Fusarium graminearum* species complex (FGSC) are the main etiological agents of Fusarium head blight (FHB) in most of the wheat growing regions of the world. In the US and Canada, *F. graminearum* is the main species infecting wheat. Additionally, our lab recently reported *F. boothii* causing FHB of wheat for the first time in the US. Other related species, such as *F. asiaticum*, *F. gerlachii*, *F. louisianense*, *F. culmorum*, and *F. cerealis*, have also been reported. Accurate species identification of isolates is important for pathogen surveillance and disease management; however, most of the species causing FHB of wheat are morphologically indistinguishable. DNA sequencing and a multilocus genotyping assay provide reliable identification, but they might not be readily available to most labs or they might be cost prohibitive for population studies. The aim of this research is to develop a simpler and more affordable PCR-based assay to identify FHB pathogens. In order to achieve our aim, we designed primers based on lineage-specific single nucleotide polymorphisms (SNPs) in the trichothecene 3-O-acetyltransferase (*TRI101*) gene. On top of the fixed SNP, an additional mismatch 1-3 bases from the 3' end of the primer was introduced using the SNAPER program (http://ausubellab.mgh.harvard.edu/) to provide better differentiation. Amplification was carried out using a two-step touchdown method. Currently, we have developed reactions to differentiate *F. graminearum* and *F. boothii* from related species. Additional studies are underway to develop assays to identify other FHB pathogens using a similar approach.

ACKNOWLEDGEMENT

This work was partially supported by the Nebraska Agricultural Experiment Station with funding from the Hatch Multistate Research capacity funding program (Accession Number 1006715) from the USDA National Institute of Food and Agriculture. Wheat surveys were funded by the Nebraska Wheat Board.

SPRAY-INDUCED GENE SILENCING TO MANAGE FUSARIUM HEAD BLIGHT IN BARLEY

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ABSTRACT

Fusarium Head Blight (FHB) in cereal crops is difficult to control and often results in significant yield losses and mycotoxin contamination of the grain. Because there are limited effective means of controlling FHB in susceptible host crops, novel strategies need to be explored to manage *Fusarium graminearum*. An exciting and emerging technology known as RNA-interference (RNAi) employs post-transcriptional gene silencing via sequence-specific mRNA degradation. This project focuses on how RNAi can be used to manage FHB in *Hordeum vulgare* (barley) through spray-induced gene silencing (SIGS) across all stages of barley production (from field to malting). SIGS can be accomplished by spraying long double-stranded RNA (dsRNA), which is sequence-complementary to fungal gene(s) of interest, directly onto living tissue. The dsRNA is taken up by the pathogen and host, processed into small RNAs, and results in knockdown of the transcripts of target genes. We have identified genes from RNA-seq experiments important to the early stages of fungal penetration of hosts. These genes have shown reduced pathogenicity in gene knockout data and are our targets for *in planta* assays of RNAi. This novel strategy is being developed for managing FHB on barley, but can be implemented across many different cropping systems and fungal organisms.

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 authors and do not necessarily reflect the view of the U.S. Department of Agriculture.

EXPLORING THE ROLE OF MATING-TYPE GENES IN *FUSARIUM GRAMINEARUM*

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ABSTRACT

Sexual development in ascomycete fungi, including *Fusarium graminearum*, is regulated by the mating type locus *MAT1*, which encodes two genes called *MAT1-1-1* and *MAT1-2-1* (Turgeon, 1998; Turgeon and Yoder, 2000). The products of these two genes interact to form heterodimers that bind DNA and regulate sexual development. In most Fusarium fungi, these two genes are separated in different individuals, and confer mating compatibility. Thus, strains encoding *MAT1-1-1* are fertile only when paired with *MAT1-2-1* individuals. *Fusarium graminearum* is unusual because it is homothallic. Both genes are present in the genome of each individual, closely linked within the MAT locus, and each individual strain is self-fertile, though retaining the ability to outcross (Bowden and Leslie, 1999; Yun et al., 2000; Martin et al., 2011). The retention of this arrangement in the lineage suggests that homothallism in *F. graminearum* provides some selective advantage. Sexual development in *Fusarium graminearum* is known to be an important pathogenicity factor because perithecia are major overwintering structures, and the sexually produced ascospores serve as primary inoculum for the establishment of epidemics (Desjardins et al., 2004). However, homothallism isn't an absolute requirement for these functions, and so we are exploring the possibility that there are other adaptive advantages of homothallism related to pathogenicity on maize and/or wheat, or resistance to environmental stresses. Given the diverse array of genes that are regulated during sexual development by the MAT idiomorphs (Sikhakolli et al., 2012), it seems possible that they also regulate genes, directly or indirectly, that impact these components of fitness.

Previous studies have demonstrated that deletion of the *MAT1-1-1* or *MAT1-2-1* genes of *F. graminearum* can produce obligately heterothallic strains (Lee et al., 2003; Zheng et al., 2013). We produced multiple independent knockout mutants (KOs) of the entire MAT1 locus, and individually of the *MAT1-1-1* and the *MAT1-2-1* genes, in the PH-1 strain of *F. graminearum* using the standard split-marker method versus double crossover gene replacement. The two methods did not differ significantly in their performance in most cases, although the split-marker method produced more KOs of the largest *MAT1* sequence. All the KOs lost their ability to produce asci and ascospores, although most still produced perithecial structures. The *MAT1-1-1* KOs, when paired with the *MAT1-2-1* KOs, formed asci and ascospores, although different transformants varied widely in their levels of interfertility. Neutral molecular markers segregated 1:1 among the progeny of the perithecia, confirming that the *MAT1-1-1* and *MAT1-2-1* KOs are heterothallic. The *MAT1* KOs were unaffected in aggressiveness to susceptible varieties of winter

wheat (Pioneer 2555), spring wheat (Wheaton), and maize (Golden Jubilee). In contrast, KOs of the individual specificity genes resulted in significant reductions in aggressiveness in most, but not all, of the transformant strains. The large amount of variation among different KO transformants in both fertility and pathogenicity was a surprise and may be due to off-site mutations that occurred during the transformation process. We will use genetic complementation, and analysis of crosses among the various KO strains, to test this hypothesis.

ACKNOWLEDGEMENT AND DISCLAIMER

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VARIETY DEVELOPMENT AND HOST PLANT RESISTANCE

HARNESSING RELATIONSHIPS TO IMPROVE GENOMIC PREDICTION ACCURACY OF FUSARIUM HEAD BLIGHT TRAITS IN WHEAT

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ABSTRACT

Maximizing the relationship between the training and breeding populations have shown to improve the accuracy of genomic prediction in crops. In this study, a leave-one-out cross-validation method in RR-BLUP was used to assess the prediction abilities of training populations selected by stratified sampling, stratified genomic relationship sampling and stratified random sampling. To achieve this, we used a set of 200 F5 lines with 45 parental lines evaluated for scab-index, visually scabby kernels and micro-test weight in St Paul, Minnesota in 2018.

The stratified sampling divided the lines into three clusters based on their genetic distance. Within each cluster, the stratified genomic relationship sampling was used to select a proportion of lines most related to the line being predicted while the stratified random sampling was used to select random lines to predict each line. The stratified genomic relationship sampling showed the highest accuracies for all traits except for scab-index while the stratified random sampling had the lowest accuracies for all traits. The stratified genomic relationship sampling tends to select the lines most related to the line that is being predicted, thus, the higher prediction abilities. In future, we would be extending this method to other traits of interest in our breeding program.

ACKNOWLEDGEMENTS AND DISCLAIMER

This material is based upon work supported by the U.S. Department of Agriculture, under Agreement No. 59-0206-8-202. 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.

MOLECULAR MECHANISMS UNDERLYING FHB RESISTANCE IN WHEAT

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ABSTRACT

In wheat, scab disease also called Fusarium Head Blight (FHB) results in lower kernel production as well as contamination of grains with poisonous mycotoxins such as Deoxynivalenol (DON). Differential DON accumulation in scab infected wheat cultivars implies a host resistance system during the course of DON production. Using, Uridine-diphosphate glycosyltransferases (UGTs) enzymes, plants convert DON into a non-toxic masked form called DON-3-glucoside (D3G). However systematic analysis of UGTs in wheat genome is still lacking and on the other hand, the metabolome of wheat genotypes varying in FHB resistance also needs investigation. In this study we have used both genomics and metabolomics approaches to identify the factors involved in host resistance during FHB occurrence. In this study, we searched the already sequenced wheat genome for putative FHB responsive *UGT* genes and also determined the metabolome responses of selected Chinese wheat cultivars to *F. graminearum* inoculation. More than 100 putative *UGT* genes, with variable length were identified and further characterized. The involvement of selected genes in FHB resistance was confirmed through RT-PCR. The LCMS study detected more than 300 compounds in the selected Chinese cultivars showing quantitative variation during FHB stress. This is a foundation work to develop resistance against *F. graminearum* and DON detoxification in cereal crops, by exploring the UGTs and the metabolites identified in this study.

ACKNOWLEDGEMENT

This work was supported by postdoctoral research program at Jiangsu Academy of Agriculture Sciences (JAAS), Nanjing, China

FUSARIUM HEAD BLIGHT BIOMASS MEASUREMENTS IN BARLEY FROM 2018 U.S. NURSERIES

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ABSTRACT

Breeding and evaluating for Fusarium head blight (FHB) resistance in the field has been limited by low correlation between severity ratings and deoxynivalenol (DON). Measurement of fungal biomass by quantitative PCR is an alternative measurement method that is attainable by most laboratories, can be high-throughput, and directly estimates fungal infection and growth. Additional benefits include establishing a pipeline for processing samples that lighten the workload on DON testing facilities by pre-grinding the samples for analysis. Winter and Spring barley samples from 2018 national FHB nurseries were evaluated for biomass (n = 2,039). Technical replication of a subset samples (n = 182) and the overall technical error was 17.4% for qPCR measurements. The coefficient of determination (R^2) was 0.6347 between log 10-transformed biomass and DON for all 2018 nurseries. The highest R^2 was 0.7215 from the Aberdeen, ID, followed by Ithaca, NY (R^2 = 0.6236), and the lowest was from Osnabrock, ND (R^2 = 0.1495). Lower R^2 were noted with coarse grinding and shipment of pre-ground samples prior to qPCR, implicating sample handling and size of grind in biomass measurements. Measurement of biomass correlated better with DON than visual ratings. Biomass measurements can improve evaluation of FHB in barley nurseries and reduce summer field labor. In remote locations, where gathering severity data is difficult to accomplish, qPCR can be a substitute for visual rating and compliment DON analysis.

ACKNOWLEDGMENTS AND DISCLAIMERS

This material is based upon work supported by the U.S. Department of Agriculture, Improvement of Barley and Oat for Enhanced Productivity, Quality, and Stress Resistance, Project Number 2050-21000-034-00-D. This is a cooperative project with the U.S. Wheat & Barley Scab Initiative and is supported by projects FY18-BA-005 and FY18-BR-029.

THE VALUE OF EARLY STAGE PHENOTYPING FOR WHEAT BREEDING IN THE AGE OF GENOMIC SELECTION

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ABSTRACT

The early stages of phenotyping involve few observations and can be inaccurate. Genomic selection (GS) could improve selection accuracy, alter resources allocation, and gains. Our objectives were 1) compare the prediction accuracy of GS and phenotyping in stage-1 and stage-2 field evaluations, and 2) to assess the use of GS in stage-1 testing. We genotyped 1769 wheat breeding lines that were phenotyped for yield and Fusarium Head Blight (FHB) resistance. The lines were in cohorts and analyses were done by cohort and results averaged. Phenotypes or GS estimated breeding values were used to determine the trait value of stage-1 lines and these values were correlated to phenotypes from stage-2 trials of the same lines. This was repeated for stage-2 to stage-3 trials. The prediction accuracy of GS and phenotypes was similar to each other. Stage-1 lines ranked superior by GS had slightly inferior phenotypes in stage-2 trials than lines ranked superior by phenotypes. A cost analysis indicated that replacing stage-1 phenotyping with GS would allow nearly three time more stage-1 candidates to be assessed and provide 0.89 to 2.22 times greater gain from selection compared to phenotyping. We conclude that GS can complement or even replace phenotyping in early stages line evaluation and selection.

