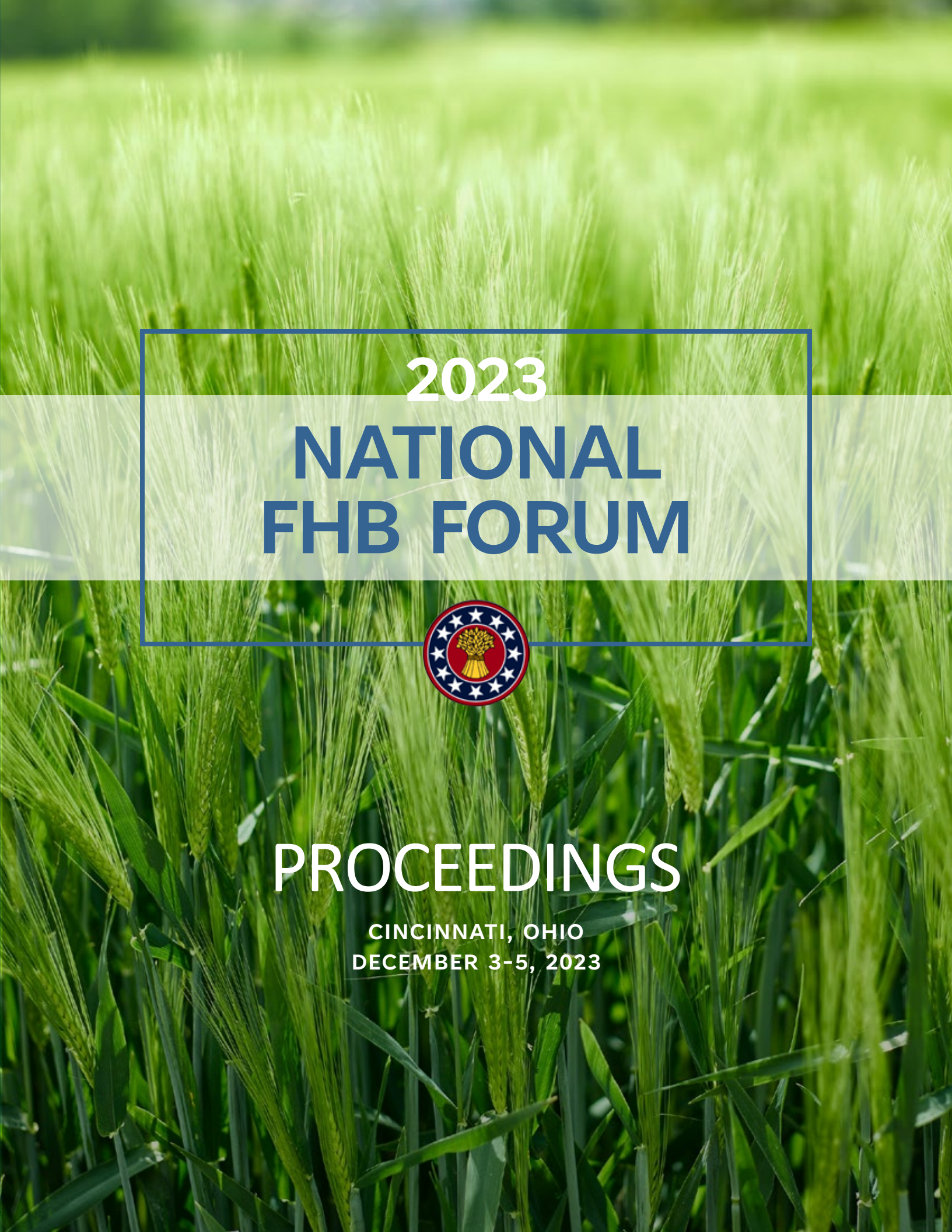

2023 NATIONAL FHB FORUM

PROCEEDINGS

CINCINNATI, OHIO
DECEMBER 3–5, 2023

Publication Title: *Proceedings of the 2023 National Fusarium Head Blight Forum*
Proceedings complied and edited by the USWBSI Networking & Facilitation Office (NFO)

