Integrated Approaches to Managing FHB

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Proceedings of the 2018 National Fusarium Head Blight Forum

Hyatt Regency St. Louis at the Arch • St. Louis, Missouri
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Six-row barley infected with FHB (left) and healthy two-row barley (right)
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MYCOTOXIN CONTROL AND MONITORING PROGRAM: ALL HANDS ON DECK

Anthony Adeuya*

Food and Drug Administration, College Park, MD 20740
*Corresponding Author: PH: 240-402-5759; Email: anthony.adeuya@fda.hhs.gov

ABSTRACT

The threat posed by mycotoxin to global food safety and food security requires involvement from all stakeholders. To protect consumers from health hazards associated with mycotoxins in food, many countries, including the United States, have established national maximum limits or recommended levels. In addition, the Codex Alimentarius (Codex) has established internationally adopted food standards and related texts aimed at protecting consumer health and fair food trade practices. Specifically, the Codex Commission works with member states to establish maximum limits for mycotoxins and develop codes of practice for prevention and reduction of mycotoxins in foods. This presentation will focus on the U.S. Food and Drug Administration's (FDA's) role in promoting and protecting consumer health, FDA's mycotoxin compliance program as well as the FDA contribution to Codex mycotoxin activities. In addition, the roles and responsibilities of the regulated industry in fighting mycotoxins will be discussed.

FHB MANAGEMENT

EFFICACY OF A NEW FUNGICIDE (MIRAVIS® ACE) FOR MANAGING FHB AND DON IN SOUTHERN IDAHO

Suzette S. Arcibal[1], Chad A. Jackson[2], Yanhong Dong[3] and Juliet M. Marshall[4]

[1]Aberdeen Research & Extension Center, University of Idaho, Aberdeen, ID 83210; [2]Department of Entomology, Plant Pathology, and Nematology, University of Idaho, Aberdeen, ID 83210; [3]Department of Plant Pathology, University of Minnesota, St. Paul, MN 55108; and [4]Department of Entomology, Plant Pathology, and Nematology, University of Idaho, Idaho Falls, ID 83402
*Corresponding Author: PH: 208-529-8376; Email: jmarshall@uidaho.edu

ABSTRACT

As part of the multi-state FHB Management Coordinated Project (MGMT_CP), we evaluated integrated FHB and DON management strategies on hard spring wheat grown in the Intermountain West, with emphasis on Miravis® Ace (Adepydin® + propiconazole). Integrated Management (IM) and Uniform Fungicide Trial (UFT) projects were conducted at the University of Idaho Research and Extension Center in Aberdeen, ID. Fungicides were applied using a CO_2 backpack sprayer with paired Teejet VS8001 nozzles, mounted at a 45-degree forward and backward angle, and calibrated to 20 gal/A. Conidial suspensions (50,000 spores/ml) were applied 24-36 hours following the anthesis fungicide treatment using VS8003 nozzles at a ground speed of 1 sec/ft at 40 psi. For the IM trial, we evaluated the integrated effects of fungicides and genetic resistance in hard spring wheat varieties, and compared the efficacy of Miravis Ace applied at heading or anthesis to that of standard anthesis applications of Prosaro®. Four varieties ('Cabernet', 'IDO1602S', 'LCS Star' and 'Rollag') were planted in a RCBD with a split-plot arrangement, with varieties as main plots and fungicide treatments as sub-plots. Miravis Ace treatments applied at heading or at anthesis were compared with inoculated and non-inoculated treatments with Prosaro applied at anthesis and untreated controls. For the UFT trial, a single variety (Cabernet) was used to compare the efficacy of a single application of Miravis Ace applied at heading or anthesis, compared to standard anthesis applications of Prosaro and Caramba® and in combination applied 4 days post-anthesis applications. To estimate the FHB index (IND), severity (percent blighted spikelets per head) of 100 heads per plot were rated 21 to 22 days after the fungicide treatment applied at anthesis. IND was low (below 10% in untreated check) but significant IM fungicide x variety interaction (P=0.0004) and UFT treatment differences (P=0.0009) were found. Treatments that included Miravis Ace applied at anthesis resulted in the lowest IND. A non-detectable FHB level (<0.01%) was observed in two-treatment applications of Miravis Ace and Caramba. Within the IM trial, fungicide-treated plots had significantly lower *Fusarium* damaged kernels (FDK) (P=0.0003) and DON (P=<.0001) compared to the untreated checks. Significant yield (P=0.0161) and test weight (P=0.0011) differences were only observed among varieties. Resistant check Rollag had the lowest disease (IND, FDK and DON) but also the lowest yield. At least another growing season is needed to determine fungicide treatment efficacies under high FHB pressure.

ACKNOWLEDGEMENT AND DISCLAIMER

This material is based upon work supported by the U.S. Department of Agriculture, under Agreement No. 59-0206-4-040 and 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.

EFFECT OF SELECTED FUNGICIDES AND THEIR APPLICATION TIMING ON FHB DISEASE INCIDENCE, SEVERITY, SEED GERMINABILITY, AND DON LEVELS OF WINTER WHEAT IN PENNSYLVANIA

Ananda Bandara[1*], Dilooshi Weerasooriya[1], Brandon Wilt[1], Chris Miller[1], Elli Tatsumi[1], Leticia Rovelo[1], Mark Antle[2], Courtney Anchor[2], James Bollinger[3], Alyssa Collins[1,3], Greg Roth[2] and Paul Esker[1]

[1]Department of Plant Pathology & Environmental Microbiology and [2]Department of Plant Sciences, The Pennsylvania State University, University Park, PA 16802; and [3]Southeast Agricultural Research & Extension Center, The Pennsylvania State University, Mannheim, PA 17545
[*]Corresponding Author: PH: 814-863-4798; Email: axb1739@psu.edu

ABSTRACT

Fusarium head blight (FHB) is a major yield limiting disease in wheat. Besides its quantitative effects on yield, FHB can directly affect seed quality. Use of most efficacious fungicides at appropriate Feekes growth stages is decisive to mitigate FHB-associated seed quality reduction. Fungicide trials were conducted at two locations (Russell E. Larson Agricultural Research Center at Rock Springs and Southeast Research Agricultural Research and Extension Center at Manheim) in Pennsylvania in 2018 using two susceptible varieties ('Seedway 63SR' = Rock Springs; 'MAS4' = Manheim). At both locations, eight treatments were evaluated (1 = untreated check; 2 = Prosaro® 421 SC/6.5 oz/Feekes 9; 3 = Delaro® / 8.0 oz/Feekes 9; 4 = Prosaro 421 SC/6.5 oz/Feekes 10.3; 5 = Miravis ACE® /13.7 oz/Feekes 10.3; 6 = Prosaro 421 SC/6.5 oz/Feekes 10.5; 7 = Miravis ACE/13.7 oz/Feekes 10.5; 8 = Delaro/6 oz/Feekes 9 followed by Prosaro 421 SC/6.5 oz/Feekes 10.5). Trials were conducted using a randomized complete block design with four replications under natural conditions for infection. Disease incidence (proportion of diseases spikes out of all sampled spikes per plot) and severity (proportion of diseased spikelets per spike) were evaluated in the field. A rolled towel assay was conducted in the lab to determine the ratio of germinated/nongeminated seeds (G:NG). Samples were submitted to the University of Minnesota for DON analyses. At Rock Springs, treatments 5, 6, and 8 significantly reduced the disease incidence compared to treatments 1, 2, 3, and 4 (range = 39 – 95 %). Similar results were observed at Manheim. Compared to treatments 1, 2, and 3, significantly lower disease severity was observed with application of treatments 5, 6, 7, 8 at Rock Springs (range = 3.25 – 33%). Similar results were observed at Manheim. While treatments 4 and 6 showed greater G:NG ratio compared to treatment 1 at Rock Springs (range = 1.12 – 3.21), treatment 8 resulted in greater G:NG ratio compared to treatments 1, 3, and 7 at Manheim (range = 0.68 – 1.24). At Rock Springs, treatments 4, 5, 6, 7, 8 resulted in significantly lower DON levels compared to treatments 1, 2, and 3 (range = 0.95 – 4.62 ppm). Similar results were observed at Manheim. For both locations, DON levels were significantly and positively correlated with disease incidence and severity. Based on these results, Feekes 10.5 (regardless of the tested fungicides) appeared to be the most critical fungicide application stage to mitigate FHB incidence, severity, and associated DON levels of winter wheat in Pennsylvania.

IMPACT OF SELECTED FHB-TARGETED FUNGICIDES AND THEIR TIME OF APPLICATION ON YIELD PERFORMANCES OF WINTER WHEAT IN PENNSYLVANIA

Ananda Bandara[1*], Dilooshi Weerasooriya[1], Brandon Wilt[1], Chris Miller[1], Elli Tatsumi[1], Leticia Rovelo[1], Mark Antle[2], Courtney Anchor[2], James Bollinger[3], Alyssa Collins[1,3], Greg Roth[2] and Paul Esker[1]

[1]Department of Plant Pathology & Environmental Microbiology and [2]Department of Plant Sciences, The Pennsylvania State University, University Park, PA 16802; and [3]Southeast Agricultural Research & Extension Center, The Pennsylvania State University, Mannheim, PA 17545
[*]Corresponding Author: PH: 814-863-4798; Email: axb1739@psu.edu

ABSTRACT

Fusarium head blight (FHB) is a devastating disease in wheat. The efficacious use of fungicides at critical growth stages is pivotal to minimize FHB-associated yield losses. Fungicide trials were conducted at two locations (Russell E. Larson Agricultural Research Center at Rock Springs and Southeast Research Agricultural Research and Extension Center at Manheim) in Pennsylvania in 2018 using two susceptible varieties ('Seedway 63SR' = Rock Springs; 'MAS4' = Manheim). Trials were conducted using a randomized complete block design with four replications under natural conditions for infection. At both locations, eight treatments were evaluated (1 = untreated check; 2 = Prosaro® 421 SC/6.5 oz/Feekes 9; 3 = Delaro®/ 8.0 0z/Feekes 9; 4 = Prosaro 421 SC/6.5 oz/Feekes 10.3; 5 = Miravis® ACE/13.7 oz/Feekes 10.3; 6 = Prosaro 421 SC/6.5 oz/Feekes 10.5; 7 = Miravis ACE/13.7 oz/Feekes 10.5; 8 = Delaro/6 oz/Feekes 9 followed by Prosaro 421 SC/6.5 oz/Feekes 10.5). Yield (bu/ac) was measured at harvested (adjusted to 14% moisture content). Additionally, 100-kernal weight (100-KW) and *Fusarium* damaged kernels (FDK, %) were measured. Significant treatment effects ($\alpha = 0.05$) were observed for all response variables at both locations. At both locations, the lowest yield (bu/ac) was obtained from the untreated check (Rock springs = 83.2, Manheim = 46.9). At Rock Springs, yields were greater in treatments 5, 6, 7, and 8 compared to treatments 1, 2, and 3 (range = 82.2 – 103.3 bu/ac), while at Manheim, greater yields were observed in treatments 4, 5, 6, 7, and 8, compared to the untreated check (range = 46.9 – 62.8 bu/ac). At both locations, the lowest 100-KW (g) was obtained from the untreated check (Rock springs = 3.6, Manheim = 3.7). At Rock Springs, 100-KW were greater in treatments 5 and 7 compared to treatments 1, 2, and 3 (range = 3.6 – 4.0 g) while the same in treatments 5, 6, 7, and 8 were greater compared to untreated check at Manheim (3.7 – 4.2 g). At both locations, the highest FDK % was observed in untreated check (Rock springs = 16.8, Manheim = 12.8) while the lowest was recorded by treatment 8 (Rock springs = 9.0, Manheim = 7.1). Integrating the different yield measures, treatments 5, 6, 7, and 8 appeared to be the best to control FHB. Therefore, in general, regardless of the fungicide, their application at either Feekes 10.3 or 10.5 appeared to be equally effective in mitigating the FHB-associated wheat yield losses in Pennsylvania.

OBSERVATIONS ON FUSARIUM HEAD BLIGHT EPIDEMICS IN ALABAMA

Kira L. Bowen*

Department of Entomology and Plant Pathology, Auburn University, Auburn, AL 36874
*Corresponding Author: PH: 334-844-1953; E-mail: BOWENKL@auburn.edu

ABSTRACT

Fusarium head blight (FHB) often caused by *Fusarium graminearum*, can be a devastating disease in winter wheat grown in Alabama. In addition to yield quantity reduction due to disease, the presence of the mycotoxin, DON (deoxynivenol), in grain can result in discounts on price received by growers. As expected, FHB may occur in one part of the state in one year, then occur in another part of the state the next year. The use of cultivars that have lower susceptibility to *F. graminearum* and appropriately timed fungicide application can contribute to reduced losses due to FHB and DON. At two study sites, Fairhope (30.5426, -87.8800) and Belle Mina (34.6908, -86.8846), several wheat cultivars have been monitored for several years. In 2016, FHB was noted at moderate to high levels at Fairhope, but was not seen at Belle Mina. Conversely, in 2018, very low levels of FHB were seen at Fairhope while the disease was readily found at Belle Mina. Rainfall patterns and distribution around the time of flowering at these locations do not readily explain FHB intensity; however, relative humidity patterns might. For example, in 2016, at both Belle Mina and Fairhope, rain events occurred 2 and 3 days, respectively, after Feekes 10.51 (early flower); however, at Fairhope, 100% relative humidity was recorded for several days before and after Feekes 10.51.

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BASELINE SENSITIVITY OF *FUSARIUM GRAMINEARUM* FROM WHEAT, CORN, SOYBEAN AND DRY BEAN TO PYDIFLUMETOFEN IN MICHIGAN

Mikaela R Breunig* and Martin I. Chilvers

Department of Plant, Soil, and Microbial Sciences,
Michigan State University, East Lansing, MI 48824
*Corresponding Author: PH: 517-353-8913; Email: breunigm@msu.edu

ABSTRACT

Historically triazole products have been the sole fungicide option for growers to manage head scab in wheat in the United States. Recently, a new SDHI product, pydiflumetofen (Syngenta), received registration in the United States for head scab control in wheat and may be marketed in the 2019 growing season as Miravis® Ace. Here, we investigate the *in vitro* sensitivity of the major head scab pathogen in Michigan, *Fusarium graminearum,* to this new chemistry pydiflumetofen. We conducted fungicide in-vitro sensitivity testing of 98 isolates of *F. graminearum* to pydiflumetofen, in order to determine effectiveness at inhibiting spore germination and mycelial growth, as well as define a baseline sensitivity before widespread use of this product. Isolates were collected from 2014-2018, from all major agricultural areas of Michigan representing 60 field sites, isolated from wheat, corn, soybean and dry bean. Effective concentration of fungicide to limit growth by 50 percent (EC_{50}) values were determined by mycelial growth assay for 98 isolates, and spore germination assay conducted on a subset of 22 isolates. EC_{50} values for mycelial growth ranged from 0.005 to 0.313 µg/ml with mean 0.069 µg/ml. The EC_{50} estimations for spore germination were generally higher than mycelial growth although all isolates still showed sensitivity, with EC_{50} ranging from 0.17 to 0.61 µg/ml, with a mean of 0.30 µg/ml. Correlation between EC_{50} values for spore germination inhibition and mycelial growth inhibition were analyzed and found to not significantly correlate ($r = 0.239$, $P = 0.2705$). The results of this study provide us with phenotypic data that can be used as a baseline for future monitoring of efficacy and indicate that *F. graminearum* populations in Michigan are very sensitive to pydiflumetofen in-vitro.

INFLUENCE OF TEMPERATURE AND RELATIVE HUMIDITY ON MYCOTOXIN PRODUCTION IN WHEAT AFTER FUSARIUM HEAD BLIGHT SYMPTOM DEVELOPMENT

Wanderson Bucker Moraes[1], Paul B. Schwarz[2], Larry V. Madden[1] and Pierce A. Paul[1*]

[1]Department of Plant Pathology, The Ohio State University, OARDC, Wooster, OH 44691, USA; and [2]Department of Plant Sciences, North Dakota State University, Fargo, ND 58108-6050, USA
[*]Corresponding Author: PH: 330-263-3842, Email: paul.661@osu.edu

OBJECTIVE

Investigate the effects of temperature (cool-20ºC, warm-25ºC, and hot-30ºC) and relative humidity during the window between Fusarium head blight (FHB) visual symptom development and harvest on deoxynivalenol (DON), deoxynivalenol-3-glucoside (D3G), and zearalenone (ZEA) in grain from spikes with known levels of FHB index.

INTRODUCTION

FHB development and DON accumulation are strongly influenced by environmental conditions before, during, and after anthesis. It is well known that major FHB epidemics and high levels of DON are associated with warm temperatures, high relative humidity, and adequate rainfall during the aforementioned periods (6, 7). However, very few studies have investigated the effects of potentially stressful environmental conditions on DON in diseased spikes during the post-anthesis window (1). In particular, the effects of different combinations of cool/hot and wet/dry conditions between visual symptom development and harvest on DON are still not fully understood. This constitutes a major knowledge gap in our understanding of the epidemiology of FHB.

Producers and researchers alike have questioned the association between low FHB index and relatively high DON or disproportionately low DON and relatively high index in some seasons. Anecdotal evidence and results from designed experiments have shown that post-anthesis environmental conditions may have contrasting effects on DON accumulation in harvested grain. For instance, in one study, moisture during the first 10 days after anthesis led to an increase in DON (2), but in a second study, a greater amount of total moisture between anthesis and harvest led to a reduction in DON (3). In addition, results from controlled-environment studies showed that post-anthesis moisture patterns may also play a role in DON exceeding critical thresholds even when FHB levels are relatively low. Andersen et al. (1) found that DON levels increased under certain patterns of intermittent moisture.

Another possible explanation for the relationship between FHB and DON breaking down under certain weather conditions could be the conversion of DON to DON-3-Glucoside (D3G), or the production of other mycotoxins such as ZEA, both of which are missed by common DON testing methods. Failure to detect DON is a major food safety concern as it may enter the food chain, and since it is heat stable, it often persists through cooking and baking. Moreover, when D3G is exposed to stomach acids, the glucose radical is cleaved, converting it back to the more toxic DON after ingestion (4). Thus, D3G may mask the toxic effects of DON in food and feed. Research such as this is needed to identify and quantify field conditions driving DON-D3G conversion in an effort to mitigate this food safety concern through screening, testing, and management well before grain/flour is processed.

MATERIALS AND METHODS

Four different experiments were performed between 2015 and 2018 to evaluate the effects of temperature and moisture after FHB visual symptom development on DON production. In all experiments, FHB-affected spikes were harvested from field-grown plants and subjected to temperature x RH treatments. For the first experiment, field plots of Bravo, an awnless, FHB-susceptible soft red winter wheat (SRWW) cultivar, were planted, whereas for the second, third and fourth experiments, plots of Cooper, an awnless, FHB-susceptible SRWW cultivar, were planted. All plots were planted at OARDC Snyder Farm near Wooster, OH into a field previously cultivated with oats, and managed according to standard agronomics practices for Ohio. In all experiments, plants were inoculated at early anthesis (Feekes 10.5.1) with a 1:1 mixture of ascospores and macroconidia (50,000 spores.ml^{-1}) from 10 Ohio isolates of *Fusarium graminearum* (5).

After visual symptom development (which occurred 18 and 21 days after inoculation), but before natural senescence, symptomatic spikes were harvested, grouped into index categories, and subjected to temperature-RH treatments. The experimental design was a randomized complete block, with a split-plot arrangement of temperature (three levels) as whole-plot, moisture (four levels) as sub-plot, and FHB index categories (five levels) as sub-sub-plot. There were 4 replicate blocks. Three programmable walk-in growth chambers set a 20, 25, and 30ºC, respectively, were used for the temperature treatments. In each chamber, different saturated salt solutions or water were used to achieve four levels of relative humidity: 70% (1:1 mixture of NaCl + KCl), 80% ($(NH_4)_2SO_4$), and 90% ($BaCl_2$), 100% (distilled water) (8, 9). A fixed volume of 250 ml of saturated salt solution or water was placed into 17-by-12-by-6-cm transparent chambers and sealed airtight to maintain the desired RH. Four arbitrarily-selected spikes in each of five index categories (8-15, 20-40, 41-60, 61-80, 90-100%) were assigned to each humidity chambers. There were 5 replicate chambers of each RH level. After four weeks, spikes were removed from the RH chambers and threshed, and kernels were ground and assayed for DON at the U.S. Wheat and Barley Scab Initiative (USWBSI)-funded laboratory at the University of Minnesota in 2015, and for DON, D3G, ZEA at the USWBSI testing laboratory at North Dakota State University in 2016 and 2017.

RESULTS AND DISCUSSION

In all cases, as expected, DON increased as mean index increased, with the highest index categories having the highest mean levels of the toxin (Fig. 1 and 2). However, DON levels varied among temperature-RH treatment combinations (Fig. 1A to E), and this variation depended on the index levels. At all three temperatures, DON levels were higher when spikes with mean index between 12 and 70% (Fig. 1A to D) were exposed to wet conditions (100% RH) compared to those exposed to relatively drier conditions (70, 80, and 90% RH). However, for spikes with a mean index above 90%, comparable levels of mean DON were observed across all temperature-RH treatment combinations (Fig. 1E). Interestingly, DON levels at 90% RH trended to be lower (at least numerically) than at 70 and 80% RH (Fig. 2A to C). This trend is better depicted in the response surfaces in Figure 2.

Temperature influenced the effect of RH on the relationship between index and DON. At 100% RH, higher levels of DON were observed at 20ºC than at 25 or 30ºC, particularly in spike with mean index between 12 and 70%. (Fig. 1 and 2). This suggested that cool and wet conditions after FHB visual symptom development may to be associated with higher DON production. Similarly, the temperature-RH treatment combinations also influenced the relationship between index and D3G. D3G levels were higher for spikes exposed to wet conditions (100% RH) than to relatively drier conditions (70, 80, and 90% RH) at 20 and 25ºC across all tested index levels, with the highest levels of D3G at 20ºC (Figure 1 and 2). DON-to-D3G conversion (based on relative amounts) varied with temperature. Contrary to what was observed

at 20 and 25°C, only marginal changes in D3G levels were observed when spikes were exposed to 100% RH at 30°C (Figure 2). DON-to- D3G conjugation also varied with index level. At 20 and 25°C and 100% RH, D3G levels increased up to 50% index and then decreased as index approached 90% (Figure 2).

Interestingly, extremely high levels of ZEA were observed in wheat spikes exposed to 100% RH across all temperatures and index levels (Figure 1 K-O). Even at the highest levels of index, low levels of ZEA (close to zero) were observed at moisture levels between 70 and 90% across all temperatures. The accumulation of ZEA under wet conditions was also influenced by temperature, with the highest mean levels observed at 20 and 25 °C compared to 30 °C, at index levels below 90%. ZEA levels trended to increase as index increased, reaching a maximum at 50% index, and then decreased at the highest levels of index at 20 and 25°C and 100% RH.

This study was the first to associate DON-D3G conversion and ZEA production during the window between FHB visual symptom development and harvest to temperature and relative humidity, and provides new information that is invaluable for understanding this complex disease-toxin system.

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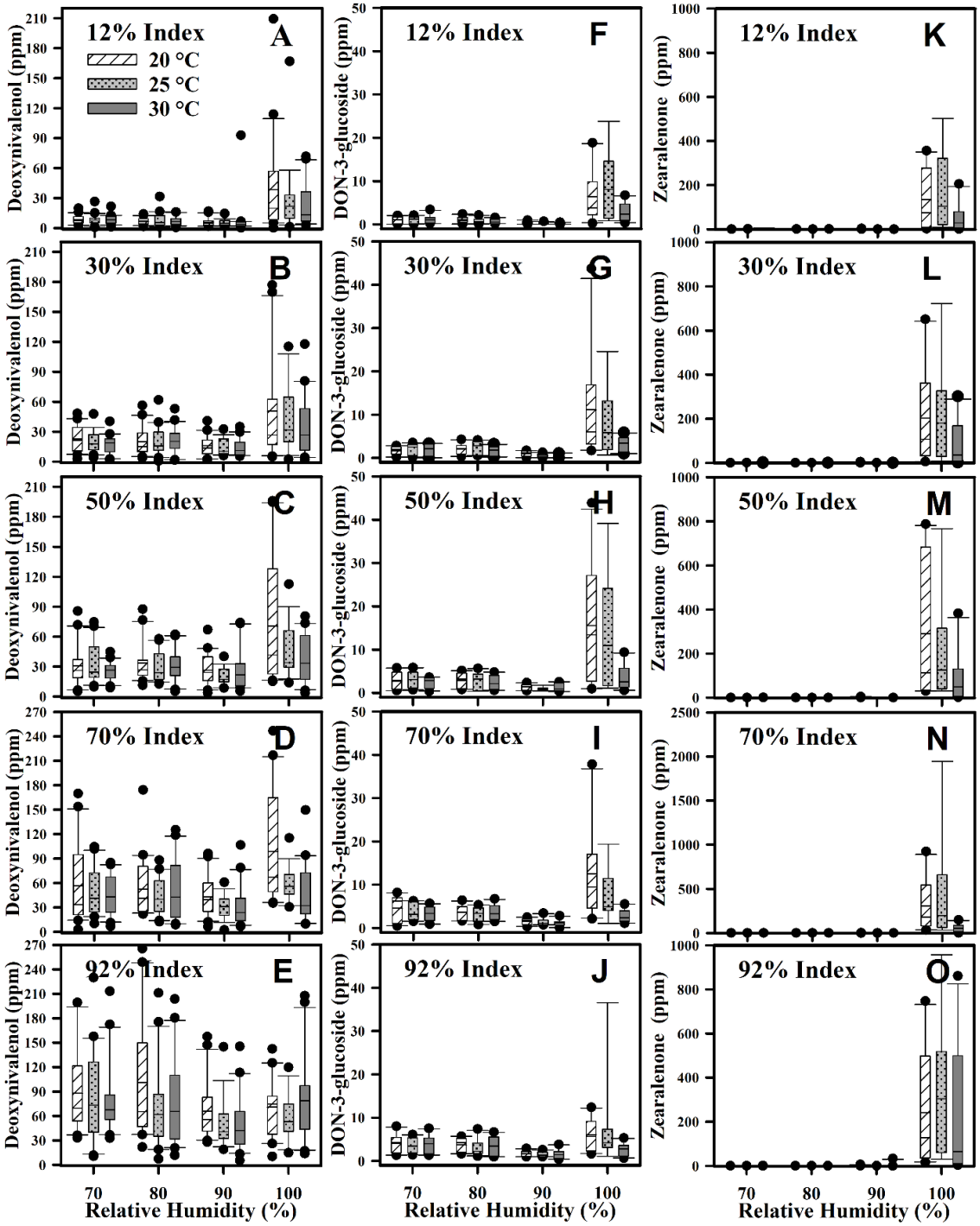

Figure 1. Box plots summarizing the distribution of mean deoxynivalenol (DON) (A-E), deoxynivalenol-3-glucoside (D3G) (F-J), and zearalenone (ZEA) (K-O) content of wheat grain (parts per million [ppm]) of spikes subjected to 70 to 100% relative humidity at 20 to 30°C after FHB visual symptom development. Detached diseased spikes were harvested from the field after FHB symptom development, split in five index categories (12% A, F, K; 30% B, G, L, 50% C, H, M, 70% D, I, N, 92% E, J, O), and placed in RH chambers. The top and bottom lines of the box represent the 75th and 25th percentiles of the data, respectively. Vertical bars extending beyond the boxes represent the 10th and 90th percentiles, and circles indicate extreme values that exceed the 10th and 90th percentiles.

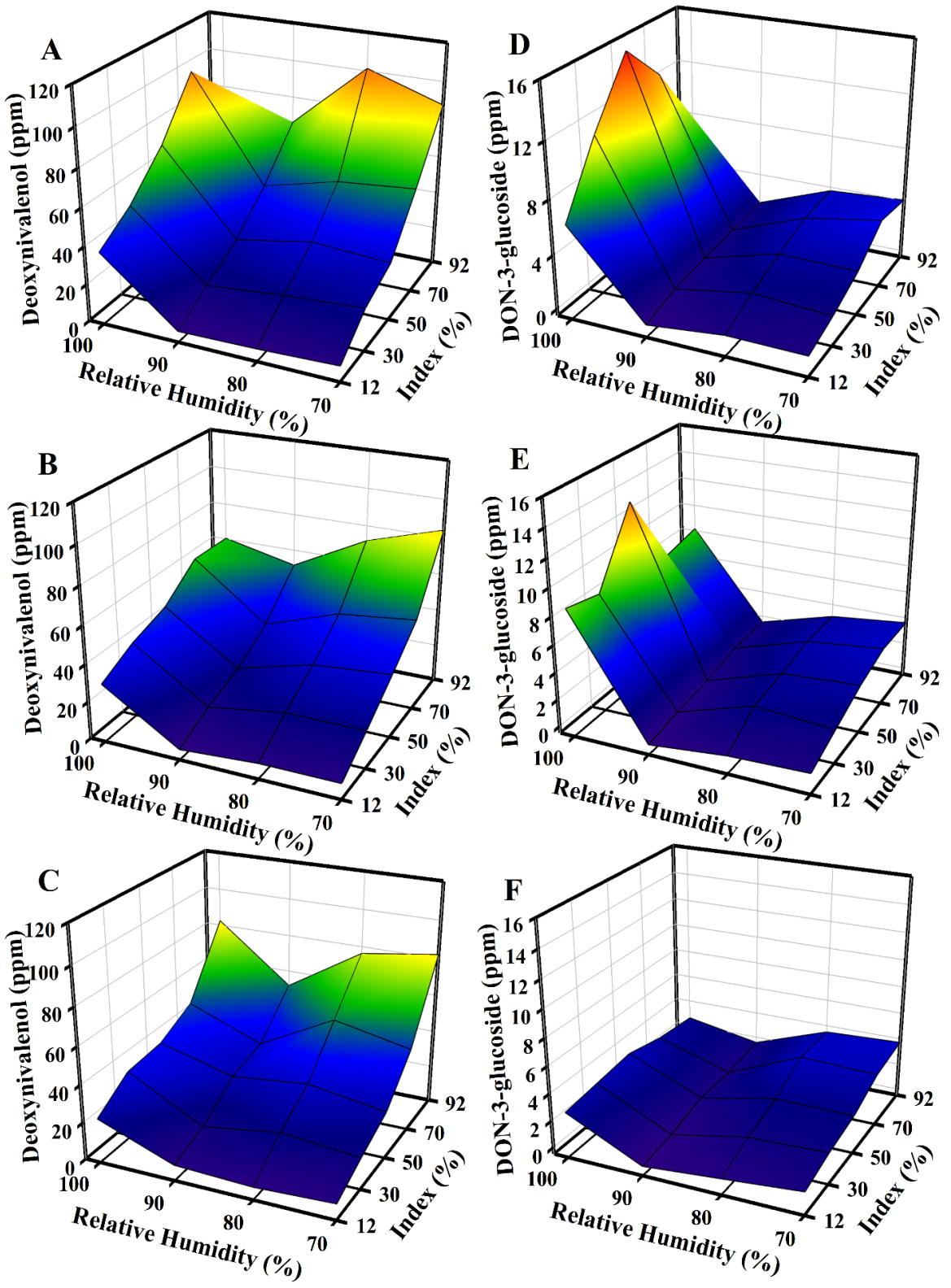

Figure 2. Response surfaces for deoxynivalenol (DON) (A-C) and deoxynivalenol-3-glucoside (D3G) (D-F) as a function of Fusarium head blight (FHB) index and relative humidity. Detached diseased spikes were subjected to 70 to 100% relative humidity at 20º (A and D), 25º (B and E) and 30°C (C and F) for 28 days. Plots were generated using values from the twenty sample replications.

UNDERSTANDING CULTURAL IMPACTS ON SCAB MANAGEMENT ADOPTION IN THE EAST

Alyssa A. Collins[1,2*] and Paul D. Esker[2]

[1]Southeast Agricultural Research & Extension Center, The Pennsylvania State University, Mannheim, PA 17545; and [2]Department of Plant Pathology & Environmental Microbiology, The Pennsylvania State University, University Park, PA 16802
[*]Corresponding Author: PH: 717-653-4728; Email: collins@psu.edu

ABSTRACT

Small grain production in the northeast and mid-Atlantic United States differs from large-scale production in both field size and shape, as well as fit within the larger cropping system. Farms in the region tend to be smaller, more diverse, and centered around dairy production. After twenty years of outreach and extension on Fusarium Head Blight management, what have we learned about how this unique group of farmers receive and use this education?

The scab-conducive weather conditions of 2018 brought into sharp focus the cultural differences among growers in their attitudes toward management intensity in small grains production. Wet, mild conditions in the mid-Atlantic persisted from mid-May through mid-June, representing the majority of anthesis timing for both barley and wheat. Low-input growers who, in the past, may have benefitted from the alignment of anthesis with minimal risk conditions were not able to avoid infection. As a consequence, increased scab and deoxynivalenol levels were reported by grain buyers across the region. In comparison, those farmers who have become proactive about small grain management anticipated scab and other quality issues in their crops in 2018 and, as a result, sought further education from consultants and Extension professionals to inform their management decisions.

What will be the impacts of a growing season that favors scab severity so uniformly? Will some farmers choose to abandon small grains production, or will this inspire different management decisions? How will already attentive growers change their approach to management? We will discuss how the conditions and harvest results of 2018 can be used as both a teaching tool for educators to use with clientele and as a learning tool for researchers to better understand how to reach farm operators with critical disease management information.

EFFECT OF THREE FUNGICIDE TIMINGS ON FHB IN WINTER BARLEY

Christina Cowger

USDA-ARS Plant Science Research Unit, North Carolina State University, Raleigh, NC 27695
Corresponding Author: PH: 919-513-7388; Email: Christina.Cowger@ars.usda.gov

ABSTRACT

In 2017-2018, a field experiment was conducted in a misted, inoculated Fusarium head blight (FHB) nursery at Raleigh, North Carolina, using three winter barley cultivars with different levels of resistance to FHB: 'Violetta' (MR), 'Thoroughbred' (MR/MS), and 'Flavia' (S). All were commercial cultivars in use in breeding programs. 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. Prosaro® (prothioconazole + tebuconazole) and Miravis® Ace (adepidyn + pydiflumetofen) were compared. There were 3 timings: 50% spike emergence (early), 100% spike emergence (medium) and 100% emergence + 6 days (late). Treatments and means across cultivars and four replicate blocks:

Fungicide	Fl oz/acre	Timing (spike emergence)	DON	TW
1 Unsprayed	--	--	3.19a	46.4a
2 Prosaro	8.0	100% emerged	1.45b	47.9b
3 Miravis Ace	11.5	100% emerged	1.01b	47.7ab
4 Miravis Ace	11.5	50% emerged	1.66b	47.9b
5 Miravis Ace	13.7	100% emerged	1.13b	47.6ab
6 Miravis Ace	13.7	50% emerged	1.99b	47.5ab
7 Prosaro	8.0	50% emerged	1.80b	47.6ab
8 Prosaro	8.0	100% emerged + 6 d	1.05b	47.5ab
9 Miravis Ace	11.5	100% emerged + 6 d	1.02b	47.3ab
10 Miravis Ace	13.7	100% emerged + 6 d	1.14b	47.8b

Flavia had significant stunting and unevenness in stem elongation and heading. Yields differed significantly among cultivars (Thoroughbred > Violetta > Flavia) but not among fungicide treatments.

DON: All fungicide treatments significantly reduced DON compared to the unsprayed control (differences significant at $P < 0.0001$). **Late applications were equal in DON reduction to medium applications.** For each cultivar, early applications were numerically higher in DON than medium or late, but the only significant timing effect was with Thoroughbred, where DON was reduced by the late application compared to the other two ($P \leq 0.01$). DON varied by cultivar ($P = 0.008$): Flavia ≥ Thoroughbred ≥ Violetta.