ACKNOWLEDGEMENT AND DISCLAIMER

This material is based upon work supported by the U.S. Department of Agriculture, under Agreement No. 59-0206-9-208. 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

GENETIC FACTORS AFFECTING FHB RESISTANCE IMPROVEMENT AND LINKAGE DRAG FROM INTROGRESSION OF EXOTIC SUMAI 3 ALLELES (INCLUDING *FHB1*, *FHB2*, AND *FHB5*) IN CANADIAN HARD RED SPRING WHEATS

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ABSTRACT

Fusarium head blight resistance genes, *Fhb1* (for Type-II resistance), *Fhb2* (Type-II), and *Fhb5* (Type-I plus some Type-II), which originate from Sumai 3, are among the most important that confer resistance in hexaploid wheat. Near-isogenic lines (NILs), in the CDC Alsask (susceptible; n = 32) and CDC Go (moderately susceptible; n= 38) backgrounds, carrying these genes in all possible combinations were developed using flanking microsatellite markers and evaluated for their response to FHB and deoxynivalenol (DON) accumulation in eight environments. The NILs along with their parents were also tested for agronomic and end-use quality traits in the yield trials in 2016 and 2017 in six site-years. NILs were haplotyped with wheat 90K iSelect assay to elucidate the genomic composition and confirm alleles' presence. Other than evaluating the effects of three major genes in common genetic background, the study elucidated the epistatic gene interactions as they influence FHB measurements; identified loci other than *Fhb1*, *Fhb2*, and *Fhb5*, in both recurrent and donor parents and examined annotated proteins in gene intervals.

Genotyping using 81,857 single nucleotide polymorphism (SNP) markers revealed polymorphism on all chromosomes and that the NILs carried <3% of alleles from the resistant donor. Significant improvement in field resistance (Type-I + Type-II) resulted only among the CDC Alsask NILs, not the CDC Go NILs. The phenotypic response of NILs carrying combinations of Sumai 3 derived genes suggested non-additive responses and *Fhb5* was as good as *Fhb1* in conferring field resistance in both populations. In addition to *Fhb1*, *Fhb2*, and *Fhb5*, four to five resistance improving alleles in both populations were identified and three of five in CDC Go were contributed by the susceptible parent. The introgressed chromosome regions carried genes encoding disease resistance proteins, protein kinases, nucleotide-binding and leucine rich repeats' domains. Complex epistatic gene-gene interactions among marker loci (including *Fhb1*, *Fhb2*, *Fhb5*) explained >20% of the phenotypic variation in FHB measurements. Among agronomic traits, introgressions resulted in lower thousand kernel weight and increased plant height with *Fhb5*. Among end-use quality traits, SDS-sedimentation volume and grain protein content were affected. In addition to *Fhb1*, *Fhb2*, and *Fhb5*, we identified 10 loci in CDC Alsask

NILs and 9 in CDC Go NILs that affected the traits measured. We found that none of these additional loci were common in both populations, indicating the presence of many alleles in exotic sources that can result in linkage drag.

Immediate Sumai 3 derivatives carry a number of resistance improving minor effect alleles, other than *Fhb1*, *Fhb2*, *Fhb5*. Results verified that marker-assisted selection is possible for the introgression of exotic FHB resistance genes, however, the genetic background of the recipient line and epistatic interactions can have a strong influence on expression and penetrance of any given gene. Improvements in FHB resistance can still be made by introgressing these major genes using marker-assisted selection and selecting rare segregants with improved agronomic and end-use quality.

ACKNOWLEDGEMENTS AND DISCLAIMER

The first author (GSB) acknowledges the University of Saskatchewan Dean's Scholarship, the Saskatchewan Wheat Development Commission Graduate Scholarship, and Monsanto's Beachell-Borlaug International Scholars Program (MBBISP) for financial support in his PhD program. Funding from Western Grains Research Foundation (WGRF) for FHB nurseries is greatly acknowledged. Funding organizations did not play direct role in the study. The results in the abstract are published as full-length manuscripts in BMC Plant Biology (1) and Molecular Breeding (2).

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MASS SELECTION FOR REDUCED DEOXYNIVALENOL CONCENTRATION USING AN OPTICAL SORTER IN SRW WHEAT

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ABSTRACT

Genetic resistance is a crucial tool when managing Fusarium head blight (FHB) in soft red winter wheat (SRWW). FHB, also known as head scab, results in discolored *Fusarium* damaged kernels (FDK) contaminated with deoxynivalenol (DON). We hypothesized that an optical sorter could be used to select breeding lines with FHB resistance (lower DON and FDK values). Mass selection (MS) using an optical sorter calibrated to reject scabby (discolored) seed and accept non-scabby seed was conducted at Lexington, Kentucky from 2016-2018. Only accepted (non-scabby) seed was used to plant subsequent generations. Final evaluation of all selection cycles was conducted in 2019 at Lexington. Selection was conducted in a disease nursery inoculated with *Fusarium* infested corn kernels. Selection candidates (1-meter rows) were arranged in a randomized complete block design (RCBD) with 2 replications; 3 replications were used in 2018. For the final evaluation in 2019, we used 6-row plots arranged in a RCBD with 2 replications, also in an inoculated disease nursery. DON levels were obtained from samples submitted to the University of Minnesota DON testing lab. FDK was estimated with the optical sorter (FDKos) using the following formula: FDKos (%) = (weight of rejected grain (g) / (weight of rejected grain (g) + weight of accepted grain (g))) *100. Using FDKos as a proxy to select for lower DON accumulation resulted in lower DON and FDK values with each additional cycle of selection; after 3 cycles of selection (C_3) DON levels were at 92% of the resistant check (KY02C-3005-25) and FDK values at 144% of KY02C-3005-25. Direct selection for lower DON resulted in final C_3 DON levels 53% of KY02C-3005-25, but DON levels did not consistently decrease each cycle of selection as had been observed with indirect selection on FDK. Direct selection for lower DON resulted in increases in FDK with each cycle of selection. An index (DON as % KY02C-3005-25 + FDK as % of KY02C-3005-25) was also used to guide selection. Index selection resulted in consistent decreases in FDK and DON, with final C_3 DON levels equal to direct selection for lower DON (53% of KY02C-3005-25) and final C_3 FDK levels at 142% of KY02C-3005-25. These findings suggest that optically sorting grain is an effective breeding strategy for lowering DON accumulation and limiting kernel damage associated with FHB in SRWW.

ACKNOWLEDGEMENT AND DISCLAIMER

This material is based upon work supported by the U.S. Department of Agriculture, under Agreement Nos. 59-0206-4-002 and 58-6070-8-020. 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.

EVALUATION OF WINTER BARLEY CULTIVAR NOMINI FOR 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 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 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. A population of 160 $F_{5:8}$ RILs derived from the cross Thoroughbred / Nomini were evaluated for FHB resistance. FHB Incidence (INC), FHB Severity (SEV), *Fusarium* damaged kernels (FDK), and deoxynivalenol (DON) were assessed in 2017-2019 at Mount Holly, VA, Kinston, NC and Lexington, KY. Preliminary QTL regions were identified on chromosomes 2H and 3H associated with FHB INC, FDK and DON and FHB INC, respectively. The 2H QTL accounted for as much as 33.7% of the phenotypic variation for DON accumulation, 24.5% for FDK, and 17.7% for FHB INC. The QTL marker region spans 164.5 to 192.5 cM with flanking markers JHI-HV50k-2016-108929 and JHI-HV50k-2016-102142. The 3H QTL regions accounted for 8.3% and 0.39% of the phenotypic variation of FHB INC, respectively. The marker regions span 118.5 to 120.5 cM and 340.5 to 342.5 cM, respectively. FHB resistance QTLs will be validated in a doubled haploid (DH) population derived from the cross Nomini / Violetta in the 2019-20 growing season, and preliminary mapping results of this population will be presented in the poster.

ACKNOWLEDGEMENT AND DISCLAIMER

This material is based upon work supported by the U.S. Department of Agriculture, under Agreement No. 59-0206-8-191. 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.

GENOME-WIDE ASSOCIATION STUDIES OF FUSARIUM HEAD BLIGHT DISEASE RESISTANCE IN SOFT RED WINTER WHEAT POPULATION

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ABSTRACT

Fusarium head blight is one of the most devastating diseases of wheat and barley. Identification of genomic regions associated with resistance to Fusarium head blight (FHB) will facilitate accelerating the development of FHB resistant cultivars. We performed genome-wide association studies (GWAS) using > 350 Purdue-bred soft red winter wheat breeding germplasm to identify genomic regions associated with Fusarium head blight (FHB). FHB nurseries were planted in randomized incomplete block designs crop in 2017-18 and 2018-19 seasons at Purdue Agronomy Farm, West Lafayette, IN, where each line was planted in a 3ft row plot. Scabby corn inoculation was conducted by using nine different isolates of *Fusarium graminearum*. For each line, we measured disease incidence (INC), disease severity (SEV), *Fusarium* damaged kernels (FDK), and FHB index (FHBi). Genotyping-by-sequencing method yielded 14,907 single nucleotide polymorphism (SNP) markers, after filtering for missing data (<20%) and minor allele frequency (>5%). GWAS was performed using FarmCPU models with four principal components as covariates in GAPIT package in in R (v 3.4.0) environment. For the four traits, twenty-four significant marker-trait associations (MTAs) were identified on 11 chromosomes including 1A, 2A, 2B, 3A, 3B, 4A, 4D, 5A, 5B, 6A, and 7A. A region on chromosome 5A (with 10 SNPs) was significantly associated with INC, SEV, is potentially the previously known QTL *Fhb5* for INC. Similarly, the MTAs on chromosome 3B associated with SEV and FDK are representing the *Fhb1* locus. Out of 24 MTAs, 11 MTAs showed lower frequencies of favorable alleles, suggesting that a greater genetic gain can be expected by increasing the frequencies of these alleles. The availability of annotated reference genome of wheat will allow us to search for candidate genes associated with the novel QTLs identified in this study. The information from this study will help characterizing the existing variation in FHB response and designing crosses to develop FHB resistance cultivars.

ACKNOWLEDGEMENT AND DISCLAIMER

This material is based upon work supported by the U.S. Department of Agriculture, under Agreement No. 59-0206-8-214. 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.

USE OF SINGLE NUCLEOTIDE POLYMORPHISM HAPLOTYPES TO AID TRANSFER OF *QFHB.RWG*-5A.2 TO WINTER WHEAT

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ABSTRACT

The fungal disease Fusarium head blight (FHB) is a worldwide threat to wheat production. Ongoing discovery, mapping and use of host resistance QTL greatly facilitate the breeding of resistant cultivars and significantly reduce crop damage. The CM82036 derived genes, *Fhb1* and *Qfhs.ifa-5A* are among the more regularly used FHB resistance QTL. In an attempt to increase and diversify the available resistance in breeding material, the NDSU hard winter wheat (HWW) breeding program aims to also acquire and use confirmed FHB resistance QTL from PI277012. In a previous attempt, only one (*Qfhb.rwg-5A.1*) of two PI277012 resistance QTL could be transferred successfully to the breeding line Novus-4. Failure to transfer *Qfhb.rwg-5A.2* was due to its absence in the donor parent (RWG21) used at the time and inadequate markers. This study re-attempts transfer of *Qfhb.rwg-5A.2* by utilizing a different spring wheat donor (GP80; a doubled haploid line from the cross Grandin/PI277012) and by supplementing existing simple sequence repeat markers with SNP haplotype data and FHB resistance phenotyping. Initially, GP80 was crossed with HWW line Novus-4 and the F_1 backcrossed to winter wheat cultivar Monument. B_1F_1 with the PI277012 allele of marker locus *Xgpw2136* were again backcrossed to three winter wheats (14Nord-01, Monument and 18Nord-114) to obtain three hybrid populations. Seventy B_2F_1 and parental controls were then genotyped with the Illumina iSelect 90K SNP array and B_2F_2 seed of each plant harvested for future FHB resistance tests. Polymorphic SNPs that are located on chromosome 5A were identified, and those that had GenTrain scores of more than 90% were selected using GenomeStudio Genotyping module V 2.0 and the data were exported to Excel. The map locations of these SNP loci were obtained from the 90K wheat consensus map.

First, a GP80 chromosome 5A map was constructed. Comparison of 114 chromosome 5A SNPs polymorphic with respect to PI277012 and Grandin (parents) with corresponding SNPs in progeny line GP80, identified regions of PI277102 and Grandin derived chromatin. The 114 SNPs occurred in 47 map positions that spanned 140 cM of the length of chromosome 5A. On average, the map locations were 2.9 cM apart (at most 11.9 cM). Apparently, GP80 chromosome 5A contains an intercalary region of Grandin chromatin within otherwise PI277012-derived chromatin. The two regions of PI277012 chromatin are: Region I that stretches from the 5AS telomere to the first crossover position, which lies in between map positions 74.8 and 82.7 cM. Region II that stretches from the second crossover position (between map positions 101.2 and 113.1 cM) to the 5AL telomere. Previous results showed that *Qfhb.rwg-5A.2* occurs within region II.

Second, comparison of the GP80 chromosome 5A haplotype map for region II with those of the respective backcross parents and three B_2F_1 populations revealed markers that are useful for tracing chromosome 5A regions that originated in PI277012. Each of the three B_2F_1 populations had a different set of four such diagnostic SNPs. In the 14Nord-01 population, the region II markers distinguished three physically

different patterns of recombined PI277012 chromatin. In each of the Monument and 18Nord-114 B_2F_1 populations, four different region II patterns of recombined PI277012 chromatin occurred. However, the 12 markers did not overlap sufficiently to reveal if the same patterns occurred in more than one progeny group. Presently, FHB resistance evaluations are underway to determine whether the different region II patterns associate with different levels of resistance. This information may reveal which recombinants carry *Qfhb.rwg-5A.2*; the relative amounts of PI277012 chromatin retained in those recombinants; and possibly identify reliable flanking markers for continued marker selection of the resistance.