Copies of this publication may be viewed at https://scabusa.org/forum23

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495 Borlaug Hall | 1991 Upper Buford Circle | St. Paul, MN 55108
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Opening Session Presentations

Thoughts from a *Fusarium* Rookie

Dan Anderson[1]
1. Anderson Wheat Farms, Haxtun, CO
Corresponding Author: Dan Anderson, dla.awf@pctelcom.coop

Abstract
Anderson Wheat Farms operates out of Haxtun, Colorado, providing high quality wheat seed to the state, region, and country. We are a certified seed dealer of PlainsGold wheat varieties developed by Colorado State University, as well as Kansas Wheat Alliance developed by Kansas State University, and Westbred. There are many factors that limit grain yield and profitability of wheat in Colorado. On a yearly basis, drought stress is more of a certainty than not, and stripe rust, other rust pathogens, and viruses vectored by wheat curl mite, can all have economic impact if not properly managed through resistant varieties or chemical and cultural control. Over the last decade, the wheat stem sawfly, has taken a toll on Colorado wheat, causing significant yield loss, lodging, and loss of residue for soil and water retention in our no-till system, with greater than $50 million in economic impact annually. The adoption of semi-solid stemmed varieties made available through significant breeding efforts by public and private programs has helped to reduce its impact. But with every year comes something new and in 2023, that something was Fusarium head blight. Colorado plants just north of 2 million acres of wheat per year, with approximately a third of those acres sown directly into corn residue. However, with a statewide average precipitation of less than 6 inches during the critical months of May-July, the environment is rarely conducive to FHB development. Near record rainfall was recorded during the late spring of 2023, with the 8th wettest June on record providing ideal conditions for the development of scab during flowering. This wet pattern continued into the summer, extending the growing season, and providing more time for scab development. However, it was not until post-harvest that the impacts of scab became most apparent, both at the elevator and from a seed certification perspective, where FHB impacted seed germination and required additional conditioning and seed treatment to improve seed viability. A season like this left this wheat grower with more questions than answers. This presentation will lay out my experience with scab, lessons learned, and questions to be answered.

Impact of *Fusarium* and DON on the Global Import/Export of Wheat

Dalton Henry[1]
1. Vice President of Policy, U.S. Wheat Associates, Arlington, VA
Corresponding Author: Dalton Henry, dhenry@uswheat.org

Abstract
The presence of DON is widely regulated by import and human health agencies around the world – who are often under pressure from domestic groups including consumer advocates and farm organizations to implement strict limits and, in some cases, regular testing. As an organization working to promote U.S. grown wheat, U.S. Wheat Associates regularly interfaces with regulators and importing country industry groups to help facilitate trade and minimize the impact of DON regulations on global wheat trade. Coordination across the U.S. wheat industry is a must to minimize those impacts and to protect free and open flow of wheat from producers to consumers.

Transformational Science Presentations

The Impact of Environment, Host Genotype and Fusarium Head Blight on Microbiome Assembly in a Barley Breeding Population

Barney A. Geddes[1], Brooke R. Benz[2], Joan Acaso[1], Abbeah R. Navasca[2], Diel D. Velasco[2], Eglantina Lopez Echartea[1], Briana Whitaker[3], and Thomas Baldwin[2]

1. North Dakota State University, Department of Plant Pathology, Fargo, ND
2. North Dakota State University, Department of Microbiology, Fargo, ND
3. USDA, Agricultural Research Service, National Center for Agricultural Utilization Research, Mycotoxin Prevention and Applied Microbiology Research Unit, Peoria, IL

*Corresponding Author*s: Barney Geddes, barney.geddes@ndsu.edu; Briana Whitaker, briana.whitaker@usda.gov