TEST WEIGHT: Test weight did not differ significantly by fungicide timing (early, medium, or late; $P \geq 0.05$). Three fungicide treatments significantly improved test weight compared to the control: Miravis Ace 11.5 oz at 50% emergence, Prosaro 8.0 oz at 100% emergence, and Miravis Ace 13.7 oz at 100% emergence + 6 d. The three cultivars differed significantly ($P < 0.0001$) in test weight: Violetta > Thoroughbred > Flavia.

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

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

Heather Darby* and Erica Cummings

University of Vermont State and Agricultural College, Burlington, VT 05405
*Corresponding Author: PH: 802-524-6501; Email: heather.darby@uvm.edu

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 2017, we observed the disease and yield impact of cultivar susceptibility, inoculation with *Fusarium graminearum*, and treatment with fungicides at two timings.

MATERIALS AND METHODS

The trial was conducted at the Borderview Research Farm in Alburgh, VT in a Benson silt loam soil planted with two spring barley varieties, 'Robust' (susceptible to FHB), 'Conlon' (moderately resistant to FHB) on 27 April 2017. 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 2017 including the surfactant Induce at 0.125% V/V. After the fungicide had dried, plots were spray-inoculated with a conidial suspension of *F. graminearum* (40,000 conidia/ ml) to augment the development of FHB. The second fungicide application occurred four days after heading on 26 June 2017 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 2017. 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 2017 growing season can be characterized as having higher than normal temperatures in April and lower than average temperatures in May, June, July, and August. Rainfall amounts were higher than average throughout the growing season resulting in 7.39 inches of precipitation more than normal.

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 significantly impact DON concentrations (Table 2). The fungicides treatments Prosaro® and Caramba® applied at heading resulted in significantly lower DON concentrations than all other fungicide treatments. The certified organic treatment of ChampION applied at heading was statistically similar to the conventional fungicide Caramba applied at heading. The barley yields did respond differently to the fungicide treatments (Table 2). Under high disease pressure, there were significant differences detected in DON concentrations among varieties (Table 3). Yield did not differ among the varieties. Even though all of the variety+fungicide+timing treatments resulted in DON concentrations above 1 ppm, it's important to note that Conlon, a moderately resistant variety, had lowest incidence of DON levels, while Rasmussen, a susceptible variety, had DON levels almost double (8.29 ppm) that of Conlon (4.53 ppm). This indicates the importance of selecting resistant cultivars to manage FHB in our region.

The application of the conventional fungicides Prosaro, Caramba, and ChampION applied at heading, reduced DON concentrations compared to the inoculated control. In general, the fungicide applications at heading resulted in lower DON concentrations than the fungicides applied 4-days after heading. Interestingly, yields did not vary significantly between fungicide type, application or variety.

ACKNOWLEDGEMENT AND DISCLAIMER

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

Fungicide treatments	Company	Fungicide active ingredient	Application rates
Control			Water
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}
Actinovate®	Novozymes	Streptomyces lydicus WYEC	6 fl oz ac^{-1}
Sonata®	Bayer CropScience	Bacillus Pumllus strain 108	2 qt ac^{-1}

Table 2. Main effect treatment on deoxynivalenol (DON) contamination and grain yield at Alburgh, VT 2017.

Fungicide treatment	DON ppm	Yield bu ac^{-1}
Non-sprayed, non-inoculated control	4.28	69.6
Inoculated FGS 10.1	7.91	58.5
Actinovate (6 fl oz) at heading	7.69	64.1
Actinovate (6 fl oz) 4 days after heading	8.15	71.3
Caramba (14 fl oz) at heading	4.80	52.4
Caramba (14 fl oz) 4 days after heading	6.57	65.3
ChampION (1.5 lbs) at heading	5.74	65.2
ChampION (1.5lbs) 4 days after heading	6.99	64.6
Prosaro SC (6.5 fl oz) at heading	3.68	58.2
Prosaro SC (6.5 fl oz) 4 days after heading	6.35	58.2
Sonota (2 qt) at heading	7.14	66.2
Sonota (2 qt) 4 days after heading	7.64	72.9
LSD (P=0.05)	1.32	NS

Table 3. Main effect of cultivar on deoxynivalenol (DON) contamination and grain yield at Alburgh, VT 2017.

Cultivar treatment	DON ppm	Yield bu ac^{-1}
Conlon	4.53	63.5
Robust	8.29	65.0
LSD (P=0.05)	0.54	NS

ALTERNATIVES OF CHEMICAL CONTROL FOR FUSARIUM IN WHEAT

Carolina Francia[1*], Cintia Palladino[2], Lucas Martella[1], Matias Passarino[1], Lucia Pareja[3] and Carlos Pérez[1]

[1]Departamento de Protección Vegetal, EEMAC, Facultad de Agronomía, UdelaR, EEMAC, Paysandú, Uruguay; [2]Polo de Desarrollo Universitario Abordaje Holístico Impactos de los Agroquímicos, Cenur Litoral Norte, UdelaR, EEMAC, Paysandú, Uruguay; and [3]Departamento de Química del Litoral, Cenur Litoral Norte, UdelaR, EEMAC, Paysandú, Uruguay
[*]Corresponding Author: PH: 598 9 2400670; Email: carofranciabent@gmail.com

ABSTRACT

The reduced diversification of crops, in addition with the direct seeding and the continuous agriculture generated propitious conditions for diseases like wheat Fusarium head blight, caused by *Fusarium graminearum*. This disease causes production and quality losses. The preventive management and the low resistance of the genotypes used, makes this disease hard to control, and necessary to use fungicides in excess. This work aims to evaluate different chemical control managements for wheat Fusarium head blight in two harvests in the locality of Paysandú.

The experiment was planted in the field during 2015/16, with two wheat cultivars (Fuste y Baguette 9), which have contrasting sanitary behavior. For the treatments it was used epoxiconazol and metconazol, as active ingredients; applied in mixture with carbendazim and tebuconazol. The application was realized at Z61, Z65 and Z61 + Z65. The evaluated parameters were: percent of incidence and severity, illness index, production, thousand grain weight, and number of grains (using blotter test).

The results show, that in both years the application of fungicides reduced Fusarium head blight; except in one case. It was observed in 'Fuste', that the triazoles applied in single or double way, were more efficient than the benzimidazol which did not show differences from the control. The application of triazoles in Z61 or mix of triazoles in Z65, allowed obtain a superior production than the treatment with benzimidazol, which again did not show differences from the control. Regarding the parameter thousand grain weight the application of benzimidazol during 2015 in 'Baguette 9', was similar from the control; but differences were observed in all the applications of triazoles. In 'Fuste', the mixed application showed better results in Z61 and Z61+Z65. Cause of the low illness levels, the results were variables, and has high variation coefficients. It was observed a negative correlation with low regression coefficients between moisture-corrected production and the illness level.

EVALUATING ADEPIDYN AND HOST RESISTANCE TO REDUCE FUSARIUM HEAD BLIGHT AND DEOXYNIVALENOL IN SPRING BARLEY

Patrick L. Gross[1], Elizabeth Bauske[1], Jessica Halvorson[1], Scott Meyer[1], Casey Schuh[1], Venkat Chapara[2], Bryan Hanson[2], Lawrence Henry[2], Travis Hakanson[2], Amanda Arens[2], Robert Brueggeman[1] and Andrew Friskop[1*]

[1]Department of Plant Pathology, North Dakota State University, Fargo, ND 58108 and [2]Langdon Research and Extension Center, North Dakota State University, Langdon, ND 58249
[*]Corresponding Author: PH: 701-231-7627; Email: andrew.j.friskop@ndsu.edu

ABSTRACT

Fusarium head blight (FHB) is a serious problem in barley production areas of the Midwest United States. New strategies involving fungicide timing are being tested to help control this devastating disease. Two barley FHB integrated management trials were established in 2018 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. 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 and FHB. Adepidyn + propiconazole applied 3 to 7 days after heading had lower DON levels than when applied at 50% heading. 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. 59-0206-4-012 and 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.

EVALUATION OF FUNGICIDE EFFICACY AND TIMING FOR MANAGEMENT OF FUSARIUM HEAD BLIGHT IN SPRING BARLEY AND HARD RED SPRING WHEAT

Jessica Halvorson[1], Elizabeth Bauske[1], Scott Meyer[1], Casey Schuh[1], Venkat Chapara[2], Bryan Hanson[2], Lawrence Henry[2], Travis Hakanson[2], Amanda Arens[2] and Andrew Friskop[1*]

[1]Department of Plant Pathology, North Dakota State University, Fargo, ND 58108; and
[2]Langdon Research Extension Center, North Dakota State University, Langdon, ND 58249
[*]Corresponding Author: PH: 701-231-7627; Email: andrew.j.friskop@ndsu.edu

ABSTRACT

The use of a well-timed fungicide application can help protect hard red spring wheat (HRSW) and spring barley from Fusarium head blight (FHB) and deoxynivalenol (DON). With funding from the U.S. Wheat and Barley Scab Initiative, three fungicide trials were conducted in 2018. The primary objective was to compare the timing and efficacy of adepidyn + propiconazole to industry standards. Research sites were established at the Langdon Research and Extension Center (Langdon) and North Dakota State University (Fargo). Trial locations were as follows; one HRSW trial in Langdon, one HRSW trial in Fargo, and one barley trial in Fargo. Trials were conducted in a randomized complete block design with four replications. All plots were sown with a susceptible cultivar relative to the target grain. Fungicides evaluated included prothioconazole + tebuconazole, propiconazole + adepidyn, metconazole, and tebuconazole. Fungicide timings evaluated in barley included Feekes 10.3 (half-head emergence), Feekes 10.5 (full-head), and 3 to 7 days after Feekes 10.5. For wheat, Feekes 10.3, Feekes 10.51 (early-anthesis), and 3 to 7 days after Feekes 10.51 were evaluated. Data from each location were analyzed individually due to differences in treatment protocol and disease development. Low to moderate disease pressure was achieved in all three trials. Most fungicide applications resulted in statistically lower DON levels than the non-treated control. Specifically, applications including a sequential application of adepidyn + propiconazole and prothioconazole + tebuconazole or metconazole resulted in the lowest DON levels. Across all locations adepidyn + propiconazole (at all timings) statistically reduced DON when compared to the non-treated control. However, the greatest reduction in DON from a single application of adepidyn + propiconazole occurred when applied at or 3 to 7 days after Feekes 10.5 (barley), Feekes 10.51 (wheat). Additional studies are needed to strengthen fungicide timing recommendations for adepidyn + propiconazole in both HRSW and barley.

ACKNOWLEDGEMENT AND DISCLAIMER

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EVALUATION OF FUNGICIDES INDIVIDUALLY OR AS PART OF AN INTEGRATED APPROACH FOR MANAGEMENT OF FUSARIUM HEAD BLIGHT IN DURUM

Audrey Kalil[1], Dimitri Fonseka[1], Tyler Tjelde[1], Joel Ransom[2], Chad Deplazes[2], Darin Eisinger[2], Blaine Schatz[3], Elizabeth Bauske[4], Jessica Halvorson[4], Scott Meyer[4], Casey Schuh[4] and Andrew Friskop[4*]

[1]Williston Research Extension Center, North Dakota State University, Williston, ND 58801; [2]Department of Plant Sciences, North Dakota State University, Fargo, ND 58108; [3]Carrington Research Extension Center, North Dakota State University, Carrington, ND 58421; and [4]Department of Plant Pathology, North Dakota State University, Fargo, ND 58108
[*]Corresponding Author: PH: 701-231-7627; Email: andrew.j.friskop@ndsu.edu

ABSTRACT

Northwest North Dakota and northeast Montana account for over 90% of the durum acreage for the United States. Fusarium head blight (FHB) is arguably the most devastating disease in these areas. With very little resistance, fungicides are an important FHB management tool. With funding from the USWBSI, three integrated management (IM) trials and two uniform fungicide trials (UFT) were conducted on durum in North Dakota at Carrington, Fargo, Nesson Valley, and Prosper. Uniform fungicide trials were conducted in a randomized complete block design and integrated management trials were designed in a randomized complete block with split-plot arrangement. For the UFT trials, a susceptible durum variety was used, while the IM trials used two durum varieties varying in FHB susceptibility. A combination of fungicides (adepidyn + propiconazole, metconazole, or prothioconazole + tebuconazole) applied at different timings (Feekes 10.3 - half-head emergence, Feekes 10.51 - early-anthesis, or 3 to 7 days after Feekes 10.51). *Fusarium* infested corn spawn was dispersed at each site to enhance FHB development and irrigation was used at Nesson Valley and Carrington. Moderate to high levels of disease occurred in two IM trials and one UFT trial. Susceptible varieties tended to have higher FHB and deoxynivalenol (DON) levels. With few exceptions, fungicide applications resulted in statistically lower DON and FHB than the non-treated control. Fungicide applications of adepidyn + propiconazole at Feekes 10.3 and Feekes 10.51 were statistically comparable to each other. Additionally, a fungicide application 3 to 7 days after Feekes 10.51 lowered DON compared to the non-treated control, but not to the extent of fungicide applications made at Feekes 10.51. With the potential labeling of a new fungicide for FHB, more studies are needed to quantify application timing recommendations.

ACKNOWLEDGEMENT AND DISCLAIMER

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INTEGRATED MANAGEMENT OF FUSARIUM HEAD BLIGHT (FHB) AND DON CONTAMINATION IN SOFT RED WINTER WHEAT IN VIRGINIA

N. Kaur, L. Byrd-Masters and H. L Mehl*

School of Plant and Environmental Sciences, Virginia Tech Tidewater AREC, Suffolk, VA 23437
*Corresponding Author: PH: 757-807-6542; Email: hlmehl@vt.edu

ABSTRACT

Fusarium head blight (FHB), a disease of small grains caused by Fusarium graminearum, causes significant yield loss and contaminates grain with deoxynivalenol (DON), thus impacting both the quantity and quality of the wheat crop. DON is a regulated mycotoxin as it affects both human and animal health. Integrated management approaches including use of resistant cultivars and an appropriate fungicide program at an optimal timing can minimize the impacts of FHB. However, for cost-effective management, growers need specific variety and fungicide recommendations. The objectives of this study were to evaluate the integrated effects of fungicides and genetic resistance on FHB and DON, compare the single and two application fungicide programs for disease control, and assess the value of next generation varieties from the Virginia wheat breeding program for integrated management of FHB. Experiments were conducted in 2016 and 2017 in Suffolk, VA, and four soft red winter wheat varieties were evaluated each year. Both experiments included an FHB susceptible ('Shirley') and moderately resistant ('Jamestown') check. New releases and lines rated moderately resistant for FHB were evaluated in the study and included 'Hilliard' (both years), 'AgriMAXX 426' (2016), and VA13W-38 (2017). The experiment was a split-plot randomized block design with four varieties (main plot), five fungicide treatments (subplot), and four replicates. An untreated control was compared to four fungicide programs which included either a single application at anthesis or an application at anthesis followed by a second application four days later. Fungicide rates per acre and application timings included Prosaro® 6.5 oz (anthesis), Prosaro 6.5 oz (anthesis) + Caramba® 14 oz (4 days after anthesis), Caramba 14 oz (anthesis) + Folicur® 4 oz (4 days after anthesis), and Proline® 5.7 oz (anthesis) + Folicur. 4 oz (4 days after anthesis). F. graminearum conidia were applied to all plots 24 hours after anthesis for all treatments. Rainfall was higher in 2016 compared to 2017, so both FHB and foliar disease were more severe in the first year of the study. However, FHB severity varied among fungicide treatments and varieties in both years. As expected, Shirley was susceptible to FHB while Jamestown, Hilliard, Agrimaxx 426, and VA13W-38 had lower disease severity. Yield varied among varieties in both years, but fungicide treatments impacted yield only in 2016 when disease severity was higher due to weather conditions. Hilliard had consistently high yields and low FHB and DON, even without a fungicide application. For the susceptible variety (Shirley), two-application fungicide programs reduced FHB and DON more than a single application. The new wheat varieties were moderately resistant to FHB and DON, and genetic resistance was more effective for minimizing FHB and DON than any fungicide program on a susceptible variety. Integrating genetic resistance with well-timed fungicide application programs provide the greatest and most consistent control of FHB and DON in wheat.

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FHB NOW WESTWARD BOUND, AND NEW STRUGGLES TO KEEP DON DOWN

J.M. Marshall[1*], S.M. Arcibal[2], P. Bregitzer[3], T. Baldwin[3] and J. Chen[2]

[1]Department of Entomology, Plant Pathology, and Nematology, University of Idaho, Idaho Falls, ID 83402; [2]Aberdeen Research & Extension Center, University of Idaho, Aberdeen, ID 83210; and [3]Small Grains and Potato Germplasm, USDA-ARS, Aberdeen, ID 83210
[*]Corresponding Author: PH: 208-529-8376; Email: jmarshall@uidaho.edu

ABSTRACT

Expansion of FHB into areas of the west has been relatively slow, but has followed the steady increase of corn production in states traditionally reserved for amber waves of grain. Production acres of corn now exceed that of potatoes in Idaho, and pivots in the Columbia basin supply irrigation to corn instead of barley, acreage of which continues to decline across many regions of the US. Economics have driven growers to choose corn, while increasing corn acreage impacts disease in small grains, further driving up the costs of grain production. Over time, testing grain delivered to local elevators for DON has become a standard practice, especially for malt barley, with increasing frequencies of barley discounted or rejected for exceeding DON tolerances. Research under irrigated production systems has increased our understanding of control strategies to reduce the impact of scab in the west. While similar to standard best management practices developed through the USWBSI, western producers have irrigation management tools to increase control of FHB and potentially mitigate damage. The use of resistant varieties without fungicide application still reduces disease and DON to within tolerance levels. However, many preferred malt barley and wheat varieties with specific end-use qualities do not have the level of resistance required to control FHB. Combinations of fungicide and irrigation management are still required and need fine-tuning for those varieties without adequate levels of host resistance. Breeding efforts in the PNW have now incorporated FHB resistance as an additional target in variety development.

INTEGRATING THE MANAGEMENT OF FUSARIUM HEAD BLIGHT AND FOLIAR DISEASES THROUGH FUNGICIDE USE AND VARIETY SELECTION TO DEVELOP PRACTICAL STRATEGIES FOR WINTER WHEAT GROWERS

Martin L. Nagelkirk

Michigan State University Extension, Sandusky, MI 48471, USA
Corresponding Author: PH: 810-404-3400; Email: nagelkir@msu.edu

ABSTRACT

Selecting fungicides and varieties that simultaneously help mitigate the risks posed by foliar fungal diseases and Fusarium head blight (FHB) has had practical and financial merit for Michigan growers. Michigan's 500,000 acres of winter wheat includes the soft red winter and soft white winter wheat subclasses. Historically, the soft white varieties have tended to be more susceptible to foliar diseases than popular soft red varieties. Further, white varieties, as a group, have been more susceptible to FHB, and its market imposes discounts for DON beginning at 1 ppm as opposed to the 2 ppm for red varieties. Consequently, at least 95 percent of the state's soft white winter acreage receives a fungicide application at flowering (Feekes 10.1,2) compared to perhaps 60 percent of soft red acres [fungicide use levels based on author's estimate].

Based on early work with triazoles, growers were often encouraged to consider applying a fungicide at flag leaf (Feekes 9) to best thwart common leaf diseases (Powdery mildew, Septoria leaf spot, Stagonospora leaf spot and Leaf rust). In more recent years, presumably because of improved varietal resistance and, in some cases, an early fungicide application at jointing (Feekes 6), this flag leaf timing is being postponed until flowering thereby syncing with the optimal application timing of Prosaro® or Caramba® against FHB. Particularly in high yield environments, this delayed timing has been effective and often results in several bushels/ac of additional yield due to the suppression of leaf diseases, while also significantly reducing the risk of elevated DON levels from FHB.

However, this application strategy did not serve growers well during the 2016 season when the region experienced a severe stripe rust epidemic. The disease developed early (jointing stages) and much more aggressively than the more common leaf diseases. Consequently, the routine fungicide application at flowering was too late to provide adequate protection against stripe rust for some fields with susceptible, moderately susceptible and moderately resistant varieties. Assuming stripe rust will occasionally strike with equal ferocity in seasons to come, researchers and industry would do well to develop more comprehensive and robust disease management recommendations, emphasizing: 1) variety selection - placing greater emphasis on avoiding varieties that are susceptible to both FHB and stripe rust; 2) fungicide use – develop alternative fungicide strategies that encompass stripe rust (as well as the more common leaf diseases) and Fusarium head blight; and 3) field scouting – emphasizing state-wide communication networks that alert industry and growers of pending threats from stripe rust in addition to FHB. It might also be helpful to create a strategy grid that helps visualize variety x fungicide schemes and vulnerabilities. The lattice could include variety susceptibility ratings for FHB and stripe rust, along with alternative fungicide schedules that could be implemented in response to levels of risk. A separate grid would need to be considered for high, medium and low grain yield potentials.

ACKNOWLEDGEMENT AND DISCLAIMER

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DETECTION OF FUSARIUM HEAD BLIGHT IN SMALL GRAINS USING HYPERSPECTRAL IMAGING

Ruicheng Qiu[1], Ce Yang[1*], Ali Moghimi[1], James Anderson[3], Brian Steffenson[2] and Peter Marchetto[1]

[1]Department of Bioproducts and Biosystems Engineering, [2]Department of Plant Pathology, and [3]Department of Agronomy and Plant Genetics, University of Minnesota, Saint Paul, MN 55108
[*]Corresponding Author: PH: 612-626-6419; Email: ceyang@umn.edu

ABSTRACT

High throughput phenotyping based on remote sensing provides more accurate, precise and faster phenotyping for many plant improvement programs. Fusarium Head Blight (FHB), also called scab, is a wheat and barley disease that is devastating with worldwide distribution. To explore the feasibility of scab disease detection using optical sensing technique, we collected hyperspectral images in the range of 400nm to 890nm in the laboratory condition. We proposed and tested hyperspectral imaging algorithms using all wavelengths and sensitive wavelengths to develop classification models. The models were evaluated and compared by their performances. The models achieved very high prediction accuracy, their running times are less than 7 seconds. Our study showed that scab disease can be detected using hyperspectral image analysis with high accuracy and efficiency.

INTRODUCTION

Scab, caused by the fungal pathogen *Fusarium graminearum*, is a widespread and devastating disease on small grain cereals. Scab is very difficult to manage completely. Therefore, breeding scab resistance varieties is imperative. Breeding for resistance against scab is extremely challenging and many of the scab disease detections are inefficient. In general, the true level of scab is assessed manually, which is time-consuming, labor-intensive, and expensive. Developing an accurate and fast assessment for scab is necessary (Cobb et al., 2013).

Over the past decade, innovations in image processing has promoted the development of phenotyping. Rapid high-throughput phenotyping exhibits great potential for detecting plant disease and reducing many problems associated with visual assessments (Mishra et al., 2017). Moreover, image processing is also amenable to automation and can handle many more samples at a much faster rate than is possible with just visual assessments.

As a commonly used sensor, hyperspectral cameras provide significantly higher spectral resolution and a wider spectral range for each pixel. A common hyperspectral imaging (HSI) system is capable of sensing hundreds of spectral bands across a wide range of the electromagnetic spectrum, including both RGB and near-infrared (400 to 1000 nm). Literatures have shown that HSI is able to detect some crop diseases. Kimuli et al. (2018) used a hyperspectral camera to detect aflatoxin B_1 of maize kernels, the validation accuracy is over 96%. Shahin et al. (2011) detected the wheat kernels infected by *Fusarium* using a visible and near-infrared hyperspectral imaging system and achieved an overall accuracy of 92% or better. The overall objective of this study was to develop methods to detect scab disease in wheat using HIS and fulfill the USWBSI's primary goal to enhance food safety and supply by reducing the negative impacts of FHB in small grain plants.

MATERIALS AND METHODS

Image Collection

In this study, a susceptible wheat line ('Wheaton') and two common wheat lines ('Alsen' and 260-4) were cultivated in greenhouse. Healthy and

inoculated spikes were collected from the field in summer 2017. Hyperspectal images with 1044×640 pixels were obtained in the darkroom using a hyperspectral camera produced by Resonon (Bozeman, Montana, USA). The spatial resolution is approximately 1 mm and the wavelengths range are 400 - 890 nm. There are 240 bands in total. The raw hyperspectral images were calibrated using a Spectralon diffuse reflectance standard panel (99%). An example is shown in Figure 1. 10 diseased images and 6 controlled images were collected for the Wheaton wheat line, 9 diseased and 11 controlled images for Alsen line, and 11 diseased and 10 controlled images for 260-4 line.

Data Pre-processing

The objective of this study was to classify healthy and FHB infected spikes. Therefore, it is essential to segment spikes from the background first and then extract the infected areas from the spikes. The reflectance differences between green bands and blue bands (G-B) were calculated, and a gray threshold was set to segment spikes from the background. And then, open operation was conducted to remove some small noises. An example hyperspectral image with color representation and the processed image are shown in figure 1.

According to figure 1, reflectance of healthy and infected spikes is different. Diseased areas are darker in the processed image. Agapiou et al. (2012) used several vegetation indexes on the images using the reflectance. In this study, a vegetation index mNDVI, which was calculated based on equation 1, was chosen to extract the infected parts. Results are shown in figure 2

$$\text{mNDVI} = \frac{NIR-Red}{NIR+Red-Blue} \quad (1)$$

where *NIR* is the reflectance on the near infrared (about 770 nm) region, *Red* is the reflectance on the red (about 680 nm) region, *Blue* is the reflectance on the blue (about 475 nm) region.

mNDVI image of spikes based on the last segmentation was processed using the maximum between-class variance (OTSU) method, the results after erosion were the infected parts of spikes. After that, pixel spectra of healthy and infected spikes were extracted respectively. As a pre-treatment process, standard normal variate (SNV) was applied to remove the spectral noise, Savitzky-Golay smoothing (SGS) filter was used to smooth the spectral data (Li et al., 2014).

Hyperspectral pixels of healthy and infected spikes were used to develop classification models. Principal component analysis (PCA) was applied to reduce the dimensionality of hyperspectral data, several principal components (PCs) were selected based on their cumulative contribution rate (Bauriegel et al., 2011). In addition, genetic algorithm (GA) was used as a feature selection algorithm to select sensitive spectral wavelengths for modeling. Then, the classification models based on support vector machine (SVM) were developed using the selected PCs or feature wavelengths as input variables. Aforementioned processing methods and classification models were developed using Matlab software (MATLAB 2014b, the MathWorks, Inc., Natick, Massachusetts, United States).

RESULTS AND DISCUSSION

Wheat Spikes Detection

As we can see in figure 1, the reflectance differences between green bands and blue bands of spikes are higher than the background. A threshold was set after segment test on all the spikes. There are many background noises after segmentation, so open operation was conducted to remove the small areas, example results are shown in figure 3.

Healthy and Infected Areas Detection

mNDVI image was processed to extract the infected parts of spikes. After segmentation, erosion was employed to remove the influence of boundary, the results were shown in figure 4.

Spectral Dataset Extraction

Spectral data of healthy pixels and infected pixels were extracted. Each pixel spectra was pre-treated using SNV and SGS. Then smooth spectral curves were generated, as shown in figure 5. It is clear that the reflectance of healthy pixels and infected pixels are different at some bands.

Calibration and Validation of Classification Models

The spectral data of 10,000 healthy pixels and 9,000 infected pixels were analyzed using PCA algorithm, and the first five principal components contained 54.84%, 28.36%, 6.56%, 4.02% and 2.61% of the variance, respectively.

Five hundred healthy and five hundred infected pixels were processed using GA. The size of population is 60, the crossover probability and mutation probability were set as 0.7, 0.01, respectively. GA was carried out 200 times, and two wavelengths (683 nm, 742 nm) that were selected most frequently were used for further processing.

Ten thousand pixels were selected randomly from 10,000 healthy pixels and 9,000 infected pixels. These pixels were employed to develop the classification models. In this study, 9,000 pixels was used to train the classification models using SVM algorithm, and 1,000 pixels were set as validation dataset. The performances of models were evaluated by 10-fold cross validation, and the mean accuracies were calculated.

The mean accuracies of classification models of the Wheaton, Alsen, and 260-4, respectively, developed using five principal components, were 98.22%, 98.66%, and 96.72%, indicating that it is feasible to use HSI to detect scab disease. The mean accuracy of the classification models based on the spectral data of 683 nm and 742 nm, are 99.16%, 99.74%, and 99.60%, respectively. It demonstrates that the two selected wavelengths are sensitive to scab disease. The results provide suggestions for detecting scab disease in a high-throughput way. But GA is much slower than PCA. Therefore, shorter running time should be the focus in the following work.

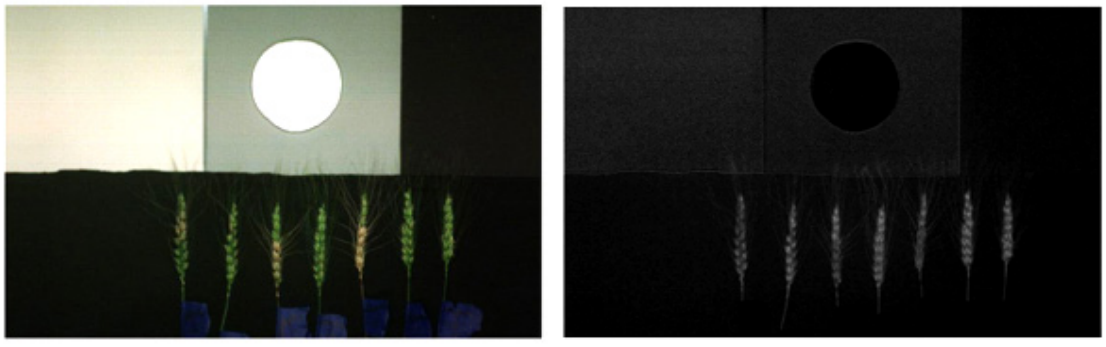

Figure 1. Segmentation of seven infected wheat spikes. The left picture is taken by the hyperspectral camera and shown as a color image. The right side is the gray image based on the reflectance differences between the green bands and blue bands.

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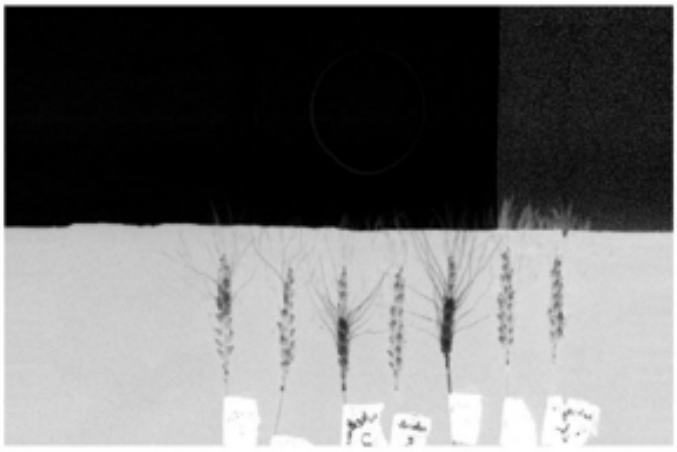

Figure 2. mNDVI image of healthy and infected spikes

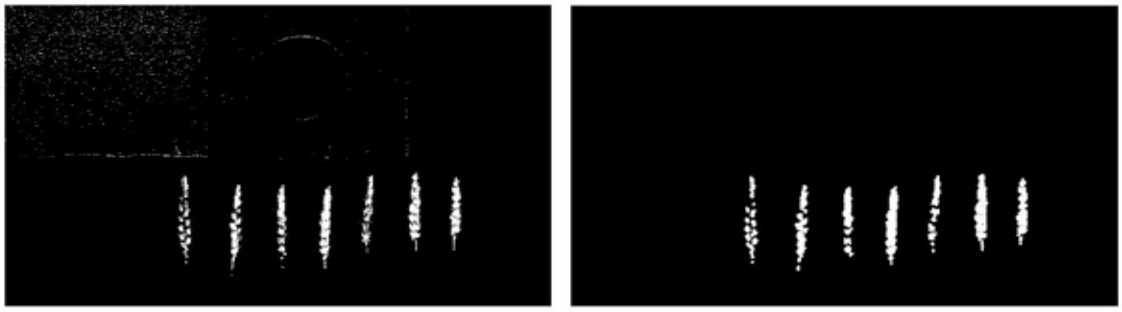

Figure 3. Segmentation of seven infected wheat spikes. The left picture is the result after segmentation. The right side is after small noise removal.

Figure 4. Segmentation of healthy and infected parts of wheat spikes. The left picture is the infected parts of spikes. The right side is the healthy parts of spikes.

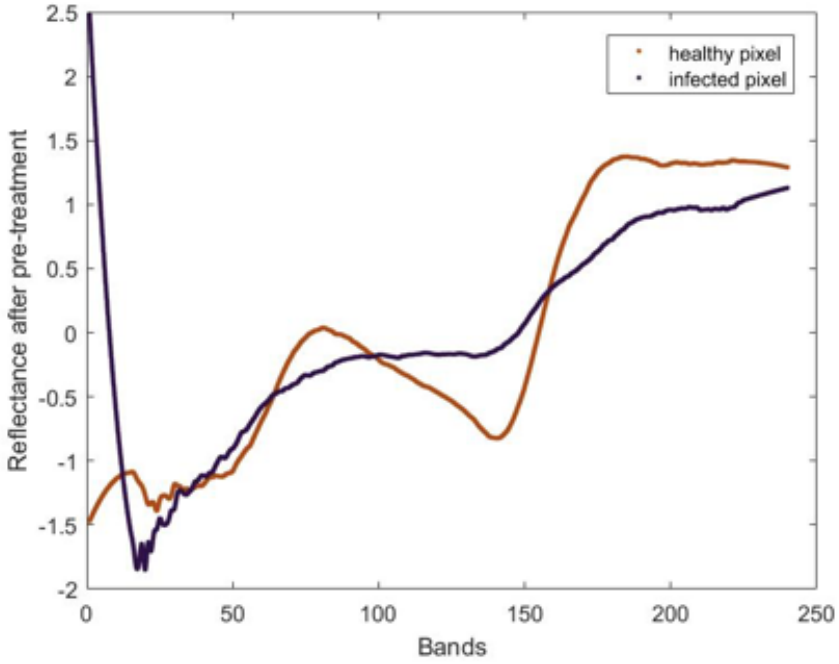

Figure 5. Spectral reflectance plots of healthy pixels and infected pixels.

VARIATION IN SPIKE EMERGENCE TIMING IN SPRING WHEAT VARIETIES SOWN AT DIFFERENT DENSITIES

Joel Ransom[1*], Andrew Friskop[1] and Ryan Buetow[2]

[1]North Dakota State University, Fargo, ND 58108; and [2]North Dakota State University Research and Extension Center, Dickinson, ND 58601
[*]Corresponding Author: PH: 701-231-7405; Email: joel.ransom@ndsu.edu

ABSTRACT

Currently recommended fungicides for Fusarium Head Blight control are most effective when applied at or near flowering. Field experiments were established near Prosper, ND and at the Dickinson Research Extension Center, Dickinson, ND to determine the variability in time to emergence of spikes (as a proxy for flowering) from four varieties of hard red spring wheat sown at four densities. Within each plot, daily spike emergence was counted within a meter-long row beginning with the first emerged spike and ending when the last spikes emerged usually from late-developing tillers. Most spikes (70%) emerged within a four-day period, regardless of seeding rate and variety. Sixteen percent of the spikes emerged more than five days after the day of peak spike emergence, with about 2% of the spikes emerging eight days after this date. The percent of spikes emerging five days after peak spike emergence was greater at the 0.75 million seeds per acre seeding rate (22%) than at higher seeding rates (an average of 18%). The highest seeding rate (2.25 million seeds per acre), did not improve the uniformity of spike emergence compared to the recommended seeding rates of 1.25 to 1.75 million seeds per acre. The relatively large number of days between the emergence of the first spike and last spike observed in these experiments may be one of the reasons that fungicide applications are not usually 100% effective in controlling Fusarium Head Blight.