ACKNOWLEDGEMENT AND DISCLAIMER:

This material is based upon work supported by the U.S. Department of Agriculture, under Agreement No. 59-0206-7-004. 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.

CHALLENGES AND EFFORTS TO MAINTAIN WINTER BARLEY AS A VIABLE CROP IN THE EASTERN U.S.

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ABSTRACT

Hybridization, breeding, genetics and development of winter barley (*Hordeum vulgare*) varieties at Virginia Tech have been conducted for more than 75 years; although, selection of pure lines from landraces was initiated in the early 1900s. The cultivar James ('Wong' / 'Bolivia') was one of the first hybrid-derived varieties release by Drs. Thomas M. Starling and Curtis W. Roane in 1961. Subsequently, they released six additional winter barley cultivars. During their tenures Drs. Roane and Starling also identified and conducted genetics research on the inheritance of resistance to *barley yellow dwarf virus*, powdery mildew (*Blumeria graminis* (DC.) E.O. Speer f. sp. *hordei* Em. Marchal), leaf rust (*Puccinia hordei* G. Otth), net blotch (*Pyrenophora teres* f. *teres* Smedeg.) and scald (*Rhynchosporium secalis* (Oudem.) J.J. Davis f. sp. *hordei*) in barley (link.lib.vt.edu/portal/Curtis-W.-Roane-History-of.../QBYi8pePgs4/). Follow the release of James, emphasis was placed on development of awnless and apically-awned varieties, trait derived from awnletted 'Hudson', due to the difficulty farmers had threshing long-awned varieties under the humid conditions in the mid-Atlantic region. While these types of varieties continue to be preferred for forage production, they produce grain having lower test weights and less plump kernels, which are not ideal for either feed or malt uses. Subsequently, varieties having short to medium-length awns, trait derived from 'Boone', and improved grain quality were developed in the program with cultivar 'Callao' being the first such variety released in 1994. This led to availability of barley varieties producing superior quality grain for both domestic and export markets. Continued interest to improve both the quality and nutritional value of barley for use in large local swine and poultry industries and the onset of local interest in ethanol production, led the program to develop hulless barley varieties, trait derived from the South Carolina variety 'H585', having significantly higher starch and lower fiber than traditional varieties. Cultivar Doyce was the first hulless variety released by Virginia Tech in 2003. A two-rowed hulless barley line VA15H-73 will be submitted for release in 2020. This line has lower FHB Index (0-100), FDK (%), ISK Index (0-100), and DON (ppm) values than the moderately resistant cultivar Eve (8.6 vs 30.2; 15% vs 30%; 29 vs 48; and 7.5 vs 11.3 ppm), respectively. A couple of anomalies were observed in cultivar 'Thoroughbred', which was derived from a cross between VA90-44-110 and the French malt barley cultivar Plaisant and released by Virginia Tech in 2003. Mark Vaughn, research specialist at EVAREC, observed while cleaning seed samples of Thoroughbred that it had very good thresh ability, and Dan Brann, former small grains extension specialist later observed that the long awns easily broke away from the spike during threshing. It was later surmised that this was due to Thoroughbred having deciduous awns. Having varieties with this trait further contributed to improvements in grain quality and plumpness. Upon emergence of interest from Copper Fox Distillery in Sperryville, VA for using local barley for distilling, the cultivar Thoroughbred had its debut as Virginia Tech's first malt barley variety. Since then, Thoroughbred also has been used for malting and production of craft beers. With decline in interest and demand for winter barley for principle use in the feed and fuel ethanol industries, emphasis has been placed on developing two-rowed winter malt barley varieties with release of the first two varieties expected in

2020. Malt barley varieties must meet significantly higher quality standards than traditional feed barley varieties, including having resistance to Fusarium head blight (FHB) and low deoxynivalenol (DON accumulation). A resistance QTL region in 'Eve' hulless barley associated with DON, FHB severity, and FDK was identified on chromosome 6H with flanking markers SCRI_RS_147342 and Bmag0613 that are 10.1 cM apart. Currently FHB resistance in 'Nomini' barley is being mapped in two populations, and preliminary results will be shared in a poster and this presentation.

ACKNOWLEDGEMENT AND DISCLAIMER

This material is based upon work supported by the Virginia Small Grains Board and the U.S. Department of Agriculture, under Agreement Nos. 59-0206-4-032 and 59-0206-8-191. 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.

WHEAT CULTIVARS LOSE NUTRITIONAL QUALITY AT ELEVATED CO_2 ALTERING *FUSARIUM GRAMINEARUM* GROWTH AND MYCOTOXIN PRODUCTION

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ABSTRACT

Increased photosynthetic carbon assimilation rate in C3 crops, such as wheat, at elevated atmospheric CO_2 concentrations can dramatically alter grain nutritional quality. Typically, growth at elevated CO_2 leads to grain with higher carbohydrate content and lower protein, mineral and lipid content. This investigation explores how changes in wheat primary metabolism due to elevated CO_2 affects *F. graminearum* growth and deoxynivalenol (DON) production. We observed a significant decline in wheat nutritional quality when grown at elevated CO_2. However, the change in nutritional quality was dependent on the cultivar and greater in the moderately resistant cultivar Alsen. The change in nutritional composition resulted in less *F. graminearum* fungal growth, but increased production of the mycotoxin DON per unit fungal biomass. Additionally, we evaluated fifteen wheat cultivars and found the moderately resistant wheat lost more protein and mineral nutrients than susceptible cultivars when grown at elevated CO_2.

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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 that produces doubled haploids can generate synergies and efficiencies. The USWBSI is supporting collaborative DH production at Oregon State University (OSU), which started in the fall of 2017. In year one, F_1s from nine pedigrees were solicited from barley researchers and received from Virginia Tech (Brooks & Griffey) and Cornell (Sorrells). From these F_1s, 338 DHs were produced for Virginia Tech and 551 DHs for Cornell. In year two, F_1s from six pedigrees were obtained from Virginia Tech (Brooks and Griffey), the USDA-ARS, Idaho (Bregitzer) and contributed by the OSU breeding program. From these F1s, 1,340 DHs were produced for Virginia Tech, 383 DHs for USDA-ARS, and 442 DHs for OSU. Currently in the third year, F_1s from three pedigrees were gathered from Virginia Tech (Brooks and Griffey) and the USDA-ARS, Idaho (Bregitzer) with the goal of producing 1,200 DHs. With the third year germplasm, we will initiate a new dimension to this project: producing seed of DH plants at OSU and providing tissue of these plants to the USDA-ARS Western Regional Small Grains Genotypic Lab (under the direction of D. See) for genotyping. Collaborators will now receive DH seed and genotype data. This germplasm, and these data, are a publicly available resource for continued progress in breeding for resistance to FHB.

ACKNOWLEDGEMENT AND DISCLAIMER

This material is based upon work supported by the U.S. Department of Agriculture, under Agreement No. 59-0206-7-156. 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.

EPIGENETIC MODIFICATIONS: A NOVEL SOURCE OF FHB RESISTANCE IN DURUM WHEAT

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ABSTRACT

Fusarium head blight (FHB) is a serious threat to wheat production globally due to its frequent outbreaks in the wheat-growing regions. Symptoms in spikelets include premature bleaching and lead to yield and quality losses. In addition, food safety can be compromised by contamination of agricultural products with mycotoxins, including deoxynivalenol (DON), that present a serious threat to consumer's health. Breeding for host resistance is considered as the most effective method to control FHB. However, the same is a challenge for durum wheat as most of the germplasm are susceptible and there is low genetic variation for this trait. Changing environments such as biotic and abiotic stresses leads to change in genomic DNA methylation patterns in plants, which ultimately results in expression of immune-responsive genes enabling resistance against the stress. To exploit this as a novel source of FHB resistance, we aimed at inducing heritable demethylation in selected durum lines.

We treated eight advanced durum-breeding lines with 5-methyl-azacytidine to remove cytosine DNA methylation. A total of 415 progeny of the treated plants were advanced to the M_4 generation. Thirty-two of the 415 M_4 lines were selected following preliminary testing. The 32 promising lines and eight parental checks were further tested for FHB resistance under greenhouse and field conditions over multiple years. Five of the 32 M_4 lines consistently showed less than 30% FHB severity, as compared with the parental lines and FHB-susceptible lines, which ranged above 30%. *Fusarium*-damaged kernels (FDK) and DON analyses on grain harvested from inoculated plants further supported the greenhouse and field disease assessments. To test the stability and inheritance of the epigenetic resistance, two of the most resistant M_4 lines were crossed to a susceptible parent and advanced for two generations without a selection pressure. The backcross-derived families were tested for FHB resistance in the third generation ($BC_1:F_3$). A number of lines showed FHB resistance similar to or better than the resistant M4 parent indicating stable inheritance. The resistant $BC_1:F_3$ lines are being further tested in greenhouse.

Global methylome level (%) analysis between select M_4 and parental lines were compared using the FASTmC method. The analysis indicated no significant difference between M4 and the parental lines. However, transcriptome analysis of a M_4 line revealed significant number of differentially expressed genes (≥2 fold change; log2 value) as compared to the susceptible parent. The differentially expressed genes belonged to signaling, secondary metabolites, proteolysis, binding, PR-proteins, cell wall components, transcription factors, and oxidative stress and have been reported to play crucial roles in FHB and other biotic stress resistance. Furthermore, several pathways, such as biosynthesis of secondary metabolites, photosynthesis, starch and sucrose metabolism, plant hormone signal transduction and plant-pathogen interaction pathways had significant number of differentially expressed genes and may

have facilitated the M_4 resistant line fight Fusarium infection. Currently biological significance of these genes is being evaluated.

ACKNOWLEDGEMENT AND DISCLAIMER

This material is based upon work supported by the U.S. Department of Agriculture, under Agreement No. 58-3640-3-008. This is a cooperative project with the U.S. Wheat and Barley Scab Initiative. Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the author(s) and do not necessarily reflect the view of the U.S. Department of Agriculture.

GENOME WIDE ANALYSIS AND PREDICTION OF FUSARIUM HEAD BLIGHT RESISTANCE IN SOFT RED WINTER WHEAT

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ABSTRACT

Fusarium head blight (FHB) is a disease in wheat caused by the fungal pathogen *Fusarium graminearum*. FHB poses potential economic losses and health risks due to the accumulation of the mycotoxin deoxynivalenol (DON) on infected seed heads. The objectives of this study were to identify novel FHB resistance loci using a genome wide association (GWAS) approach and determine if the use of fixed marker effects in genomic selection models improve prediction accuracies for FHB resistance traits in a training population consisting of 354 soft red winter wheat (SRWW) lines. The population was evaluated in inoculated, misted FHB nurseries in Fayetteville and Newport, AR and Winnsboro, LA in a randomized complete block design from 2014-2017. Lines were phenotyped for resistance traits including DON accumulation, *Fusarium* damaged kernels (FDK), incidence, and severity. Fifty SNP markers were significantly ($p \leq 0.0001$) associated with the resistance traits across 19 chromosomes using the FarmCPU model. Thirteen significant SNPs were identified for DON, notably on chromosome 7AS. Ten were identified for FDK, notably on chromosomes 3AL, 3BL, 4BL, and 7DL. Twelve were identified for incidence, notably on chromosomes 2BS, 5DS, 4AL, and 7BL. While 15 were identified for severity, notably on chromosomes 3BL and 4BL. The naïve genomic selection model outperformed the fixed effect model for all four traits. Genomic prediction accuracies (r) for the naïve model were 0.607, 0.535, 0.342, and 0.550 for DON, FDK, incidence, and severity, respectively. Results from this study will facilitate the development of SRWW cultivars with improved resistance to FHB.

ACKNOWLEDGEMENT AND DISCLAIMER

This work was supported by the United States Wheat and Barley Scab Initiative and in collaboration with SunGrains. DON analysis was conducted by the USDA Mycotoxin Diagnostic Laboratory in the Department of Plant Pathology Department at the University of Minnesota under the direction of Dr. Yanhong Dong.