Abstract

There is an emerging understanding that plants under biotic stress can recruit beneficial microbes in a "cry for help" strategy, and there is ample evidence that this is occurring in the case of *Fusarium*. Disease-recruited microbes in response to *Fusarium* pathogens have proven to be readily culturable and potent biocontrol agents in laboratory and greenhouse settings. Unfortunately, despite decades of work towards deploying microbes as biocontrol agents, promising laboratory and greenhouse studies have proven to be difficult to translate to the field. To overcome this limitation, we were funded by the USWBSI Transformational Sciences program to identify biocontrol microbes that are both recruited under Fusarium head blight (FHB) biotic stress and responsive to plant genotype. Our long-term goal is to use an understanding of these ecological interactions to develop an integrated management strategy leveraging microbiome-based approaches to reduce FHB on barley. The ability to breed crops with an elite ability to recruit protective microbes or microbial communities against FHB would be transformative to modern agriculture and could act in synergy with the application of biocontrols. In this presentation, I will discuss results from our massive amplicon-based metagenomic profiling of barley heads under disease pressure from FHB. This study involved sampling approximately 800 diseased and non-diseased barley heads from ten random training population genotypes grown in four nurseries across the U.S. (Fargo ND, Ithaca NY, St. Paul MN and Kimberly ID). Amplicon sequencing profiles of 16S and ITS were used to assess the bacterial and fungal microbial community compositions, respectively. Overall, our data indicates a significant response of the microbial communities to both barley genotype and disease, supporting our initial rational. It also enables us to establish the core microbiome from barley heads in diseased and non-diseased states. I will also discuss ongoing and future directions of our research to: 1) design metagenomics-informed, screenable markers for breeding to improve FHB-antagonistic microbiome associations, 2) establish a diseased and non-diseased barley spike microbiome culture collection, and 3) develop experimentally-tractable Synthetic Communities to unravel the rules of barley microbiome assembly in the context of disease.

Rover Based Field Detection and Quantification of FHB

Cory D. Hirsch[1]
1. University of Minnesota, Department of Plant Pathology, Saint Paul, MN
Corresponding Author: Corey Hirsch, cdhirsch@umn.edu

Abstract
Fusarium head blight (FHB) is an economically impactful fungal pathogen of wheat. Although there are numerous strategies to limit the effects of this disease, one important approach is to develop resistant varieties. Currently, phenotyping is a limiting step in the development of resistant varieties as it requires a lot of labor, time, is not standardized across the community, and is subjective. We aimed to reduce these phenotyping limitations by using a novel, high-throughput phenotyping rover to capture in-field RGB images of inoculated wheat spikes. The images acquired from the rover were manually annotated for spikes and regions of pathogen symptoms on the spikes and used in deep neural networks to locate and quantify FHB on each spike. The inference models were validated at the spike and plot level with manual disease scores from five raters on images and in the field. The combination of the rover and image models exceeded conventional rating methods and can reduce many of the limitations of conventional FHB phenotyping. The plot level scores from in-field raters and from image-based inferences were strongly correlated. Likewise, a high correlation was found in comparisons of image-based individual spike FHB disease scores from the models and raters. The developed image-based FHB disease detection methods continued to perform well across environments, image types, and levels of disease. These results show that current field FHB phenotyping methods can be improved through precise and efficient quantification of disease symptoms on a plot and individual spike throughout the growing season.