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EVALUATING SOFT RED WINTER WHEAT AGRONOMIC PRACTICES TO REDUCE GRAIN DEOXYNIVALENOL (DON) CONTAMINATION

Katherine S. Rod[1*], Carrie A. Knott[2] and Carl A. Bradley[3]

[1]Department of Plant and Soil Sciences, University of Kentucky, Lexington KY 40546; [2]Department of Plant and Soil Sciences, and [3]Department of Plant Pathology, University of Kentucky, Princeton KY 42445
[*]Corresponding Author: PH: 815-830-1508; Email: katherine.rod@uky.edu

ABSTRACT

Deoxynivalenol (DON) contamination of wheat grain, caused by *Fusarium graminearum,* is a major concern for soft red winter wheat producers and millers. Current agronomic practices to reduce DON contamination in wheat grain includes planting cultivars with moderate resistance to *F. graminearum* and applying efficacious fungicides at beginning of anthesis (Feekes 10.5.1). The objectives of this study are to determine the effect of DON contamination and grain yield when wheat is harvested at different grain moisture concentrations and when phosphorus is applied as an in-furrow application at planting. Two field trials were established in Princeton, KY in the fall of 2016 and 2017. Each year, one trial was mist irrigated with *F. graminearum* infected corn kernels spread throughout the plot area to promote *F. graminearum* infection. A second trial was planted following a corn crop. Both trials were conducted as a randomized complete block design. Treatments includes two planting dates (mid-October and mid-November), two harvesting timings (20-22% grain moisture [early] and 13-15% grain moisture [normal]), two soft red winter wheat cultivars (moderately resistant to FHB cultivar and a susceptible to FHB cultivar), and two phosphorous applications applied in-furrow at planting (0 kg/ha P_2O_5 and 47 kg/ha P_2O_5). Each inoculation type was analyzed separately due to a year x treatment interaction. In the ambient locations, the treatment with the least DON contamination (0.08 ppm) was wheat planted in October and harvested at normal grain moisture. The October planted wheat harvested early had similar DON contamination levels (0.38 ppm) as the November planted wheat harvested at normal timing (0.39 ppm). The highest DON contamination level was seen in the November planted wheat harvested early (1.1 ppm). Similar trends were seen in the *F. graminearium* inoculated locations. The lowest DON contamination (1.4 ppm) was seen in the October planted wheat at normal harvest timing followed by the October planted wheat harvested early (2.3 ppm), November planted wheat harvested at normal timing (3.1 ppm) and the November planted wheat harvested early (4.9 ppm). In the ambient conditions the October planted wheat had similar yields as the early harvest timing (6.0 mt/ha) and the normal harvest timing (6.05 mt/ha), compared to the November planted wheat with yields of 5.03 mt/ha for the early harvest timing and 5.52 mt/ha for normal harvest timing. Similar trends were seen in the *F. graminarium* inoculated locations. The October planted wheat had similar grain yields, 4.15 mt/ha for early harvest and 3.92 mt/ha for normal harvest timings, while the November wheat had lower yields of 3.32 mt/ha for early harvest and 3.65 mt/ha for normal harvest timings. The phosphorus treatments did not affect DON contamination or grain yield across the two years.

EFFICACY AND CURATIVE EFFECTS OF FUNGICIDES FOR FHB AND DON MANAGEMENT IN OHIO

J.D. Salgado[1], J.P. Edwards[2], LV. Madden[1] and P.A. Paul[1*]

[1]The Ohio State University/OARDC, Wooster, OH 44691; and
[2]Instituto de Patología vegetal; CIAP- INTA, Córdoba, Argentina
[*]Corresponding Author: PH: 330-263-3842; Email: paul.661@osu.edu

ABSTRACT

Effective management of Fusarium head blight (FHB) and deoxynivalenol (DON) depends on the use of resistant cultivars and application of triazole fungicides such as Prosaro or Caramba between early anthesis and 6 days post-anthesis. However, adverse weather and field conditions, and variable crop development (maturity, flowering date, and tillering habit) may prevent applications from being made at the recommended growth stage. This study aimed to characterize the efficacy of post-anthesis fungicide applications by quantifying the curative effects of Prosaro and Caramba on FHB intensity and DON contamination on primary, early-flowering, and secondary, late-flowering tillers. Controlled-environment experiments were conducted in 2015 and 2016 and field experiments in 2016 and 2017. Prosaro® and Caramba® were applied at regular intervals between anthesis and soft dough (up to 20 days post-anthesis) to a moderately resistant and a susceptible cultivar in the field, and between anthesis and 14 days post anthesis to a susceptible cultivar in the greenhouse. Experimental units were inoculated with a spore suspension of *Fusarium graminearum* between 24 to 48 hours after the anthesis treatments were applied. FHB incidence (INC), severity (SEV), index (IND) and *Fusarium* damaged kernels (FDK) were visually estimated, and DON contamination of grain was quantified in all experiments. In addition, field experiments included systematic assessments and sampling to quantify these responses in primary and secondary tillers. Results from both sets of experiments showed that anthesis and post-anthesis treatments, particularly those applied between 2 and 6 days after anthesis, consistently reduced FHB and DON relative to the non-treated check. However, the effects of post-anthesis treatments applied between 6 and 20 days varied with cultivar and fungicide, and between greenhouse and field experiments. Fungicide treatment effects were greater on the susceptible cultivars and provided greater reduction in FHB and DON relative to the non-treated check. Results from field experiments showed that post-anthesis treatments (2, 4 and 6 days) resulted in lower mean IND, FDK and DON in secondary, late-flowering tillers, than in primary, early-flowering tillers. Both Prosaro and Caramba show evidence of curative effects when applied up to 6 days after infection but not later.

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This material is based upon work supported by the U.S. Department of Agriculture, under Agreement Nos. 59-0206-4-018 and 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.

EFFICACY OF MIRAVIS® ACE FOR FHB AND DON MANAGEMENT ACROSS ENVIRONMENTS AND GRAIN MARKET CLASSES: A PROGRESS REPORT

J.D. Salgado[1], G. Bergstrom[2], C. Bradley[3,4], K. Bowen[5], E. Byamukama[6], A. Byrne[10], A. Collins[7], C. Cowger[8], J. Cummings[2], V. Chapara[9], M.I. Chilvers[10], E. De Wolf [21], R. Dill-Macky[12], H.M. Darby[13], P.D. Esker[7], A. Friskop[9], J. Halvorson[9], N. Kleczewski[3,14], L.V. Madden[1], J. Marshall[15], H. Mehl[16], M. Nagelkirk[10,11], J. Starr[2], J. Stevens[17], D. Smith[18], M. Smith[12], S. Wegulo[17], K. Wise[4,19], D. Yabwalo[6], H.M. Young-Kelly[20] and P.A. Paul[1*]

[1]The Ohio State University/OARDC, Wooster 44691; [2]Cornell University, Ithaca, NY 14853; [3]University of Illinois, Urbana, IL 61801; [4]University of Kentucky, Lexington, KY 40506; [5]Auburn University , Auburn, AL 36849; [6]South Dakota State University, Brookings, SD 57007; [7] The Pennsylvania State University, University Park, PA 16802; [8]North Carolina State University/ USDA-ARS, Raleigh, NC 27695; [9]North Dakota State University, Fargo, ND 58102; [10]Michigan State University Extension, Sandusky, MI 48471; [11]Michigan State University, East Lansing, MI 48824;[12]University of Minnesota, St. Paul, MN 55108; [13]The University of Vermont, Burlington, VT 05405; [14]The University of Delaware, Newark, DE 19719; [15]University of Idaho, Idaho Falls, ID 83402; [16]Virginia Tech, Suffolk VA 23437; [17]University of Nebraska-Lincoln, NE 68588; [18]University of Wisconsin, Madison, WI 53706; [19]Purdue University, West Lafayette, IN 47907; [20]The University of Tennessee at Knoxville, Jackson, TN 38301.[21] Kansas State University, Manhattan, KS 66506
[*]Corresponding Author: PH: 330-263-3842; Email: paul.661@osu.edu

OBJECTIVES

1. Evaluate the integrated effects of fungicide treatment and genetic resistance on FHB and DON in all major grain classes, with emphasis on a new fungicide, Miravis ®Ace.

2. Compare the efficacy of Miravis Ace when applied at heading or at anthesis to that of a standard anthesis application of Prosaro® or Caramba®.

INTRODUCTION

Results from previous uniform fungicide trials (UFTs) and management coordinated projects (IM_CP) showed that Demethylation Inhibitors (DMI) fungicides such as Prosaro and Caramba are the most effective against FHB and DON. Applications made at or up to 6 days after anthesis to moderately resistant cultivars provide more than 70% reduction of both FHB index and DON, relative to a non-treated, susceptible check (2,4,7). Results from UFTs also showed that Quinone Outside Inhibitor (QoI) fungicides consistently led to an increase in DON accumulation in harvested grain, particularly when applied close to anthesis (3). Based on these findings, DMIs are the most widely used fungicides for FHB and DON management, but this is not a good fungicide resistance management strategy. Isolates of *Fusarium graminearum* with resistance to tebuconazole have been reported (1,5), and a recent quantitative synthesis of 20 years of data from UFTs (Madden et al. *personal communication*) suggests that the efficacy of this and other DMIs may be declining in some areas. Preliminary results from a few pilot studies showed

that Miravis Ace, a new Succinate Dehydrogenase Inhibitor + propiconazole fungicide, provides comparable levels of FHB and DON reduction to that of Prosaro and Caramba when applied at anthesis (Feekes 10.5.1). Moreover, this new fungicide is reported to be just as effective as the industry standards when applied at Feekes 10.3.

MATERIALS AND METHODS

Two sets of field experiments were conducted in 11 US wheat-growing states in 2018 to evaluate the performance if Miravis Ace. **For objective #1 (IM_CP)**, fungicide treatments (sub-plot) consisting of a non-treated check (**CK**), Prosaro at 6.5 fl. oz./A applied at 50% early anthesis (**I**), and Miravis Ace at 13.7 fl. oz./A applied at 50% early anthesis (**II**) or at 50% heading (Feekes 10.3, **III**) were applied to replicate plots (3-6 blocks) of susceptible (S), moderately susceptible (MS), and moderately resistant (MR) cultivars (whole-plot). **For objective #2 (UFT)**, plots of a susceptible cultivar were planted in 3-6 replicate blocks and subjected to various combinations of the following fungicide programs: **1**) a non-treated check; **2**) Miravis Ace at 11.5 fl. oz./A (low rate) at Feekes 10.3-10.5; **3**) Miravis Ace at 13.7 fl. oz./A (high rate) at Feekes 10.3-10.5; **4**) Miravis Ace at 11.5 fl. oz./A at anthesis; **5**) Miravis Ace at 13.7 fl. oz./A at anthesis; **6**) Prosaro at 6.5 fl. oz./A at anthesis; and **7**) Caramba at 13.5 fl. oz./A at anthesis. In addition, we evaluated two-treatment programs consisting of an application of Miravis Ace (at low or high rate) at anthesis followed by an application of Prosaro at 6.5 fl. oz./A (**8**), Caramba at 13.5 fl. oz./A (**9**), or Folicur at 4 fl. oz./A (**10**) at 3-5 days post anthesis. Separate post-anthesis (3-5 days) applications of Prosaro at 6.5 fl. oz./A (**11**) and Miravis Ace at 13.7 fl. oz./A (**12**), and a pre-anthesis (Feekes 10.3-10.5) application of Prosaro (**13**) were also included in some trials.

All plots were artificially inoculated with either *F. graminearum*-colonized grain spawn or a spore suspension of the fungus sprayed approximately 24-36 hours after the anthesis treatments were applied. Some locations were naturally infected. FHB index (IND) was rated or calculated as previously described (6) on 60-100 spikes per plot at approximately Feekes 11.2. Grain 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 management program and treatment effects.

RESULTS AND DISCUSSION

DON data were not available for some environments at the time of this report. For **objective 1**, trial-level mean IND and DON in the non-treated susceptible check (S_CK) ranged from 2.3 to 46.4 % and 0.5 to 8.6 ppm, respectively. All cultivar x fungicide treatment combinations had significantly ($p < 0.05$) lower mean IND (**Fig. 1A**) and DON (**Fig. 1B**) than S_CK. When applied at 13.7 fl. oz/A at Feekes 10.5.1 (**II**) or at Feekes 10.3-10.5 (**III**), Miravis Ace was not significantly different from Prosaro at 6.5 fl oz/A applied at anthesis (**Fig. 1A** and **B**).

For **objective 2**, trial-level mean IND and DON in the non-treated check (CK) ranged from 0.22 to 42.1 % and 1.3 to 38.7 ppm, respectively. All fungicide programs that included an anthesis application resulted in significantly ($p < 0.05$) lower mean IND and DON than the check (**Fig. 2**). For IND, means were not significantly different among Prosaro, Caramba, and Miravis Ace, and applications made at Feekes 10.3-10.5 were not significantly different from those made at 50% Feekes 10.5.1, or 4 days after 50% early anthesis, in the case of Miravis Ace. Two-treatment programs consisting of Miravis Ace applied at 50% early anthesis followed by Prosaro, Caramba, or Folicur applied 4 days after resulted in the lowest overall mean IND, with the Miravis Ace + Folicur and Miravis Ace + Caramba combinations having significantly lower means than the anthesis-only Prosaro or Caramba treatments (**Fig. 2A**). Two-treatment programs also outperformed all other tested programs in terms of mean DON reduction, however, treatments applied at between Feekes 10.3 and 10.5 (MIR_H and PRO_H) were not

significantly different from the non-treated check (**Fig. 2B**). In particular, the anthesis and post-anthesis applications of Miravis Ace (MIR_A and MIR_A+4) had significantly lower mean DON than MIR_H.

Although based on a relatively small sample size (6-16 trials for the MGMT_CP and 2-12 for the UFT, depending on the treatment and the response), these results are very promising. When applied at anthesis, Miravis Ace was just as effective as Prosaro and Caramba in terms of FHB and DON reduction. However, when applied between Feekes 10.3 and 10.5, the performance of this new fungicide was not consistent between the MGMT_CP and the UFT in terms of efficacy against DON. A second year of data will be collected in 2019, and a more complete set of analyses will be performed.

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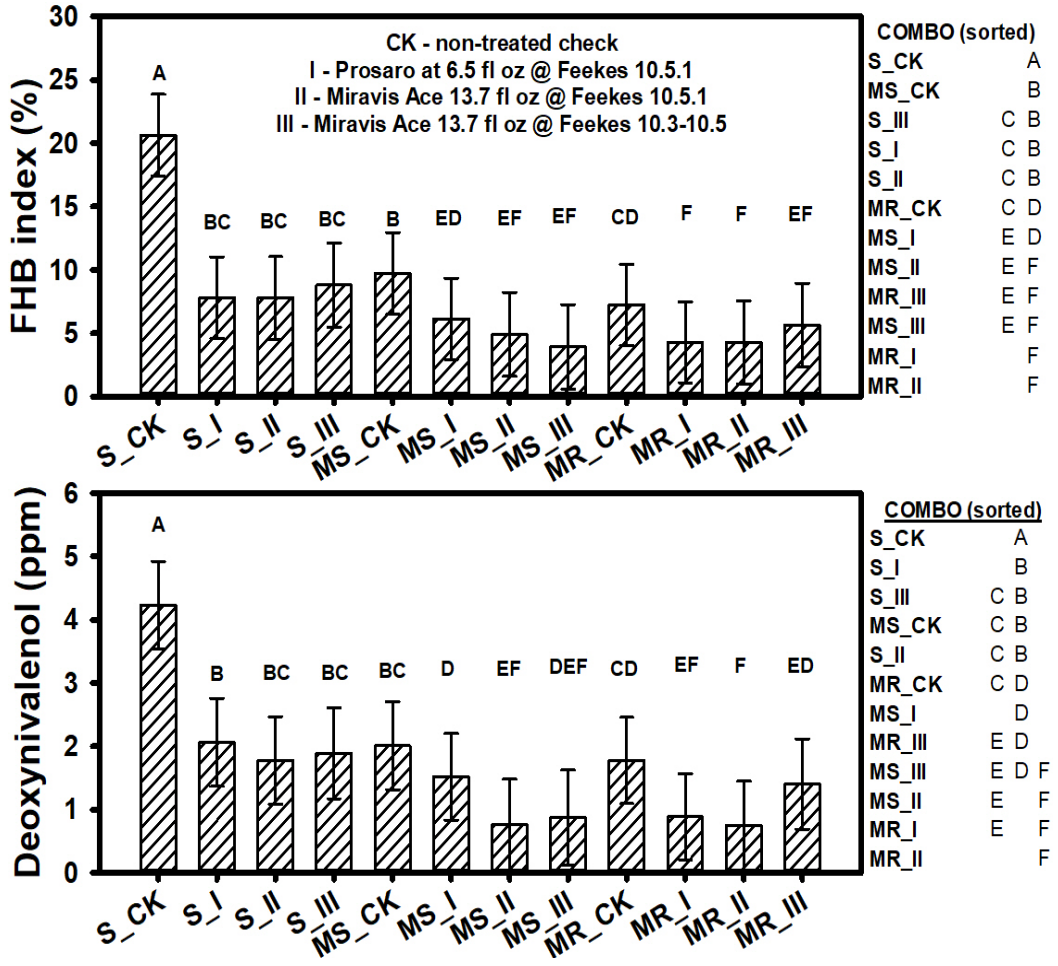

Fig. 1. Mean FHB index (**A**) and DON (**B**) for different FHB management combinations (COMBO) consisting of fungicide treatments (I, II, and III) applied to susceptible (S), moderately susceptible (MS), and moderately resistant (MR) cultivars. Analyses were done of arcsine square root-transformed IND and log-transformed DON. ***A lower rate of Miravis Ace (11.5 fl. oz/A) was tested in some trials but was not included in these analyses.***

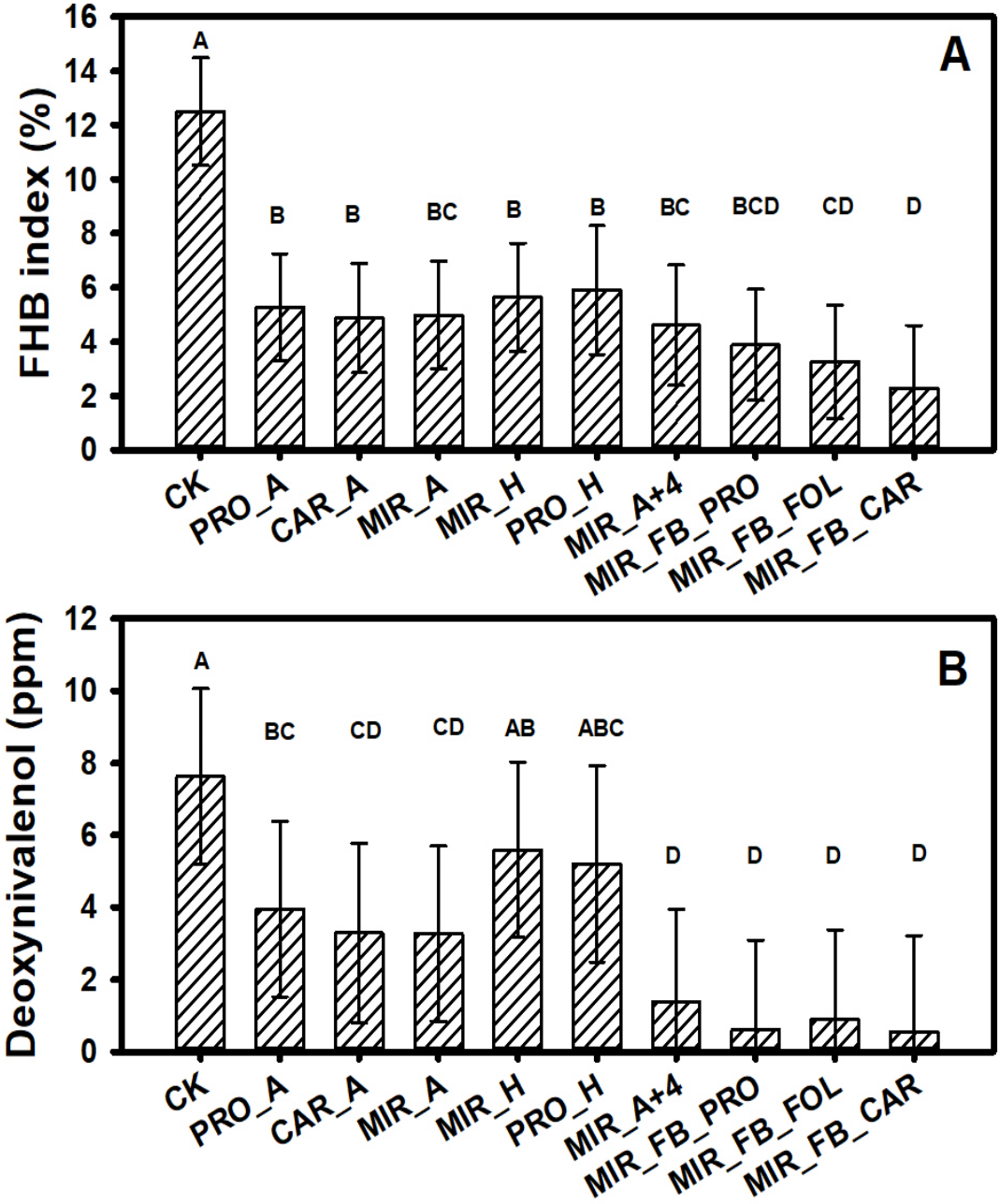

Fig. 2. Mean FHB index (A) and DON (B) for different FHB management programs consisting of the application of Prosaro at 6.5 fl. oz/A (**PRO**), Caramba at 13.5 fl.oz/A (**CAR**) or Miravis Ace at 13.7 fl. oz/A (**MIR**) at anthesis (abbreviated as **A**); Miravis Ace or Prosaro between Feekes 10.3 and 10.5 (abbreviated as **MIR_H** and **PRO_H**); Miravis Ace at 3-7 days after anthesis (abbreviate as **MIR_A+4**); or sequential application of MIR at 50% early anthesis followed by PRO, CAR, or Folicur (4 fl oz/A) applied 4 days after. Analyses were done of arcsine square root-transformed IND and log-transformed DON. ***A lower rate of Miravis Ace (11.5 fl. oz/A) was tested in some trials but was not included in these analyses.***

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

J.D. Salgado[1], G. Bergstrom[2], C. Bradley[3,4], K. Bowen[5], E. Byamukama[6], A. Byrne[10], A. Collins[7], C. Cowger[8], J. Cummings[2], V. Chapara[9], M.I. Chilvers[10], R. Dill-Macky[12], H.M. Darby[13], A. Friskop[9], N. Kleczewski[3,14], L.V. Madden[1], J. Marshall[15], H. Mehl[16], M. Nagelkirk[10,11], J. Stevens[17], D. Smith[18], M. Smith[12], S. Wegulo[17], K. Wise[4,19], D. Yabwalo[6], H.M. Young-Kelly[20] and P.A. Paul[1*]

[1]The Ohio State University/OARDC, Wooster 44691; [2]Cornell University, Ithaca, NY 14853; [3]University of Illinois, Urbana, IL 61801; [4]University of Kentucky, Lexington, KY 40506; [5]Auburn University , Auburn, AL 36849; [6]South Dakota State University, Brookings, SD 57007; [7]Southeast Agricultural Research & Extension Center The Pennsylvania State University, Manheim, PA 17545; [8]North Carolina State University/USDA-ARS, Raleigh, NC 27695; [9]North Dakota State University, Fargo, ND 58102; [10]Michigan State University Extension, Sandusky, MI 48471; [11]Michigan State University, East Lansing, MI 48824;[12]University of Minnesota, St. Paul, MN 55108; [13]The University of Vermont, Burlington, VT 05405; [14]The University of Delaware, Newark, DE 19719; [15]University of Idaho, Idaho Falls, ID 83402; [16]Virginia Tech, Suffolk VA 23437; [17]University of Nebraska-Lincoln, NE 68588; [18]University of Wisconsin, Madison, WI 53706; [19]Purdue University, West Lafayette, IN 47907; and [20]The University of Tennessee at Knoxville, Jackson, TN 38301
[*]Corresponding Author: PH: 330-263-3842; Email: paul.661@osu.edu

ABSTRACT

A single well-timed application of a triazole fungicide at anthesis is recommended for effective management and reduction of Fusarium head blight (FHB) deoxynivalenol (DON). However, later applications made up to 6 days after anthesis have been shown to be just as effective as an anthesis application in terms of percent FHB and DON control. Therefore, field experiments were conducted in 16 US wheat-growing states in 2016 and 2017 to determine whether combining an anthesis and a late application (two-treatment program) would provide higher levels of FHB and DON control than single-treatment programs. Plots of susceptible (S), moderately susceptible (MS), or moderately resistant (MR) cultivars were established, artificially inoculated with either *F. graminearum* colonized grain spawn or a spore suspension, and then subjected to different single- or two-treatment fungicide programs. FHB index (IND) was assessed during the soft dough stage of grain development. Milled grain samples were sent to a USWBSI-supported laboratory for mycotoxin analysis. Percent control was estimated for IND and DON for each cultivar x fungicide program combination relative to the non-treated susceptible check, for each of 57 trials/environments, representing 28 soft red winter, four soft white winter, six hard red winter, nine hard red spring, two hard white spring, two soft white spring wheat, and five durum market classes. Mean IND and DON in the non-treated susceptible checks ranged from 0 to 63% and 0 to 38 ppm, respectively. Relative to the checks, fungicide treatments applied at anthesis (I) to MR cultivars (MR_I) resulted in the highest mean percent control of IND (78%) followed by MS_I (62%) and S_I (47%). Similarly, mean percent control of DON was 70% for MR_I, 64 % for MS_I and 45 % for S_I cultivars. Overall, percent control values were higher for fungicide programs that combined an anthesis and a late application (II, III and IV) than programs with an anthesis-only

application (I) or MS or MR alone. Averaged across environment and cultivars, percent control of IND with an application of Prosaro® at anthesis followed by Caramba® after anthesis (II) ranged from 69 to 85%, Caramba followed by Folicur® (III) ranged from 63 to 83%, and Proline® followed by Folicur (IV) ranged from 60 to 87%. Similarly, percent reduction in DON content ranged from 67 to 79%, 58 to 75%, and 53 to 73% for II, III and IV, respectively. Moderately resistant cultivars alone offered higher mean percent control of both IND and DON (75 and 67%, respectively) than MS cultivars alone (65 to 56%, respectively). Based on these results, there is evidence suggesting that the combination of a "late" or "post-anthesis" and an anthesis fungicide application, coupled with MS or MR cultivars can be more effective at reducing FHB and DON than an anthesis-only application. A more comprehensive analysis of the data as well as a cost-benefit assessment of all FHB management programs will be presented.

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RESPONSE OF OAT GRAINS TO *FUSARIUM* INFECTION AND MYCOTOXIN CONTAMINATION

M. Tabassum, M. Banik and X. Wang*

Morden Research and Development Centre, Agriculture and Agri-Food Canada, 101 Route 100, Morden, MB, R6M 1Y5, Canada
*Corresponding Author: PH. 204-822-7533; E-mail: xiben.wang@canada.ca

ABSTRACT

Fusarium head blight (FHB) on small grain cereals, caused by a complex of *Fusarium* species, is a serious threat to the global food safety. In oats, the recent increase of Fusarium head blight severity has been noted in western Canada which has caused the concern of the oat industry. In addition to *F. graminearum*, *F. poae* has been frequently isolated from commercial oat fields. *F. graminearum* produces several toxic secondary metabolites, among which deoxynivalenol (DON) and zearalenone (ZEN) are the most closely monitored due to their high detection rates and strong toxicity. *F. poae* can produce a wide range of type A and B trichothecene mycotoxins as well as several non-trichothecene mycotoxins. To date, very little is known about Fusarium species complex infecting oats and the oat resistance against these pathogens. In this study, we surveyed *Fusarium* species infecting oats in Manitoba from 2016 to 2018. *Fusarium* infection in contaminated oat grains was assessed by conventional and real time qPCR. Additionally, we evaluated the level of resistance to *F. graminearum* in commercial oat cultivars grown in western Canada in a mist-irrigated artificially-inoculated FHB nursery at Morden, Manitoba. Our results indicate that *Fusarium* species infecting oats are more diverse than *Fusarium* species infecting wheat. *F. poae*, *F. graminearum* and *F. sporotrichioides* are the three most common *Fusarium* species found in commercial oat fields in western Canada. Deoxynivalenol was detected in all commercial oat varieties tested in Morden nursery (3.4 to 46.4 μg/g of DON in contaminated oat grains). It is concluded that Fusarium mycotoxin could be a potential problem for oat production under high disease pressures in western Canada. The severity of this problem needs to be assessed by extensive monitoring of mycotoxin level under natural conditions. Additionally, oat genotypes with different level of FHB resistance have been identified and will be used in the future genetic analysis of FHB resistance in oats.

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INDEPENDENT IMPACT OF METCONAZOLE VERSUS TEBUCONAZOLE+PROTHIOCONAZOLE ON FUSARIUM HEAD BLIGHT AND DEOXYNIVALENOL UNDER MODERATE DISEASE PRESSURE

Dalitso N. Yabwalo[1], Shaukat Ali[1], Kay Ruden[2] and Emmanuel Byamukama[1*]

[1]South Dakota State University, Agronomy, Horticulture and Plant Science Department, PO 2108, Brookings SD 57007; and [2]Bayer Crop Science Division, BCS-RD-DEVE-PD H-BRP, Field Operations North, 307 First Street, PO Box 425, Bruce, SD 57220
[*]Corresponding Author: PH: 605-688-4521; Email: emmanuel.byamukama@sdstate.edu

ABSTRACT

Metconazole and tebuconazole+prothioconazole fungicides have been used for years to manage Fusarium head blight (FHB) of wheat and barley that is primarily caused by *Fusarium graminearum*. Few studies have investigated the independent impact of metconazole and prothioconazole+tebuconazole based fungicides applied at 13.5fl oz/acre and 6.5fl oz/acre at Feekes 10.5.1, respectively, at moderate FHB levels. A study was developed to determine the independent effect of two fungicides; a metconazole based (Caramba®; BASF Corp.) and tebuconazole+prothioconazole based (Prosaro®; Bayer CropScience) fungicide on FHB incidence, disease index, *Fusarium* damaged kernels (FDK) and deoxynivalenol (DON) concentration in 2017. Two hard red spring wheat cultivars, Brick (FHB resistant) and Samson (susceptible) were set up in a split plot arrangement where cultivars were the main plot and subplots included; (i) untreated, (ii) metconazole and (iii) tebuconazole+prothioconazole. All plots were inoculated with *F. graminearum* in the form of infested corn spawn at Feekes 9, and a misting system was installed to maintain moist conditions for the pathogen to thrive and infect. No significant differences between Caramba and Prosaro were observed in terms of DON concentration, FHB incidence, severity and disease index in both cultivars at $p<0.05$. However, Prosaro showed a 5bu/acre higher yield than Caramba in the susceptible cultivar, $p = 0.024$. Treatment effects did not generate significant changes from the original p-values with a 10000 bootstrap procedure for 95% confidence interval. A Pearson's product moment correlation for DON concentration revealed a strong positive association ($r = 0.99$, $p < .0001$) with FDK and negative associations with 1000KW, test weight and yield. These preliminary results suggest that there is no significant difference using either fungicide for managing FHB index, FDK, and DON concentration. However, inclusion of more diverse environments and high FHB disease pressure would generate concrete conclusions.

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MULTI-ENVIRONMENT ASSESSMENT OF HOST GENETICS, ARTIFICIAL INOCULATION AND PRITHIOCONAZOLE+TEBUCONAZOLE IN *FUSARIUM GRAMINEARUM* MANAGEMENT STUDIES

Dalitso N. Yabwalo[1], Shaukat Ali[1], Karl Glover[1], Kay Ruden[2] and Emmanuel Byamukama[1*]

[1]South Dakota State University, Agronomy, Horticulture and Plant Science Department, PO 2108, Brookings SD 57007; and [2]Bayer Crop Science Division, BCS-RD-DEVE-PD H-BRP, Field Operations North, 307 First Street, PO Box 425, Bruce, SD 57220-0425
[*]Corresponding Author: PH: 605-688-4521; Email: emmanuel.byamukama@sdstate.edu

ABSTRACT

Fusarium head blight (FHB), caused by *Fusarium graminearum* continues to cause significant grain yield and quality losses throughout the wheat growing regions in the USA. Management practices for FHB include host resistance and application of fungicides such as prothioconazole (P) and tebuconazole (T). These fungicides belong to triazoles, a group of systemic fungicides that weaken fungal cell membrane integrity by inhibiting demethylation during sterol biosynthesis. Studies on FHB management usually adopt artificial inoculation methods and misting systems to increase the chance of *F. graminearum* to colonize the host. This study evaluated the efficacy of prothioconazole+tebuconazole (Prosaro®; Bayer CropScience) application and host genotype. Treatments included; inoculated+untreated, noninoculated+untreated, and inoculated+treated to three hard red spring wheat (*Triticum aestivum* L.) cultivars namely; 'Brick' (FHB resistant), 'Prevail' (FHB moderately resistant) and 'Samson' (FHB susceptible). All three cultivars were inoculated with *F. graminearum* using infested corn spawn at Feekes 9.0 and misting at 5 of the 10 environments whereas the rest were left under natural inoculum. The study was set up as a split plot where cultivars were the main plots while inoculated+treated with Prosaro (T1), inoculated+untreated (T2), and noninoculated+untreated (T3) were subplots in 10 environments (5 years by 2 locations). T2 had significantly high *Fusarium* damaged kernels (FDK) compared to T3 across all genotypes. There were no significant differences between T1 and T2 for Brick and Prevail. Similarly, no significant differences ($p<0.05$) were observed between T2 and T3 for Brick and Prevail ($p<0.05$). T2 plots were significantly ($p<0.0001$) different from T3 plots for Samson. Fungicide application prevented high FDK levels in all three cultivars. In addition, plots sprayed with fungicide had lower DON levels compared to non-treated plots. These results underscore the importance of artificial inoculation for better FHB fungicide or cultivar evaluation in areas where FHB disease forecast is low. These results also reiterate the importance of using host resistance in FHB management.

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FOOD SAFETY AND TOXICOLOGY

BIOPROSPECTING FOR ENZYMES TO TRANSPORT AND MODIFY DON FROM LIBRARIES OF DON-DETOXIFYING MICROORGANISMS

Celia Jimenez-Sanchez[1*], Nina Wilson[1], Nicole McMaster[1], Dash Gantulga[1], Ryan Senger[2] and David G. Schmale III[1]

[1]School of Plant and Environmental Sciences and [2]Department of Biological Systems Engineering, Virginia Tech, Blacksburg, VA 24061
[*]Corresponding Author: PH: 540-231-0733; Email: celia84@vt.edu

ABSTRACT

New strategies are needed to mitigate the mycotoxin deoxynivalenol (DON) in wheat and barley. We screened a library of microbial fragments for enzymes to transport and modify DON. These fragments were cloned into a PCR8/TOPO vector, and recombined into the yeast vector, pYES-DEST52. Transformants were screened in 100 ppm DON to find candidates that were able to grow better than the DON-sensitive yeast strains in the presence of DON. Resulting candidates were plated on a selective media in order to isolate singles colonies containing single microbial fragments. These fragments were sequenced, and most were similar (identity around 90% or higher) to known enzymes or transporters. Additional assays were conducted with a transport inhibitor (ferulic acid), to inhibit the putative transporters. An exciting candidate (4D) was inhibited by ferulic acid, and appears to be similar to an ABC transporter. The ultimate goal of our work is to reduce mycotoxin contamination in wheat and barley used for feed and food. Future work could extend to the development of treatments to reduce mycotoxins in the field prior to harvest, during grain storage, and/or during milling and processing.