GA09129-16E55 (AGS 3015), A NEW SOFT RED WINTER WHEAT CULTIVAR ADAPTED TO THE US SOUTHEAST WITH IMPROVED FHB RESISTANCE

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ABSTRACT

Genetic resistance is the most efficient and economic, and environment friendly approach to control wheat diseases including scab or Fusarium Head Blight (FHB). Adapted germplasm of Soft Red Winter Wheat (SRWW) to the US Southeast (SE) region have been crossed with various sources of FHB resistance (Neuse and Jamestown) to introduce FHB resistant QTL into the University of GA (UGA) adapted SRWW genetic backgrounds. Currently, advanced and elite UGA lines possess various levels of FHB resistance while being well adapted the SE regions. In 2019, The University of Georgia (UGA) in collaboration with the SUNGRAINS breeding programs, a new UGA line, GA09129-16E55, was released and licensed to AGSouth Genetics (AGS) Company under the name of AGS 3015 to complement previously released cultivars arsenal (AGS 3030, GA JT141-14E45 and AGS 30340, GA 051207-14E53, to combat FHB in GA and the SE region. It was selected from the cross of GA991109-6E8 *2/ IL00-8530. AGS 3015 has wide adaptation covering many regions in the SE and it combines high grain yield, excellent test weigh and diseases/insects resistances including FHB. AGS 3015 is an awned, with excellent test weight, and medium height cultivar. It has several FHB QTLs including Fhb-1B and 6A from Jamestown and Fhb-1A and Fhb-4A from Neuse. These QTLs provide AGS 3015 good resistance to FHB compared to most previously GA cultivars. It shows relatively low disease severity and lowers levels of Deoxynivalenol (DON) toxin, FHB Index, and *Fusarium* damaged/scabby Kernels (FDK/FSK). AGS 3015 also has the *Yr17*/*Lr37*/*Sr38* genes that protect it from most prevalent races of rusts in the SE regions. In addition, AGS 3015 has field resistance to Hessian fly and *Sbm1* gene for soil born mosaic virus resistance. AGS 3015 along with many cultivars and elite lines with FHB resistance derived from Truman/Bess, Neuse, MD08-27-E9 and Jamestown, showed excellent performance when evaluated under Georgia's field conditions during 2018-19 for FHB resistance and agronomic performances. In addition, several double haploid lines with good levels of FHB resistance derived from Jamestown, Truman, Neuse or derived *FHB1* lines were also identified with high grain yield potential are being evaluated and are potential for release in 2021. It is important to emphasize

that MAS/GS, double haploid development and field phenotyping have enhanced substantially FHB resistance in our program.

ACKNOWLEDGEMENT AND DISCLAIMER

This material is based upon work supported by the U.S. Department of Agriculture, under Agreement No. 59-0206-8-207. 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.

EVALUATION OF ABERDEEN BARLEY (*HORDEUM VULGARE*) GERMPLASM FOR FUSARIUM HEAD BLIGHT RESISTANCE

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ABSTRACT

In response to increased incidence of Fusarium head blight (FHB) in commercial barley fields in Idaho, the USDA-ARS barley research program has increased efforts to breed for resistance to FHB disease. Our objectives include characterization of FHB disease in Aberdeen spring and winter barley germplasm, the identification of quantitative trait loci (QTL) in this germplasm, and the initiation of a genomic selection program for spring malting barley. In 2017-18, selected winter-habit barley lines (150) were evaluated in Aberdeen, ID, and Mt. Holly, VA; and in 2018-19 testing was expanded to include 200 lines and two additional nurseries (Kimberly, ID and Ithaca, NY). All tests were conducted in replicated, inoculated and misted FHB nurseries. Results indicated that some of the Aberdeen winter germplasm possessed useful levels of FHB resistance. In 2019 a genomic selection program was initiated, made possible in part by increased funding for FHB research at Aberdeen. As a first step, a 248-line two rowed spring barley training population (TP) was selected for FHB evaluation in the misted and inoculated FHB nursery in Aberdeen, ID. The TP includes parents, breeding lines and cultivars selected from the founder population for our genomic selection breeding program. The TP was phenotyped by scoring for visual severity and correlated well with agronomic data, including lodging and height. Preliminary genome-wide association study (GWAS) indicates the potential of using genomic selection in our breeding program to advance lines selected for desired agronomic traits along with FHB resistance.

ACKNOWLEDGEMENT AND DISCLAIMER

This material is based upon work supported by the U. S. Department of Agriculture, USDA-ARS Project 2050-21000-034-00. This is a cooperative project with the U.S. Wheat & Barley Scab Initiative (Project FY18-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.

THE 2019 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, resistance to Hessian fly, milling and baking quality and agronomic characteristics. Genotypic analyses identify major QTL alleles present at numerous important loci. In addition, we provide Genomic Estimated Breeding Values (GEBV) for resistance traits in nursery entries to research the utility of genomic selection approaches to breeding for FHB resistance. These were estimated from a training population of nursery entries from 2011 to 2018. A combined mixed model analysis of the phenotypic data from 2011 to 2018 was performed using SAS 9.3 and BLUEs for each genotype were recorded. The number of SNP markers utilized was 19,897. The genotypic selection model utilized Ridge Regression BLUP through the R-package RR-BLUP (ver. 4.6) to predict GEBVs for individuals in the 2018 nursery. GS model accuracy was evaluated by Pearson correlation between GEBVs and best linear unbiased estimate (BLUE) for the 2018 entries. Correlation varied between 0.69 for FHB Rating to 0.68 for FDK and 0.78 for DON.

The 2018-19 nursery comprised 44 advanced generation breeding lines and six check cultivars, Ernie, Bess, Jamestown (partially resistant) and 'Coker 9835', 'SS 8641'and 'AGS 2035' (susceptible). 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 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 2019 National Fusarium Head Blight Forum and subsequently on line at the USWBSI web site: https://scabusa.org/.

ACKNOWLEDGEMENT AND DISCLAIMER

This material is based upon work supported by the U.S. Department of Agriculture, under Agreement No. 59-0206-8-209. 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. Phenotypic means across locations, correlations between GEBV and phenotypic means and genotypic content of regions associated with FHB resistance.

	Cultivar/ Designation	FHB Rating		FHB Incidence		FHB Severity		FHB Index		FDK		ISK		DON	
			Rank		Rank		Rank		Rank		Rank		Rank		Rank
1	ERNIE	3	2	61	1	22	1	15	3	16	1	32	5	4	7
2	COKER9835	7	49	99	50	65	49	65	49	60	49	71	49	18	49
3	BESS	3	2	71	12	30	13	21	8	16	1	37	14	4	7
4	JAMESTOWN	3	2	67	7	29	8	18	4	21	10	37	14	3	3
5	SS 8641	7	49	97	49	72	50	70	50	68	50	72	50	21	50
6	AGS 2035	5	38	92	47	44	36	41	42	45	45	67	48	16	47
7	NC14-23372	3	2	79	31	29	8	24	15	28	23	38	18	8	34
8	DH13SRW023-201	4	19	79	31	28	6	23	13	29	26	40	22	8	34
9	NC11546-14	3	2	72	14	30	13	24	15	26	20	34	9	5	16
10	NC11331-38	4	19	78	29	44	36	37	38	28	23	43	28	8	34
11	NC15-21834	3	2	75	22	43	35	32	31	22	11	43	28	7	27
12	NC15-21835	3	2	78	29	41	30	31	29	25	18	42	25	8	34
13	NC15-21836	3	2	76	27	37	24	28	24	30	30	47	33	7	27
14	NC12164-25T	5	38	84	39	55	47	46	46	42	43	57	44	8	34
15	NC12642-81	3	2	73	16	36	20	27	22	19	5	32	5	4	7
16	NC12753-139	5	38	86	42	46	41	40	41	39	39	55	43	9	41
17	AR11289-8-1	4	19	79	31	40	27	32	31	25	18	46	32	8	34
18	ARLA09179UC-1-1	5	38	73	16	34	19	24	15	24	16	42	25	7	27
19	ARGA09485-10-1	4	19	84	39	40	27	32	31	39	39	54	40	11	43
20	AR11255-10-3	5	38	94	48	51	45	48	47	42	43	66	47	13	45
21	ARFHBDH2_72	3	2	67	7	41	30	28	24	26	20	38	18	3	3
22	ARFHBDH2_75	4	19	69	9	36	20	24	15	28	23	37	14	6	23
23	GA15VDH-FHB-MAS33-18LE46	5	38	80	36	45	38	35	36	39	39	53	38	5	16
24	GA15VDH-FHB-MAS23-18LE43F	3	2	62	3	24	3	14	1	20	7	27	2	4	7
25	GA15VDH-FHB-MAS30-18ESc43F	3	2	72	14	26	4	19	6	30	30	36	12	4	7
26	GA15VDH-FHB-MAS22-18ESc-41F	4	19	75	22	45	38	36	37	33	37	45	31	3	3
27	GA15VDH-FHB-MAS30-18EDH29F	3	2	75	22	29	8	22	11	24	16	35	11	3	3
28	GA15VDH-FHB-MAS27-18ADH33F	4	19	76	27	32	15	25	20	30	28	40	22	7	27
29	KWS202	4	19	81	37	52	46	42	44	30	30	49	36	7	27
30	KWS207	5	38	86	42	45	38	39	39	47	46	53	38	13	45
31	KWS219	3	2	68	9	28	6	21	8	18	3	27	2	5	16
32	KWS240	4	19	84	39	47	42	39	39	32	35	54	40	10	42
33	KWS242	4	19	82	38	49	44	41	42	32	35	54	40	7	27
34	LES 172093	2	1	71	12	29	8	21	8	19	5	37	14	5	16
35	LES 167851	4	19	68	9	27	5	18	4	20	7	32	5	6	23
36	LES 70022	4	19	63	3	36	20	24	15	18	3	28	4	4	7
37	LA08080C-31-1	6	47	90	45	48	43	43	45	52	47	61	45	12	44
38	LA13197SC-46	6	47	91	46	58	48	53	48	54	48	64	46	16	47
39	LA15203-LDH112	4	19	79	31	41	30	34	34	30	28	43	28	8	34
40	LA15VDH-FHB-MAS10-16	3	2	61	1	23	2	14	1	23	13	26	1	2	1
41	LA15VDH-FHB-MAS10-18	3	2	66	5	29	8	20	7	22	11	32	5	2	1
42	LANCDH11558-109	5	38	73	16	40	27	30	28	31	33	36	12	5	16
43	13VTK434-89	3	2	73	16	39	26	29	26	20	7	39	21	5	16
44	DH13SRW021-70	4	19	74	21	42	33	34	34	23	13	34	9	5	16
45	VA11MAS2-68-4-1-3	4	19	79	31	32	15	25	20	32	34	42	25	6	23
46	VA17W-74	4	19	64	5	33	17	22	11	27	22	38	18	4	7
47	VA17W-75	4	19	73	16	33	17	23	13	23	13	41	24	4	7
48	VA17W-176	4	19	75	22	38	25	27	22	33	37	49	36	6	23
49	15VDH-FHB-MAS33-30	5	38	75	22	42	33	29	26	29	26	48	35	4	7
50	13VTK429-3	4	19	86	42	36	20	31	29	36	39	47	33	7	27
	Mean	4		77		39		31		30		42		7	
	LSD (0.05)	3		24		21		22		25		15		8	
	CV%	34.0		16.1		26.9		36.7		41.7		17.4		61.0	
	Correlations with Predic.	0.69		.		.		0.7		0.68		.		0.78	