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PATTERNS OF FUNGAL DISTRIBUTION IN *FUSARIUM* INFECTED BARLEY, RYE AND TRITICALE GRAIN AND MALT

Zhao Jin[1], Shyam Solanki[2], Ruoling Tang[1], James Gillespie[1], John Barr[1], Robert Brueggeman[2] and Paul Schwarz[1*]

[1]Department of Plant Sciences and [2]Department of Plant Pathology, North Dakota State University, Fargo, ND 58108
[*]Corresponding Author: PH: 701-231-7732; Email: paul.schwarz@ndsu.edu

ABSTRACT

Increases in DON following the malting of grain that had relatively low initial levels, and also had been stored for several months are seen as aberrant behavior. This behavior was seen in the 2016 crop with barley samples from upper Midwest and Prairie provinces of Canada), rye and triticale samples from North Dakota, Minnesota and New York. Maltsters have speculated that in barley, this may relate to internal vs external infection with *Fusarium* species. The objective of the current study was to investigate the development of *Fusarium* distribution in seeds during the malting of these Fusarium Head Blight (FHB) infected small grains. Cross-sections from the middle of the grain and malt were stained with WGA-Alexa Fluor 488 which specifically binds with fungal hyphae, and then were analyzed with confocal laser scanning microscopy (CLSM). Microscopic evaluation showed that fungal hyphae were mainly present in the husk (spongy parenchyma and cementing layer) of barley. However, hyphae thickly distributed in the husk of malt, and in some cases, penetrated the pericarp and testa, aleurone layer, and even slightly into the interspace of starchy endosperm. *Fusarium* Tri5 DNA increased from 0.71 pg/g in barley to 9.25 pg/g in malt, and DON increase from 1.20 μg/g to 6.42 μg/g in these samples. In addition, extensive growth of hyphae was observed in pericarp, testa, alerone layer and the central endosperm of rye and triticale, which illustrated internal infection in rye, triticale and their malts. A dramatic increase of DON and Tri5 DNA levels were observed following the malting of these rye and triticale samples. In contrast, fungal hyphae were not observed or only extremely small amount in relatively clean grain and malt samples, in which DON levels were non-detectable or below 0.30 μg/g and Tri5 DNA were below 0.10 pg/g.

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QUANTIFICATION OF DON IN SORGHUM USING A STABLE ISOTOPE DILUTION ASSAY

Nicole McMaster[1*], Bhupendra Acharya[2], Jan Grothe[1], Hillary Mehl[2] and David G. Schmale III[1]

[1]School of Plant and Environmental Sciences, Virginia Tech, Blacksburg, VA 24060; and [2]School of Plant and Environmental Sciences, Virginia Tech Tidewater Agricultural and Research Extension Center, Suffolk, VA 23437
[*]Corresponding Author: PH: 540-231-0733; Email: niki@vt.edu

ABSTRACT

Sorghum has gained popularity with consumers as a grain source with its gluten-free and high protein dietary characteristics. Acreage is increasing in the mid-Atlantic partially due to the demand for an alternative feed source for poultry and swine. Little is known about the potential for mycotoxin contamination in sorghum. New methods and approaches are needed to accurately detect and quantify mycotoxins in sorghum. A traditional method to quantify DON (solid phase extraction chromatography with C18, followed by GC-MS), produced inconsistent DON values following controlled spiking and recovery experiments with different sorghum lines. Consequently, we developed a new method using a stable isotope (d1-DON) as an internal standard. This method (Stable Isotype Dilution Analysis, or SIDA) was used to accurately determine DON levels in 196 sorghum samples representing 98 different lines. Of the 98 lines tested (two samples per line), 76 of the lines had DON levels that were greater than the limit of detection for both methods (0.20 ppm). For a regulatory limit of 1 ppm, about one third of all of the lines (26/76) had at least 20 percent more DON using the SIDA method. For a regulatory limit of 5 ppm, about seven percent of all the lines (5/76) had at least 20 percent more DON using the SIDA method. Using SIDA, the amount of DON in a sorghum sample can be accurately and reliably quantitated by basing calculations on the recovery of d1DON, and may find application in future samples with complex matrices.

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ZEARALENONE AND RELATED METABOLITES IN SWINE FEED AND REPRODUCTIVE TISSUES

Erica Pack[1], Jacob Stewart[2], Holly Newberne[2], Michelle Rhoads[2], James Knight[2], Raffaella De Vita[3], Sherrie Clark[4] and David Schmale[1*]

[1]School of Plant and Environmental Sciences, [2]Department of Animal and Poultry Sciences, and [3]Department of Biomedical Engineering, and Mechanics, and [4]Department of Large Animal Clinical Sciences, Virginia-Maryland College of Veterinary Medicine, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061
[*]Corresponding Author: PH: 540-231-6943; Email: dschmale@vt.edu

ABSTRACT

The mycotoxin zearalenone (ZON) is a common contaminant of corn and small grains. Swine feed is often supplemented with co-products from the production of fuel and beverage ethanol, herein referred to as "ethanol co-products". These co-products may contain concentrated amounts of mycotoxins, including ZON. ZON is known to have potent estrogenic effects in swine, contributing to costly reproductive issues such as prolapse, vulva swelling, decreased litter size, and anestrous. Though the effects of ZON exposure are well documented, it is still not yet known which reproductive organs are most vulnerable to ZON's estrogenic effects. To determine the reproductive organs most vulnerable to ZON exposure, we designed and conducted an Institutional Animal Care and Use Committee (IACUC)-approved feeding study wherein pubertal gilts were exposed to ZON-contaminated feed. A total of 27 pubertal gilts were assigned one of three treatments: (A) 6mg ZON/day for 21 days (n=10), (B) 6mg ZON/day for 7 days followed by non-contaminated feed for 14 days (n=8), (C) non-contaminated (control) feed for 21 days (n=9). Non-contaminated feed was sampled daily in order to determine naturally occurring ZON content in feed prior to contamination. Reproductive tracts were harvested at slaughter after 21 days of feeding. Concentrations of ZON and the related metabolite α-zearalenol (α-ZOL) are being quantified in feed and tissues using gas chromatography – mass spectrometry (GC-MS). Results of this study should shed some light on how swine feed contaminated with ZON may contribute to reproductive anomalies in the swine production industry.

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ANALYTICAL METHODOLOGIES FOR THE DETERMINATION OF FUNGICIDE RESIDUES USED FOR THE CONTROL OF FUSARIUM HEAD BLIGHT IN WHEAT GRAIN

Cintia Palladino[1*], Carolina Francia[2], Lucas Martella[2], Matias Passarino[2], Carlos Pérez[2] and Lucia Pareja[3]

Polo de Desarrollo Universitario Abordaje Holístico Impactos de los Agroquímicos, Cenur Litoral Norte, UdelaR, EEMAC, Paysandú, Uruguay; [2]Departamento de Protección Vegetal, EEMAC, Facultad de Agronomía, UdelaR, EEMAC, Paysandú, Uruguay; and [3]Departamento de Química del Litoral, Cenur Litoral Norte, UdelaR, EEMAC, Paysandú, Uruguay
[*]Corresponding Author: PH: 598-9-826-4734; Email: cintiapalladino@fagro.edu.uy

ABSTRACT

Fusarium head blight (FHB) is one of the diseases that causes the most economic losses in wheat. The most used management is the preventive chemical control, which can lead to unnecessary applications if the conditions for the development of the disease are not given generating residues of the fungicides in grains. These can be an obstacle in the world markets, which are very demanding with the maximum residue limits (MRL). The aim of this work is to validate a method for the determination of fungicide residues in grain obtained under different chemical treatments for FHB in wheat. The method was developed by comparing different variations of extraction and clean-up methodologies based on the QuEChERS method. The confirmation and quantification of the residues was by liquid chromatography coupled to tandem mass spectrometry. The method was applied on two varieties of wheat treated with different chemical management (M1: metconazole+epoxiconazole and M2: tebuconazole), at the beginning of flowering. The level of FHB was quantified by the disease index (incidence * severity). The method presented percentages of recovery in the range 70-120% for the selected compounds even at low concentration levels and percentages of relative standard deviation less than 20%. The linearity in solvent and matrix of the compounds showed a linear behavior (R^2 greater than 0.99). Metconazole was the only compound that presented matrix effect. The limits of detection and quantification were lower than the MRLs established by European regulations. Preliminary data show that the evaluated managements presented differences in the index of the disease, being M1 the most efficient. Moreover, for both varieties none of the evaluated treatments showed residue levels higher than the MRLs. The validated method allows verifying the compliance of MRLs in wheat grain in the country, a necessary requirement for a safe and environmentally-friendly production of wheat.

ENRICHMENT CULTURES WITH THE ABILITY TO BIOTRANSFORM DEOXYNIVALENOL

Hina Shanakhat[1], Susan P. McCormick[2] and Matthew G. Bakker[2*]

[1]Centre for Food Innovation and Development in the Food Industry, University of Naples, Federico II, Naples, Italy; and [2]Mycotoxin Prevention & Applied Microbiology, US Department of Agriculture, Peoria, IL 61604
[*]Corresponding Author: PH: 309-681-6589; Email: Matt.Bakker@ars.usda.gov

ABSTRACT

The production and accumulation of trichothecene mycotoxins is responsible for much of the negative economic impact associated with Fusarium head blight. A variety of biochemical transformations to trichothecenes have been described, some of which result in a less toxic product. We expect that continued prospecting will reveal additional microbial transformations to trichothecenes, and eventually microbial enzymes having utility in plant protection or in restoring value to contaminated grain. We have developed methods for producing enrichment cultures in which complex microbial consortia (e.g., seeded from soil dilutions) are directed towards the transformation of deoxynivalenol (DON). We can now reliably produce enrichment cultures that transform DON. However, deriving from these communities a pure culture of an organism that transforms DON has remained elusive. Here we describe our procedure for producing enrichment cultures, and the microbial communities that develop in these enrichment cultures over time.

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CROPPING FACTORS: THE KEY FOR SUSTAINABLE MYCOTOXIN MANAGEMENT IN CEREALS

Susanne Vogelgsang[1*], Marco Beyer[2], Matias Pasquali[3], Eveline Jenny[1], Tomke Musa[1], Torsten Schöneberg[1], Dimitrios Drakopoulos[1], Alejandro Gimeno Sierra[1], Thomas D. Bucheli[1], Felix E. Wettstein[1] and Hans-Rudolf Forrer[1]

[1]Agroscope, 8046 Zürich, Switzerland, [2]Luxembourg Institute of Science and Technology (LIST), Luxembourg, and [3]University of Milan, Milano, Italy
[*]Corresponding Author: P H: 41-58-468-7229; Email: susanne.vogelgsang@agroscope.admin.ch

ABSTRACT

Over an eight-year period, 686 winter wheat grain samples and information on their cropping history were obtained from Swiss growers. To estimate the risk of Fusarium head blight (FHB), grains were examined for *Fusarium* species incidence, mycotoxin content as well as the abundance of *F. graminearum* (FG) and *F. poae* (FP) DNA and three chemotypes, 15-acetyl-deoxynivalenol (15ADON), 3-acetyl-deoxynivalenol (3ADON) and nivalenol (NIV). Of all *Fusarium* species, FG and FP were predominant, and the average abundance of the FG DNA was three times higher compared with that of FP. In addition, the average detection of the 15ADON chemotype was twice as high as those of 3ADON and NIV, respectively. Deoxynivalenol (DON), zearalenone (ZEA) and nivalenol (NIV) were the most frequently detected toxins. For DON, 11% and for ZEA, 7% of all samples exceeded the European maximum limits for unprocessed cereals (1). Furthermore, NIV was most likely produced by four different *Fusarium* species, including FP, FG as well as *F. cerealis* and *F. culmorum*. A multiple correspondence analysis revealed that high levels of FG and DON were mainly observed in grain samples from fields with the previous crop maize, reduced tillage, cultivars with poor FHB resistance and strobilurin-based fungicides. Other previous crops and/or ploughing decreased the DON content by 78 to 95%. ZEA showed a similar pattern. In contrast, high levels of FP and NIV were associated with samples from ploughed fields and the previous crop canola (2). These findings and the negative correlations between FP DNA and FG incidence, ZEA and DON suggest a different ecological niche for FP or diverging requirements for infection. Moreover, the effect of cropping factors on FG infection and DON contamination in wheat was quantified to develop the forecasting system FusaProg. This internet-based system employs plot-specific cropping, growth stage and regional weather data (3). FusaProg was successfully validated with more than 600 wheat samples.

The barley survey (2013-2014 and ongoing) showed similar patterns as those in wheat except that tillage did not have a significant effect on the DON content (4). In oats (2013-2015), T-2/HT-2 toxins were detected in 91% of all samples. Samples of the winter variety 'Wiland' or from fields with pre-crop cereals contained significantly higher T2-/HT-2 contents compared with other varieties or pre-crops (5).

For growers that depend on a maize-wheat rotation, supplementary strategies are needed. The fungal antagonist *Clonostachys rosea* applied during maize harvest onto crop residues appears to be highly promising. Currently, different *C. rosea* formulations and strains are examined to ensure competitiveness under field conditions. Inter-/cover crops in maize-wheat rotations as well as biofumigation could also

reduce FG inoculum through physical barriers and/or through antifungal properties. Preliminary results from these experiments will be presented.

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GENE DISCOVERY AND ENGINEERING RESISTANCE

HOST-INDUCED GENE SILENCING (HIGS) FOR ENHANCING RESISTANCE TO *FUSARIUM GRAMINEARUM*

Syeda T. Alam[1], Neerja Tyagi[2], Harold N. Trick[2] and Jyoti Shah[1*]

[1]Department of Biological Sciences and BioDiscovery Institute, University of North Texas, Denton, TX 76203; and [2]Department of Plant Pathology, Kansas State University, Manhattan KS 66506
[*]Corresponding Author: PH: 940-565-3535; Email: Jyoti.Shah@unt.edu

ABSTRACT

Fusarium graminearum (*Fg*) is the major causative agent of Fusarium head blight (FHB) disease in wheat and barley. *Fg* also causes seedling blight disease. Current methods to control FHB severity include planting partially resistant varieties combined with fungicide application. Strategies that target the expression and/or activity of fungal pathogenicity genes offer an alternative approach to control FHB severity. The goal of this project is to utilize host-induced gene silencing (HIGS) as a mechanism to target the transcripts of *Fg* pathogenicity genes. As part of HIGS a double stranded RNA (dsRNA) designed to a fungal gene is expressed in plants. The resultant dsRNA will be processed into small RNAs in the plant. Upon uptake by the fungus, these small RNAs are expected to destabilize transcripts of the targeted fungal gene. We have utilized HIGS to target a secretory lipase FGL1, and a secretory hydroxylase that putatively limits accumulation of salicylic acid and/or phenolics in infected plants. dsRNA targeting these *Fg* genes when expressed in Arabidopsis enhanced resistance against *Fg* infection, thus confirming the effectiveness of HIGS as a strategy to control *Fg* infection. Next up, we will test the effectiveness of HIGS-mediated silencing of these genes in promoting FHB resistance in wheat.

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

Syeda Alam[1], Bhavit Chhabra[2], Nidhi Rawat[2*] and Jyoti Shah[1*]

[1]Department of Biological Sciences and BioDiscovery Institute, University of North Texas, Denton, TX 76203; and [2]Department of Plant Science and Landscape Architecture, University of Maryland, College Park, MD 20742
[*]Corresponding Authors: Jyoti Shah - PH: 940-565-3535; Email: Jyoti.Shah@unt.edu, Nidhi Rawat - PH: 301-405-9744; Email: nidhirwt@umd.edu

ABSTRACT

Fusarium graminearum (*Fg*) is the principal causative agent of Fusarium head blight (FHB) in cereal grains like wheat and barley. 9-lipoxygenases (9-LOXs) have been identified as susceptibility factors in Arabidopsis and wheat interaction with *Fg*. RNA-interference (RNAi)-mediated knockdown of 9-LOXs in the hexaploid wheat cv 'Bobwhite' and knockout of 9-LOXs in Arabidopsis confer enhanced resistance against the fungus (Nalam et al. 2015). *Fg* infection in 9-LOX-silenced wheat was mostly confined to the inoculated spikelet. *Lpx3* is one of the wheat 9-LOX's that contribute towards susceptibility to *Fusarium graminearum*. As a non-GMO approach, TILLING lines with nonsense and/or missense *Lpx3* variants were identified in hexaploid wheat variety 'Cadenza' and tetraploid wheat variety 'Kronos'. A dCAPS (derived cleaved amplified polymorphic sequence) strategy was utilized to develop homeolog-specific co-dominant markers that could distinguish between the wild-type and mutant *Lpx3* alleles. FHB incidence was reduced in some of these lines. This non-GMO strategy will facilitate the integration of these FHB-resistant 9-LOX alleles into exiting wheat breeding programs.

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TOWARDS UNDERSTANDING THE FUNCTION OF THE BARLEY UDP-GLUCOSYL TRANSFERASE UGT13248 IN DISEASE RESISTANCE

Gerit Bethke[1], Xin Li[1], Yadong Huang[1], Goetz Hensel[2], Jochen Kumlehn[2], Silvio Salvi[3] and Gary Muehlbauer[1*]

[1]University of Minnesota, Department of Agronomy and Plant Genetics, Saint Paul, MN 55108, USA; [2]Leibniz Institute of Plant Genetics and Crop Plant Research, Plant Reproductive Biology, Gatersleben, Germany; and [3]University of Bologna, Department of Agricultural and Food Sciences, Bologna, Italy

[*]Corresponding Author: PH: 612-625-6228; Email: muehl003@umn.edu

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 predominantly by accumulation of trichothecene mycotoxins, e.g. deoxynivalenol (DON), in grain. DON production is part of the fungal infection strategy and increased DON accumulation coincides with the switch of fungal lifestyle from the biotrophic to the necrotrophic stage. Glycosylation of DON to DON-3-glucose (D3G) is thought to be catalyzed by UDP-glucosyl transferases (UGT). In barley, at least 10 UGT genes are upregulated upon *F. graminearum* infection or DON treatment. One of those, UGT13248, was shown to convert DON to D3G in yeast, Arabidopsis and wheat. In wheat, expression of UGT13248 decreased disease severity of FHB. Our goal in this project is to understand the role of UGT13248 in barley FHB resistance. Overexpression of UGT13248 lead to decreased DON sensitivity of barley roots. Currently, we are investigating TILLING lines with amino acid changes close to the UDP-sugar binding site of UGT13248 and RNAi lines. Preliminary results suggest that mutations close to the UDP-sugar binding site of UGT13248 result in increased sensitivity of barley roots to DON-containing media. In the future, we will test fungal growth and DON to D3G conversion rates in these lines. Taken together, our data suggest that UGT13248 is important for DON resistance in barley and hence might affect pathogen susceptibility.

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CRISPR/CAS9 GENOME EDITING TECHNOLOGY FOR FHB RESISTANCE IMPROVEMENT IN WHEAT

Hui Chen[1], Zhenqi Su[1], Bin Tian[2], Harold N. Trick[2] and Guihua Bai[1,3*]

[1]Department of Agronomy and [2]Department of Plant Pathology, Kansas State University, Manhattan, KS 66506, USA; and [3]Hard Winter Wheat Genetics Research Unit, USDA-ARS, Manhattan, KS 66506, USA
[*]Corresponding Author: PH: 785-532-1124; Email: guihua.bai@ars.usda.gov

ABSTRACT

Fusarium head blight (FHB) is a destructive disease of wheat and barley in the US and many other countries. Growing resistant cultivars is the most effective measure for FHB control. *Fhb1* is a gene from a Chinese wheat cultivar Suma 3 and many Chinese wheat landraces and shows the largest effect on FHB resistance among all quantitative trait loci (QTLs)/genes reported to date. Recently, we cloned *TaHRC* (a histidine-rich calcium binding protein) as the key determinant of *Fhb1* and showed that the wild type *TaHRC* is a susceptible allele and a large mutation in the start codon region is the causal mutation for *Fhb1* resistance. This finding suggests that knocking out the susceptible allele of *TaHRC* can improve FHB resistance in wheat. The clustered regularly interspaced short palindromic repeats CRISPR/Cas9 genome editing technology developed recently can be used to modify plant traits by changing DNA sequence at a specified genome location. In this study, we knocked out the susceptible gene *TaHRC* by editing the gene sequence using conventional CRISPR/Cas9 system and significantly improved FHB resistance of transgenic wheat. However, currently we are only be able to transform the varieties 'Bobwhite' and 'Fielder' for gene editing due to low efficiencies in gene editing and poor regeneration rates of many wheat genotypes. These factors limit the application of gene editing as a routine tool in many breeding programs. More recently, we developed a novel *Barley stripe mosaic virus*-mediated CRISPR/Cas9 genome editing system that can bypass the routine transformation and regeneration steps as used in the conventional wheat gene transformation process. This new genome editing system shows significant improvement from current wheat transformation technique and can edit genes in many wheat backgrounds for gene function validation and wheat breeding.

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GENOME-WIDE ASSOCIATION ANALYSIS AND ALLELE FREQUENCY DIFFERENTIATION TO MAP QTL FOR DON IN BARLEY

Sintayehu Daba[1], Richard Horsley[2] and Mohsen Mohammadi[1*]

[1]Department of Agronomy, Purdue University, West Lafayette, IN 47907; and
[2]Department of Plant Sciences, North Dakota State University, Fargo, ND 58108
[*]Corresponding Author: PH: 765-496-6851; Email: mohamm20@purdue.edu

ABSTRACT

The disease Fusarium Head Blight (FHB) causes considerable grain yield and quality loss in small grains. It produces the mycotoxin deoxynivalenol (DON) that is toxic to both humans and animals. It is hypothesized that when a breeding population is under selection, the genomic regions controlling the trait of interest are expected to differentiate for allele frequency between individuals with higher and lower levels of trait expression. We tested this hypothesis by applying allele frequency differentiation (AFD) and genome-wide association studies (GWAS) to identify and map loci that are associated with DON. The DON measurements from three experiments in North Dakota (Langdon in 2012 and 2013, and Osnabrock in 2012) on four groups of germplasm (Ethiopian landrace, Ethiopian breeding lines, ICARDA breeding lines, and NDSU breeding lines) were used in this study. For the AFD approach, two groups of germplasm contrasting for DON were formed, i.e.; one group with high DON (> 56.6 ppm) consisted of 47 lines and one group with low DON (< 16.3 ppm) consisted of 51 lines. With threshold of AFD ≥ 0.70, 15 SNP markers associated with DON were located in chromosomes 2H, 3H, 4H, and 7H. A total of 33 genes were located close to these 15 loci. These genes were related to response to biotic stress (Tubby-like protein and MLO-like protein), plant-type cell wall organization (Expansin-like protein), carbohydrate metabolic process, and sulfotransferase activity. The GWAS result (with $-log(p) \geq 4.0$) revealed three loci in chromosome 2H and one locus in chromosome 7H. A total of 28 genes were found near these loci. These genes were related to oxidation-reduction process, sulfotransferase activity, and photosystem II. We detected no shared SNPs by using the two methods. Two regions were detected in chromosme 2H one by AFD and one by GWAS method and were 354.5 Mbp apart.

TESTING TRANSGENIC SPRING WHEAT AND BARLEY LINES FOR REACTION TO FUSARIUM HEAD BLIGHT: 2018 FIELD NURSERY REPORT

Ruth Dill-Macky[1*], Amar M. Elakkad[1], Beheshteh Zargaran[1], Gary J. Muehlbauer[2], Gerit Bethke[2], John McLaughlin[3], Nilgun Tumer[3] and Deanna Funnell-Harris[4]

[1]Department of Plant Pathology, and [2]Department of Agronomy and Plant Genetics, University of Minnesota, St. Paul, MN 55108; [3]Department of Plant Biology and Pathology, Rutgers University, New Brunswick, NJ 08910; and [4]USDA-ARS, Wheat, Sorghum and Forage Research Unit, Lincoln, NE 68583
[*]Corresponding Author: PH: 612-625-2227, Email: ruthdm@umn.edu

ABSTRACT

The 2018 field screening nursery consisted of 98 wheat and 15 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 (46 wheat lines + 'Rollag' and 'Linkert'; 13 barley lines + 'Rasmusson'), Rutgers University (16 wheat lines + 'RB07' and 'Bobwhite') and the USDA (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 Rollag and 'Sumai 3' and the susceptible cultivars 'Norm' and 'Wheaton'. The barley checks were the moderately resistant cultivar 'Quest' and the susceptible cultivar Rasmusson. Individual plots were 2.43 m long single rows. The trial was planted on May 22, 2018. All plots were inoculated twice. The first inoculation was applied at anthesis for wheat (July 2) and at head emergence (July 10) 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 20. 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 July 2 through July 27 to facilitate FHB development. FHB incidence and severity were assessed visually 20-24 d.a.i. for wheat and 13-14 d.a.i. for barley 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 on August 13 and 14. Approximately sixty heads where harvested from each plot, threshed and the seed cleaned manually. 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 untransformed parental wheat checks Bobwhite, CB037, Linkert, RB07, and Rollag were 22%, 36%, 6%, 11% and 9%, respectively. The mean FHB severity for the susceptible wheat check Wheaton was 31%. The mean FHB severity for the untransformed parent barley check Rasmusson was 26% while the susceptible barley check 'Lacey' had a mean FHB severity of 34% and the moderately resistant line Quest had an FHB severity of 13%. The FHB severity data indicated that resistance was improved in some transformed lines compared to

the untransformed checks. The harvested grain is currently being analyzed for VSK and DON. The data are not yet available, although they will be included in the poster presented at the forum.

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RESPONSE OF WHEAT CONSTITUTIVELY EXPRESSING LIGNIN GENES TO FUSARIUM HEAD BLIGHT

Zachary Duray[1,2], Deanna L. Funnell-Harris[1,2*], Robert A. Graybosch[1,3], Scott E. Sattler[1,3], Stephen N. Wegulo[2] and Thomas E. Clemente[3]

[1]USDA-ARS, Wheat, Sorghum and Forage Research Unit Lincoln, NE 68583;
[2]Department of Plant Pathology, and [3]Department of Agronomy and Horticulture, University of Nebraska, Lincoln, NE 68583
[*]Corresponding Author: PH: 402-472-9099; Email: Deanna.Funnell-Harris@ars.usda.gov

ABSTRACT

The goal of this research is to identify resistance to Fusarium Head Blight (FHB) in wheat through increased monolignol biosynthesis, which produces the subunits of lignin. Monolignols are secreted into plant cell walls to provide structural support, and this pathway also is induced upon pathogen attack. The spring wheat 'CB037' was transformed with constitutive expression constructs containing the gene for the sorghum transcription factor *SbMyb60*, or a gene encoding a sorghum monolignol pathway enzyme [caffeoyl-CoA 3-O-methyltransferase (*SbCCoAOMT*), 4-coumarate-Coenzyme A ligase (*Sb4CL*), or *p*-coumarate 3-hydroxylase (*SbC3H*)], each under control of the cauliflower mosaic virus E35S promoter. Greenhouse-grown plants of lead transgenic events, CB037, and the checks, 'Sumai 3' (FHB moderately resistant) and 'Wheaton' (FHB susceptible), were either spray-inoculated with a *Fusarium graminearum* conidial suspension to assess Type I resistance (to initial infection), or point-inoculated to assess Type II resistance (to pathogen spread). Disease severity, determined at multiple time points up to 21 days after inoculation, was used to calculate Area Under the Disease Progress Curve (AUDPC). Proportion of *Fusarium* Damaged Kernels (FDK) and deoxynivalenol (DON) levels were also determined. In two assays, FDK following point inoculations showed that transgenic events carrying *SbC3H* (one of two) and *SbCCoAOMT* (one of two) were not significantly different from Sumai 3. However, after a third assay conducted under warmer conditions, FDK of these transgenic lines were significantly greater than Sumai 3. For the three assays, *SbC3H* and *SbCCoAOMT* lines had reduced AUDPC as compared with *Sb4CL* and *SbMyb60*, but not significantly different from Wheaton or CB037 in both point and spray assays. Disease measurements for *Sb4CL* and *SbMyb60* lead events, following three assays with point and spray inoculations, were similar to Wheaton (FDK and DON) or even significantly greater (AUDPC). DON levels in all overexpression lines were similar to Wheaton and CB037. The enzymes C3H and CCoAOMT and intermediates in the monolignol biosynthesis pathway have been associated with resistance against pathogen infection in other systems. Genetic crosses of CB037 carrying *SbCCoAOMT* and *SbC3H* overexpression constructs are being performed with Sumai No. 3 and other spring wheat lines with moderate FHB resistance to stack multiple resistance strategies.

ACKNOWLEDGEMENT AND DISCLAIMER

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

FINE MAPPING OF FHB QUANTITATIVE TRAIT LOCI ON CHROMOSOMES 6H AND 2H IN BARLEY

Yadong Huang[1], Shane Heinen[1], Brian Steffenson[2], Kevin P Smith[1] and Gary J. Muehlbauer[1,3*]

[1]Department of Agronomy and Plant Genetics, [2]Department of Plant Pathology, and [3]Department of Plant Biology, University of Minnesota, St. Paul, MN 55108
[*]Corresponding Author: PH: 612-625-6228; Email: muehl003@umn.edu

ABSTRACT

Fusarium species cause Fusarium head blight (FHB) disease in wheat and barley. 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 detected two consistent QTL on chromosome 2H and 6H, respectively. The 2H FHB QTL coincided with a heading date QTL and the 6H FHB QTL coincided with QTL for grain protein content (GPC) and kernel discoloration (KD). Parents with introgressions of Chevron alleles in both QTL intervals were developed. The aims of the current studies are to (1) develop recombinant near-isogenic lines (rNILs) for both QTL regions and characterize their disease and correlated agronomic phenotypes; and (2) fine-map both QTL and identify candidate genes. For fine mapping the 2H QTL, an F_2 population of 2,038 plants was generated from a cross of 'Gen1-001' (resistant parent) to 'M69' (a susceptible breeding line) and was genotyped with SNP markers flanking the introgression (~26 cM). A total of 489 recombinants were identified which were further genotyped with 32 SNP markers spanning the introgression. This resulted in 17 recombinant classes and homozygous $F_{2:3}$ plants were identified and phenotyped for disease in field and greenhouse conditions from 2016-2018. Significant variations among rNILs and environments were detected. Some lines exhibited lower disease severities than Gen1-001 and could be used as parents for further fine mapping. For mapping the 6H QTL, an F_2 population of 2,082 plants was derived from crossing two lines carrying Chevron alleles in the 6H QTL region with the susceptible cultivar 'Lacey'. Recombinants identified from the population were genotyped with 34 SNP markers covering the target region (~3.0 cM), which resulted in the identification of sub-recombinants representing 12 recombinant classes. Selected homozygous $F_{2:3}$ rNILs were tested for FHB and GPC in field and greenhouse conditions from 2016-2018. Multiple lines showed lower FHB severities than the resistant parents in some environments. Preliminary results suggested that GPC and FHB resistance are controlled by tightly linked loci. The resistant lines with lower GPC could be used in breeding for malting barley cultivars.

ARABIDOPSIS AND WHEAT NON-SPECIFIC LIPID TRANSFER (NSLTP) PROTEINS INHIBIT *FUSARIUM GRAMINEARUM* AND CONFER ENHANCED RESISTANCE TO FHB: GREENHOUSE, FIELD AND *IN VITRO* VIDENCE

John E. McLaughlin[1], Neerja Tyagi[2], Harold N. Trick[2], Susan McCormick[3], Ruth Dill-Macky[4] and Nilgun E. Tumer[1*]

[1]Department of Plant Biology and Pathology, School of Environmental and Biological Sciences, Rutgers University, New Brunswick, NJ 08901; [2]Department of Plant Pathology, Kansas State University, Manhattan, KS 66506; [3]Bacterial Foodborne Pathogens and Mycology Unit, USDA-ARS-NCAUR, Peoria, Illinois 61604; and [4]Department of Plant Pathology, University of Minnesota, St. Paul, MN 55108
[*]Corresponding Author: PH: 848-932-6359; Email: tumer@aesop.rutgers.edu

ABSTRACT

Non-specific lipid transfer proteins are involved in plant defense and abiotic stress responses. These small, basic, cysteine-rich proteins have been shown to be upregulated in plants in response to the application of both trichothecenes and fungi. Previous research in our laboratory has found that overexpressing nonspecific lipid transfer proteins in *Arabidopsis* enhances resistance to trichothecenes by reducing ROS stress. Our primary research goal here is to determine if overexpression of two non-specific lipid transfer proteins (nsLTPs) AtLTP4.4 (AT5G55450) and TaLTP3 (AY226580) enhance resistance of wheat to FHB. We have characterized transgenic wheat expressing AtLTP4.4 and TaLTP3 in transgenic 'Bobwhite' and 'RB07' lines. Confocal and Western analysis showed high levels of expression of Ubi:AtLTP4.4:GFP and Ubi:TaLTP3:GFP in Bobwhite and Rb07 wheat. Transgenic wheat lines expressing AtLTP4.4:GFP and TaLTP3:GFP showed significant reductions in fungal growth based on both visual assessment and via a qPCR bioassay that measures fungal DNA relative to wheat DNA. Results from the greenhouse show that overexpression of both TaLTP3 and AtLTP4.4 significantly enhance resistance to FHB when wheat floral tissue (at flowering, Feekes growth stage 10.5.1) is spot inoculated and later evaluated at 7, 14, and 21 days after inoculation (DAI). Results from the 2018 field test in Rosemount, Minnesota show reduced FHB severity of two of the AtLTP4.4 overexpressing lines in the RB07 genetic background (non-significant mean reduction). Field tests of nsLTP overexpressing lines in the Bobwhite genetic background revealed one AtLTP4.4 overexpressing line which shows a non-significant mean reduction in FHB severity and two TaLTP3 overexpressing lines which show significantly reduced FHB severity. To investigate the function of nsLTPs, we isolated the AtLTP4.4 protein from *Pichia pastoris* and show, using an *in vitro* liquid culture assay, that this nsLTP is able to inhibit *Fusarium graminearum* growth. Exogenous application of a wheat TaLTP9 (9 kD nsLTP) also showed anti-fungal properties. However, not all nsLTPs have antifungal properties. For instance, TaLTP7 (7 kD nsLTP) had no effect on *F. graminearum* growth when applied in liquid culture. These results suggest some nsLTPs may have differential roles in protecting plants against trichothecene virulence factors and the fungus itself.

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EXPRESSION OF PHYTOHORMONE RELATED WHEAT DEFENSE GENES IN *PFT*-MEDIATED RESISTANCE AGAINST *FUSARIUM GRAMINEARUM*

Lovepreet Singh[1], James Anderson[2], Bikram S. Gill[3] and Nidhi Rawat[1*]

[1]Department of Plant Science and Landscape Architecture, University of Maryland, College Park, MD 20742; [2]Department of Agronomy and Plant Genetics, University of Minnesota, St. Paul, MN 55108; and [3]Department of Plant Pathology, Kansas State University, Manhattan, KS 66506
[*]Corresponding Author: PH: 301-405-9744; Email: nidhirwt@umd.edu

ABSTRACT

Fusarium Head Blight (FHB) is a devastating disease of small grains impacting their yield and quality. Growing disease resistant cultivars is the most economical and sustainable strategy to manage FHB. *Fhb1* is the most stable large effect quantitative trait locus (QTL) that confers broad spectrum resistance against FHB. Recently, a pore-forming toxin-like (*PFT*) gene was identified as the underlying resistance gene for this QTL. Although role of various phytohormones in regulating defense against FHB has been reported, defense signaling specific to *Fhb1* is still elusive. The objective of this study was to elucidate role of Salicylic Acid (SA), Jasmonic Acid (JA), and Ethylene (ET) signaling in *PFT*-mediated resistance. The time course gene expression experiment was performed between resistant genotype: HR58, carrying *PFT* gene, and a knock out mutant of PFT gene, pft[1958]. The expression of SA, JA, and ET related genes was analyzed at 0, 6, 12, 24, and 48 hours post-inoculation (hpi). Significant differences in expression of JA and SA related genes were observed at 48 hpi between HR58 and pft[1958]. The expression of SA responsive genes, *PR1* and *PR2*, and JA biosynthesis gene *TaAOS1* significantly increased in HR58 as compared to pft[1958] at 48 hpi. Our results suggest potential role of SA signaling and JA biosynthesis in *PFT*-mediated resistance during early infection in wheat.

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WHOLE GENOME SEQUENCES OF WHEAT BRING A NEW ERA FOR GENE DISCOVERY AND BREEDING

Sean Walkowiak[1*], Jemanesh Haile[1], Amidou N'Diaye[1], Brook Byrns[1], Kirby Nilsen[1], Andrew Sharpe[2], Luigi Cattivelli[3] and Curtis Pozniak[1]

[1]Crop Development Centre, University of Saskatchewan, Saskatoon, Canada; [2]Global Institute for Food Security, University of Saskatchewan, Saskatoon, Canada; [3]CREA Research Centre for Genomics and Bioinformatics, Fiorenzuola d'Arda, Italy
*Corresponding Author: PH: 306-250-0359; Email: sean.walkowiak@usask.ca

ABSTRACT

Genomics is particularly useful for marker development, QTL discovery, and understanding the root cause of important traits, including complex traits such as resistance to Fusarium head blight (FHB). To date, over 20 chromosomal regions have been identified that contribute to FHB resistance; however, the underlying genes that confer the resistance are largely unknown. With the recent completion of the genome sequence of Chinese Spring, we now have new resources at our fingertips to refine these genetic intervals and identify gene candidates for resistance. Unfortunately, Chinese Spring does not carry FHB resistance genes of interest to North American breeders; therefore, additional genomic resources are required to identify FHB resistance genes. These additional genomic resources will not only generate improved markers for breeding, but also help uncover how wheat can best defend itself against FHB fungi. In our wheat breeding programs at the University of Saskatchewan, we are using the latest sequencing and assembly technologies to generate several whole genome assemblies for both bread (hexaploid) and durum (tetraploid) wheat, with the goal to uncover the genetic cause of important traits in wheat, including resistance to FHB. Genome sequencing and assembly has been performed at different levels of quality and depth, including >10 high-quality reference genome assemblies at a quality level similar to Chinese Spring, as well as >100 mid-low coverage reference-guided genome assemblies. Results demonstrating the application of these genomic resources for gene discovery and breeding will be presented.

CHARACTERIZATION OF FUSARIUM HEAD BLIGHT RESISTANCE IN A WHEAT MUTANT DERIVED FROM HARD WINTER WHEAT JAGGER USING GENOTYPING-BY-SEQUENCING (GBS) MARKERS

Yuzhou Xu[1], Amy Bernardo[2] and Guihua Bai[1,3*]

[1]Department of Agronomy, [2]Department of Plant Pathology, Kansas State University, Manhattan, KS 66506; and [3]Hard Winter Wheat Genetics Research Unit, USDA-ARS, Manhattan, KS 66506
[*]Corresponding Author: PH: 785-532-1124; Email:guihua.bai@ars.usda.gov

ABSTRACT

Fusarium head blight (FHB) is one of the most destructive diseases of cereal crops worldwide. Three major types of FHB resistance (Types I, II and III) have been reported in wheat. Among them, resistance to spread of FHB symptoms within a spike (type II resistance) is the most stable type of resistance and has been extensively investigated. Jagger is a moderately susceptible hard winter wheat cultivar from Kansas. We mutagenized Jagger seeds with EMS to create a mutant population. This population was screened for type II FHB resistance in greenhouses and three mutant lines were identified to have high FHB resistance. Sequence analysis indicated that those mutants carry susceptible alleles for *Fhb1*. One of the mutants, JaggerR1097, was crossed to Jagger and another highly susceptible cultivar Overley to develop recombinant inbred line (RIL) populations (JaggerR1097/Overley and JaggerR1097/Jagger). The two RIL populations and their parents were inoculated with *Fusarium graminearum* in the greenhouses to evaluate their FHB resistance and genotyped using genotyping-by-sequencing (GBS). Two linkage bin maps were constructed using 816 (JaggerR1097/Overley) and 303 (JaggerR1097/Jagger) unique SNPs. Using those maps, four QTLs were detected for Type II resistance on the chromosomes 1B, 2D, 4A and 6B and explained 7.9%, 7.8%, 9.2 % and 5.0% of the phenotypic variation, respectively. Among them, two QTLs (2D, 4A) were from mutant parent. Three QTLs on chromosomes 2D, 4B and 5D were detected for increased plant height, and explained 7.8, 13.9% and 14.5% of the phenotypic variations. The preliminary results indicated that wheat response to FHB infection may be regulated by susceptibility genes and the loss-of-function mutations in those susceptibility genes increase wheat FHB resistance.

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A QUANTITATIVE PROTEOMIC VIEW OF MECHANISMS OF THE *QFHB1*-CONTROLLED FHB RESISTANCE IN WHEAT

Yang Yen

Department of Biology and Microbiology, South Dakota State University, Brookings, SD 57007
Corresponding Author: PH: 605-688-4567; Email: yang.yen@sdstate.edu

ABSTRACT

Quantitative trait locus *Qfhb1* (syn. *Qfhs.ndsu.3BS*, *Fhb1*) is the most utilized source of FHB resistance in wheat-breeding programs, but very little is known about its resistance mechanism. A quantitative proteomic study using 2D-DIGE and MALDI-TOF/TOF technologies was carried out to understand the prospective resistance mechanism brought by this QTL. In this study, proteomic signatures of *Qfhb1* in a pair of wheat near-isogenic lines (NIL) contrasting *Qfhb1* were elucidated after 24 hours of infection of wheat florets by *Fusarium graminearum*.

Statistical comparisons of the abundances of protein spots on the 2D-DIGE gels of the NILs enabled us to select 80 high-ranking differentially accumulated protein (DAP) spots. An additional evaluation confirmed that 44 DAPs were specific to the fhb1-NIL (44 spots), and seven DAPs to the fhb1+NIL. The proteomic data also suggest that the absence of *Qfhb1* makes the fhb1-NIL vulnerable to *Fusarium* attack by constitutively impairing several mechanisms including sucrose homeostasis by enhancing starch synthesis from sucrose. In the absence of *Qfhb1*, *Fusarium* infection severely damaged photosynthetic machinery, altered the metabolism of carbohydrates, nitrogen and phenylpropanoids, disrupted the balance of proton gradients across relevant membranes, disturbed the homeostasis of many important signaling molecules induced the mobility of cellular repair; and reduced translational activities. These changes in the fhb1-NIL led to strong defense responses centered on the hypersensitive response (HSR), resulting in infected cells suicide and the consequent initiation of FHB development. Therefore, the results of this study suggest that *Qfhb1* largely functions to either alleviate HSR or to manipulate the host cells to not respond to *Fusarium* infection.

PATHOGEN BIOLOGY AND GENETICS

SHARING MUTANTS AND EXPERIMENTAL INFORMATION PREPUBLICATION USING FGMUTANTDB

Thomas Baldwin and Phil Bregitzer*

[1]USDA-ARS Small Grains and Potato Germplasm Research, Aberdeen, ID 83210
*Corresponding Author: PH: 208-397-4162; Email: Phil.Bregitzer@usda.gov

ABSTRACT

FgMutantDb (https://scabusa.org/FgMutantDb) was created as a community-driven and community-curated web accessible resource that offers easy to navigate records on the location of mutants and experimental data generated by researchers studying *Fusarium graminearum.* Prior to the advent of this resource, an informal spread sheet was passed around between investigators to compile information on mutants and their location within the global *F. graminearum* research community. Development of a web-based resource for access to information on mutants created and their phenotypes was identified as a desired output on the USWBSI - PBG committee as far back as 2007. This initiative started with Dr. Jin-Rong Xu by accumulating mutants on an excel sheet and we further developed it into simple web-based accessible spreadsheet database for storing phenotypes, unpublished information, and communicating the current locations of mutants. Additionally, the FgMutantDb cross-references different *Fusarium graminearum* assemblies, links out to and shares data with *F. graminearum* genomic resources. To date, 1,248 comments were uploaded to FungiDB sharing information on mutants and publications via FgMutantDb. The database is accessed weekly and internationally from the UK, France, Germany, Australia, and other countries. FgMutantDb aids researchers by promoting the sharing of information and material for *F. graminearum* mutant strains. A new web-interface for FgMutantDb was developed for a better experience of accessing data and will be implemented in early 2019.

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FIELD PATHOGENOMICS OF FUSARIUM HEAD BLIGHT REVEALS PATHOGEN TRANSCRIPTOME DIFFERENCES DUE TO HOST RESISTANCE

Leigh Ann Fall[1], Melissa M. Salazar[1], Jenny Drnevich[2], Jessica R. Holmes[2], Meng-Chun Tseng[2], Frederic L. Kolb[1] and Santiago X. Mideros[1*]

[1]University of Illinois at Urbana-Champaign, Department of Crop Sciences, 1102 S. Goodwin Ave., Urbana, IL 61801; and [2]High Performance Biological Computing and the Roy J. Carver Biotechnology Center, University of Illinois, 1206 W. Gregory Dr., Urbana, IL 61801
[*]Corresponding Author: PH: 217-265-6340; Email: smideros@illinois.edu

ABSTRACT

Fusarium head blight (FHB) is caused by a diverse group of *Fusarium* species. *Fusarium graminearum*, the most common, is also diverse with distinct isolates varying on their levels of aggressiveness. The variability of the pathogen suggests that distinct strains of the pathogen population could be better positioned to cause disease on specific wheat genotypes. In addition, the different levels of host resistance could exert a selection pressure on the pathogen population. In this study, we used field pathogenomics to investigate gene expression and population structure of isolates collected from natural infection of wheat lines with varying resistance levels (susceptible, intermediate, and resistant). Differential gene expression was found among isolates collected from different host genotypes. Candidate gene sets were identified for both *F. graminearum* infection of specific host genotypes and general infection of wheat. Population structure of isolates from different resistance level sources was the same, with all isolates belonging to the NA1 population. The development of precision disease management techniques to control FHB requires improved methods of surveillance as well as a better understanding of pathogen aggressiveness.

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FUSARIUM GRAMINEARUM POPULATIONS AT THE INTERSECTION OF WHEAT AND WILD GRASS COMMUNITIES IN NEW YORK

Michael R. Fulcher*, James B. Winans, Menchus Quan and Gary C. Bergstrom

Cornell University, School of Integrative Plant Science, Plant Pathology and Plant-Microbe Biology Section, Ithaca, NY 14853
*Corresponding Author: Phone: 703-577-7293; Email: mrf236@cornell.edu

ABSSTRACT

Wild grasses are known to harbor *Fusarium graminearum* asymptomatically, and their overwintered stems support ascospore production that could contribute to head blight epidemics in wheat and barley. These gramineous reservoirs may contain diverse pathogen genotypes and serve as sites for sexual recombination, but there is no information about the population structure of isolates recovered from cultivated and non-cultivated hosts. We compared the genotypic diversity of isolates collected from agricultural and non-agricultural field sites in two regions of New York, central and northeastern, known to contain different ratios of *F. graminearum* chemotypes. Isolates of the pathogen recovered from wheat spikes, wild grass spikes, and overwintered grass debris were genotyped at eight microsatellite loci and the TRI12 locus associated with chemotype (n = 500). Individuals with a 3-ADON genotype were then compared at the TRI1 locus predictive of NX-2 production. Chemotype proportions were comparable across hosts, and, as found previously in wheat and corn stubble, the 3-ADON and NX-2 chemotypes occur at significantly higher frequencies in northeastern New York. Based on preliminary results, genotypic diversity measured using microsatellites is high across all hosts and field sites, whether agricultural or natural. No evidence of genetic differentiation was seen between wheat and grass derived isolates in central New York, but work to characterize the population in northeastern New York is ongoing. Finally, isolates collected from wheat and wild grass (n = 12 per host source) at a single site in northeastern New York were compared across six measures of fitness, including virulence on wheat. Wheat-derived isolates produced a greater number of conidia and had longer conidia ($P \leq 0.036$). No other phenotypic differences were observed. These results suggest that *F. graminearum* inhabiting wild grass is capable of causing disease on wheat and that this generalist pathogen's population is not structured by host species.

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SPECIES COMPOSITION, TOXIGENIC POTENTIAL AND AGGRESSIVENESS OF FUSARIUM ISOLATES CAUSING HEAD BLIGHT OF BARLEY IN URUGUAY

Gabriela Garmendia[1], Lucia Pattarino[1], Camila Negrin[2], Adalgisa Martinez-Silveira[1], Silvia A. Pereyra[2*], Todd J. Ward[3] and Silvana Vero[1]

[1]Universidad de la República, Facultad de Química, Montevideo, Uruguay; [2]National Institute for Agricultural Research (INIA), La Estanzuela, Colonia, Uruguay; and [3]United States Department of Agriculture, Agricultural Research Service, Peoria, IL, USA
[*]Corresponding Author: PH: 59 845748000; Email: spereyra@inia.org.uy

ABSTRACT

Fusarium Head Blight (FHB) represents one of the major constraints for barley production in Uruguay and significantly decreases grain yield and quality. FHB is also a major food safety concern because causal agents contaminate grains with trichothecenes and other mycotoxins. DNA sequence-based analyses and in-vitro toxin assessments were used to characterize species and trichothecene chemotype composition of FHB pathogens on barley grains in Uruguay. *Fusarium graminearum* was the dominant species (89.7%), and three other members of the *F. graminearum* species complex (FGSC) were identified as FHB pathogens of barley in Uruguay for the first time. Other minor FHB species included *F. poae, F. avenaceum, F. pseudograminearum* and an unnamed species of the *F. incarnatum-equiseti* species complex (FIESC). Most isolates (89.7%) had the 15-acetyldeoxynivalenol (15-ADON) trichothecene type. Yet, results from this study expanded the known area of occurrence within Uruguay for the nivalenol (NIV) toxin type, which was observed among isolates from three species of the FGSC, *F. pseudograminearum*, and *F. poae*. Isolates with the 3-acetyldeoxynivalenol (3-ADON) or NX-2 toxin types were not observed, although a previously published multilocus genotyping assay was updated to identify NX-2 strains. Analyses of population structure and comparisons with FHB isolates from wheat in Uruguay indicated that *F. graminearum* constitutes a single genetic population with no evidence of population differentiation related to the sampled hosts. Inter and intraspecific differences were observed in aggressiveness toward four barley genotypes with different levels of resistance to FHB, and in general nivalenol producers were the least aggressive isolates. Sensitivity to metconazole was approximately 10 times higher than the one detected for tebuconazole. This is the first report regarding tebuconazole and metconazole sensitivity for Fusarium species causing FHB in barley in Uruguay and establishes an important starting point for monitoring temporal or spatial changes in FGSC sensitivity, which is critical to define FHB management practices.

FUSARIUM GRAMINEARUM EFFECTORS SUPPRESS PLANT IMMUNITY

Guixia Hao*, Martha M. Vaughan and Susan McCormick

Mycotoxin Prevention and Applied Microbiology Research Unit, NCAUR, USDA-ARS, Peoria, IL
*Corresponding Author: PH: 309-681-6520; Email: Guixia.hao@ars.usda.gov

ABSTRACT

Fusarium head blight (FHB) caused by *Fusarium graminearum* is one of the most devastating wheat and barley diseases worldwide. The disease causes significant yield loss and grain contamination with harmful trichothecenes. Pathogens frequently produce effectors that can suppress plant immunity and promote disease. *F. graminearum* is predicted to secrete hundreds of effectors, which may play a critical role during interactions with host plants. However, only a few *F. graminearum* effectors have been functionally characterized. In the current study, we selected 30 effector candidates and evaluated their expression during wheat head infection. Following whole head inoculation, tissue was collected and the expression of candidate effector genes was evaluated at multiple time points. Quantitative PCR revealed that four of the effectors were upregulated immediately after inoculation. Three effectors were induced at 36 h and reached the peak at 3 or 5 d after inoculation. The expression of FGSG_12160 was induced over 2,400-fold at 3 d after inoculation, sharing a profile similar to that of *TRI5* (trichodiene synthase). Additionally, transient expression assays in *Nicotiana benthamiana* showed that several highly induced effectors suppressed reactive oxygen species production which is typically part of the plant defense response. Our results suggest that these highly induced effectors play a role in suppressing plant immunity which in turn promotes pathogenesis. Further investigations via mutagenesis are currently underway to determine the role of these effectors in FHB development.

HARNESSING ANTIVIRAL DEFENSE REACTIONS TO DEFEAT FUSARIUM HEAD BLIGHT

Cornelia Heinze[1], Christine Blum[1], Michael Mentges[1], Anke Brockmann[1], Arne Alder[1], Svenja-Kim Landt[1], Brian Josephson[2], Daniela Indenbirken[3], Michael Spohn[3], Birte Plitzko[4], Sandra Loesgen[4], Michael Freitag[2], Wilhelm Schäfer[3] and Jörg Bormann[1,2*]

[1]University of Hamburg, Institute of Plant Science and Microbiology, Department of Molecular Phytopathology, Hamburg, Germany; [2]Department of Biochemistry and Biophysics, Corvallis, Oregon State University, Oregon, USA; [3]Heinrich Pette Institute, Leibniz Institute for Experimental Virology, Hamburg, Germany; and [4]Department of Chemistry, Oregon State University, Corvallis, Oregon, USA
[*]Corresponding Author: PH: 49-421-218-62880; Email: joerg.bormann@uni-bremen.de; ORCID: https://orcid.org/0000-0002-3493-2450

ABSTRACT

Mycoviruses are ubiquitously found in all phyla of the true fungi. Very often, mycovirus infections remain symptomless, but some infections suppress asexual and sexual propagation, mycelial growth, and most notably, virulence, a phenomenon known as "hypovirulence". Hypovirulence-inducing mycoviruses, therefore, represent a powerful mean to defeat fungal epidemics on crop plants but the poor understanding of the molecular basis of disease symptom development after mycovirus infections limits their application. Infection with FgV-ch9, a dsRNA chrysovirus-like mycovirus, debilitates *Fusarium graminearum*. A mutant which shares a high degree of similarity to a FgV-ch9 infection with respect to growth, reproduction, and virulence was identified. In this mutant, a gene coding for an mRNA-binding protein, named virus response 1 (*vr1*), was deleted. Gene expression analysis revealed a drastic downregulation of *vr1* in the presence of virus and in mutants expressing the viral structural protein coded on segment 3 (P3), suggesting a causal link between virus-induced symptom development and *vr1* gene expression. In turn, P3 expression causes virus-infection like symptoms. Mutants with constitutive expression of *vr1* display asymptomatic growth and virulence, despite harboring FgV-ch9 or expressing P3. The results show that *vr1* represents a fundamental host factor for the expression of virus-related symptoms and helps to understand the underlying mechanisms of hypovirulence. Ultimately, the *vr1*-mediated debilitation of the fungus limits virus spread. Therefore, facilitating augmented horizontal FgV-ch9 transmission in infected virus donor strains or exploiting the potential of the symptom-inducing coat protein "P3" to debilitate *F. graminearum* may point the way to novel biocontrol strategies.

IDENTIFICATION OF MARKERS ASSOCIATED WITH INCREASED LEVELS OF AGGRESSIVENESS AND TOXIGENICITY IN TRANSGRESSIVE PROGENY FROM A CROSS OF TWO GENETICALLY AND PHENOTYPICALLY SIMILAR *FUSARIUM GRAMINEARUM* STRAINS

Franklin Machado[1+], Sladana Bec[1,4+], Mark Farman[1], Scott Schwartz[2,5], Richard Metz[2], Charles Johnson[2], Emerson Del Ponte[3] and Lisa Vaillancourt[1*]

[1]Department of Plant Pathology, University of Kentucky, Lexington KY 40546; [2]Texas A&M AgriLife, Genomics & Bioinformatics Services, College Station, TX 77845; [3]Departamento de Fitopatologia, Universidade de Viçosa, Viçosa MG Brasil; [4]UF/IFAS Plant Diagnostic Center, University of Florida, Gainesville FL 32603; and [5]Department of Integrative Biology, University of Texas, Austin TX 78712. [+]First two authors contributed equally.
[*]Corresponding Author: PH: 859-218-0731; Email: vaillan@uky.edu

ABSTRACT

Fusarium graminearum is a homothallic ascomycete that causes Fusarium Head Blight (FHB) of wheat and small grains, resulting in yield and quality losses. Harvested grain is frequently contaminated with trichothecene mycotoxins that are harmful for human and animal health. Co-infection by multiple fungal strains could result in out-crossing, which can produce transgressive progeny that are more aggressive and toxigenic than either parent. Two genetically well-characterized laboratory strains of *F. graminearum*, PH-1 (NRRL 31084) and Gz3639 (NRRL 29169), were used in this study. PH-1 grew faster than Gz3639 on carboxymethylcellulose medium (CMC), potato dextrose agar (PDA), and mung bean agar (MBA). PH-1 produced more macroconidia on CMC, whereas Gz3639 produced more on PDA, and both strains produced similar numbers of conidia on MBA. PH-1 produced more perithecia and ascospores on carrot agar than Gz3639. Both strains were equally aggressive on the susceptible soft red winter wheat (SRWW) variety Pioneer 2555. However, Gz3639 was more aggressive on the more resistant SRWW varieties 'Pioneer 25R18' and 'Truman'. Gz3639 produced more DON than PH-1 *in planta* on 'Pioneer 2555', and *in vitro* on rice and in liquid media. PH-1 and a green-fluorescent transformant strain of Gz3639 were crossed using the mycelial plug method. After about 3 weeks, mature perithecia with green-fluorescent cirrhi were recovered from the PH-1 side of the plate. A total of 95 single-ascospore progeny were recovered from four different perithecia (23-24 from each). The strains were checked by using molecular markers to confirm that they were the product of outcrossing. Segregation and recombination of two unlinked molecular markers occurred in the expected ratios. The progeny were quite variable in appearance on PDA. Some strains were much more fertile than the parents, and some produced up to ten-fold more macroconidia. The strains displayed a broad range of aggressiveness on Pioneer 2555. Four transgressive progeny were significantly more aggressive than either parent, and one was significantly less aggressive. Two of the most aggressive strains, and two of the least aggressive were chosen for further characterization. Analysis of DON levels in blighted heads revealed that the most aggressive strains produced amounts of DON that were up to 50-fold greater 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) of

chromosome 2 seemed to be tightly linked to aggressiveness. This region contains approximately 40 genes, but it does not include the trichothecene metabolite cluster.

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MUTATIONS IN GENES FOR *FUSARIUM* TRANSPORTERS RESULT IN REDUCED DON ACCUMULATION AND VIRULENCE

Sean O'Mara[1], Yanhong Dong[1] and H. Corby Kistler[2*]

[1]University of Minnesota, St. Paul, MN 55108; and [2]USDA-ARS Cereal Disease Laboratory, St. Paul, MN 55108
[*]Corresponding Author: PH: 612-625-9774; Email: corby.kistler@ars.usda.gov

ABSTRACT

Fusarium head blight pathogens produce trichothecene mycotoxins such as deoxynivalenol (DON) that are critical for determining the outcome of plant disease interactions. While much progress has been made in understanding the enzymatic pathways for DON biosynthesis, little is known about how toxins are exported from fungal cells and delivered to the host. The specific goal of our research is to examine several candidate multidrug transporters in *Fusarium graminearum* for their potential role in trichothecene export and fungal virulence. We have identified four co-regulated multidrug resistance transporters that, along with the trichothecene efflux pump Tri12, may be essential for maximum trichothecene export. Each of the five genes have been individually deleted and the mutant alleles have been combined by sexual recombination to create strains with deletions in combinations of two and three per strain. These genotypes have been tested for their ability to accumulate DON *in vitro* and *in planta* and for their effect on fungal virulence. The Δtri12 deletion mutant shows reduced DON accumulation *in vitro* and *in planta*, but no significant reduction in virulence. The Δabc1 deletion mutant is reduced in virulence and DON accumulation *in planta*, and the Δabc6 deletion mutant is reduced in DON accumulation *in planta.* Certain combinations of two transporter mutations reduce virulence more than the individual mutations separately. Transporter genes are being expressed in yeast to determine their ability to allow for DON resistance when expressed in this heterologous host. This information is potentially useful because trichothecene exporters that deliver toxin to the plant when expressed in Fusarium, may allow for resistance to DON if expressed in plants. Our ultimate goal is to develop transgenic wheat and barley lines with increased trichothecene tolerance achieved by expression of Fusarium proteins conferring resistance to DON. This transgenic approach may represent a novel strategy by which small grain crops may escape the toxic effects of pathogen-produced small molecules.

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GENETICS OF DIFFERENTIAL DEFENSE RESPONSES TO *FUSARIUM GRAMINEARUM*

Rebecca Shay[1] and Frances Trail[1,2*]

[1]Department of Plant Biology and [2]Department of Plant, Soil, and Microbial Sciences, Michigan State University, East Lansing, MI 48824
[*]Corresponding Author: PH: 517-432-2939; Email: trail@msu.edu

ABSTRACT

Understanding the interactions between plant pathogenic fungi and host plants is vital for the management of disease symptoms. *Fusarium graminearum* is a fungal plant pathogen and the primary causal agent of Fusarium Head Blight (FHB) on wheat and barley, which reduces quality and quantity of grain yields. FHB is partially controlled by fungicides, with no strong resistance available in host crops. Because of the inefficiency of fungicides in controlling some plant diseases, new methods for disease prevention and treatment need to be established. Plant penetration and colonization are two important stages of infection, which is where we focused our study. Barley has a moderate resistance response where *F. graminearum* cannot spread from individual infection sites. The plant produces a focal accumulation of plant defense compounds (cellulose and lignin) at the infection sites of trichomes on the surface of barley florets in response to *F. graminearum* inoculation. Previous work has shown greater numbers of focal accumulations (foci) on barley varieties with small, dome trichomes than varieties with long, prickle-like trichomes. The genetic basis of the differential response seen in trichome morphologies is being investigated through the use of near-isogenic barley varieties. *In vitro* and *in planta* assays characterize important plant and fungal responses during infection stages leading to disease development. A locus has been identified in barley as important for the defense response to *F. graminearum*. Light and confocal laser scanning microscopies are used to visualize these interactions. Genes of interest have been identified as important for *F. graminearum* – barley interactions. The molecular interactions studied in this work provide new areas to develop disease management tools.

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EFFECT OF SILICA ON THE INTERACTIONS OF *FUSARIUM GRAMINEARUM* WITH BARLEY

Ben Smith[1] and Frances Trail[1,2*]

[1]Department of Plant Biology and [2]Department of Plant, Soil, and Microbial Sciences, Michigan State University, East Lansing, MI
[*]Corresponding Author: PH: 517-432-2939; E-mail: trail@msu.edu

ABSTRACT

We are investigating the role that silicon (Si) and its derivatives play in host interactions with *Fusarium graminearum.* We have previously identified two important stages of the life cycle that are influenced by the presence of specific host cells that accumulate silica: the "silica cells" associated with nodes and the stomates of internodes and florets support conidial production and perithecium development; and the trichomes support fungal penetration. Our objectives are to (1) test the effect of silica on the growth of *F. graminearum* in culture, (2) compare methods to grow barley in low silica conditions and (3) test the influence of Si levels in barley florets on the pathogenicity and perithecium development of *F. graminearum.* This project is designed to determine how Si influences the infection of hosts by *F. graminearum* and sporulation in association with silica cells on crop residues.

We found that silica amended medium increases production of the mycotoxin aurofusarin, suggesting a relationship between mycotoxins and silica. Perithecium production increased in low silica plants. Physical changes were apparent in low-silica plants, reflecting the importance of silica as a structural element in the plant. In summary, there appears to be a balance of positive and negative effects of silica on the plant that affect scab development.

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FIRST REPORT OF *FUSARIUM BOOTHII* CAUSING HEAD BLIGHT OF WHEAT IN THE UNITED STATES

Esteban Valverde-Bogantes[2], Stephen N. Wegulo[1*], Carlos Bolanos-Carriel[1], Heather Hallen-Adams[2], Andreia Bianchini[2], Nicole McMaster[3] and David G. Schmale III[3]

[1]Department of Plant Pathology and [2]Department of Food Science and Technology, University of Nebraska–Lincoln, Lincoln, NE; and [3]Department of Plant Pathology, Physiology, and Weed Science, Virginia Tech, Blacksburg, VA
[*]Corresponding Author: PH: 402-472-8735; Email: swegulo2@unl.edu

ABSTRACT

Fusarium head blight (FHB) occurs sporadically in Nebraska due to a variable climate. Severe epidemics affected the state in 2015 causing 30% to 50% yield loss in grain production fields and up to 100% loss in some seed production fields. As part of a larger survey study, symptomatic wheat heads were collected in 2015 from 23 different locations in Nebraska. Surface-sterilized kernels were plated on Nash and Snyder media for the isolation of *Fusarium* spp. After incubation, typical *Fusarium* colonies were single spored and transferred to PDA plates. DNA was extracted from the isolates using the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. For species identification, a portion of the translation elongation factor 1-α (*EF-1α*) gene was amplified and sequenced from both directions by Sanger sequencing. The sequences were analyzed and assembled in MEGA7, and BLAST queries were performed in Fusarium-ID and Fusarium MLST. Three isolates, from Chase County in the southwest (isolates NE16-15Fb and NE19-15Fb) and Box Butte County (isolate NE21-15Fb) in the northwest, had 100% similarity with the reference strain *F. boothii* NRRL 29105. All the other isolates were identified as *F. graminearum*. Trichothecene genotype was determined in a multiplex PCR and all isolates had the 15-ADON genotype. In order to fulfill Koch's postulates, FHB-susceptible spring wheat cultivar Wheaton was grown in the greenhouse. Spikes were spray-inoculated at anthesis with approximately 1×10^5 spores/mL of *F. boothii* isolates NE16-15Fb and NE19-15Fb and a single isolate of *F. graminearum* (NE20-15Fg) collected in the same survey in Kimball County in the southwest, or not inoculated. The inoculated spikes were covered with transparent bags for 72 h to maintain high humidity and favor infection. A randomized complete block design with five or six replications was used in two replicate experiments. FHB symptoms were observed 3 days after inoculation (dai) in all three isolate treatments. At 21 dai, FHB severity (percentage of symptomatic spikelets on a spike) ranged from 18 to 87%. No symptoms developed in the check treatment. Deoxynivalenol (DON) and its acetylated derivatives were quantified in the grain using gas chromatography/mass spectrometry. In agreement with their trichothecene genotype, all three isolates had the 15-ADON chemotype. Single-spored cultures of the three isolates were recovered on PDA from symptomatic kernels obtained from both experiments and re-sequenced to confirm species identity. The three isolates produced fertile perithecia on carrot agar after incubation at room temperature for 7 weeks. To our knowledge, this is the first report of *F. boothii* causing head blight of wheat in the United States. Additional studies are underway to determine the aggressiveness of these *F. boothii* isolates compared to *F. graminearum* isolates.

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FUSARIUM GRAMINEARUM POPULATION-SPECIFIC DIFFERENCES DURING WHEAT INFECTION

Martha M. Vaughan[1†*], Todd Ward[1†], Miroslava Cuperlovic-Culf[2], Susan P. McCormick[1] and Matthew G. Bakker[1]

[1]Mycotoxin Prevention and Applied Microbiology Research Unit, National Center for Agricultural Utilization Research, USDA ARS, Peoria, IL, USA; and [2]National Research Council Canada, Ottawa, Canada [†]Contributed equally to this research
[*]Corresponding Author: PH: 309-681-6295; Email: Martha.Vaughan@ars.usda.gov

ABSTRACT

Fusarium graminearum (*Fg*) is the primary fungal pathogen responsible for Fusarium head blight (FHB), a devastating disease of wheat and barley worldwide. FHB reduces crop yield and contaminates grain with trichothecene mycotoxins that are harmful to plant, human and animal health. Three genetically distinct populations of *Fg*, each associated with a different trichothecene chemotype, have been identified in North America (NA1, NA2, and NA3). To determine how this population-level diversity influences pathogenesis and mycotoxin contamination, we inoculated moderately resistant hard red spring wheat variety 'Alsen' with 15 representative strains from each NA population and evaluated disease progression and mycotoxin accumulation. Additionally, we evaluated *Fg* population-specific differences in induced host defense responses.

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VARIETY DEVELOPMENT AND HOST PLANT RESISTANCE

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

R.K. Acharya[1*], J.M. Sarinelli[1], J.H. Lyerly[1], P. Tyagi[1], G. Brown-Guedira[2] and J.P. Murphy[1]

[1]Department of Crop and Soil Sciences, Box 7629, North Carolina State University, Raleigh, NC 27695; and [2]USDA-ARS, Box 7620, North Carolina State University, Raleigh, NC 27695
[*]Corresponding Author: PH: 919-869-0401; Email: rkachary@ncsu.edu

ABSTRACT

The Uniform Southern Soft Red Winter Wheat Scab Nursery is evaluated annually in up to nine locations to determine the resistance of advanced generation breeding lines relative to the check cultivars. Disease development varies across locations and years, so selection of environments for inclusion in training populations for Genomic Selection is necessary. An environment was defined as a single location within a year. The objectives of this research were to compare five methods of choosing environments for inclusion in Training Populations and evaluate the impact on the prediction accuracy of scab resistance traits. The methods were: 1) all environments included, 2) selection using PCA (i.e. PCA1 and PCA2) based pairwise correlation between environments, 3) correlated environment selection (r>0.90) after year-wise environment discrimination in GGEbiplot using all entries, 4) correlated (r>0.90) environment selection after environment discrimination using checks in GGEbiplot, and 5) environment selection based on relative differences of checks where mean *Fusarium* Damaged Kernels (FDK) and DON were greater than 15% and 6 ppm respectively and the susceptible check Coker 9835 was greater than or equal to 1.5 times the disease score of the resistant checks Ernie, Bess and Jamestown for both FDK and DON. For FHB Severity in *method 5*, environments having Severity>20% were selected. The check varieties ranked as Coker9835>>Ernie=Jamestown>Bess in the susceptibility-resistance continuum of Severity, FDK and DON. A total of 52, 32 and 26 environments with 2922, 1862, 1440 datapoints were available for Severity, FDK and DON TP development respectively. After environment selection, genotype least square means were calculated for each TP using mixed model in R studio. The five methods were compared based on their prediction accuracy and correlation obtained from cross-validation (5 folds, 50 cycles) analysis using 'rrblup' package in R. 49,441 SNPs were utilized after running the GBS pipeline, SNP filtering and missing marker imputation. The *Method 5* selected TP accuracies were slightly higher (+3, +1 and +2 units for Severity, FDK and DON) than other methods. Therefore, Method Five selected TP was used to study the impact of number of environments per year on prediction accuracy. There was a gradual increase in prediction accuracy as more environments per year were included in the TP. Despite few environments per year (i.e. 5.7, 4.9 and 2.7 for Severity, FDK and DON), the prediction accuracy was as high as 0.77, 0.72, 0.72 respectively when all the selected environments were used. Scab traits may need at least 3 good environments per year to achieve the minimum prediction accuracy of 0.66 for all traits.