Table 1. Continued

	Cultivar/ Designation	Head. Date		Plant Ht.		Flour Yield %		Softness Equiv. %		Hessian Fly			Fhb Massey 3BL	Fhb 5A_Ning	Fhb 2DL Wuhan 1/W14	Bess 2B	Bess 3B	Jamestown 1B	NC-Neuse 1A	NC-Neuse 6A
			Rank		Rank		Rank		Rank	Bio. L	H13	Fhb1								
1	ERNIE	116	2	34	23	69.3	34	58.5	25	0-20	no	no	F3BM	no	no	no	het	no	F1AN	F8AN
2	COKER9835	117	10	34	23	69.7	38	65.4	49	0-18	no	no	no	no	no	no	no	no	no	no
3	BESS	118	18	35	30	67.5	11	57.4	19	0-18	no	no	no	no	no	F2BB	F3BB	no	F1AN	het
4	JAMESTOWN	115	1	30	2	68.5	24	59.9	34	0-14	no	no	no	no	no	no	no	F1BJ	F1AN	no
5	SS 8641	118	18	33	16	68.2	18	57.7	20	3-13	no	no	no	no	no	no	no	no	no	no
6	AGS 2035	116	2	34	23	71.9	50	59.2	30	-	-	-	-	-	-	-	-	-	-	-
7	NC14-23372	120	35	35	30	67.2	8	55.1	6	18-2	no	Fhb1	no	no	no	no	no	F1BJ	F1AN	F8AN
8	DH13SRW023-201	118	18	35	30	65.1	1	49.7	1	0-18	no	no	no	no	no	no	no	F1BJ	F1AN	no
9	NC11546-14	120	35	36	38	67.4	9	61.2	37	14-0	H13	het	no	no	no	no	no	F1BJ	F1AN	no
10	NC11331-38	119	28	36	38	67.8	16	56.5	13	16-1	het	no	no	no	no	no	no	F1BJ	F1AN	F8AN
11	NC15-21834	122	49	37	47	66.4	4	55.5	9	0-17	no	no	no	no	no	no	no	no	het	no
12	NC15-21835	120	35	35	30	67.5	11	56.8	17	0-19	no	no	no	no	no	no	no	no	F1AN	no
13	NC15-21836	120	35	36	38	66.3	3	54.7	5	0-19	no	no	no	no	no	no	no	no	F1AN	no
14	NC12164-25T	121	45	36	38	69.2	31	53.6	4	8-9	no	Fhb1	no	no	no	no	no	no	no	no
15	NC12642-81	117	10	35	30	69.0	30	59.4	31	21-0	H13	Fhb1	no	no	no	no	no	het	F1AN	het
16	NC12753-139	117	10	36	38	67.7	15	58.9	28	9-5	no	no	no	no	no	no	no	no	no	no
17	AR11289-8-1	120	35	37	47	68.9	28	55.2	7	0-16	no	no	no	no	no	no	no	no	no	no
18	ARLA09179UC-1-1	117	10	33	16	71.4	48	58.9	28	0-19	no	no	no	no	no	no	no	no	no	no
19	ARGA05485-10-1	118	18	37	47	69.9	39	58.5	25	0-18	no	no	no	no	no	no	no	no	no	no
20	AR11255-10-3	118	18	37	47	70.2	42	58.8	27	0-17	no	no	no	no	no	no	no	no	no	no
21	ARFHBDH2_72	117	10	32	10	65.4	2	52.8	3	0-15	no	no	no	no	2DL	no	no	F1BJ	F1AN	no
22	ARFHBDH2_75	119	28	32	10	66.5	5	56.3	12	0-19	het	no	no	F5ANg	no	no	no	no	het	no
23	GA15VDH-FHB-MAS33-18LE46	117	10	32	10	68.3	19	64.4	46	16-1	no	no	F3BM	no	no	no	no	F1BJ	no	no
24	GA15VDH-FHB-MAS23-18LE43F	118	18	31	6	68.3	20	58.0	22	0-18	no	Fhb1	no	F5ANg	no	no	no	no	no	no
25	GA15VDH-FHB-MAS30-18ESc43F	117	10	30	2	69.3	34	64.1	45	0-15	no	Fhb1	no	no	no	no	no	no	no	no
26	GA15VDH-FHB-MAS22-18ESc41F	118	18	32	10	70.3	43	64.0	44	17-0	H13	het	no	no	no	no	no	no	no	no
27	GA15VDH-FHB-MAS30-18EDH29F	116	2	30	2	69.6	37	64.5	47	0-21	no	Fhb1	no	no	no	no	no	no	no	no
28	GA15VDH-FHB-MAS27-18ADH33F	119	28	36	38	68.4	22	59.4	31	0-17	no	het	het	F5ANg	2DL	no	no	no	het	no
29	KWS202	119	28	35	30	70.5	45	65.3	48	0-17	no	no	no	no	no	no	no	no	F1AN	F8AN
30	KWS207	121	45	31	6	69.2	31	63.7	43	0-19	no	no	no	no	no	no	no	no	no	no
31	KWS219	119	28	30	2	67.6	14	56.2	11	0-18	no	no	F3BM	no	no	no	no	no	F1AN	no
32	KWS240	120	35	36	38	68.5	24	61.7	40	0-15	no	no	no	no	no	no	no	F1BJ	no	no
33	KWS242	121	45	33	16	70.0	40	66.7	50	0-18	het	no	no	no	no	no	no	no	no	no
34	LES 172093	122	49	35	30	68.6	27	63.5	41	0-17	no	no	no	no	no	no	F3BB	F1BJ	F1AN	F8AN
35	LES 167851	120	35	34	23	70.6	47	55.4	8	0-14	no	no	no	no	no	no	no	no	het	no
36	LES 70022	116	2	34	23	71.6	49	52.3	2	0-17	no	no	no	no	no	no	no	no	F1AN	het
37	LA08080C-31-1	120	35	32	10	68.9	28	61.2	37	16-0	H13	no	no	no	no	no	no	F1BJ	het	no
38	LA13197SC-46	119	28	32	10	69.3	34	57.9	21	0-16	no	no	no	no	no	no	no	no	F1AN	no
39	LA15203-LDH112	118	18	34	23	69.2	31	56.7	16	12-3	no	no	no	no	no	no	no	het	F1AN	no
40	LA15VDH-FHB-MAS10-16	120	35	36	38	66.6	6	56.1	10	12-4	no	Fhb1	no	F5ANg	no	no	no	F1BJ	no	no
41	LA15VDH-FHB-MAS10-18	121	45	36	38	67.9	17	59.5	33	0-21	no	Fhb1	no	no	no	no	no	no	no	no
42	LANCDH11558-109	116	2	33	16	70.4	44	61.4	39	0-18	no	no	no	no	no	no	no	no	het	no
43	13VTK434-89	118	18	35	30	68.5	24	61.0	36	0-18	no	no	no	no	no	no	no	het	no	no
44	DH13SRW021-70	119	28	31	6	70.1	41	63.5	41	0-25	no	no	no	no	no	no	no	no	no	no
45	VA11MAS2-68-4-1-3	116	2	29	1	70.5	45	58.2	24	20-0	het	no	no	no	no	no	het	no	het	no
46	VA17W-74	116	2	33	16	67.4	9	56.6	14	0-20	no	no	no	no	no	no	no	no	F1AN	F8AN
47	VA17W-75	116	2	33	16	67.5	11	56.6	14	0-22	no	no	no	no	no	het	no	no	F1AN	F8AN
48	VA17W-176	118	18	33	16	68.4	22	57.1	18	0-19	no	no	no	no	no	no	no	no	no	no
49	15VDH-FHB-MAS33-30	117	10	31	6	66.6	6	58.1	23	0-19	no	Fhb1	F3BM	no	2DL	no	no	F1BJ	no	no
50	13VTK429-3	120	35	34	23	68.4	21	60.8	35	0-19	no	no	no	no	no	no	no	F1BJ	F1AN	no
	Mean	118		34		68.6		58.9												
	LSD (0.05)	7		.		.		.												
	CV%	3.0		.		.		.												

RAPID GENERATION ADVANCEMENT AND GENOMIC SELECTION ACCELERATE GENETIC GAIN FOR RESISTANCE TO FUSARIUM HEAD BLIGHT

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ABSTRACT

The MSU wheat breeding and genetics program implements genomic selection in wheat variety development for the early identification of lines with predicted high grain yield and resistance to Fusarium head blight (FHB). Each year ~500 populations are developed with emphasis on grain yield and FHB resistance traits including DON and visual FHB severity. Populations are advanced in the greenhouse using "speed breeding" methods. Inbred lines are space planted in the field and undergo visual selection for maturity, agronomic type and disease resistance. Selected plants are genotyped and breeding values are estimated for yield, DON and visual FHB severity. Lines with predicted high grain yield and low FHB resistance traits are increased in single plots that can be combine-harvested and are evaluated under heavy disease pressure in the FHB nursery. A training population of 250 to 300 lines is comprised of all parental lines used to develop breeding populations and advanced breeding lines in replicated yield testing. The training population is evaluated for FHB traits and grain yield in three locations each year. The composition of the training population evolves each year as new parents are moved into the crossing program and new lines enter replicated yield testing. High prediction accuracies are possible for DON mycotoxin. Modest accuracies are possible for grain yield and visual FHB severity. Using rapid generation advancement in the greenhouse and genomic predictions in combination with visual selection, only three years are required to advance from F_1 seed to replicated yield testing.

ACKNOWLEDGEMENT AND DISCLAIMER

This material is based upon work supported by the U.S. Department of Agriculture, under Agreement No. 59-0206-6-003. 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.

EVALUATION OF SELECT BARLEY ACCESSIONS FOR RESISTANCE TO FUSARIUM HEAD BLIGHT AND DON ACCUMULATION IN MULTI-YEAR, MULTI-ENVIRONMENT TRIALS IN THE UPPER MIDWEST

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ABSTRACT

Fusarium head blight (FHB) has devastated the once-thriving malting barley industry in the Upper Midwest and is now threatening other production areas in the western and northeastern United States. *Fusarium graminearum* is the primary causal agent of the disease and produces a number of harmful mycotoxins during infection, most notably deoxynivalenol (DON). Since the reemergence of this disease as a major threat to barley production in the early 1990s, extensive germplasm screening efforts have been made in the field to identify sources of FHB resistance. Over 25,000 *Hordeum* accessions accessions (i.e. barley cultivars, breeding lines, landraces and the two wild relatives of *H. vulgare* ssp. *spontaneum* and *H. bulbosum*) have been screened over the past 20 years. These lines were sourced from many gene banks including the USDA-ARS National Small Grains Collection (NSGC, Aberdeen, ID USA), the N. I. Vavilov All-Russian Scientific Research Institute of Plant Industry (VIR, St. Petersburg Russia), the Nordic Gene Bank (NGB, Alnarp Sweden), the Institute for Cereal Crops Improvement (ICCI, Tel Aviv Israel), the International Center for Agricultural Research in Dry Areas (ICARDA, Aleppo Syria), Plant Genetic Resources of Canada (PGRC, Saskatoon Canada), Leibniz-Institut für Pflanzengenetik und Kulturpflanzenforschung (IPK) (Gatersleben, Germany), and various barley breeding programs around the world. During each season, selections of the most resistant 6-rowed and 2-rowed accessions were made based on the respective resistant controls of Chevron and CIho 4196. The objective of this study was to evaluate all of the barley accessions selected as resistant from past experiments in head-to-head comparisons over multi-year, multi-environment field trials. The ultimate goal of this research was to identify accessions that perform consistently well with respect to FHB resistance and DON accumulation under the Upper Midwest conditions. Evaluations of selected accessions were conducted from 2016-2019 at the University of Minnesota Northwest Research and Outreach Center (NWROC) in Crookston, MN and the Minnesota Agricultural Experiment Station (MAES) in St. Paul, MN. For each accession, data were collected for FHB severity (%), DON accumulation (ppm), and relevant agro-morphological traits (e.g. heading date, height, etc.). Thirty-eight accessions were evaluated in all seven environments (unique year and location combination), while 155 accessions were evaluated in at least four environments. Of the 38 lines evaluated in all seven environments, eight had a mean relative FHB severity (i.e. percentage of infected kernels as compared to the susceptible 6-rowed control Stander averaged over all environments) below 50% and 33 had a mean relative DON accumulation (i.e. percent of DON accumulation in ppm of Stander) below 50%. Of the 155 lines evaluated in at least four environments, 33 had a mean relative FHB severity below 50% and 130 had a mean relative DON accumulation below 50%. This panel of select resistant accessions (176 lines) was genotyped with the 50k Illumina Infinium iSelect genotyping array for barley to provide a comprehensive set of

markers to be used for future haplotype analysis of previously reported FHB resistance quantitative trait loci (QTL). These data will be useful for selecting diverse parental sources for enhancing FHB resistance in breeding programs.

ACKNOWLEDGEMENT AND DISCLAIMER

This material is based upon work supported by the U.S. Department of Agriculture, under Agreement No. 59-0206-6-006. 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.

MOLECULAR MAPPING OF QUANTITATIVE TRAIT LOCI FOR FUSARIUM HEAD BLIGHT RESISTANCE IN THE BRAZILIAN SPRING WHEAT CULTIVAR 'SURPRESA'

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OBJECTIVE

To identify novel quantitative trait loci (QTL) for resistance to Fusarium head blight (FHB) and DON accumulation in the Brazilian spring wheat cultivar Surpresa, and to develop SNP markers for marker-assisted selection (MAS) of FHB resistance in wheat breeding programs.

INTRODUCTION

Host resistance to FHB is a complex quantitative trait governed by quantitative trait loci (QTL) and strongly affected by environmental conditions. No immunity to FHB has been discovered so far, although sources with partial resistance have been identified through extensive germplasm evaluations. Genetic variation in wheat gene pools from diverse geographic regions is valuable to detect sources of FHB resistance and develop locally adapted cultivars with improved FHB resistance. Currently, a few sources of FHB resistance, including Sumai3 and its derivatives, are commonly utilized in wheat breeding programs (Bai and Shaner 2004; Buerstmayr et al. 2009). Extensive utilization of only one or a few sources of resistance over large crop production areas poses vulnerability to resistance breakdown and risk of severe disease epidemics. Therefore, diverse sources of resistance need to be identified and used to develop wheat varieties with a high level of FHB resistance.

'Surpresa' (NSGC accession # PI 185843) is a Brazilian spring wheat cultivar that was identified to have moderate resistance to FHB and DON accumulation from screening over 1000 common wheat accessions in the National Small Grains Collection (NSGC) (Zhang et al. 2008). It was derived from the cross between cultivar Alfredo Chaves-6-21 and Polyssu to withstand aluminum toxicity in the Brazilian acid soil (Rajaram et al. 1988). Without known sources of FHB resistance in the pedigrees, Surpresa may carry a new set of genes for resistance to FHB and DON accumulation. Understanding the genetic basis of FHB resistance in Surpresa will be useful for its utilization in wheat breeding programs.