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OPTIMIZING TRAINING POPULATION SIZE TO IMPROVE PREDICTION ACCURACY OF FUSARIUM HEAD BLIGHT TRAITS IN WHEAT

Emmanuel Adeyemo*, Prabin Bajgain and James Anderson

Department of Agronomy and Plant Genetics, University of Minnesota, St Paul, MN 55108
*Corresponding Author: PH: 612-625-6794; Email: adeye017@umn.edu

ABSTRACT

The choice of training population (TP) is an important criterion in genomic selection. In this study, 200 F_5 wheat lines were selected based on a k-means clustering method, partitioned into three groups (K-1, K-2, and K-3), to represent a portion of the TP. An additional 300 F_5 lines selected based on pedigree, and 45 parental lines were also included to make a TP with a total size of 545 lines. Our objective was to assess the prediction accuracies in each subset using cross validation method in RR-BLUP model for four traits associated with Fusarium Head Blight. The TP was evaluated for disease incidence (INC), disease severity (SEV), visually scabby kernels (VSK) and micro-test weight (MTWT) in St Paul, Minnesota in 2018. Prediction accuracies (r_{MP}) ranged from 0.12 in INC to 0.39 in MTWT when cross-validation was performed on the entire TP. Among the three groups, r_{MP} was the highest when cross-validation was performed on the K-2 subset including parents (n = 91) with values ranging from 0.25 for SEV to 0.60 for MTWT. We are looking into some of the genetic characteristics of K-2 that might have contributed to its high r_{MP} values as we speculate that choosing a TP with similar genetic makeup as K-2 will improve the efficiency of genomic selection in our wheat breeding program.

FHB SUSCEPTIBILITY OF SPRING BARLEY AND WHEAT CULTIVARS IN THE INTERMOUNTAIN WEST

Suzette S. Arcibal[1], Thomas T. Baldwin[2], Phil P. Bregitzer[2] and Juliet M. Marshall[3*]

[1]University of Idaho, Aberdeen, ID 83210; [2]USDA-ARS, Aberdeen, ID 83210; and [3]University of Idaho, Idaho Falls, ID 83402
[*]Corresponding Author: PH: 208-529-8376; Email: jmarshall@uidaho.edu

ABSTRACT

The majority of the wheat and barley varieties grown in the Intermountain West were not bred for resistance to Fusarium head blight (FHB). For the past several years, FHB has become a regularly occurring disease due to the increase in corn acreage and changes in irrigation methods and crop rotation. Therefore, resistance levels of widely grown varieties and advanced lines must be determined to provide the best management recommendations under arid, irrigated production systems. Since the establishment of FHB screening nurseries at the University of Idaho in 2014, hundreds of Pacific Northwest wheat (hard red, hard white, soft white and durum) and barley (two-rowed feed, two-rowed malt and six-rowed) entries have been evaluated for FHB susceptibility. For all growing seasons, small plots consisting of two rows were planted in blocks and replicated twice. Since 2015, planting dates were delayed to late April or early May to increase disease pressure. Wobbler sprinklers were installed for irrigation and corn inoculum was spread in the field within 5 to 6 weeks after planting, to ensure disease development. Barley varieties were supplemented with conidial inoculum applied at head emergence and 7 days thereafter. FHB index (IND) values were estimated by arbitrarily selecting 20 spikes per plot for head severity ratings at soft dough. At least 100 grams of grain per variety was sent to University of Minnesota for deoxynivalenol (DON) testing. Wheat samples were also rated for *Fusarium* damaged kernels (FDK). Data were analyzed using the GLIMMIX procedure in SAS 9.4. At least 40 barley and 29 wheat entries had data to report as three-year averages (2015 to 2017). In barley, there were 11 entries (25%) with mean IND values over 5% and only 6 varieties (10%) with mean DON accumulation above 5 ppm. In wheat, there was a wide range of IND values from undetectable to over 50%. Although all wheat entries had lower than 10% FDK, all but one (HRS3419) have varying levels of susceptibility. There were 22 entries (73%) with mean DON accumulation above 5 ppm, wherein 9 entries had over 10 ppm. Through the years, we incorporated additional site-specific methods such as adding fine mist sprinkler systems and increasing conidial inoculum concentrations for optimum FHB development. Identifying valuable disease parameters and weather variables are necessary to better understand FHB risk and develop forecasting tools specific to Idaho and neighboring states.

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CORRELATION BETWEEN GENOMIC ESTIMATED BREEDING VALUES AND OBSERVED PHENOTYPIC VALUES IN WHEAT BREEDING

M. Nelly Arguello-Blanco, Dan Borrenpohl, Mao Huang and Clay Sneller*

The Ohio State University, 1680 Madison Avenue, Wooster, OH 44691
*Corresponding Author: PH: 330-263-3944; E-mail: sneller.5@osu.edu

ABSTRACT

The use of genomic selection (GS) in breeding programs is becoming a routine. GS estimates the value (called the genomic estimated breeding values (GEBVs) of genotyped individuals that have not been phenotyped by using data from a set of relatives that have been phenotyped and genotyped (called the training population (TP)). A cycle of GS can be completed very quickly as phenotyping is not required to select the best parents to initiate a new breeding cycle. The OSU wheat program has conducted five cycles for GS primarily for yield, but also for FHB resistance initiated from one TP (YLDTP), and three cycles of GS primarily for FHB resistance initiated from a second TP (FHBTP). The objective of this research is to assess the ability of GS to predict the FHB phenotype of lines derived from cycles of GS executed in each TP. The FHB resistance of the YLDTP was assessed for two years in Wooster Ohio in an inoculated nursery while the FHBTP was phenotyped for three years in multiple states. Varying number of F2-derived progeny from cycles of GS from each TP were assessed for FHB index in inoculated nurseries from 2015 to 2018. The cross-validation accuracy of FHB index within the YLDTP was 0.39 and was 0.49 in the FHBTP. The GS model trained on the YLDTP was used to calculate GEBVs for F2 derived families from all cycles derived from the YLDTP. Currently only lines from cycles 1, 2, and 3 have been phenotyped. This was done in 2015, 2016, 217 and 2018. The correlation of the phenotype of these F2-derived families with their GEBVs was 0.35 in 2015, 0.42 in 2016, 0.61 in 2017, and 0.28 in 2018. Data from phenotyping F2-derived lines from the cycles of GS from the FHBTP will be presented. In conclusion, rapid cycling GS seemed to produce GEBV for F2 derived families would be useful in selecting for improved resistance to FHB.

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FUSARIUM BIOMASS MEASUREMENTS EVALUATED AS A SELECTION TOOL

Thomas Baldwin[1], Suzette Arcibal[2], Juliet Marshall[3] and Phil Bregitzer[1*]

[1]USDA-ARS Small Grains and Potato Germplasm Research, Aberdeen, ID 83210; [2]University of Idaho, Aberdeen, ID 83210; and [3]University of Idaho, Idaho Falls, ID 83402
[*]Corresponding Author: PH: 208-397-4162; Email: Phil.Bregitzer@usda.gov

OBJECTIVE

To investigate the relationship between visual severity rating, deoxynivalenol, and *Fusarium* biomass by qPCR.

INTRODUCTION

Selecting Fusarium head blight (FHB) resistant barley germplasm has been difficult. The lack of correlation between severity ratings or FHB index (IND) and deoxynivalenol (DON) limits breeding solely by visual scoring. Breeders are left to rely on DON measurements via mass spectrometry analyses that are expensive, time consuming, and environmentally hazardous. A simple method for quantifying *Fusarium* biomass through qPCR may allow for rapid and high throughput selection of FHB resistant barley cultivars, complimenting both IND and DON measurements.

MATERIALS AND METHODS

Sampling seeds and DNA extraction. The 2017 FHB barley screening nursery in Aberdeen, ID was sampled. Each of the barley varieties had two replications and were previously rated for IND and tested for DON. Approximately 4 g of seed from each of 128 samples (two replicates, 64 barley cultivars or breeding lines) was pulverized for 7.5 min at 1250 rpm with a Geno-grinder 2000 (SPEX, Metuchen, NJ, USA) in a 50 mL polycarbonate cryovial (SPEX, Metuchen, NJ, USA) with a ¾" chrome/steel bearing ball (Bearing Ball Store, Orlando, Fl, USA). DNA was obtained from 50 mg of pulverized seed extracted using the Plant/Fungi DNA Isolation kit (Norgen Biotek Corp., Thorold, ON, Canada).

Quantitative PCR. Fusarium graminearum biomass was estimated using primers Tri5QF (TGAGGGATGTTGGATTGAGCAGTAC) and Tri5QR (TGCTTCCGCTCATCAAACAGGT) from Bluhm et al., 2007 (MPMI 20:627-636). Relative amounts were measured using the barley DNA as a reference with actin primers Actin F2 (GTTCTCGACTCTGGTGATGG) and Actin R2 (CAAACGAAGAATGGCATGGG). 100 ng/µL of purified *F. graminearum* DNA and 100 ng/µL barley DNA (cv Golden Promise) were combined and serial diluted to generate a standard curve. Primer efficiencies were measured at E=101.3%, R^2= 1.000 for *Fusarium TRI5*, and for barley actin primers was E= 105.0, R^2= 0.989. Duplicate qPCR reactions were performed for each sample using Sso Advanced Sybr Green Supermix according to the manufacturer's protocol (BioRad, Hercules, CA USA) with 25 ng of extracted DNA and 500 nM of primer. Samples were run on a CFX Connect thermocyler (Bio-Rad). The thermal cycler program was: 95° C for 30 seconds (s), 40 cycles of a melting step at 95° C for 5 s and an annealing/extension step at 60° C for 5 s.

Data analysis. Each data set (DON, IND, and Biomass) was Log10 transformed to meet normality requirements. Pearson correlations were applied to "DON v. *Fusarium* biomass" and "DON v. IND" and the R^2 values are reported. A Spearman correlation was performed by taking a rank sum for each data set and re-applying a Pearson correlation.

RESULTS AND DISCUSSION

Input required to attain *Fusarium* biomass measurements include time for processing samples, and costs for DNA extraction and qPCR. Processing time in the Genogrinder was estimated at 1 min and 40 s per sample. Per sample costs for DNA extraction with the Norgen Biotek kits cost $3.13 and $1.73 for qPCR (including the super mix, 96 well plates, Microseal®, and pipette tips), for a total per sample cost of $4.86. This cost could be dramatically decreased by non-kit DNA extraction and developing a probe-based duplex qPCR assay. Furthermore, remnant pulverized seed can be used for DON analysis without further preparation, Subsequent to processing the seed samples reported here, the use of a Udy cyclone grinder (Udy Corporation, Fort Collins, CO) has been investigated for increased processing speed and ability to increase representative sampling from 4 g to 20 g of seed.

The correlation of DON and *Fusarium* biomass by qPCR was 0.89 (R^2 = 0.79; Fig 1). Restricting the analysis to samples with lower DON concentrations decreased the R^2 for *Fusarium* biomass but correlations to DON were higher than with IND (Table 1). These results suggest *Fusarium* biomass estimation by qPCR is a closer proxy to DON than IND, especially in the 1–10 ppm range where differentiating resistant and susceptible lines are difficult.

Most samples fall within the 1–10 ppm range, thus we suspected that the tails might have undue influence on the correlations. A Spearman's correlation was implemented to remove the influence of the tails and the "DON v. *Fusarium* biomass" R^2 was reduced to 0.60 (Fig 2) and 0.46 for "DON v. IND" (data not shown). Separating samples in this way revealed 18 lines in the resistant (R) group (R^2 = 0.73) and 30 lines in the susceptible (S) group (R^2 = 0.79) (Fig. 2). There were 16 lines that were unplaced because replicates appeared in both groups. This method of grouping was supported by the field ratings for IND, e.g. moderately resistant (MR) cultivars tended to occur below the line, and moderately susceptible (MS) cultivars tended to be above the line (data not shown). The S group had higher DON:*Fusarium* biomass ratios (above the corr. line), and vice versa for the R group (below the corr. line). Interestingly, certain cultivars with a history of susceptibility to FHB (e.g. 'Stander') had relatively high DON:biomass ratios; whereas, relatively resistant cultivars (e.g. 'Quest') had much lower ratios even when mean DON levels were similar. If biomass is indicative of infection severity, qPCR-based estimation of biomass may reveal propensity to accumulate DON (and thus susceptibility to economic damage) even when infection variability within studies results in a low infection in particular plots. Furthermore, the newly-released high beta-glucan barley cultivar 'Goldenhart' (Hu et al., unpublished), categorized as susceptible based on field IND scores, appears to have some resistance to DON accumulation.

CONCLUSION AND FUTURE PLANS

Breeding for FHB resistance from measuring DON and IND has disadvantages in terms of time and expense. Utilizing qPCR for *Fusarium* biomass measurements is fast and can be quite cheap, and might even be used to eliminate the most infected lines—presumably with very high DON—from further analysis. Combining qPCR, DON, and IND may provide a better selection tool for differentiating susceptible and resistant cultivars. Further investigation of the potential utility of qPCR estimation of fungal biomass is underway, and we plan to evaluate samples from multiple 2018 nurseries.

ACKNOWLEDGEMENTS AND DISLAIMER

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(University of Minnesota) for DON analysis of barley samples; and Dr. Bill Price (University of Idaho) for his recommendations for statistical analysis. 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 (Projects FY18-BA-005 and FY6-MA-036). 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: Log10 Comparison of R^2 values for *Fusarium* infection in grain grown at Aberdeen, 2017, estimated via qPCR vs IND

DON level (ppm)	≤5	≤10	≤20	≤40	≤80
No. of Cultivars	55	100	118	126	128
visual (IND) R^2	0.23	0.32	0.46	0.51	0.56
qPCR R^2	0.66	0.60	0.68	0.76	0.79

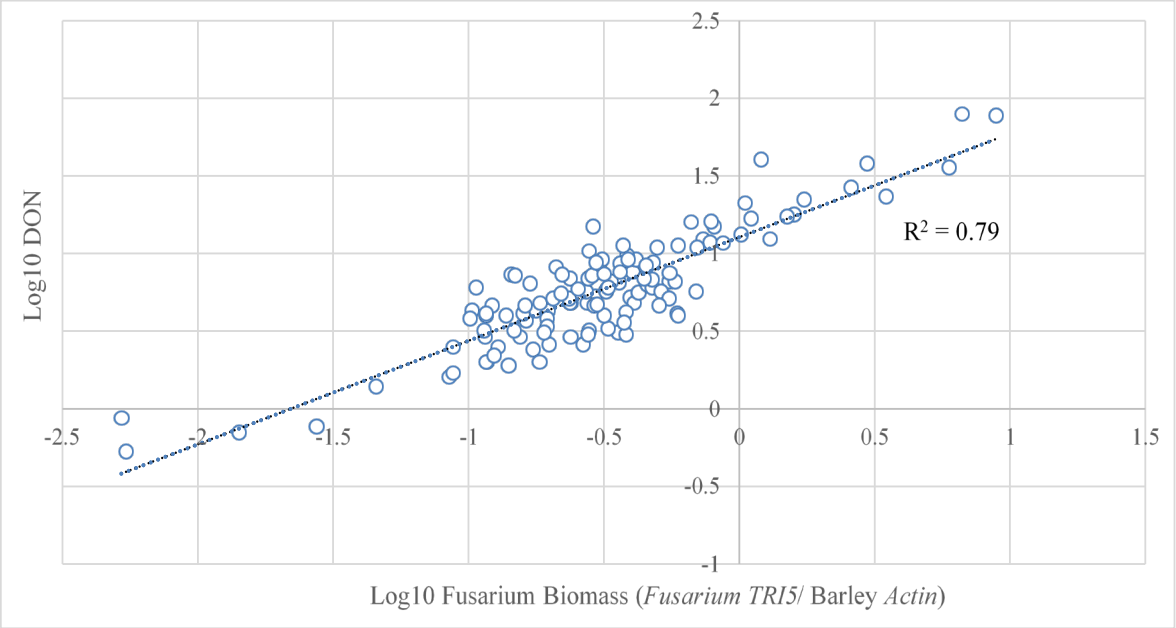

Figure 1: Correlation between DON and *Fusarium* biomass (qPCR) for the spring 2017 barley head blight nursery n=128.

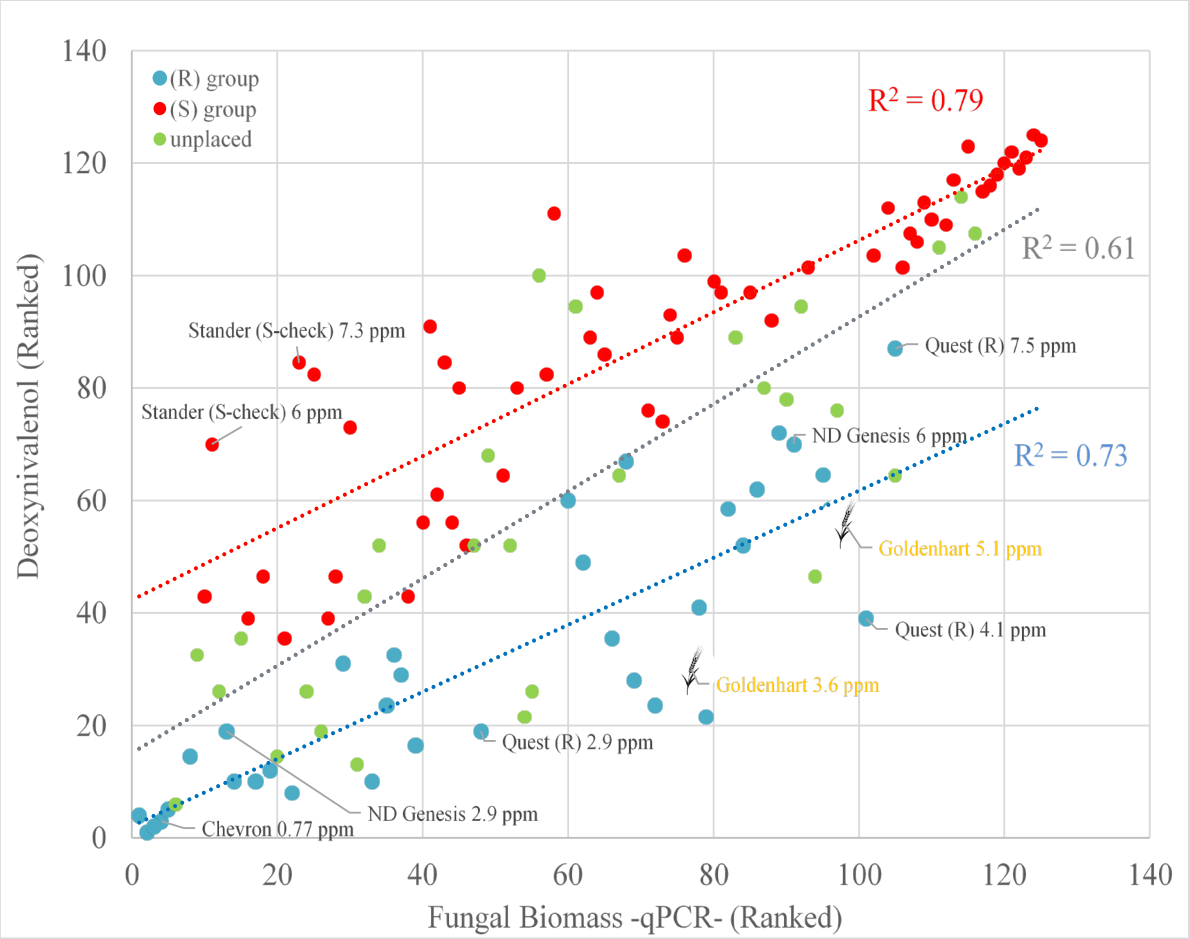

Figure 2: Spearman correlation of DON and *Fusarium* biomass (qPCR) between All sample (grey line), the susceptible group (red) (n=60), and the resistant group (blue) (n=38). The barley cultivars that could not be placed are shown in green (n=32).

DETERMINING THE OPTIMUM INOCULUM CONCENTRATION AND SPIKE BAGGING PERIOD FOR DISCRIMINATING BETWEEN FHB-SUSCEPTIBLE AND -RESISTANT WHEAT CULTIVARS UNDER GREENHOUSE CONDITIONS

Carlos Bolanos-Carriel[1], Stephen N. Wegulo[1*], Heather Hallen-Adams[2], P. Stephen Baenziger[3], Kent Eskridge[4] and Deanna Funnell-Harris[4]

[1]Department of Plant Pathology, [2]Department of Food Science and Technology, [3]Department of Agronomy and Horticulture, and [4]Department of Statistics, University of Nebraska-Lincoln, Lincoln, NE 68583; and [5]Wheat, Sorghum and Forage Research Unit, United States Department of Agriculture USDA- ARS, Lincoln, NE
[*]Corresponding Author: PH: 402-472-8735; Email: swegulo2@unl.edu

ABSTRACT

Fusarium Head blight (FHB), caused mainly by *Fusarium graminearum*, causes devastating economic losses in small grain cereal crops. In regions where FHB occurs frequently, breeding for resistance to the disease is a priority in small grain breeding programs. Screening lines or cultivars for resistance to FHB under controlled conditions is necessary due to the sporadic nature of the disease under field conditions. However, screening for resistance is challenging due to the quantitative nature of resistance to FHB and the variability in aggressiveness of pathogen isolates. Too high or too low inoculum concentration or too much humidity can lead to inaccurate results. Greenhouse experiments were conducted to determine the optimum inoculum concentration and spike bagging period following inoculation for discriminating between a susceptible and a moderately resistant spring wheat cultivar. The cultivars used were 'Samson' (susceptible) and 'Glenn' (moderately resistant). In one experiment, spikes were inoculated at anthesis with the standard spore concentration of 1 x 10^5 *F. graminearum* spores/mL and 1/2, 1/4, 1/8, and 1/16 of the standard concentration. In a second experiment, spikes were inoculated at anthesis with the standard spore concentration of 1 x 10^5 *F. graminearum* spores/mL and covered with Ziplock® bags for 12, 24, 36, 48, or 72 hours. In both experiments, FHB severity was visually assessed seven times at 3-day intervals following inoculation. The percentage of *Fusarium*-damaged kernels (FDK) and deoxynivalenol (DON) concentration were determined after harvest. FHB severity was the best variable for discriminating between the two cultivars. FHB severity results showed that 1/16 and 1/8 of the standard spore concentration discriminated between the two cultivars whereas higher concentrations did not. The best discrimination between the two cultivars was achieved by bagging spikes for either 48 or 72 hours following inoculation. The results from this study indicate that for screening wheat cultivars for resistance to FHB under greenhouse conditions, lower concentrations of *F. graminearum* spores (6.25 x 10^3 or 1.25 x 10^4 spores/mL) are better than higher concentrations, and the optimum spike bagging period following inoculation is 48 to 72 hours.

ACKNOWLEDGEMENTS AND DISCLAIMER

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IMPLEMENTING GENOMIC SELECTION IN PRELIMINARY YIELD TRIALS

Dan Borrenpohl, Mao Huang,
M. Nelly Arguello-Blanco and Clay Sneller

Ohio Agricultural Research and Development Center,
The Ohio State University, Wooster, OH 44691
*Corresponding Author: PH: 618-972-2229; Email: borrenpohl.1@osu.edu

ABSTRACT

Preliminary yield trials (PYT) are a ubiquitous feature in plant breeding programs where many lines are assessed in few environments with limited replication. The cost of growing and phenotyping a PYT is high as there are many entries. The data from a PYT though is often of poor quality and is a poor predictor of future performance, especially for complex traits. We propose that genomic selection (GS) will be an effective tool in predicting future performance of lines in PYTs. We can build a GS model using high-quality phenotypic and genotypic data from widely replicated lines from past and current trials. The GS model can then predict future performance of PYT lines using genotypic data alone by calculating genomic estimated breeding values (GEBV). We can then assess the prediction accuracy of GEBVs by correlating them to phenotypic data for the same PYT lines that are advanced to multi-environment trials (MET). The prediction accuracy of PYT phenotypic data can be assessed in the same manner, and the prediction accuracies of GEBV and PYT phenotypic data can be compared. We expect GEBV's to have a higher prediction accuracy than PYT phenotypic data. We will present data on FHB resistance in our breeding program to demonstrate how this GS approach can be implemented for any trait of interest in PYTs.

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RECURRENT PHENOTYPIC SELECTION AUGMENTED BY GENOMIC SELECTION FOR HEAD SCAB RESISTANCE

Jesse Carmack and David A. Van Sanford*

Department of Plant and Soil Science, University of Kentucky, Lexington, KY, 40546
*Corresponding Author: PH: 859-338-2409; Email: dvs@uky.edu

ABSTRACT

Head scab or Fusarium head blight (FHB) of wheat *(Triticum aestivum)*, caused by *Fusarium graminearum*, is a devastating disease that results in discolored grain, mycotoxin production, and significant decreases in yield. Plant breeders utilize many different selection schemes to generate cultivars with enhanced FHB resistance. Recurrent phenotypic selection is a classic example, and a breeding scheme that relies on data gathered using intensive and slow in-field phenotyping to gradually increase the frequency of favorable alleles. Another more recent breeding scheme, genomic selection, relies on a computational model developed by associating genomic polymorphisms with trait expression in a training population, to predict phenotypes using genotypes in a validation population. The objectives of this study were: (i) utilize a high throughput optical seed sorter in a recurrent mass selection scheme to generate breeding populations with enhanced FHB resistance; and (ii) develop a genomic selection model using the extensive phenotyping data obtained with the optical sorter and SNP calls obtained with sequence-based genotyping to accurately predict plant performance. Three hundred breeding lines from 5 unique 3-way crosses were used in an experiment conducted over several years in Lexington, KY. Each population was grown in 1-meter rows in an inoculated, irrigated scab nursery with 2 replications. Heading date, plant height, and a visual FHB rating were taken in the field. *Fusarium* damaged kernels (FDK) and deoxynivalenol (DON) concentration were calculated post-harvest. After two cycles of mass selection with the seed sorter the average population-level FDK value decreased from 312 to 163% of the resistant check -- DON concentration in ppm decreased from 143 to 135% of the resistant check. Raw sequence reads for all individuals in two of the five populations (120 lines) were obtained using reduced representation sequence-based genotyping. 7,393 SNPs were identified after raw reads were processed, aligned to a reference genome, filtered, and imputed. Results and implications of average trait prediction accuracies for breeding programs will be discussed.

ACKNOWLEDGEMENT AND DISCLAIMER

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IDENTIFICATION OF QTLS FOR RESISTANCE TO FUSARIUM HEAD BLIGHT (*FUSARIUM GRAMINEARUM*) IN A DOUBLED HAPLOID POPULATION BETWEEN AGS 2060 AND AGS 2035

Alejandro Castro[1*], Kelly Arceneaux[1], Niranjan Baisakh[1], Stephen Harrison[1], Esten Mason[2] and Paul Price[3]

[1]School of Plant, Environmental, and Soil Sciences, Louisiana State University Agricultural Center, Baton Rouge, LA; [2]Crop, Soil, and Environmental Sciences, University of Arkansas Fayetteville, AR; and [3]Macon Ridge Research Station, Louisiana State University Agricultural Center, Baton Rouge, LA
[*]Corresponding Author: PH: 225-384-9125; Email: acastro@agcenter.lsu.edu

ABSTRACT

Fusarium head blight (FHB), caused by the fungus *Fusarium graminearum*, is a disease that attacks several grass species, including wheat. FHB emerged as a major disease of wheat across the southeastern US in the past fifteen years. 'AGS 2060' is a soft red winter wheat variety released by the Louisiana State University in 2004 (PVP 200800412). AGS 2060 is moderately susceptible to FHB but is found in pedigrees of several resistant breeding lines and varieties. 'AGS 2035' (PVP200900420) is susceptible to FHB. The objective of this study is to identify QTLs for resistance to FHB using 192 doubled haploid (DH) lines derived from a cross between varieties AGS 2060 and AGS 2035 (FHB susceptible). The cross LA12016 was made in 2012 and DHs were produced by Heartland Plant Innovations. DNA samples from each line were analyzed with a 90K SNP chip. Replicated field trials were conducted at Baton Rouge, LA and Winnsboro, LA in 2017 and 2018. The two-row headrow plots were replicated twice and inoculated with *Fusarium*-infected corn seed that was evenly distributed throughout the nursery. The nursery was misted twice nightly for 20 minutes from flag leaf through mid-dough stages. At maturity, plots were individually harvested with rice knives and threshed with minimal air flow to reduce loss of shriveled and scabby kernels. Rows were rated during late grain filling for FHB symptoms (0-9 scale). Kernels were rated for percentage of *Fusarium* diseased kernels (FDK) by comparing to standard samples of known FDK percent. Seed samples were submitted to the USDA Mycotoxin Diagnostic Laboratory at the University of Minnesota for deoxynivalenol (DON) quantification. ANOVA results indicated highly significant differences between lines in the population for FDK rating and DON content across all locations and years. QTL analysis was conducted individually for each location and year. Thirteen QTLs were identified for FDK: one each on 1D, 2A, 2B, 2D, 5D, 6A, and 7B, and two each on 3A, 5B, and 7A. Only one QTL on chromosome 6A was consistent in Baton Rouge and Winnsboro, whereas the QTL on chromosome 7B was consistently expressed in both 2017 and 2018 in Baton Rouge. Three QTLs on chromosome 2D, 6A, and 7B were identified for DON in Baton Rouge. Of these, the QTLs on chromosome 2D and 6A were also expressed in Winnsboro. QTLs on chromosome 2B, 2D, 3A, 6A and 7A were consistently expressed in both locations and years. QTLs for DON in Winnsboro 2018 were found in 1A, 1B, 2B, 2D, 3A, 3D, 5B, and 6A. Chromosome 6A contained QTLs in five out of the six tests. Chromosome 2B, 3A, and 7B contained QTLs in four out of the six tests. Results indicate that resistance to FHB contributed by AGS2060 is controlled by QTLs on several chromosomes. Further field trials and fine-resolution mapping will be conducted to more precisely identify the genes controlling FHB resistance.

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Test and results are based on work supported by SUN Grains collaborative work. 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.

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GENOME-WIDE ANALYSIS OF GRAIN YIELD IN PURDUE GERMPLASM: PROTECTING PUBLIC GERMPLASM DURING TENURE TRANSITION

Rupesh Gaire[1], Gina Brown-Guedira[2], Herbert Ohm[1] and Mohsen Mohammadi[1*]

[1]Department of Agronomy, Purdue University, West Lafayette, IN, 47907; and
[2]Department of Crop and soil sciences, North Carolina State University, Raleigh, NC, 27695
[*]Corresponding Author: PH: 765-496-6851; Email: mohamm20@purdue.edu

ABSTRACT

After inheriting Purdue germplasm from Dr. Herb Ohm, we have single-seed descended 437 lines for increasing the level of homozygosity. DNA was collected from leaf tissues and genotyped by using genotyping-by-sequencing method. After filtering markers for missing data (< 50%) and minor allele frequency (> 5%), a total of 38,336 markers were produced. Three hundred lines with enough seed were tested in a single-replicate yield trial in an augmented design in 2017-18 season. Association mapping did not identify any significant markers, with -logP value exceeding the 5% FDR threshold that are associated with yield. The maximum –logP observed was 3.62. A genomic selection model was fitted using the rrBLUP package in R v.3.4.0. Accuracy of the model was determined by the average of Pearson's correlation between the GEBV and the observed phenotypic data across 100 cycles. Prediction accuracy of yield on a 60%:40% training-to-validation dataset was 0.47. The germplasm was planted again in October 2018 for a second-year of data collection.

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GENOME-WIDE ASSOCIATION STUDIES OF FUSARIUM HEAD BLIGHT DISEASE RESISTANCE IN PURDUE PANEL

Rupesh Gaire[1], Gina Brown-Guedira[2], Herbert Ohm[1] and Mohsen Mohammadi[1*]

[1]Department of Agronomy, Purdue University, West Lafayette, IN, 47907; and [2]Department of Crop and Soil Sciences, North Carolina State University, Raleigh, NC, 27695
[*]Corresponding Author: PH: 765-496-6851; Email: mohamm20@purdue.edu

ABSTRACT

The Purdue soft red winter wheat population is well-known for its rich exotic introgression that confers disease and pest resistance. Our objective is to evaluate the response to Fusarium head blight (FHB) disease in over 350 Purdue-bred experimental lines. A single-replicated FHB nursery was planted following a previous corn crop in 2017-18 season, where each line was planted in a 3 ft row plot. Common check germplasm INW0411, INW0412, 'Monon', and 'Patterson' with known reactions were also planted. Scabby corn and spray inoculation were done by using nine different isolates of *Fusairum graminearum*, from Illinois, Indiana, and Ohio. For each line we measured disease incidence (DI), disease severity (DS), *Fusarium* damaged kernels (FDK), and calculated FHB index (FHBi). Genotyping-by-sequencing method yielded 38,336 markers after filtering the raw SNPs for missing data (< 50%) and minor allele frequency (> 5%). GWAS was performed using the rrBLUP package in R (v 3.4.0) accounting for population structure and background polygenic effects. A region associated with all traits was identified (-logP > 4.0) on chromosome 2B. In addition, other regions were identified on chromosome 5A for DI and chromosome 7D for DS and FHBi, and chromosomes 1A and 7A for FDK. QTL for disease severity on 7D is probably due to use of 7D (7E) *Thinopyrum ponticum*-translocated wheat lines as a source of disease resistance. The information from this study will help with characterizing the existing variation in FHB response and guiding how to develop FHB resistance cultivars.

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BREEDING FOR FHB RESISTANCE IN NORTH DAKOTA: MORE QUESTIONS THAN ANSWERS

Andrew Green[1], Justin Leier[1], Ying Lin[1], Shaobin Zhong[1], Xuehui Li[1], Andrew Friskop[1], Steven Xu[2], Xiwen Cai[1], Richard Frohberg[1], Robert Stack[1] and Mohamed Mergoum[3]

[1]North Dakota State University, Fargo, ND 58108; [2]USDA-ARS Northern Crop Science Laboratory, Fargo, ND 58108; [3]University of Georgia, CAES Campus. Griffin, GA 30223
[*]Corresponding Author: PH: 701-231-8478; Email: andrew.j.green@ndsu.edu

ABSTRACT

Breeding for Fusarium head blight (FHB) resistance has been a primary objective of the North Dakota State University Spring Wheat program for over 20 years. Great progress has been made, and the integration of resistance genes is on ongoing effort. As perhaps with any scientific process, more questions remain than answers.

Fusarium head blight has long been thought of as a problem for production environments in eastern North Dakota where annual rainfall is higher. However, in recent years, FHB outbreaks have caused problems across the state, taking many by surprise. These environments typically show lower yield potential, so genetic disease resistance is even more crucial from an economic standpoint. This brings about the question of how we balance selection for diverse environments where environmental conditions, abiotic stress factors, and acceptance for chemical inputs all vary.

The landscape of wheat varieties in North Dakota continues to change. The lifespan of varieties in the marketplace appears to be shortening, accompanied by a sharp increase in acres planted to varieties developed by private seed companies. New varieties have a wide range in FHB resistance. So, in order to provide timely information to producers, the question is, how do we test genotypes in a limited number of environments and confidently report their level of resistance? And what data do we provide to them? Visual scores are easily understood, but don't always correlate with Deoxynivalenol (DON) or *Fusarium* Damaged Kernel (FDK) levels, which can be highly variable as well.

'ND2710' (PI 633976) was one of the very first derivatives of *Sumai-3* developed by Dr. Frohberg in the 1990's. Its level of resistance is still almost unmatched in our released varieties, prompting the question of why?

'Glenn' (PI 639273), an important variety in our program due to its statewide adaptability and extremely high baking quality, does not amplify for *Fhb1*. Yet, it has shown a high level of resistance to FHB. As such, a genome-wide association analysis of advanced and elite breeding lines revealed that *Fhb1* appears in fewer than half of the lines in our program, yet it is considered our most widely used resistance gene. This prompted the question, where does the resistance in Glenn comes from, and how did it get there?

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MINING WATKINS COLLECTION OF WHEAT FOR RESISTANCE AGAINST FUSARIUM HEAD BLIGHT (FHB), TAN SPOT AND SEPTORIA NODORUM BLOTCH (SNB)

Jyotirmoy Halder, Shaukat Ali, Jinfeng Zhang and Sunish K. Sehgal*

Department of Agronomy, Horticulture & Plant Science, South Dakota State University, SD 57007
*Corresponding Author: PH: 605-688-5709; Email: sunish.sehgal@sdstate.edu

ABSTRACT

In the late 1920s, A. E. Watkins collected several thousand landrace cultivars (LCs) of bread wheat (*Triticum aestivum* L.) from 32 different countries around the world. A higher genetic diversity was reported in the LCs suggesting a potential of identifying new alleles or genes for biotic and abiotic stress resistance. In this study, a core set of 121 accessions, which captures the majority of the genetic diversity of the Watkins collection, was evaluated for identifying novel sources of resistance against three economically important diseases in wheat namely, Fusarium Head Blight (FHB), Tan spot, and Septoria nodorum blotch (SNB) caused by *Fusarium graminearum*, *Pyrenophora tritici-repentis* and *Parastagonospora nodorum,* respectively. To identify the germplasm(s) resistant to FHB, we evaluated the core set of LCs in the field under mist-irrigated and inoculated FHB field nursery. Mean FHB disease index (DI) was 34% with a range of 17.1% to 56.6%. Five (4%) of 121 accessions (LCs) demonstrated a moderately resistant response (DI 13.4% - 25.3%) to FHB under field conditions and were further evaluated in the greenhouse using point inoculations. Four of the five accessions showed the percent spikelet severity (PSS) ranging from 8.6-10.2% suggesting a moderate level of resistance to FHB. We also evaluated the core set of LCs against *Pyrenophora tritici-repentis* races 1 (PTR1) and 5 (PTR5) and their corresponding toxins Ptr ToxA and Ptr ToxB, respectively and against *Parastagonospora nodorum* in the greenhouse. LCs showed diverse reactions ranging from susceptible to resistant against both the races of *P. tritici-repentis* and *P. nodorum*. The majority of LCs exhibit susceptibility to PTR1, while more than 90% of the LCs were either resistant or moderately resistant against PTR5. Of the 121 accessions, 1(0.8%), 8(6.6%), 75(62.0%) and 37(30.6%) accessions were resistant, moderately resistant, moderately susceptible and susceptible against PTR1, respectively, whereas, 35(28.9%), 75 (61.9%) and 11 (9.1%) accessions were resistant, moderately resistant, and moderately susceptible against PTR5, respectively. On the other hand, 6 (5.0%), 60 (50%), 52 (43%), and 3 (2.5%) accessions were found resistant, moderately resistant, moderately susceptible, and susceptible against *P. nodorum*. Three accessions (2.48%) were either resistant or moderately resistant to both *P. tritici-repentis* races (PTR1 and PTR5) and *P. nodorum*. Our results suggest that the LCs are a valuable source of resistant genes/ alleles against fungal diseases and the resistant accessions can readily be used in breeding programs.

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FHB RESISTANCE OF USDA-ARS BARLEY BREEDING MATERIALS IN IDAHO

Gongshe Hu[1*], Phil Bregitzer[1], Kathy Satterfield[1], Chris Evans[1] and Juliet Marshall[2]

[1]Small Grains and Potato Research, USDA-ARS, Aberdeen, Idaho 83210; and [2]Department of Entomology, Plant Pathology, and Nematology, University of Idaho, Idaho Falls, ID 83402
[*]Corresponding Author: PH: 208-397-4162; Email: gongshe.hu@ars.usda.gov

ABSTRACT

Fusarium head blight (FHB) disease is very damaging to barley yield, quality, and commercial values. With climate change and the increase of corn planting, FHB infected plants were identified in Idaho barley fields in recent years. This is a worrisome problem for Idaho barley growers in the normally high quality, virtually disease free, barley production state. As a first step to deal with the potential disease, we have screened 100 of our spring elite or advanced breeding materials in two screening nurseries at North Dakota State University, in 2014 and 2015, and in Aberdeen, Idaho, 2015. Five year-location averaged data for infection and DON showed that there are some elite lines from the Aberdeen breeding program with promising resistance in both measurements of infection rate and DON. Compared to the 2-row resistant check of 'Conlon', 62 of the 100 lines have lower infection index and 44 lines showed lower DON content on average in five year-locations. Two of our Plant Scale Testing lines, 2Ab04-X01084-27 and 2Ab07-X031098-31, showed lower DON than Conlon. The results indicate that Aberdeen elite breeding lines likely contain resistant genetic resources which should positively impact the breeding program in developing FHB resistant cultivars. In 2018, we expanded FHB testing to include 150 winter habit lines that were evaluated in Idaho and Virginia. Initial observations indicate variability for infection severity among lines and cultivars.

ACKNOWLEDGEMENT AND DISCLAIMER

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EPIGENOME MODIFICATION IN DURUM WHEAT PROVIDES FHB RESISTANCE

Jitendra Kumar[1], Steven Xu[2], Elias M. Elias[3], Ruth Dill-Macky[1] and Shahryar Kianian[4*]

[1]Department of Plant Pathology, University of Minnesota, St. Paul 55108, MN; [2]USDA-ARS Cereal Crops Research, Fargo, ND 58102; [3]Department of Plant Sciences, North Dakota State University, Fargo, ND 58108; and [4]USDA-ARS Cereal Disease Laboratory, St. Paul, MN 55108
[*]Corresponding Author: PH: 612-624-4155; Email: shahryar.kianian@ars.usda.gov

ABSTRACT

Fusarium head blight (FHB), caused by *Fusarium* sp., is one of the most serious and economically damaging diseases affecting wheat in the U.S. and throughout the world. FHB severely affects wheat production by causing yield loss, quality and food safety by mycotoxins infested grains. Improving host genetic resistance is an effective way to achieve control over FHB, which is a continuous challenge for durum wheat as most of the germplasm are susceptible with low genetic variation for this trait. Biotic and abiotic stresses lead to demethylation of several immune-responsive genes in plants. Our project aims at inducing heritable demethylation in durum lines as a novel source of FHB resistance.

We treated advanced durum breeding lines with 5-methyl-azacytidine that removes CG methylation. Around 500 treated progenies were advanced to the M4 generation. A total of 32 promising of the 500 M4 lines were selected following preliminary testing against FHB. The 32 promising lines and eight parental checks were further tested for FHB resistance under greenhouse and field conditions. Five of the 32 selected lines tested showed less than 30% FHB severity as compared with the parental lines and FHB susceptible lines, which ranged from 50-100%. The analysis of *Fusarium*-damaged kernels (FDK) and deoxynivalenol (DON) on grain harvested from inoculated plants further supported the greenhouse and field disease assessments. The five best performing lines, together with their respective parental lines and susceptible checks, were examined to determine the overall percentages of epigenetic change that were responsible for the enhanced FHB resistance observed. Global methylome level analysis did not show significant differences between the five best performing lines and the parental lines from which they were derived; however, transcriptome analysis (with a mean library size of ≈ 200 bp, quality scores of ≥ Q30 and approximate coverage of 32 - 50 million reads per samples) of the selected lines (treated, parental and susceptible checks) revealed some highly up- and down- regulated (≥ 5 folds; log2 value) novel candidate genes in the M4 lines (E.25.10 and E.25.30) as compared to a susceptible check ('Ben'). In addition, several known genes such as, lipid transfer protein, protein binding, Osmotin/thaumatin-like protein and oxidoreductase were ≥ 5 folds up regulated in the M4 lines as compared to the check. The novel candidate genes are being characterized using bioinformatics analysis and reverse genetics approach. We have advanced the two most resistant M4 lines by backcrossing, to the parental cultivar, with an aim of testing the stability and inheritance of the resistance. The F3 plants were tested in the field together with the resistant M4 lines and the susceptible checks. Fifty backcross F3 lines, of the 400, showed FHB resistance similar to the M4 lines. The backcross-derived F3 lines are being further tested in greenhouse.

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FUSARIUM HEAD BLIGHT RESISTANCE IN HYBRID HARD RED SPRING WHEAT

Justin Leier and Andrew Green*

Department of Plant Sciences, North Dakota State University, Fargo, ND 58108
*Corresponding Author: PH: 701-231-8478; Email: andrew.j.green@ndsu.edu

ABSTRACT

Wheat varieties are typically bred and released as inbred lines. However, advances in chemical hybridizing agents (CHAs) and cytoplasmic male sterility (CMS) systems have reopened the possibility of hybrid wheat breeding and production. When used efficiently, a hybrid system may generate a yield advantage that will be required to feed the increasing population. Fusarium Head Blight (FHB) is a detrimental wheat disease caused by several *Fusarium* species. Direct production losses can reach hundreds of millions of dollars annually without resistance genes or management, and a combination of both strategies most efficiently reduces the impact of FHB (Wilson et al., 2017). For hybrid breeding, knowledge of resistance gene action can help formulate crossing strategies. Complete dominance of all FHB resistance genes would be desirable to hybrid breeders, since crosses could be made to susceptible parents to produce resistant F_1 hybrid progeny. In our study, a common susceptible parent ('2398') and four resistant parents ('Alsen,' 'Faller,' 'SY Soren,' & 'Glenn') with differing resistance genes were crossed to create F_1 hybrids. 'Alsen' carries *Fhb1*, *Fhb5*, and a QTL on chromosome 3A that is derived from 'Frontana.' 'Faller' contains both *Fhb1,* and *Fhb5*. 'SY Soren' only carries *Fhb1,* and 'Glenn' is not confirmed to contain any of these genes. '2398' contains no resistance and is rated as highly susceptible. The experiment also contained the long-term resistance check 'ND2710' and a local susceptible check, 'WB-Mayville.' The experiment in 2018 contained all reciprocal crosses except for one, which was unavailable due to seed production issues. F_1 genotypes were tested in inoculated field trials at Prosper and Langdon, ND during the summer of 2018. The fields were mist irrigated and inoculated using Fusarium colonized corn grain spread at early jointing and boot stage for a total rate of 61g/m^2. Fields were organized in a randomized complete block design with six replicates. Incidence was scored 21 days post-anthesis by counting the number of infected heads in ten randomly selected heads. Severity was determined by counting infected spikelets out of total spikelets on ten infected heads. FHB index was calculated using the incidence and severity values. Test weight, DON, and *Fusarium* damaged kernels (FDK) were recorded post-harvest. FDK values were scored as percentage of damaged kernels in the sample. The Langdon, ND location had to be abandoned due to lack of FHB infection. Data were analyzed using Proc GLM with SAS 9.4. There were no differences found between reciprocal crosses made with the same parents, so reciprocal hybrid crosses were analyzed as a single genotype. In almost all response variables analyzed, the mean value of the F_1 hybrid was intermediate to the mean of its respective parents, but closer to that of the resistant parent. Using LSD mean separation by trait, F_1 hybrids were almost always different ($p < 0.05$) than either parent. The FHB index values ranged from 19% (Glenn) to 90% (2398). Average FDK values ranged from 6%, (ND2710) to 77%, (2398). Hybrid genotype FDK values ranged from 45-70% of the value its resistant parent. Test weight ranged from 41bs/bu (2398) to 57 lbs/bu (ND2710) and the hybrid average ranged from 70-90% of the mean of its resistant parent. These initial results suggest that the various genetic sources of FHB resistance we examined do not exhibit complete dominance gene action. The experiment will be repeated at three locations in North Dakota in 2019.

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PYRAMIDING WHEAT FUSARIUM HEAD BLIGHT RESISTANCE GENES FROM DIFFERENT SOURCES USING MARKER-ASSISTED BACKCROSSING AND LOW-COST SNP MARKERS FOR BACKGROUND SELECTION

Yaoguang Li[1], Amy Bernardo[1], Paul St. Amand[2] and Guihua Bai[1,2*]

[1]Kansas State University, Manhattan, KS 66506; and [2]USDA-ARS,
Hard Winter Wheat Genetic Research Unit, Manhattan, KS 66506
[*]Corresponding Author: PH: 785-532-1124; Email: Guihua.bai@ars.usda.gov

ABSTRACT

Fusarium head blight (FHB), also called scab, is a devastating disease of wheat, barley and other cereals in the world. FHB can result in severe losses in grain yield and quality. *Fusarium graminearum* is a major pathogen for FHB in the USA. Mycotoxins, especially deoxynivalenol (DON), produced by the pathogen are harmful to human and animal health. Although the application of fungicides can reduce FHB damage, improvement of wheat FHB resistance is the most effective approach to reduce the losses. Unfortunately, most cultivars currently used in production in the U.S. Great Plains are highly susceptible to FHB. Most FHB resistance sources have poor agronomic traits and are undesirable as parents in breeding programs. Marker-assisted backcrossing (MAB) is an effective method for pyramiding genes from different sources into new cultivars and removing undesired genetic backgrounds. *Fhb1* is a major gene for FHB resistance identified from a Chinese cultivar 'Sumai 3'. This gene shows a major stable effect on FHB resistance in almost all backgrounds. Recently two other genes on wheat chromosome 5A were identified from (PI277012) and also show a significant effect on FHB resistance. Pyramiding the three major genes for FHB resistance in hard winter wheat backgrounds will provide useful germplasm for improvement FHB resistance in hard winter wheat. Multiplex restriction amplicon sequencing (MRASeq) is a new low-cost PCR and next-generation-sequencing-based genotyping method for various breeding applications. In this study, we used MAB to transfer *Fhb1* and two genes on chromosome 5AS, one on the short arm (*Qfhs.ifa-5A*) and the other on the long arm of 5A into two Kansas hard winter wheat cultivars, 'Everest' and 'Overland'. Additionally, MRASeq was used for background selection to develop locally adapted FHB-resistant hard winter wheat germplasm lines. After two backcrosses, about 100 lines with eight possible homozygous combinations of the three genes were selected using gene-linked markers in each background. The phenotypic results from the first year field experiment revealed that lines positive for all three QTLs have the greatest resistance in both backgrounds. Our first greenhouse test indicated that the effects of FHB resistance of different gene combinations were different between the two backgrounds. In the 'Everest' background, selected lines with all three positive genes and lines with only *Fhb1* showed significantly better resistance than others. In the 'Overland' background, lines with *Fhb1* and the positive gene on chromosome 5AL showed significantly better resistance than others. Those selected germplasms with a high level of FHB resistance and improved agronomic traits will be released as FHB resistant germplasm for genetic improvement of FHB resistance in hard winter wheat breeding programs.

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GENOME WIDE ASSOCIATION ANALYSIS AND PREDICTION OF FHB RESISTANCE IN SOFT WINTER WHEAT

R. Esten Mason[1*], Dylan Larkin[1] and Stephen Harrison[2]

[1]Crop, Soil, and Environmental Sciences, University of Arkansas Fayetteville, AR 72701; and [2]School of Plant, Environmental, and Soil Sciences, Louisiana State University Agricultural Center, Baton Rouge, LA 70803
[*]Corresponding Author: PH: 479-387-8899; Email: esten@uark.edu

ABSTRACT

Fusarium head blight (FHB) is a disease of small grains 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 (GWA) approach and determine genomic selection prediction accuracies for FHB resistance traits in a training population consisting of 360 soft red winter wheat lines. The population was evaluated in inoculated misted FHB nurseries in Fayetteville and Newport, AR and Winnsboro, LA (2017 only) in a randomized complete block design from 2014-2017. At all locations, lines were sown in two row plots, inoculated with *F. graminearum* infected corn (*Zea mays L.*) and overhead misted throughout the months of April and May to provide optimal conditions for FHB infection. Lines were phenotyped for the major resistance traits including resistance to initial inoculum (incidence); resistance to spread within the head (severity); resistance to DON accumulation; and resistance to *Fusarium* damaged kernels (FDK). A total of 13 SNP markers exceeded the Bonferroni threshold ($p = 0.05$) using FarmCPU, including four for incidence located on 1B, 1D and 4B, three for severity located on 7B and 7D, five for FDK located on 3B, 4B, 5D and 7A and one for DON located on 4A. Genomic prediction accuracies (r) were 0.39, 0.58, 0.46 and 0.57 for incidence, severity, FDK and DON, respectively. Current work is focused on estimating genome estimated breeding values in new breeding lines and confirming the efficacy of prediction models for selecting resistant lines. Results from this study will facilitate the development of SRWW cultivars with improved resistance to FHB.

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COLLABORATIVE DOUBLED HAPLOID BREEDING FOR FUSARIUM HEAD BLIGHT RESISTANCE IN BARLEY

Brigid Meints*, Tanya Filichkin, Laura Helgerson, Scott Fisk and Patrick M. Hayes

Deptartment of Crop and Soil Science, Oregon State University, Corvallis, OR 97333
*Corresponding Author: PH: 541-231-6747; Email: brigid.meints@oregonstate.edu

ABSTRACT

Breeding barley is a long-term process that requires multiple cycles of self-pollination to achieve complete homozygosity. Doubled haploid (DH) production leads to complete homozygosity in a single generation, thus bypassing the complications of field, greenhouse, or off-season generation advance. Completely homozygous material facilitates the phenotyping of complex traits and simplifies integration of phenotype with genotype for gene discovery and characterization. A collaborative network in which multiple investigators provide germplasm of interest and a central facility produces doubled haploids can generate synergies and efficiencies. The USWBSI is supporting collaborative DH production at Oregon State University (OSU), which started in the fall of 2017. In year one, F_1's from nine pedigrees were solicited from barley researchers and received from Cornell (Sorrells) and Virginia Tech (Brooks & Griffey). From these F_1's, 1139 plantlets were produced for Cornell and 908 were produced for Virginia Tech. DH seed and plantlets will be shipped back to donors for increase and testing. In year two, F_1's from six pedigrees were obtained from Virginia Tech (Brooks and Griffey) and the USDA-ARS, Idaho (Bregitzer) and contributed by the OSU breeding program. Additionally, naked barley DH's created at OSU for other projects are being analyzed in FHB nurseries in New York and Minnesota based on reports from the literature that naked barley shows lower accumulation of deoxynivalenol (DON) than hulled barley because a large portion of the mycotoxins build up in the hull, which remains in the field after harvest.

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GOING WEST – ESTABLISHING A WHEAT BREEDING PROGRAM IN THE US

Jana Murche

KWS Cereals USA, Champaign IL 61822
Corresponding Author: PH: 330-234-0302; Email: jana.murche@kws.com

ABSTRACT

KWS is an independent and family-owned company based in Germany and focusing on plant breeding, with activities in about 70 countries. The product range includes seed varieties for sugar beet, corn, cereals, rapeseed and potatoes.

In 2011, the company's cereals business unit started its first breeding "adventure" outside of Europe by acquiring and merging two small Eastern winter wheat breeding companies. With this acquisition access to adapted winter wheat breeding material was ensured and a SRW and SWW breeding program for the Eastern US was established in Wooster, OH. After a "tough" start under challenging conditions, the program was re-located to Champaign, IL where we found near ideal conditions for our main breeding location and activities. Since 2013, the team size has increased, new equipment to support our activities has been acquired, collaborations have been established and the use of new breeding technologies has increased. Today, the breeding program has grown to a desired size and is starting to produce competitive, broadly adapted and regional varieties for the Eastern US wheat market.

Over the past 8 years, the primary breeding targets of high yield performance and low FHB susceptibility have been addressed. The trial network has been increased to ca. 30 locations in pre-commercial testing. Within our internal trial system we were able to achieve an average yield increase of 1.7% per year. The development of KWS lines in the regional uniform nursery yield trials demonstrates a trend to establish a position within the 5 best ranked lines each year. To target an improved FHB resistance, KWS is actively participating in the collaborative regional scab nurseries. It shows in the different nurseries, that for different FHB parameters, KWS started out on a susceptible to moderately-susceptible level. Through intensive breeding efforts over the past years, we lowered the susceptibility level of our material to be in the range of the moderately-resistant check varieties with single lines reaching the resistance level of Truman. Not surprisingly, the most challenging part lies in identifying breeding lines possessing the combination of commercially competitive yields with good FHB resistance. To address this, we continue to phenotypically screen our most advanced high yielding lines in an internal scab nursery, as well as in the regional scab nurseries. We also utilize marker data provided by the USDA-ARS genotyping lab in Raleigh to track individual marker loci. In addition we use genome-wide SNP data in the implementation of Genomic Selection to receive additional performance indications, which proves especially valuable in years with low scab disease pressure. The combination of field-based observations and marker-based predictions will aid in the overall improvement of new wheat varieties for yield and FHB resistance.

Overall, it has been a rewarding first 8 years and together with the wheat community, we strive to improve the perception of "wheat" and increase the acreage of high performing varieties combined with a competitive disease resistance package.

THE 2018 UNIFORM SOUTHERN SOFT RED WINTER WHEAT SCAB NURSERY

J.P. Murphy[1*], J.H. Lyerly[1], R. Acharya[1], B. Ward[2] and G. Brown-Guedira[2]

[1]Department of Crop Science, North Carolina State University, Raleigh, NC 27695; and [2]USDA-ARS, North Carolina State University, Raleigh, NC 27695
[*]Corresponding Author: PH: 919-610-0100; Email: paul_murphy@ncsu.edu

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 2017. A combined mixed model analysis of the phenotypic data from 2011 to 2017 was performed using SAS 9.3 and BLUEs for each genotype were recorded. The number of SNP markers utilized was 36,018. 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 0.56 for FHB Severity to 0.53 for *Fusarium* damaged kernels (FDK) and 0.49 for DON.

The 2017-18 nursery comprised 45 advanced generation breeding lines and four check cultivars, Ernie, Bess, Jamestown (partially resistant) and 'Coker 9835' (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 2018 National Fusarium Head Blight Forum and subsequently on line at the USWBSI web site: https://scabusa.org.

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

	Cultivar/ Designation	FHB Rating	RANK	FHB Incidence	RANK	FHB Severity	RANK	FHB Index	RANK	FDK	RANK	ISK	RANK	DON	RANK
1	ERNIE	4	16	61	13	42	22	26	19	12	4	32	11	12	16
2	COKER9835	7	48	84	48	77	48	64	48	55	49	60	48	30	49
3	BESS	3	1	56	7	34	8	19	4	11	3	28	5	13	22
4	JAMESTOWN	4	16	68	26	38	16	25	18	16	16	32	11	9	4
5	NC13-21213	5	38	78	44	55	40	45	42	36	43	51	44	12	16
6	NC14-23372	3	1	66	23	47	29	32	29	15	11	33	16	10	9
7	NC14-23373	3	1	65	22	36	14	24	16	15	11	33	16	14	26
8	AR09006-10-2	4	16	74	41	48	31	38	35	24	35	45	37	20	41
9	AR09009-8-3	4	16	73	35	51	33	43	41	23	32	52	45	21	43
10	AR09045-4-2	4	16	73	35	52	37	39	38	23	32	43	34	27	47
11	ARLA09218C-5-2	5	38	69	29	45	27	33	30	21	29	38	28	16	33
12	ARLA09238C-6-3	4	16	73	35	51	33	38	35	24	35	47	41	11	13
13	ARLA09179UC-9-3	6	44	81	46	65	46	53	47	19	25	45	37	13	22
14	ARLA09137UC-17-2	6	44	74	41	70	47	50	45	30	41	40	31	17	36
15	ARLW08160D-20-1	4	16	68	26	52	37	36	32	17	19	36	23	13	22
16	GA13VA-FHB-DH83-17EL53	3	1	68	26	23	1	16	1	12	4	27	2	6	1
17	GA091034-17EL44	8	49	86	49	84	49	71	49	53	48	62	49	25	46
18	GA10654-17LE46	4	16	63	21	40	20	27	20	16	16	34	19	10	9
19	GA10389-17LE56	4	16	70	30	51	33	36	32	19	25	43	34	12	16
20	GA111005-17A3	6	44	81	46	58	44	50	45	33	42	53	46	17	36
21	GA121086-LDH20-17A24	5	38	73	35	51	33	38	35	21	29	46	40	9	4
22	GA091537-17A29	3	1	61	13	35	9	20	5	13	7	27	2	8	2
23	GA1035-DH49-17LE52	4	16	60	11	35	9	23	13	10	1	28	5	11	13
24	KWS154	3	1	54	3	35	9	21	8	15	11	35	21	9	4
25	KWS192	4	16	62	17	38	16	24	16	15	11	36	23	13	22
26	KWS193	3	1	60	11	35	9	22	12	17	19	35	21	15	28
27	L11815	4	16	61	13	42	22	27	20	18	22	39	30	10	9
28	L11820	4	16	54	3	33	6	21	8	18	22	32	11	9	4
29	L11811	4	16	62	17	41	21	27	20	37	44	45	37	21	43
30	LA14066DH-147	5	38	78	44	55	40	46	44	39	46	50	42	20	41
31	LA08277C-P5-3-1	4	16	66	23	43	25	28	24	20	28	36	23	16	33
32	LA11289C-57-4	5	38	71	33	53	39	39	38	42	47	54	47	15	28
33	LA12120SB-56-4	3	1	61	13	47	29	28	24	17	19	31	8	9	4
34	LA14076-LDH6	3	1	58	8	28	2	17	2	24	35	31	8	12	16
35	LA14066DH-172	6	44	73	35	62	45	45	42	37	44	50	42	14	26
36	NC14-20369	4	16	55	6	38	16	23	13	15	11	34	19	11	13
37	NC14-22588	4	16	59	9	46	28	28	24	16	16	37	27	12	16
38	NC11546-14	3	1	62	17	32	5	20	5	13	7	27	2	10	9
39	NC15-23047	3	1	62	17	36	14	23	13	18	22	32	11	15	28
40	NC15-21787	4	16	66	23	55	40	36	32	22	31	44	36	18	39
41	NC11331-6	3	1	54	3	31	4	21	8	10	1	23	1	8	2
42	DH12SRW057-081	4	16	53	1	33	6	20	5	12	4	33	16	12	16
43	13VA-FHB-DH131	3	1	70	30	38	16	27	20	13	7	32	11	15	28
44	VA15W-70	4	16	74	41	55	40	41	40	25	39	42	33	18	39
45	VA16W-31	4	16	70	30	43	25	31	28	23	32	38	28	28	48
46	VA16W-202	5	38	73	35	48	31	35	31	24	35	40	31	15	28
47	12VTK10-156	4	16	72	34	42	22	30	27	25	39	36	23	17	36
48	DH13SRW023-201	3	1	59	9	35	9	21	8	19	25	31	8	16	33
49	DH13SRW025-14	3	1	53	1	30	3	17	2	14	10	28	5	21	43
	Mean	4		67		45		32		22		39		15	
	LSD (0.05)	2		21		23		20		17		17		7	
	CV%	27.4		15.7		25.1		32		39.0		22.5		22.8	
	Correlations with Prediction	0.54		.		0.56		0.59		0.53		.		0.48	

Table 1. Continued

	Cultivar/ Designation	Heading Date	RANK	Plant Height	RANK	Flour Yield %	RANK	Softness Equiv. %	RANK	Hessian Fly Bio. L	H13	Fhb1	Fhb Massey 3BL	Fhb 5A_Ning	Fhb 2DL Wuhan 1/W14	Bess 2B	Bess 3B	Jamestown 1B	NC-Neuse 1A	NC-Neuse 6A
1	ERNIE	125	3	32	8	65.9	18	58.7	17	0-17	no	no	F3BM	no	no	no	no	no	F1AN	F6AN
2	COKER9835	128	36	31	3	64.7	36	60.3	11	0-14	no	no	no	no	no	no	no	no	no	no
3	BESS	126	10	36	33	65.1	31	59.2	14	0-17	no	no	no	no	no	no	F3BB	F1BJ	het	no
4	JAMESTOWN	124	2	33	12	62.7	47	56.5	28	0-18	no	no	no	no	no	no	no	F1BJ	F1AN	no
5	NC13-21213	126	10	33	12	62.9	45	54.7	39	17-0	H13	no	no	no	no	no	no	F1BJ	F1AN	no
6	NC14-23372	129	47	36	33	67.0	10	56.4	29	0-16	no	Fhb1	no	no	no	no	no	F1BJ	F1AN	F6AN
7	NC14-23373	130	49	36	33	65.9	19	54.8	38	13-2	no	Fhb1	no	no	no	no	no	F1BJ	F1AN	F6AN
8	AR09006-10-2	127	21	37	41	62.9	46	58.2	23	0-18	no	no	no	no	no	no	no	no	F1AN	no
9	AR09009-8-3	127	21	37	41	64.5	38	55.7	35	0-22	no	no	no	no	no	no	no	no	F1AN	no
10	AR09045-4-2	128	36	37	41	63.7	41	57.8	25	0-13	no	no	no	no	no	no	no	no	F1AN	no
11	ARLA09218C-5-2	128	36	35	25	64.9	33	55.8	34	0-11	no	no	no	no	no	no	no	no	no	no
12	ARLA09238C-6-3	129	47	37	41	65.9	20	58.5	18	0-19	Het?	no	no	no	no	no	no	no	F1AN	no
13	ARLA09179UC-9-3	127	21	33	12	68.6	2	60.6	8	0-16	no	no	no	no	no	no	no	no	no	no
14	ARLA09137UC-17-2	126	10	36	33	63.9	40	59.0	16	14-0	no	no	no	no	no	no	no	no	no	no
15	ARLW08160D-20-1	126	10	37	41	64.8	34	55.5	36	0-15	no	no	no	no	no	no	no	no	F1AN	no
16	GA13VA-FHB-DH83-17EL53	123	1	34	21	66.1	16	58.3	22	0-14	no	no	no	no	no	no	no	no	no	no
17	GA091034-17EL44	127	21	35	25	66.1	17	56.8	27	0-15	no	no	no	no	no	no	no	no	no	no
18	GA10654-17LE46	125	3	35	25	63.0	44	53.6	44	0-17	no	no	no	no	no	no	F3BB	F1BJ	het	het
19	GA10389-17LE56	125	3	35	25	65.3	29	57.7	26	18-0	H13	no	no	no	no	no	no	F1BJ	no	no
20	GA111005-17A3	126	10	33	12	62.4	48	54.5	40	17-0	H13	no	no	no	no	no	no	no	no	no
21	GA121086-LDH20-17A24	125	3	31	3	63.5	43	53.4	45	17-0	H13	no	no	no	no	no	no	F1BJ	F1AN	no
22	GA091537-17A29	128	36	36	33	66.7	11	50.1	48	0-19	no	Fhb1	no	no	F2DLW	F2DLW	no	no	no	no
23	GA1035-DH49-17LE52	125	3	35	25	63.7	42	55.9	33	17-0	no	Fhb1	no	no	no	no	no	no	no	F6AN
24	KWS154	127	21	37	41	67.3	8	61.3	6	0-15	no	no	F3BM	no	no	no	no	no	F1AN	F6AN
25	KWS192	127	21	36	33	67.1	9	58.3	21	18-0	H13	no	no	no	no	no	no	no	no	no
26	KWS193	128	36	34	21	64.3	39	53.7	43	15-0	H13	no	no	no	**	**	no	no	no	no
27	L11815	127	21	35	25	67.8	4	60.4	9	0-18	no	no	het	no	no	no	no	F1BJ	F1AN	no
28	L11820	125	3	34	21	65.3	30	61.0	7	0-15	no	no	no	no	no	no	no	F1BJ	F1AN	no
29	L11811	128	36	33	12	68.2	3	56.3	32	0-18	no	no	no	no	no	no	no	no	no	no
30	LA14066DH-147	127	21	34	21	67.6	5	52.9	46	15-0	H13	no	no	no	no	no	no	no	F1AN	no
31	LA08277C-P5-3-1	125	3	33	12	65.0	32	62.7	3	0-18	no	no	no	no	no	no	no	no	no	no
32	LA11289C-57-4	126	10	33	12	61.4	49	56.4	31	0-19	no	no	no	no	no	no	no	F1BJT	no	no
33	LA12120SB-56-4	128	36	40	49	65.4	27	61.5	5	17-0	H13	no	no	no	no	no	no	F1BJT	F1AN	F6AN
34	LA14076-LDH6	127	21	33	12	66.2	15	59.3	13	0-18	no	Fhb1	no	no	F2DLW	F2DLW	no	no	no	no
35	LA14066DH-172	126	10	32	8	67.6	6	54.3	41	19-0	H13	no	no	no	no	no	no	F1BJT	F1AN	no
36	NC14-20369	127	21	39	47	67.6	7	61.8	4	18-0	H13	no	het	no	no	no	no	F1BJT	F1AN	no
37	NC14-22588	126	10	35	25	64.7	37	58.5	20	0-16	no	no	no	no	no	no	no	no	F1AN	no
38	NC11546-14	126	10	36	33	65.7	23	59.1	15	17-0	H13	het	het	no	no	no	no	1BJT_h	F1AN	no
39	NC15-23047	127	21	36	33	65.5	25	58.5	19	2-14?	no	no	no	no	no	no	F3BB	F1BJT	F1AN	F6AN
40	NC15-21787	128	36	39	47	65.9	21	65.6	1	15-0	H13	Fhb1	no	no	no	no	no	no	no	F6AN
41	NC11331-6	128	36	30	2	65.4	28	60.4	10	16-0	H13	Fhb1	no	no	no	no	no	F1BJT	no	F6AN
42	DH12SRW057-081	127	21	31	3	70.8	1	59.7	12	0-17	no	no	het	no	no	no	no	F1BJT	F1AN	no
43	13VA-FHB-DH131	127	21	32	8	66.3	14	53.9	42	0-13	no	no	no	no	no	no	no	F1BJT?1	AN_h	no
44	VA15W-70	128	36	31	3	66.4	13	56.4	30	4-4	no	no	no	no	no	no	no	no	no	no
45	VA16W-31	128	36	29	1	65.9	22	55.1	37	0-18	no	no	no	no	no	no	no	no	no	no
46	VA16W-202	126	10	31	3	65.7	24	63.4	2	18-0	H13	no	no	no	no	no	no	no	no	no
47	12VTK10-156	127	21	33	12	66.5	12	57.9	24	16-0	H13	no	no	no	no	no	no	no	F1AN	no
48	DH13SRW023-201	127	21	35	25	65.5	##	50.8	47	0-19	no	no	no	no	no	no	no	F1BJT	F1AN	no
49	DH13SRW025-14	126	10	32	8	64.8	35	49.2	49	0-15	no	no	no	no	no	no	no	no	no	no
	Mean	127		34		65.4		57.5		.	.									
	LSD (0.05)	3		2		.		.		.	.									
	CV%	1.2		3.6		.		.		.	.									
	Correlations with Predictions	.		0.53		.		.		.	.									

USING GENOMEWIDE MARKERS AND SIMULATED POPULATIONS TO PREDICT GENETIC VARIANCE AND CORRELATION FOR FUSARIUM HEAD BLIGHT RESISTANCE IN BARLEY

Jeffrey L. Neyhart and Kevin P. Smith*

Department of Agronomy and Plant Genetics, University of Minnesota, St. Paul, MN 55108
*Corresponding Author: PH: 612-624-1211; Email: smith376@umn.edu

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 quantitative traits among lines in bi-parental populations; and 2) validate these predictions empirically using contemporary breeding populations phenotyped for Fusarium head blight severity, heading date and plant height.