MATERIALS AND METHODS

For mapping FHB resistance QTL in Surpresa, a bi-parental mapping population containing 187 recombinant inbred lines (RILs) ($F_{2:7}$) was developed from the cross between Surpresa and the FHB-susceptible spring wheat cultivar Wheaton (PI 469271) using a single-seed descent method. The RILs and parents were evaluated for type II resistance in three greenhouse and four field experiments between 2016 and 2018 using the procedures described by Chu et al. (2011). Alsen (PI 615543), having a known *Fhb1* locus, was used as a resistant check in all experiments. All field evaluations were conducted in the FHB nursery located in Fargo, ND. Overhead misting

arrangements were made to maintain optimum relative humidity. Experimental design and disease assessments were done using the protocol described in Zhao et al. (2018). For DON evaluation, infected spikes of all RILs and parents were harvested from both greenhouse and field experiments, threshed, ground to a fine powder, and sent to the USWBSI-supported laboratory for DON analysis.

The RILs and parents were genotyped using two-enzyme genotyping-by-sequencing (GBS) following the protocol described in Liu et al. (2019). Reads generated from GBS were processed by TASSEL-GBS (Glaubitz et al. 2014) to detect SNPs among RILs. The SNPs were filtered to keep those with *MAF* greater than 0.05 and missing data less than 30%. A genetic linkage map with the GBS-SNP markers was constructed in JoinMap® version 5.0 (Van Ooijen 2018) with default settings. The minimum logarithm of odds (LOD) threshold of 3 was used to determine linkage groups. Composite Interval Mapping (CIM) was used to detect QTL in QGene v.4.4 (Joehanes and Nelson 2008). LOD threshold for claiming significant QTL at $P < 0.05$ was determined by performing 1000 permutation.

RESULTS AND DISCUSSION

Continous distribution of FHB severity (type II resistance) and DON accumulation (type III resistance) in the RIL population indicated that these traits were quantitatively inherited (Figures 1 and 2). Transgressive phenotypes were observed for both traits. Surpresa showed moderate resistance to FHB while Wheaton was susceptible, and Alsen was consistently resistant than both parents. RILs had higher FHB severity and DON in greenhouse experiments than in field experiments (Table 1). Such disparity might be due to conditions more conducive for disease development in the greenhouse than in the field. The ANOVA showed a significant genotype and genotype-by-year interaction for FHB severity in all experiments (Table 2). Broad-sense heritability was moderate (0.57) in the greenhouse experiments but was low (0.15) in the field experiments across years. The result indicated a significant influence of genotype-by-year interaction in the reproducibility of FHB severity assessments. DON accumulation in the moderately resistant parent Surpresa varied between 3.4 to 10.3 ppm while it ranged between 5.9 to 47.10 ppm in the susceptible parent Wheaton. Average DON accumulation ranged between 0.33 to 202.40 ppm among RILs across three experiments (Figure 3). Consistent with disease severity, DON accumulation was also higher in greenhouse compared to field experiments. In field experiments, relatively higher DON accumulation was found in corn-spawn inoculated samples than in the point-inoculated samples.

Genotyping-by-sequencing (GBS) analysis of the RIL population identified a total of 5681 SNP markers. After data filtration, 5370 markers were used for linkage map construction and QTL analyses. Four QTL originating from Surpresa for type II (disease) resistance were detected on chromosomes 2A (*Qfhb.ndwp-2AS* and *Qfhb.ndwp-2AL*), 3B (*Qfhb.ndwp-3BL*), and 4D (*Qfhb.ndwp-4D*) explaining 11.1-15.8% of the phenotypic variation (Figure 4). A lower proportion of phenotypic variance accounted for by these QTLs could be due to the effect of significant environment and genotype-by-environment interaction. The QTL detected on chromosome 4D (*Qfhb.ndwp-4D*) showed the largest effect explaining 15.8% of the phenotypic variation. This QTL was delineated to a 3.47 cM interval between SNPs S4D_68970439 and S4D_234703979 and co-localized with the *Rht-D1* locus conferring plant height phenotype. Previous studies have shown co-localization of FHB resistance (type II) QTL with the *Rht-D1* locus (Draeger et al. 2007; Srinivasachary et al. 2008), suggesting that some QTL for FHB resistance may be due to pleiotropic effects of plant height. No significant QTL detected for type III (DON accumulation) resistance.

The two QTL (*Qfhb.ndwp-2AS* and *Qfhb.ndwp-2AL*) on chromosome 2A were mapped approximately 28 cM apart from each other. *Qfhb.ndwp-2AS* was mapped to a 4.08 cM interval flanked by markers S2A_50055119 and S2A_51983004 while *Qfhb.ndwp-2AL* was mapped to a 2.72cM interval

with a peak at SNP S2A_494977419. Based on the physical locations of the markers, both *Qfhb.ndwp-2AS* and *Qfhb.ndwp-2AL* are likely novel QTL because they were not in the genomic regions with QTL detected in previous studies (Angelica et al. 2016; Gervais et al. 2003; Ma et al. 2006; Yi et al. 2018).

Qfhb.ndwp-3BL was mapped to a 6.28 cM interval on the long arm of chromosome 3B. Several QTL for FHB resistance have been identified on chromosome 3B; however only two of them have been mapped onto chromosome 3BL (Bourdoncle and Ohm 2003; Cai et al. 2016). *Qfhb.ndwp-3BL* is not located in the same regions with previously reported QTL for FHB resistance on 3BL, implying that it is likely a new QTL.

The QTL for FHB resistance detected in Surpresa appears to be not stable across different environments. This may be due to the strong genotype by environment effect. Overall, several QTL with small to moderate effect appear to govern resistance to FHB in Surpresa. Based on this study, the genetic basis underlying FHB resistance in Surpresa is complex, and further studies are needed to validate the QTL so that they can be used in wheat breeding programs.

ACKNOWLEDGEMENT AND DISCLAIMER

This material is based upon work supported by the U.S. Department of Agriculture, under Agreement No. 59-0206-7-153. 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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Table 1. Phenotypic data and broad-sense heritabilities for FHB severity and DON content in parents and Wheaton/Surpresa RIL population

Trait	Environments	Parents			RILs		
		Alsen	Surpresa	Wheaton	Mean±SD	Range	H^2
FHB severity	16GH	0.27	na	0.86	0.73 ± 0.19	0.13 – 1.00	
	17GH	0.25	0.401	0.89	0.60 ± 0.16	0.17 – 0.95	
	18GH	0.24	0.36	0.91	0.61 ± 0.15	0.22 – 0.97	0.57
	16FAR	0.19	0.28	0.86	0.50 ± 0.17	0.14 – 0.97	
	17FAR	0.31	0.59	0.85	0.37 ± 0.15	0.10 – 0.84	
	18FAR_P	0.24	0.30	0.66	0.46 ± 0.10	0.19 – 0.71	0.15
	18FAR_C	0.27	0.35	0.76	0.55 ± 0.09	0.31 – 0.76	-
DON content (ppm)	18GH	1.17	7.35	47.10	37.45 ± 30.81	0.33 - 202.4	
	18FAR_P	9.30	3.40	5.90	11.42 ± 6.78	1.00 - 49.90	
	18FAR_C	8.30	10.30	39.80	23.30 ± 12.48	6.80 - 72.10	-

H^2, broad-sense heritabilities; FHB severity, mean of the symptomatic proportions of infected spikes; FAR, field nursery at Fargo location; GH, greenhouse; P, point inoculation; C, corn-spawn inoculation

Table 2. Variance components of FHB severity across environments for the Wheaton/Surpresa population

	Greenhouse			Field		
Source	df	MS	*F*-value	df	MS	*F*-value
Genotype (G)	186	0.15	6.69***	18	0.05	1.80***
Year (Y)	2	0.05	2.76	2	0.22	7.96
G×Y	370	0.05	2.12***	364	0.03	1.23**
Error	1099			740		

MS mean squares, *** P<0.0001, ** P<0.001

Table 3. Summary of QTL in the Wheaton/Surpresa RIL population

Name	Chr	ENV	Flanking SNP markers	LOD	R2	Add.	Association
Qfhb.ndwp-2AS	2A	17GH	S2A_50055119 - S2A_51983004	6.2*	0.144	-4.76	-
Qfhb.ndwp-2AL	2A	16GH	S2A_473904223 - S2A_498098498	5.8*	0.133	-6.33	-
Qfhb.ndwp-3BL	3B	18FAR_C	S3B_792570263 - S3B_807079831	4.3*	0.101	-1.16	-
Qfhb.ndwp-4D	4D	17GH	S4D_68970439 - S4D_234703979	6.3*	0.158	4.90	*Rht-D*

* LOD significant at P<0.05; Chr, chromosome; ENV, experiment in which the QTL was detected; GH, greenhouse; FAR_C, field experiment inoculated with corn inoculum

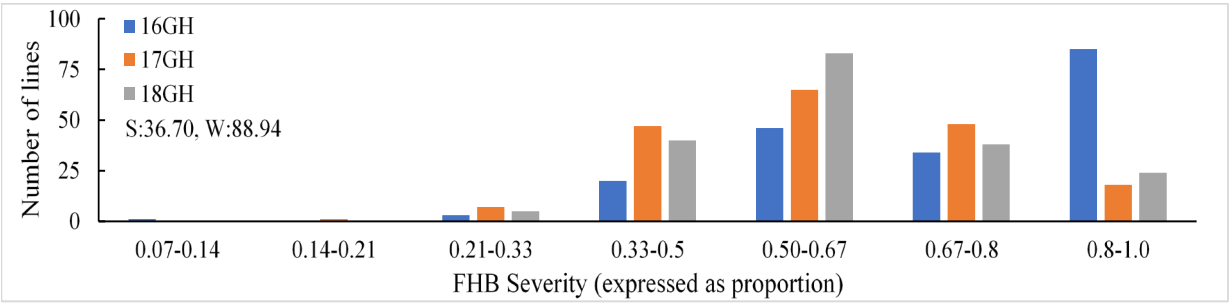

Figure 1. Frequency distribution of FHB severity in Wheaton/Surpresa RILs across greenhouse experiments. (GH, Greenhouse; S, Surpresa; W, Wheaton).

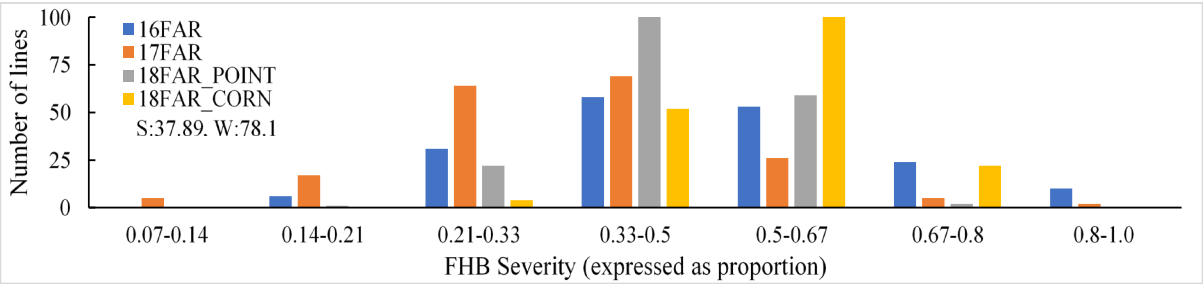

Figure 2. Frequency distribution of FHB severity in Wheaton/Surpresa RILs in field experiments. (16FAR, 2016 Field; 17FAR, 2017 Field; 18FAR_POINT, 2018 Field with point inoculation; 18FAR_CORN, 2018 field with corn-spawn inoculum; S, Surpresa; W, Wheaton).

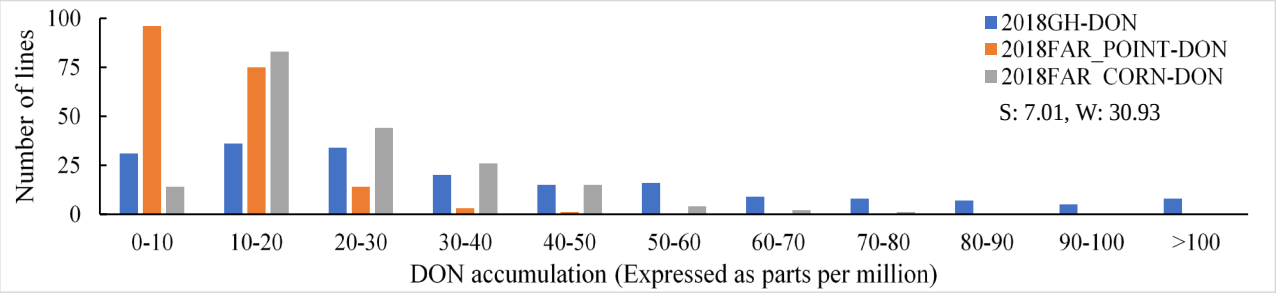

Figure 3. Frequency distribution of DON accumulation in Wheaton/Surpresa population. (GH-DON, DON content on samples collected from greenhouse; FAR_POINT-DON, DON content on samples collected from field experiment with point inoculation; FAR_CORN-DON, DON content on samples collected from field experiment with corn-spawn inoculum; S, Surpresa; W, Wheaton).