INTRODUCTION

Improving Fusarium head blight (FHB) resistance in barley, a highly quantitative trait, relies on maintaining the genetic response to selection, a function of trait heritability, selection intensity, and genetic variance (Falconer and Mackay, 1996). While research has focused on better selection of individuals within established populations, little work has addressed the selection of parent combinations to create populations with greater genetic variance. The benefit of populations with greater genetic variance is formalized in the usefulness criterion and the related superior progeny mean (Zhong and Jannink, 2007), or the mean value of the selected fraction of individuals in a population. The superior progeny mean (μ_{sp}) of a population can be derived from the expected population mean (μ) and genetic variance (V_G), assuming a normally distributed trait and given selection intensity: $\mu_{sp} = \mu + \text{sqrt}(V_G)$. Simply, among populations with similar mean, those with larger genetic variance will yield superior progeny with a more favorable mean.

While this equation has utility, it addresses only a single trait, and breeders routinely consider many more traits when making selections. Further, quantitative traits are often genetically correlated with each other. Well-known examples include grain yield and plant height in maize (*Zea mays* L.) (Chi et al., 1969) and protein content and oil content in soybean (*Glycine max* L. Merr) (Johnson et al., 1955). In barley, FHB resistance is often unfavorably correlated with heading date and plant height, where early-flowering and shorter plants tend to be more severely diseased (Mesfin et al., 2003; Massman et al., 2011).

Knowledge of the genetic variance or genetic correlation (r_G) expected in a cross would be useful before making said cross. The advent of genomewide selection (Meuwissen et al., 2001) and the availability of affordable computing power has led to a new method of predicting genetic variance and genetic correlation that is based on simulated bi-parental populations (Bernardo, 2014; Mohammadi et al., 2015). While this method may be a promising tool for breeders, empirical validation is needed in the context of a contemporary breeding program.

MATERIALS AND METHODS

A training population (TP) of 175 two-row barley lines was used for genomewide prediction. The TP was phenotyped in Crookston, MN and St. Paul, MN in 2014 and 2015 (four location-year environments total) for FHB severity (% diseased kernels), heading date (days after planting), and plant height (cm). Offspring of the TP, 831 lines in total, were considered parental candidates. The

TP and all parental candidates were genotyped for 6,361 single nucleotide polymorphism (SNP) markers using genotyping-by-sequencing (GBS). Genetic positions for these markers were obtained by linear interpolation, using 3,072 Barley Oligonucleotide Pool Assay SNP markers as anchors (Close et al., 2009). Observations on the TP were analyzed using univariate mixed-models to estimate genetic variance and heritability and bi-variate mixed-models to estimate genetic correlations.

Using the PopVar packaged in *R* (Mohammadi et al., 2015), we predicted the mean (μ), genetic variance (V_G), superior progeny mean (μ_{sp}), and genetic correlation (r_G) for each of $C(831, 2) = 330{,}072$ possible non-reciprocal crosses. Predictions of the mean, genetic variance, superior progeny mean were made for both FHB severity, heading date, and plant height individually, while pairwise predictions of the genetic correlation were made between these traits. Twenty-seven parent combinations were selected from these predictions. These crosses were made and progeny were advanced to the $F_{3:4}$ stage via single-seed descent. The developed families ranged in size from 28 to 160 lines, with a median of 90 and a total of 2,661 lines across all families. We will refer to these populations as validation families (VF).

The VF were phenotyped in Crookston, MN and St. Paul, MN in 2017 and 2018 (four location-year environments total) for FHB severity, heading date, and plant height. As in the analysis of the TP, we modeled the effect of lines in each family as random in a univariate mixed-model to calculate the observed family mean and genetic variance. The genotype means of lines were used to calculate the observed superior progeny mean in each family for each trait. Bi-variate mixed-models were used to estimate the observed genetic correlation between traits. We measured prediction accuracy as the correlation between the predicted and observed values for the family mean, genetic variance, superior progeny mean and genetic correlation. Bootstrapping (1,000 replicates) was used to calculate a 95% confidence interval about each correlation coefficient.

RESULTS AND DISCUSSION

Estimates of broad sense heritability in the training population (TP) were moderate to high, with $H = 0.45$ for FHB severity, $H = 0.96$ for heading date, and $H = 0.52$ for plant height. Genetic variance was significant for all traits ($P < 6 \times 10^{-7}$; likelihood ratio test), as was genotype-environment interaction variance ($P < 0.05$), particularly for FHB severity ($P < 5 \times 10^{-18}$). These results are consistent with prior heritability estimates for these traits (Hockett and Nilan, 1985; Massman et al., 2011). Genetic correlations varied in magnitude and direction. FHB severity was negatively correlated with heading date ($r_G = -0.99$) and plant height ($r_G = -0.61$). These associations are highly unfavorable (low disease severity, early flowering, and short stature are all ideal), but are consistent with previous observations (Massman et al., 2011). Heading date and plant height were positively correlated ($r_G = 0.38$).

As in previous studies (Bernardo, 2014; Mohammadi et al., 2015; Lado et al., 2017), we observed a triangular relationship between the predicted μ and V_G, where crosses with more extreme μ were accompanied by low V_G, and vice versa (Fig. 1). Among crosses with a common parent, this relationship was approximately linear, as exemplified in Fig. 1. A similar, albeit more complex pattern was observed between the predicted μ for two traits and the predicted r_G (Fig. 2). Crosses with intermediate predicted μ for both traits were often associated with a predicted r_G of higher magnitude. Additionally, we observed that the correlation between predicted μ for two traits reflected the genetic correlation estimated in the TP (Fig. 2). These results highlight the additive nature of the prediction model and the underlying theory that parents with similarly extreme phenotypes will likely share alleles at most QTL influencing a trait (Zhong and Jannink, 2007). Sharing fewer alleles (i.e. at more intermediate phenotypes) is predicted

to beget greater genetic variance within a trait and stronger covariance between traits.

Heritability estimates across all individuals in the validation families (VF) were 0.11 for FHB severity, 0.78 for heading date, and 0.74 for plant height. Family-wise estimates of heritability mirrored these overall estimates, but some variability was present among families. For FHB severity, the mean *H* (and range) was 0.10 (0, 0.28), while that for heading date was 0.49 (0.16, 0.84) and that for plant height was 0.41 (0, 0.76). Genetic and genotype-by-environment variances were significant for all traits ($P < 0.005$; likelihood ratio test), and the relative contribution of these variance components was consistent with observations in the TP.

Estimates of predictive ability are summarized in Tables 1 and 2. The predictive ability for family mean was moderate to high for all traits (r_{MP} = 0.46-0.62). On a per-trait basis, these measurements were consistent with the estimates of heritability in the TP and the VF, where lower heritability corresponded to lower predictive ability, as expected from genomewide selection theory (Daetwyler et al., 2008). The predictive ability for genetic variance was always lower than that for μ, ranging from 0.01 (FHB severity; not significant) to 0.48 (plant height) (Table 1). This is not unexpected, since any error associated with the predicted marker effect will more strongly influence V_G than μ (Zhong and Jannink, 2007; Lado et al., 2017). The predictive abilities for μ_{sp} for FHB severity (r_{MP} = 0.69) and plant height (r_{MP} = 0.62) were greater than those for μ. Finally, the prediction ability for r_G was low to moderate for all trait pairs, ranging from -0.01 (FHB severity and plant height) to 0.41 (heading date and plant height) (Table 2). This trend indicates that the heritability of both traits is important for accurate predictions of r_G.

We have shown that, under typical breeding program conditions, the mean, genetic variance, superior progeny mean, and genetic correlations in potential crosses can be predicted to varying degrees of accuracy using genomewide markers and simulated populations. The favorable results observed for three relevant quantitative traits in barley indicate that this prediction method may be generalized across traits of varying complexity, though we note that heritability, a usual suspect, is again crucial for accurate predictions. With reliable phenotypic data, breeders may use this tool to inform their selection of crosses to improve FHB resistance and other highly complex traits.

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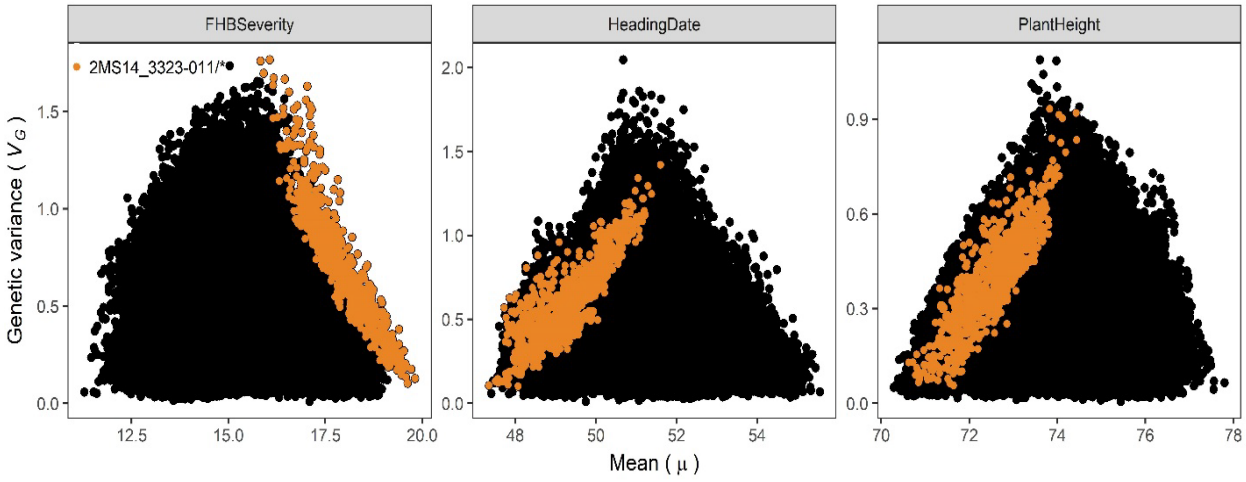

Figure 1. Across 330,072 possible bi-parental crosses and the three focal traits, the relationship between predicted family mean (μ) and genetic variance (VG) formed a triangular pattern. This pattern is composed of many linear relationships within crosses sharing a common parent. For instance, crosses sharing line "2MS14_3323-011" are highlighted as orange points.

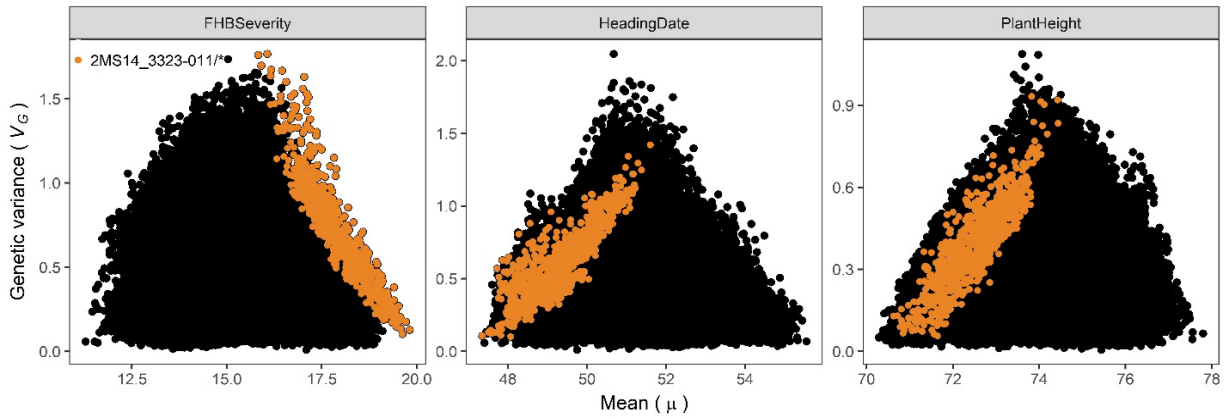

Figure 1. Across 330,072 possible bi-parental crosses and the three focal traits, the relationship between predicted family mean (μ) and genetic variance (VG) formed a triangular pattern. This pattern is composed of many linear relationships within crosses sharing a common parent. For instance, crosses sharing line "2MS14_3323-011" are highlighted as orange points.

Table 1. Estimates (and 95% confidence interval) of the predictive ability for the family mean, genetic variance, and superior progeny mean for FHB severity, heading date, and plant height. Included is the number of families (*N*) used to estimate predictive ability.

		Predictive ability		
Trait	*N*	Family mean (μ)	Genetic variance (V_G)	Superior progeny mean (μ_{sp})
FHB Severity	14	0.46 (0.07, 0.85)	0.01 (-0.36, 0.56)	0.69 (0.28, 0.89)
Heading Date	26	0.62 (0.45, 0.76)	0.39 (0.03, 0.77)	0.56 (0.38, 0.73)
Plant Height	26	0.53 (0.26, 0.74)	0.48 (0.18, 0.7)	0.62 (0.39, 0.8)

Table 2. Estimates (and 95% confidence interval) of the predictive ability for genetic correlations between FHB severity, heading date, and plant height. Included is the number of families (*N*) used to estimate predictive ability.

			Predictive ability
Trait 1	Trait 2	*N*	Genetic correlation
FHB Severity	Heading Date	14	0.24 (-0.30, 0.67)
FHB Severity	Plant Height	14	-0.01 (-0.55, 0.62
Heading Date	Plant Height	26	0.41 (-0.061, 0.71)

VARIETAL RESPONSE OF MALT BARLEY TO FUSARIUM HEAD BLIGHT IN MONTANA

Cecilia Peluola[1], Jamie Sherman[2], Amber Ferda[1], Liz Elmore[2], Bob Brueggerman[3], Paul Schwarz[3] and Frankie Crutcher[1*]

[1]Montana State University, Eastern Agriculture Research Center, Sidney, MT 59270;
[2]Montana State University, Plant Science and Plant Pathology, Bozeman, MT 59715;
and [3] North Dakota State University, Plant Pathology, Fargo, ND 58108
[*]Corresponding Author: PH: 406-433-2208; Email: frankie.crutcher@montana.edu

ABSTRACT

For the last several years, grain has been rejected in different regions of Montana due to high levels of deoxynivalenol (DON) caused by Fusarium head blight (FHB). Although FHB is not a regular problem in Montana, increased corn acreage, no-till practices, increased irrigation and a changing environment have led to epidemics. Varietal development and screening for host resistance is a major tool in mitigating and managing FHB in barley (*Hordeum vulgare*) production. The purpose of this work is to screen progeny from crosses between barley varieties that have more resistance to FHB and elite regional varieties to evaluate for resistance as well as decreased mycotoxin production. An in-field screening trial was set up between April and September 2018. A total of 50 progeny, the parent lines Quest, Kutahya, and Conlon and susceptible variety Conrad were planted. *Fusarium graminearum* infested corn spawn was applied to the field three weeks prior to heading and one application of conidial inoculum was applied at heading. Seedling vigor, disease incidence, severity, *Fusarium* diseased kernels (FDK) and grain yield were assessed. Sufficient precipitation and irrigation favored disease development in this trial. Seven lines (14%) as follows: 39-9, 41-8, 42-17, 43-1, 47-7, 47-8, 81-4, 81-6 and Quest showed significantly lower incidence and severity of FHB. Similarly, eight lines (16%), 41-7, 41-8, 43-1, 45-7, 45-15, 47-7, 69-3, 80-23, and Quest also had lower FDK comparable to resistant line Conlon. Severity of FHB increases with incidence when severity was regressed against incidence $p < 0.001$. For seed production about 30 lines (60%) were comparable with resistant control. Comparison of FHB severity among 26 progenies screened in Fargo and Langdon North Dakota in 2017 with the same progeny tested in Sidney in 2018, showed that eight (31%), nine (34%) and sixteen (61%) lines from Fargo, Langdon and Sidney, respectively, were significantly lower in severity comparable to resistant line Conlon. Out of the 26 progenies, four lines 42-17, 44-3, 44-6 and 45-7 showed comparably lower severities similar to Conlon across the three locations. These results indicated that some of the progeny evaluated may be useful for developing elite regional varieties for control against FHB. Further screening of these lines and repeat trials to confirm this study will be necessary in an effort to establish suitable resistant lines in the state and across the region.

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MAPPING QUANTITATIVE TRAIT LOCI FOR FUSARIUM HEAD BLIGHT IN RIL POPULATION DERIVED FROM HARD WINTER WHEAT EVEREST X OVERLAND

Umara Sahar Rana[1*] and Guihua Bai[2]

[1]Kansas State University, Manhattan, KS 66506; and [2]USDA-ARS,
Hard Winter Wheat Genetics Research Unit, Manhattan KS 66506
[*]Corresponding Author: PH: 785-317-7306; Email: umararana@ksu.edu

ABSTRACT

Fusarium head blight (FHB), caused by *Fusarium graminearum* (Schw), is one of the most important fungal diseases of wheat. FHB drastically reduces not only grain yield but also grain quality due to mycotoxins, in particular deoxynivalenol (DON), that is produced by the pathogen during infection. Consumption of DON contaminated grain is a major health concern for animal and human. Host resistance is the most effective way to combat the disease. Exotic sources of FHB resistance are available for breeding, but poor agronomic traits in those sources prevent direct use of them as parents in breeding. In this study, we used genotyping-by-sequencing (GBS) markers and an $F_{6:7}$ recombinant inbred line (RIL) population developed from a cross between 'Overland' and 'Everest' to identify native quantitative trait loci (QTLs) associated with FHB resistance in the locally adapted hard winter wheat two cultivars. Overland is moderately resistant, and Everest is moderately susceptible to FHB. The RIL population and parents were evaluated for FHB type-II resistance in one field and three greenhouse experiments using randomized complete block design with two replications. The RIL population and parents were genotyped using genotyping-by-sequencing (GBS) markers. Composite interval mapping (CIM) identified three QTLs for resistance to FHB spread within a spike on the chromosome arms 7AS, 3BS and 4BS from Everest and four QTLs on 5AL, 4BS, 4AS and 2DS from Overland. The QTL on the chromosome arm 4BS was consistently significant in the three experiments and showed significant association with a reduced plant height gene. Single nucleotide polymorphism (SNPs) markers tightly linked with QTLs were identified and will be converted into breeder-friendly competitive allele specific PCR (KASP) assays to be used in marker-assisted breeding in wheat.

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WHEAT BREEDING IN SOUTHERN BRAZIL: FOCUSING ON FUSARIUM HEAD BLIGHT RESISTANCE

André Rosa* and Paulo Kuhnem

Biotrigo Genética Ltda., Estrada do Trigo, 1000, Passo Fundo, RS 99025-030, BRAZIL
*Corresponding Author: PH: 55 54 98141-2858; Email: andre@biotrigo.com.br

ABSTRACT

Wheat (*Triticum aestivum*) is one of the most important staple food crops throughout the world. In Brazil, despite the importance of this crop as a staple food and its agri-benefits related to the system, the cultivated area of wheat has declined from over 4 million hectares in the 1990s to approximately 2.1 million hectares per year in the last 5 years, providing just around half of the annual Brazilian consumption (12 million ton). This reduction can be partly explained because of a very challenging disease, Fusarium head blight (FHB). This is one of the major problems of wheat production in Southern Brazil, where most of the production is concentrated. It causes direct losses to yield and quality in addition to the losses caused by deoxynivalenol (DON) contamination. Epidemics have been reported in Southern Brazil approximately every 7 out 10 years, due to the fact that flowering occurs usually under warm and wet conditions favoring FHB development. In this context, FHB resistance is an important trait in any plant breeding program in Southern Brazil. Over the years, progress in FHB resistance in Brazil were made mainly by phenotyping and selection under natural field conditions. This conventional breeding techniques allowed breeders to increase FHB resistance derivated mostly from "native" sources such as the cultivar Frontana (based mainly on type I resistance). Some Chinese e CIMMYT sources such as Sumai#3, NyuBai, Nabeokabozu and Sha/Catbird where also used in the last 50 years by different breeding programs – few genes or QTL from these sources seem to have been kept in the germplasm. Despite of the interesting level of resistance obtained so far, it is not enough to sustain production every year and, much less, to meet the requirements of the newer Brazilian mycotoxin legislation. In the past years, our breeding program has increased its efforts to improve FHB resistance by incorporating "new" genes and QTL (quantitative trait loci), especially *Fhb1*, from different sources, specially from Sumai#3. Marker assistant selection (MAS) was adopted to improve selection for these traits. After some years of crosses and backcrosses, genotyping and phenotyping in the field, some improvement was made, but no cultivar was yet released by this effort. We believe that, to be more successful, we will need to reinforce levels of Type I and II, together. Currently, lines combining "native" sources associated with *Fhb1* and other QTL are under development using natural and artificial infection. We keep getting closer to a commercial release that offers a substantial improvement over a large area. While breeding efforts continue, the increased attention will be given to other tolls that are readily available and poorly used, such as, improved fungicides and the technologies that surround its use.

MARKER VALIDATION FOR FHB RESISTANCE IN DURUM WHEAT

Yuefeng Ruan[1*], Samia Berraies[1], Ehsan Sari[2], Ron E. Knox[1], Richard D. Cuthbert[1], Isabelle Piché[1], Maria Antonia Henriquez[3], Santosh Kumar[4], Andrew J. Burt[5], David J. Konkin[2], Heather L. Campbell[1], Lin Li[1], Raja Ragupathy[1], Wentao Zhang[2] and Pierre R. Fobert[2]

[1]Swift Current Research and Development Centre, Agriculture and Agri-Food Canada, Swift Current, SK, Canada, S9H 3X2; [2]Aquatic and Crop Resource Development Centre, National Research Council Canada, Saskatoon, SK, Canada, S7N, 0W9; [3]Morden Research and Development Centre, Agriculture and Agri-Food Canada, Morden MB, Canada, R6M 1Y5; [4]Brandon Research and Development Centre, Agriculture and Agri-Food Canada, Brandon, MB, Canada, R7A 5Y3; and [5]Ottawa Research and Development Centre, Agriculture and Agri-Food Canada, Ottawa ON, Canada, K1A 0C6
[*]Corresponding Author: PH: 306-770-4504; Email: yuefeng.ruan@canada.ca

ABSTRACT

Fusarium head blight (FHB) is the most serious disease in durum wheat as there is a lack of resistance in adapted breeding germplasm. Marker-assisted breeding has been considered an effective way to increase resistance to FHB by selecting favorable genotypes. The objective of this study was to understand the appropriateness of selecting the favorable alleles of the 3BS QTL (*Fhb1*) from 'Sumai 3', 1A QTL from 'Blackbird' and 2B QTL from 'Strongfield.' A set of 360 advanced breeding lines were screened with one 3BS, three 1A, and four 2B QTL KASP markers. The breeding lines were evaluated for FHB incidence, severity and index calculated based on ratings from artificially inoculated FHB nurseries located near Morden, Brandon, and/or Carman MB in two years. Lines carrying *Fhb1* combined with the 1A QTL and/or 2B QTL showed better FHB resistance than lines carrying only the 1A QTL and/or 2B QTL. *Fhb1* combined with the 2B QTL increased FHB resistance compared to the three QTL combination in the breeding lines. The results of this study justified the use of the *Fhb1* marker for marker assisted selection in breeding. The FHB resistance level could be affected by QTL combination. The selection of lines carrying *Fhb1* either combined with the 1A QTL or 2B QTL will enhance FHB resistance in durum wheat.

QFHS.IFA-5A AND *QFHB.RWG-5A.1* - ALLELES OF THE SAME CHROMOSOME 5AS LOCUS?

Hongbin Tao* and Francois Marais

Department of Plant Sciences, North Dakota State University, ND 58102
*Corresponding Author: PH: 701-231-8169; E-mail: hongbin.tao@ndsu.edu

ABSTRACT

The NDSU HRWW breeding program is transferring FHB resistance from spring wheat (CM82036 and PI277102) into winter wheat germplasm. Both donor sources carry chromosome 5AS resistance QTL, i.e. *Qfhs.ifa-5A* (CM82036) and *Qfhb.rwg-5A.1* (PI277012). Published genetic maps suggest that the broad chromosome regions containing *Qfhs.ifa-5A* and *Qfhb.rwg-5A.1* overlap; however, the exact relationship between the two loci is not clear. From a breeding perspective, we wished to determine whether we should continue to pursue the two loci as different breeding targets (both have already been incorporated into winter wheat). Therefore, cross 15K353 was made between winter wheat breeding lines Novus-4 (homozygous *Qfhb.rwg-5A.1*) and 14K456-K-1 (homozygous *Fhb1* and *Qfhs.ifa-5A*). (a) Eighteen F_2's that were homozygous for *Fhb1* plus *Qfhs.ifa-5A* (detected using the *Xbarc186-1* allele from 14K456-K-1), and (b) 16 F_2's that were homozygous for *Fhb1* plus the *Xbarc186-2* allele from Novus-4, were selected. Four F_3 plants were sampled per family (total = 144) for 9K SNP analyses. Polymorphic SNP markers were identified using GenomeStudio Genotyping Module V 1.0. With respect to the 144 F_4 sub-families, 4 replications consisting of 5 plants each were included in a greenhouse FHB trial to test type II resistance. SNP haplotype analysis showed no indication that a (Novus-4 derived) resistance QTL located outside the general *Qfhs.ifa-5A* region contributed to the resistance of the best performing lines. Moreover, frequency distributions of infection percentages of the two groups (*Fhb1* plus *Xbarc-186-1*, and *Fhb1* plus *Xbarc186-2*) were very similar with mean values of 23.4% and 24.3%, respectively. We also did not find recombinants that were more resistant than *Fhb1* in combination with either locus. It appears that each of the two 5AS QTL complements the *Fhb1* resistance and to the same extent. Thus, the genes appear similar enough not to merit their individual selection.

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GWAS FOR FUSARIUM HEAD BLIGHT TRAITS IN THE ELITE EASTERN WHEAT MAPPING PANEL

Elisane Tessmann and David Van Sanford*

University of Kentucky, Department of Plant and Soil Science, Lexington, KY, 40546
*Corresponding Author: PH: 859-338-2409; Email: dvs@uky.edu

ABSTRACT

Fusarium head blight (FHB) is an important disease in wheat (*Triticum aestivum*) and other small grains, causing billions of dollars in losses in the past decades. *Fusarium graminearum* is the causal agent and symptoms such as shriveled grains, reduction in grain weight and yield and toxin production are observed for this disease. Despite an extensive breeding effort, there are no wheat cultivars completely resistant to FHB. Multiple loci are involved in conferring resistance which is further complicated by environmental conditions. The objectives of this study were to: (i) evaluate phenotypic response to FHB in a large diverse soft red winter wheat (SRWW) mapping panel; and (ii) identify promising QTL associated with FHB resistance based on a genome wide association study (GWAS). During the growing seasons of 2014-2015 and 2015-2016, 256 cultivars and breeding lines were grown in an irrigated and inoculated scab nursery near Lexington, KY. Traits evaluated were: heading date (Julian), plant height (cm), FHB rating (0 – 9), severity (%), incidence (%), index (%), *Fusarium* damaged kernel (FDK, %) and deoxynivalenol (DON, ppm). There were significant ($p < 0.05$) differences among genotypes for all traits evaluated. Overall, disease levels were lower in 2016 than in 2015, with the exception of DON with higher levels in 2016. Broad sense heritability values were relatively high for FDK (0.69) and DON (0.77). GWAS identified 16 significant ($p < 0.001$) SNPs associated with FHB traits on multiple chromosomes. SNP effects ranged from -2.14 to 4.01% of the mean of a given trait. For FDK and DON, SNPs were detected on chromosomes 4A, 5B and 6B that were associated with DON reductions of 1.3, 1.9 and 3.1 ppm, respectively. Our study demonstrated that even small-effect QTL can potentially decrease disease levels and thus be useful in breeding programs.

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EVALUATION FOR FUSARIUM HEAD BLIGHT (SCAB) RESISTANCE BY DETACHED LEAF ASSAY IN BACKCROSS POPULATIONS OF WHEAT

Fang Wang[1], Vikas Belamkar[1], Stephen Wegulo[2] and P. Stephen Baenziger[1*]

[1]University of Nebraska-Lincoln, Lincoln, NE 68583-0915; and
[2]University of Nebraska-Lincoln, Lincoln, NE 68583-0722
[*]Corresponding Author: PH: 402-472-1538, Email: pbaenziger1@unl.edu

ABSTRACT

Fusarium head blight (Scab), mainly caused by *Fusarium graminearum*, is a destructive disease of wheat in the humid and semi-humid areas worldwide. *Fhb1* is a major QTL explaining up to 60% of the phenotypic variation. Backcross breeding is an effective strategy to improve FHB resistance to identify native resistant lines followed by the introgression of major FHB QTLs to elite lines. In our research, eight backcross populations were constructed with elite NE winter wheat lines and donor parent (Overland_Fhb10 which contains the *Fhb1* gene). In addition, as part of our efforts to incorporate genomic selection and genome wide association studies to produce scab tolerant cultivars, we have studied the detached leaf assay to evaluate our wheat genotypes for scab resistance. The winter wheat resistance by detached leaf assay was generally consistent with spike inoculation (except for 'Camelot' and 'Freeman', both susceptible, but reversed in the detached leaf assay). The result of spring wheat was with greater resistance reversed between detached leaf assay and spike inoculation. On the basis of these results, for genomic selection we have decided to expand our adult plant phenotypic efforts to provide the necessary phenotypic data to build our genomic selection training datasets and algorithms, while continuing to study the detached leaf assay to determine if it can provide additional information that may be valuable to reduce the devastating effects of scab in hard winter wheat.

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The line Overland_FHB10 was developed in a cooperative HWWCP project with Dr. Guihua Bai, USDA-ARS, Manhattan, KS and is the source of *Fhb1* in an adapted background for this research. Dr. Mary Guttieri, USDA-ARS, Manhattan, KS, was also instrumental in purifying Overland_FHB10 for general release to the wheat breeding community.

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GENOTYPING-BY-SEQUENCING MARKERS FOR DETECTING ALLELES OF THE *FHB1* QUANTITATIVE TRAIT LOCUS IN SOFT WINTER WHEAT

Brian P. Ward[1*], Gina Brown-Guedira[2] and J. Paul Murphy[1]

[1]Department of Crop Science, North Carolina State University, Raleigh, NC 27695;
and [2]USDA–ARS Plant Science Research Unit, Raleigh, NC 27695
[*]Corresponding Author: PH: 919-513-7926; Email: bpward2@ncsu.edu

ABSTRACT

The *Fhb1* QTL, first described in Chinese wheat cultivar 'Sumai 3', produces the most stable and effective Type II resistance to Fusarium head blight of the 50 or more resistance QTLs discovered to date. Recently, two candidate genes within *Fhb1* have been described. One, *TaHRC*, is a putative histidine-rich calcium binding protein. The other, *PFT*, is a pore-forming toxin-like protein, structurally similar to agglutinin. Cultivar Chinese Spring contains an inactive version of *Fhb1*. Currently, the USDA-ARS Eastern Regional Small Grains Genotyping Laboratory performs regular genome-wide genotyping using genotyping-by-sequencing (GBS) markers, but must perform a separate targeted assay using LGC Genomics KASP technology to determine the presence or absence of *Fhb1*. We sought to determine whether GBS markers could serve as a proxy for the current KASP assay. We identified the location of the inactive *Fhb1* locus in the recently-published Chinese Spring reference genome, spanning a region from approximately 8Mb to 10Mb on the short arm of chromosome 3B. We then performed GBS genotyping on a panel of 209 Eastern soft winter wheat lines consisting of half *Fhb1*-positive and half *Fhb1*-wild-type lines, appending the *Fhb1* sequence from CM-82036 to the Chinese Spring genome. Although it has been reported that *Fhb1* is more similar in gene content and arrangement to portions of Chinese Spring chromosome 3D, we found no SNPs on 3D that were in significant LD with the current KASP assay. The most predictive SNPs were located in a cluster from approximately 10Mb to 10.7Mb on 3B, and exhibited r^2 values with the current KASP assay of between 0.7 and 0.8. This cluster of SNPs was not in close proximity to any genes sharing high sequence similarity to the genes present on the CM-82036 copy of *Fhb1*. One SNP was located within intron 2 of the Chinese Spring copy of *TaHRC*, though it exhibited low LD ($r^2 = 0.33$) with the KASP assay. On the CM-82036 copy of *Fhb1*, the most predictive GBS SNPs ($r^2 \approx 0.74$) were exonic within a putative cytochrome P450 enzyme, and within a putative tRNA (guanine-N1)-methyltransferase. While no GBS SNP was perfectly predictive of the current KASP assay, the results indicate that haplotypes of multiple SNPs may be viable as suitable proxies for the current assay.

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MAPPING QUANTITATIVE TRAIT LOCI FOR FUSARIUM HEAD BLIGHT RESISTANCE IN A CHINESE WHEAT POPULATION YANGMAI 158 X ZHENGMAI 9023

Pingping Zhang[1,2], Chenjin Guo[1,3], Amy Bernardo[1], Hongxiang Ma[2] and Guihua Bai[1,4*]

[1]Department of Agronomy, Kansas State University, Manhattan, KS 66506; [2]Jiangsu Academy of Agricultural Sciences, Nanjing, Jiangsu 210014, China; [3]Hebei Agricultural University, Baoding, Hebei 071001, China; and [4]USDA-ARS, Hard Winter Wheat Genetics Research Unit, Manhattan KS 66506
[*]Corresponding Author: PH: 785-532-1124; Email: Guihua.bai@ars.usda.gov

ABSTRACT

Fusarium head blight (FHB) is one of the prevalent fungal diseases of wheat worldwide. Its epidemics can cause significant reduction in grain yield and quality. Wheat FHB resistance is a quantitative trait. To date, many quantitative trait loci (QTLs) for FHB resistance have been located on all 21 chromosomes. Only *Fhb1* on chromosome 3B consistently showed a stable major effect and explained 10-50% of the phenotypic variance for type II resistance across different genetic backgrounds and testing environments. However, *Fhb1* alone is not sufficient for preventing FHB damage in severe FHB epidemic years. Thus, pyramiding *Fhb1* with other FHB resistance QTLs in adapted genetic backgrounds can achieve acceptable level of FHB protection and other desirable agronomic traits. Exploring new FHB resistance QTLs in locally adapted wheat cultivars is a critical step for such pyramiding. Some Chinese winter wheat cultivars showed a moderate level of FHB resistance, but QTLs in those adapted cultivars remain unknown. In this study, we developed a population of 231 $F_{2:7}$ recombinant inbred lines (RILs) using two popular Chinese wheat cultivars with moderate FHB resistance, 'Yangmai 158' and 'Zhengmai 9023', to identify the QTLs for type II FHB resistance. The population was evaluated for FHB resistance in two greenhouse experiments using single floret injection. Percentage of symptomatic spikelets per spike (PSS) were recorded for each inoculated spike on the 15th day after inoculation. Genomic DNA was extracted from leaf tissue at the three-leaf stage, and genotyping-by-sequencing (GBS) libraries were constructed using MspI and PstI restriction enzymes and sequenced in an Ion Proton sequencer. Single nucleotide polymorphism (SNP) markers were called using a TASSEL pipeline. A SNP linkage map was constructed using JoinMap v4.0 for QTL analysis using WinQTLCart v2.5. Mean PSS of Yangmai 158 and Zhengmai 9023 were 33.2% and 28.4%, respectively. Frequency distribution of PSS from the RIL population is continuous and shown obvious transgressive segregation, suggesting both parents contributed resistance alleles. A linkage map was constructed with 1,067 SNPs. Using the map, seven QTLs for FHB resistance were mapped, and four of them were repeatable in both experiments. Zhengmai 9023 contributed the resistance alleles at two repeatable QTLs, QTL.4A1 and QTL.7D, whereas Yangmai 158 contributed the two other repeatable QTLs, QTL.3A and QTL.2D1. Replacement of single resistance alleles at each locus of the four repeatable FHB resistance QTLs significantly decreased PSS. QTLs from the two parents appear to be additive and accumulation of the resistance alleles from all four repeatable QTLs showed the highest resistance. Thus, pyramiding those QTLs could significantly improve FHB resistance of new cultivars.

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