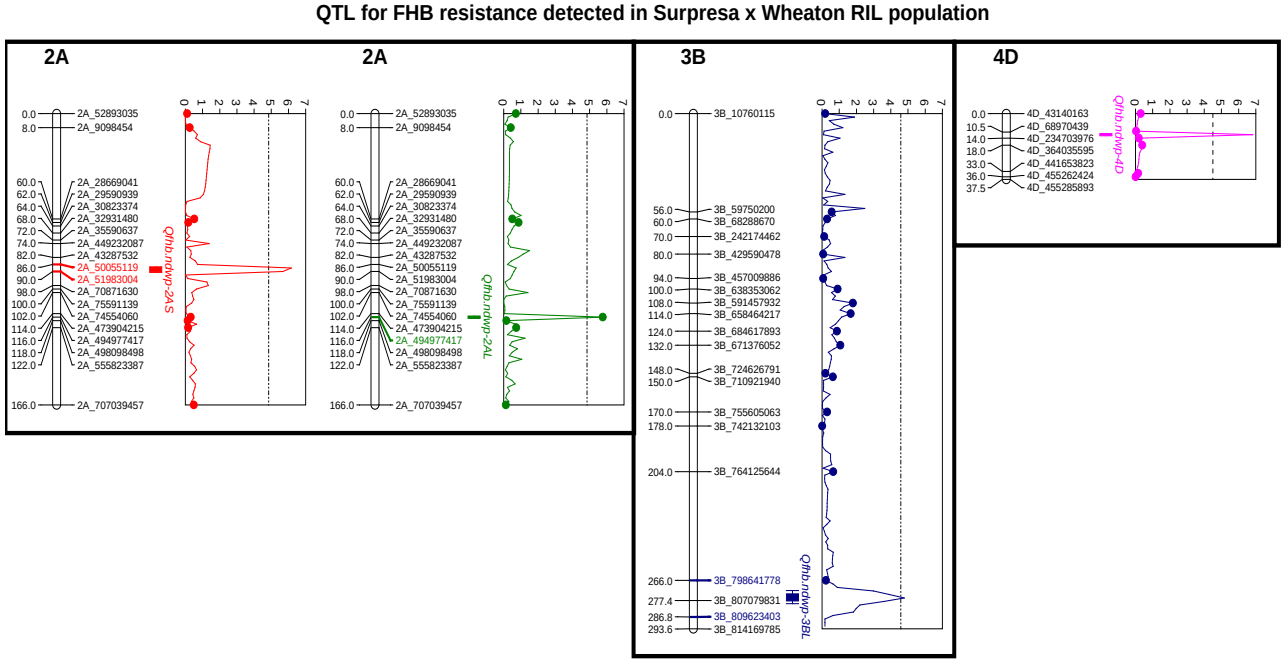

Figure 4. Linkage maps of chromosomes 2A, 3B, and 4D showing respective QTL for type II resistance (disease severity) derived from Wheaton/Surpresa RIL population.

JOINT LINKAGE AND ASSOCIATION ANALYSIS OF FHB RESISTANCE FROM SYNTHETIC HEXAPLOID WHEAT

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ABSTRACT

Fusarium Head Blight (FHB) in wheat, caused by *Fusarium graminearum*, is a fungal pathogen that results in the decrease of yield and quality of small grains including wheat, barley, and rye. The presence of FHB can also lead to the formation of DON in wheat, which is toxic to humans and livestock. Synthetic Hexaploid wheat (SHW), produced by crossing *Aegilops tauschii* (DD) with tetraploid *Triticum turgidum* (AABB) has been used for introgression of resistance to pathogens of hexaploid wheat, *Triticum aestivum*. The SHW lines RL5406, SW-8 and SYN-172 were identified that carry the D genomes of FHB-resistant *Ae. tauschii* accessions TA1599, TA1703 and TA2477, respectively. The SHW lines were crossed with a common wheat line, WA8214 and F_5-derived recombinant inbred lines were developed. A set of 385 RILs were evaluated in a misted and inoculated FHB nursery. Sequence-based genotyping was done to generate genome-wide markers. QTL analysis is being carried out at the whole population and family level to identify genomic regions associated with resistance from the D genome of *Ae. tauschii*. The QTL identified in this work will be valuable in the development of FHB-resistant wheat varieties. Linked markers will facilitate the pyramiding of QTL from *Ae. tauschii* with other large effect QTL present in wheat breeding germplasm.

MOLECULAR MAPPING OF HEXAPLOID WHEAT-DERIVED FUSARIUM HEAD BLIGHT RESISTANCE IN DURUM WHEAT

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ABSTRACT

The complex inheritance of Fusarium head blight (FHB) resistance in the durum background has limited resistance deployment in durum germplasm and varieties. In addition, wheat D genome may play a role in the expression of FHB resistance genes. We have found that the hexaploid-derived FHB resistance genes exhibit different inheritance patterns in durum. Also, we have observed significant variation in FHB resistance among the durum D-genome chromosome substitution and addition lines, suggesting the effect of D-genome chromosomes on FHB resistance. We developed a large RIL population (n=234) from the crosses of FHB-resistant hexaploid wheat 'PI 277012' with FHB-susceptible 'Langdon' (LDN) durum. Chromosome constitutions of the RILs were analyzed using D-genome chromosome-specific STS or SSR markers. Fourteen of them contained 1-7 D-genome chromosomes. The RIL population was evaluated for Type II FHB resistance in three greenhouse seasons using the point inoculation method. High-throughput 90K SNP assay led to the construction of the linkage maps covering the entire A and B subgenomes in the RIL population. The genome-wide QTL analysis identified and mapped five FHB resistance QTL on chromosomes 1A, 3A, 3B, 5A, and 7A. They explained 8.8%, 13.8%, 7.2%, 7.8%, and 5.2% phenotypic variation, respectively. Two FHB resistance QTL (*Qfhb.rwg-5A.1* and *Qfhb.rwg-5A.2*) were previously identified in the FHB-resistant parent PI 277012 and mapped to the short and long arm of chromosome 5A. In this study, we detected *Qfhb.rwg-5A.2* on 5AL, but could not detect *Qfhb.rwg-5A.1* on 5AS in the RIL population. Also, we identified new FHB resistance QTL on other chromosomes (1A, 3A, 3B, and 7A), which were all derived from PI 277012. These QTL analysis results indicated that some durum-derived genetic factors or D-genome chromosomes might play a role in FHB resistance under this particular genetic background. Further studies of the resistance QTL are in progress.

ACKNOWLEDGEMENT AND DISCLAIMER

This material is based upon work supported by the U.S. Department of Agriculture, under Agreement No. 59-0206-7-002. 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.

IMPLEMENTING CROSS SELECTION USING GENOMEWIDE PREDICTIONS FOR SUPERIOR PROGENY MEAN AND TRAIT CORRELATIONS WITH FUSARIUM HEAD BLIGHT SEVERITY

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OBJECTIVES

The objectives of this research were to 1) use genomewide markers and simulated populations to predict the mean, genetic variance, superior progeny mean, and genetic correlation for Fusarium head blight FHB severity, heading date (HD), grain yield and plant height (PH) for possible parent combinations in our breeding program; and 2) compare our current cross combination selection strategy to one informed by these predictions of superior progeny mean and trait correlation.

INTRODUCTION

A major obstacle for improving FHB resistance in barley is its unfavorable association with other agronomic traits. Mapping studies have repeatedly shown an association between low disease severity and late heading or tall plant height (eg. Massman, 2011; Mesfin, 2003). In some cases, there are QTL that are coincident for the unfavorable trait suggesting pleiotropy. In other cases, the correlation may be due to linkage. If the correlation is based on linkage, it should be possible to select parent combinations that would produce progeny in which the unfavorable correlation is less unfavorable. In this study, we use genomewide marker effects combined with simulation of crosses to determine the "best" crosses to make from a select pool of parents. We used the R package PopVar and other code to calculate the superior progeny mean for FHB severity and the correlations among FHB severity, heading date, plant height, and yield. To simultaneously implement and evaluate this crossing strategy we use a set of 23 advanced breeding lines from the University of Minnesota two-row barley breeding program as our parent pool. We imposed two selection strategies to determine the specific cross combinations to make among the 253 possible crosses. The first strategy (traditional) used available trait data from the parents to select parent combinations. The second strategy was informed by the additional information about superior progeny mean and predicted trait correlation.

MATERIALS AND METHODS

The parent candidate pool was selected from our 2019 breeding lines entered into second year evaluations. We selected these parents based on all available phenotypic data, which included two environments of malting quality data, six environments of agronomic data, and four environments of FHB severity data. From the 51 entries in our 2019 second year trial, we selected 23 to be used as parents in our fall 2019 crossing block. Our crossing block included other parents, however for the purposes of this comparison we only considered the crosses that are derived from these 23 parents.

A training population (TP) of 781 two-row barley breeding lines was used for genomewide prediction. These lines were entered into first year yield trials in 2017, 2018, and 2019. Phenotypic data available for these lines was used to train the prediction models. In first year screening, these lines were evaluated in three environments for agronomic traits and two FHB nurseries for severity. Those that advanced to second year

trials were evaluated in the same number of environments for each trait. All of the breeding lines were genotyped with the Illumina 50K SNP barley chip. Using the PopVar packaged in R (Mohammadi et al., 2015), we predicted the mean (μ), genetic variance (VG), superior progeny mean (μsp), and genetic correlations (rG) for each of 253 possible non-reciprocal crosses. Predictions of the mean, genetic variance, and superior progeny mean were made for both FHB severity, yield, HD, and PH individually, while pairwise predictions of the genetic correlation were made between FHB and the other three traits.

RESULTS AND DISCUSSION

The correlations between FHB severity and PH, HD, and yield in the TP were -0.30, -0.36, and -0.04, respectively. This is consistent with previous observations of lower FHB severity associated with taller plants and later flowering. Yield was not significantly correlated; however, we retained it as a factor in our trait correlation predictions.

Traditional Selection Cross Combinations
We selected specific cross combinations considering all available trait information and identifying combinations where one parent had favorable characteristics that complemented another parent. The top 18 cross combinations were selected for crossing. Four of these overlapped with the prediction informed cross combinations (see below).

Prediction Informed Cross Combinations
The distribution of the trait correlations for the crosses reflected the correlations among the traits *per se* in the TP with yield skewed only slightly negatively, and HD and PH skewed more negatively (Figure 1). The range of FHB μsp for all crosses was 16.5% to 31.8% (Figure 2). We selected specific cross combinations by first removing the crosses that exceeded a threshold value of 23% for FHB μsp. This first truncation resulted in retaining 80 crosses (31%). The second selection criteria was based on the evenly weighted favorable direction of the trait correlations for HD, PH, and yield, resulting in 18 cross combinations (Figure 3). As indicated above, four of these overlapped with the traditional method, leaving a total of 32 crosses in this experiment.

Future Work We will make all of these crosses and advance them in our breeding program using our standard procedures. Lines derived from these crosses could be selected to enter first-year yield trials in 2021. We will compare the two parent selection methods by examining the number of lines advanced to first year trials as well as the average performance of lines from each selection criteria. Similarly, we will follow performance of any lines that advance to second year trials. We will initiate another round of this experiment in the fall of 2020 and again in 2021.

ACKNOWLEDGEMENTS AND DISCLAIMER

We thank Ed Schiefelbein, Guillermo Velasquez, and Karen Beaubien for technical support during population development. Thanks go to Madeline Smith and Joseph Wodarek for managing the FHB trial in Crookston, MN. Resources from the Minnesota Supercomputing Institute were used to complete this project. This research was supported by the U.S. Wheat and Barley Scab Initiative under USDA Agreement # 59-0206-4-020, the Minnesota Department of Agriculture, the American Malting Barley Association, and USDA-NIFA Grant #2018-67011-28075. Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the author(s) and do not necessarily reflect the view of the U.S. Department of Agriculture.

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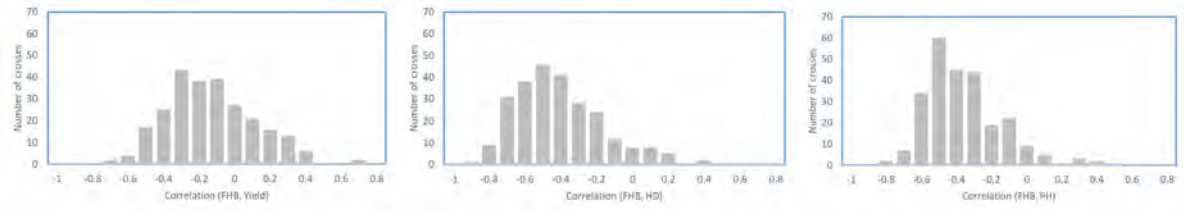

Figure 1. Distribution of correlations between FHB severity and yield, heading date (HD), and plant height (PH) for 253 possible cross combinations among 23 parents

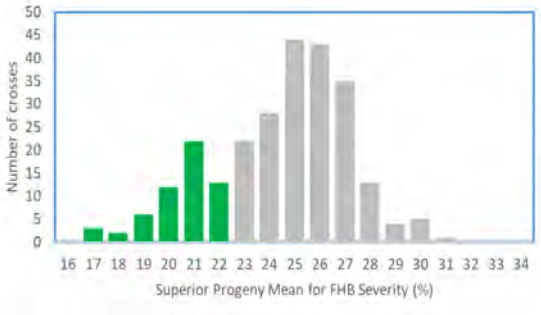

Figure 2. Distribution of FHB superior progeny mean (μsp) for 253 possible cross combinations among 23 parents. Green bars represent the 80 crosses that were selected.

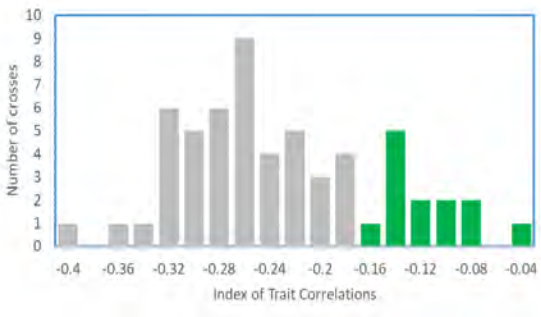

Figure 3. Distribution of the trait correlation index value for the 80 crosses selected by FHB superior progeny mean (see Figure 2).

Index = (-Corr(FHB, Y) + Corr(FHB, HD) + Corr(FHB,PH))/3

Larger values are desirable. Green bars represent the 18 crosses that were selected.

APPLICATION OF GENOMIC SELECTION AT PRELIMINARY YIELD TRIAL STAGE: TRAINING POPULATION DESIGN TO PREDICT UNTESTED LINES

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ABSTRACT

Fusarium Head Blight (FHB) resistance is quantitative and the disease is difficult to evaluate in the field. Genomic selection (GS) could be an excellent tool to calculate genomic estimated breeding values (GEBV) for the different FHB traits, like *Fusarium* Damaged Kernels (FDK) and deoxynivalenol (DON) concentration. In this study, we used data from the Northern Uniform and Southern Uniform scab nurseries to comprise the Training Population (TP). The selection candidates were a total of 360 lines belonging to advanced yield trials from the UK wheat breeding program. The lines were evaluated in a FHB screening nursery, at Lexington, KY, in 2017. We defined sets of 50 lines, randomly, to become the validating populations and we designed different training populations to evaluate size of the TP, performance of the NUS vs SUS, and training population selection methods (Random, Two Tails and PEV) on prediction ability. Overall our results show moderate prediction accuracies, with the SUS being a better training population to predict DON accumulation in the KY lines. To predict FDK, no significant advantage was found using either nursery as the TP, nor were the different TP selection methods significantly different.

ACKNOWLEDGEMENT AND DISCLAIMER

This material is based upon work supported by the U.S. Department of Agriculture, under Agreement Nos. 59-0206-4-002 and 58-6070-8-020. 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.

GENOMIC SELECTION FOR FUSARIUM HEAD BLIGHT (SCAB) RESISTANCE IN NEBRASKA WINTER WHEAT

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ABSTRACT

Fusarium head blight (FHB) is a destructive disease of wheat worldwide that results in severe yield loss, reduced quality and mycotoxin production. Breeding for FHB resistance is one of the major objectives in Nebraska wheat breeding program with the predictive increase of humidity. In order to accelerate scab breeding progress, marker-assisted selection was performed in the backcross populations constructed with elite NE lines and resistant donor parent. On the other hand, genomic selection analysis is applied in the advanced ($F_{3:7}$) and elite ($F_{3:8}$ ~ $F_{3:12}$) yield trials in 2015~2018. Severity and incidence were obtained from phenotypic screening in the misted FHB nursery in Mead, NE. Genotypic data were provided by genotype-by-sequencing (GBS). The average of severity and incidence of elite trials were lower than advanced trials in 2016~2018. The broad-sense heritability of severity was 0.14 ~ 0.74 while 0.30 ~ 0.65 for incidence. The cross validation will be performed in our observation yield trial ($F_{3:5}$) which only have GBS data with unknown FHB performance. In Nebraska, the weather is inconstant that already went through wet, drought and heat since 2015. Due to scab's sensitivity to environment, we expanded 200 lines from NE preliminary yield trial ($F_{3:6}$) to Wooster OH in 2020 which provides a more consistent environment for screen and reliable phenotypes. We will report our results in the poster presentation.

ACKNOWLEDGEMENT AND DISCLAIMER

This material is based upon work supported by the U.S. Department of Agriculture, under Agreement Nos. 59-0206-4-011 and 58-3020-8-027. 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.

QTL PYRAMIDING TO IMPROVE FUSARIUM HEAD BLIGHT RESISTANCE IN DURUM WHEAT

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ABSTRACT

Fusarium head blight (FHB) resistance in wheat is a complex trait controlled by many genes. Pyramiding multiple major QTL is a promising way to improve FHB resistance. QTL mapping had been previously conduced in three durum mapping populations, BP025 (Ben x PI41025), J10Ae (Joppa x 10Ae564), and DP527 (Divide x PI272527). A total of three QTL were identified in the BP025 population and located on chromosomes 2A, 3A, and 5A, respectively; five QTL were identified in the DP527 population and located on chromosomes 1B, 2A, 3A, 5A, and 7B, respectively; six QTL were identified in the J10Ae population and located on chromosomes 1B, 2A, 5A, 6B, and 7A, respectively. A consensus linkage map containing 17,197 SNP markers was first assembled using the three individual linkage maps. To detect redundant QTL, meta-QTL analysis was performed using the 14 initial QTL identified from the three mapping populations and the resulted consensus linkage map. In total, four meta-QTL were detected, including MQTL-*Fhb*-1B, MQTL-*Fhb*-2A, MQTL-*Fhb*-3A, and MQTL-*Fhb*-5A. KASP markers were designed for the detected FHB resistance QTL and meta-QTL. Top five FHB resistance lines with desirable resistant alleles of the identified QTL/meta-QTL were selected from the J10Ae population and were intercrossed with the selected top five lines from the other two populations. A total of 1000 F_2 progenies were obtained from the intercrosses. Marker-assisted selection will select F_2 individuals with resistant alleles of the complementary QTL identified from different populations. We expect to develop durum wheat lines with improved FHB resistance through the marker-assisted QTL pyramiding.

ACKNOWLEDGEMENT AND DISCLAIMER

This material is based upon work supported by the U.S. Department of Agriculture, under Agreement No. 59-0206-7-157. 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.

MACHINE LEARNING MODELS FOR PREDICTING DEOXYNIVALENOL CONCENTRATION FROM GRAIN IMAGING DATA

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ABSTRACT

Resistance to Fusarium head blight (FHB) is a critical trait in many wheat growing regions. However, accurate quantification of FHB resistance is difficult to obtain. Measurement of grain deoxynivalenol (DON) content is quantitative and objective, but expensive and time-consuming. Visual counts of *Fusarium*-damaged kernels are generally highly correlated with DON levels, but data on this trait is subjective and laborious to collect. Grain imaging data presents an attractive alternative to both of these measurements, if it can be used to generate accurate predictions of DON concentrations. We tested 103 doubled-haploid lines from biparental population Catawba × NC12-22844, grown in headrows with two replications in Raleigh, NC during the 2018-2019 winter wheat growing season. All headrows were rated for heading date (HD), FHB incidence (INC) and severity (SEV). DON concentrations of milled grain samples were assessed at the University of Minnesota. Approximately 15mL of threshed seed from each headrow was imaged on a Vibe QM3 grain analyzer. Color data (lightness, hue, and chromaticity) and grain dimension data (pixels/grain, grain length, and grain width) were averaged across all grains for each sample. Multiple linear regression (MLR) and nonlinear random forest regression (RFR) models were used to generate predictions for grain DON content. 50 repetitions of 5-fold cross-validation were performed to assess model generalization ability and overfitting. DON heritability was 0.72, while heritabilities for field-collected data ranged from 0.51 for SEV to 0.97 for HD. Heritabilities for imager-collected data ranged from 0.81 for lightness to 0.96 for grain length. A MLR model fit with HD, INC, and SEV as predictors had a coefficient of determination (R^2) of 0.63. The root mean square error (RMSE) of this model in cross-validation was 4.56 parts per million (ppm) DON. The RFR model generated using the same predictors had a R^2 value of 0.55, and a RMSE of 4.7 ppm DON. A MLR model fit using heading date and imaging data as predictors exhibited a R^2 value of 0.55, and a RMSE of 5.17 ppm DON. Finally, a RFR model fit using the same predictors exhibited a R^2 value of 0.78, and a RMSE of 5.0 ppm DON. These results suggest that grain imaging data can come close to reproducing the accuracy of DON predictions generated using field-collected FHB trait data. The slightly better performance of the RFR model using imaging data suggests the presence of some non-linear relationships between imaging traits and grain DON concentration.

ACKNOWLEDGEMENT AND DISCLAIMER

This material is based upon work supported by the U.S. Department of Agriculture, under Agreement No. 59-0206-7-009. 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.

PRELIMINARY MAPPING OF FUSARIUM HEAD BLIGHT RESISTANCE IN NC13-20076 SOFT RED WINTER WHEAT

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ABSTRACT

Fusarium head blight (FHB) infection causes yield loss, quality degradation, and the production of hazardous mycotoxins in common bread wheat (*Triticum aestivum* L). Genetic resistance to FHB is environmentally ethical and effective through marker assisted selection; therefore, it is in the best interest of the wheat breeding community to identify quantitative trait loci (QTL) that contribute to genetic resistance. NC13-20076 (Jamestown // GA951231-4E28 / NCAG11G / AGS2026) has demonstrated high levels of FHB resistance over two seasons in the Uniform Southern Soft Red Winter Wheat Scab Nursery. Nevertheless, it does not contain previously identified FHB resistance QTL found in Southern US germplasm. A population of 185 double haploid lines from the cross of GA06493-13LE6, a susceptible line, and NC13-20076 was phenotyped in three misted and inoculated nurseries in North Carolina and Virginia during the 2018-19 season. *Fusarium* damage on the head was recorded on a 0 to 9 scale during grain filling; *Fusarium* damaged kernels (FDK) and deoxynivalenol (DON) accumulation were estimated post-harvest. Heading date was recorded at head emergence. A linkage map consisting of 2,047 single nucleotide polymorphic markers on 21 linkage groups was constructed. Major-effect QTL were identified for FDK on chromosomes 2B, 3B, 4A, 5A, and 7A; FHB rating on chromosomes 2A, 2B, 5A, 6A, and 7A; and HD on chromosomes 4A and 5A. QTL consistent for FDK and FHB were identified on chromosome 7A and had significant ($p<0.05$) likelihood of odd scores of 7.41 and 6.99 at their peak markers, respectively. The QTL found on 7A accounted for 7.09% of the variance in FDK and 6.07% for FHB ratings. This study is being repeated during the 2019-20 season.

OTHER

SCREENING OF DON DISEASES FOR DIFFERENT GENETIC LINES OF WHEAT AND BARLEY SEEDS BASED ON HYPERSPECTRAL IMAGING

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ABSTRACT

Deoxynivalenol (DON) is a naturally occurring mycotoxin produced by fungi, which has a tendency to cause lighter weight kernels and contaminate agricultural commodities. The absence of visible fungi does not mean that toxins are not present. It is necessary to find an easy and toxin sensitive way to test DON in wheat and barley seeds from different genetic lines. Thus, hyperspectral imaging was used to detect barley seed kernels samples of 642 genetic lines tested in the experimental field on the Saint Paul campus of the University of Minnesota. DON values of these seed kernel samples were tested by Gas Chromatography-Mass Spectrometer (GC-MS) method as ground truth data, ranging from less than 0.05 ppm to 73.7 ppm. The spectral range of the hyperspectral images is from 367 nm to 1048 nm with 580 wavebands. The hyperspectral images were batch preprocessed for image enhancement to help segment images into background and foreground. Average reflectance of seed kernels was calculated from each hyperspectral cube after preprocessing steps. Partial least squares regression (PLSR) models were then used to predict DON value based on the average reflectance. Correlation between average reflectance spectrum and the GC-MS method for DON value were 0.7673 and 0.6813 for calibration and test set, respectively, while the correlation between DON value and FHB severity is only 0.3238. It indicates that reflectance spectra are more suitable for predicting DON value than testing FHB severity by eyesight. The results marked with plot numbers and genetic lines could also help to find the DON sensitive genetic lines.

ACKNOWLEDGEMENT AND DISCLAIMER

This work was supported in part by Education Department of Fujian Province, and in part by the USDA-ARS U.S. Wheat and Barley Scab Initiative (Agreement No. 58-5062-8-018). 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 project is also partially funded by the National Natural Science Foundation of China (Grant No. 31971786) and the Industrial Partnership Grants from MnDRIVE Robotics, Sensors and Advanced Manufacturing Initiative

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