Haplotype-Informed Associations of FHB Resistance in U.S. Wheat Breeding

Katherine W. Jordan[1], Lawrence Tidakbi[2], Jessica Rupp[2], and Guihua Bai[1]
1. USDA-ARS, Manhattan, KS
2. Kansas State University, Department of Plant Pathology, Manhattan, KS
Corresponding Author: Katherine W. Jordan, katherine.jordan@usda.gov

Abstract
In conjunction with wheat breeders from the Great Plains and Eastern regions of the United States, we have assembled an association mapping panel of ~290 well-adapted cultivars and advanced breeding lines. This panel consists of mostly winter wheat varieties (both hard and soft), but also includes some spring and synthetic varieties. We sequenced this panel to ~15x coverage with wheat exome capture and used the genomic data to construct a wheat practical haplotype graph (PHG) representing the diversity in the panel. More than 1 million high quality segregating variants (MAF > 1%) were identified and combined with two years of field data measured at the Kansas State University FHB research station in Manhattan, Kansas. Initial GWAS results reveal ~50 significant associations across many wheat chromosomes for FHB severity, area under the disease progressive curve (AUDPC), FDK, and DON accumulation, including both known and novel regions for winter wheat associations. Validation of these associations is underway while we are waiting for a third year of field phenotypes. Descriptions of the panel makeup, PHG database construction, initial results, and how we as a community can use this data will be presented.

Marker-Free Transfer of *Fhb7* to Barley

Wanlong Li[1]
1. South Dakota State University, Brookings, SD
Corresponding Author: Wanlong Li, Wanlong.li@sdstate.edu

Abstract
Scab is a devastating disease in wheat and barley. Significant progress has been made in understanding and improving host resistance in wheat with molecular cloning of the major QTL *Fhb1* and *Fhb7*; however, similar research with barley has lagged due to the lack of highly resistant genotypes, which makes it difficult to effectively control FHB and DON contamination. Supported by the USWBSI-TSCI program, we are developing marker-free transfer of *Fhb7*, encoding a glutathione S-transferase functioning in the detoxification of mycotoxins including DON, to barley via CRISPR-mediated targeted gene insertion. We first developed an all-in-one construct including the CRISPR/Cas9, sgRNA targeting the *mlo* locus, and *Fhb7* donor DNA and transformed into 'Golden Promise' by *Agrobacterium* mediation, which produced 39 transgenic plants. Detached leaf assay showed *Fhb7* function in resistance to *Fusarium graminearum*, but no targeted insertions were detected in the T1 populations possibly due to low copy number of the donor DNA. Subsequently, we use chemically modified *Fhb7* donor DNA and a CRISPR–Cas9 construct targeting the *mlo* locus to transform the immature embryos of the elite two-rowed malting barley cultivar 'Excelsior Gold.' From about 300 T0 plants, we identified 13 *Fhb7* insertion lines. The *Fhb7* gene transmitted to the T1 generation. We are in the process of characterizing the insertion junctions. I will discuss the detailed progress of this TSCI project in my presentation.

Graphene Quantum Dots (GQDs) Mediated dsRNA Delivery for the Control of Fusarium Head Blight in Wheat

Binod Gyawali[1], Rahim Rahimi[2], and Mohsen Mohammadi[1]
1. Department of Agronomy, Purdue University, West Lafayette, IN
2. School of Materials Engineering, Purdue University, West Lafayette, IN
Corresponding Author: Mohsen Mohammadi, mohamm20@purdue.edu

Abstract
Spray-induced gene silencing (SIGS) is a non-transgenic RNA interference (RNAi) strategy used for the control of various pathogen and pest. *Fusarium graminearum* (*Fg*) is the pathogen responsible for Fusarium head blight (FHB) disease in wheat. We aimed to silence those genes of *Fg* which are responsible for the growth and pathogenicity. Given the instability of RNA molecules, the hypothesis is that the nanoparticle coating offers stability and slow-release to double-stranded RNA (dsRNA) molecules with continuous inhibition of *Fg* during the critical time of infection. In this study, we designed native and synthetic dsRNA against MGV1 and RAS1, and six other genes, by using pssRNAit web server. The synthetized segments were cloned into L4440 double-T7 plasmid and in-vivo dsRNAs were produced in the RNAase III-deficient *E. coli* strain HT115(DE3). GQDs were synthesized by pyrolyzing citric acid and they were surface functionalized by using branched polyethyleneimine (bPEI). After dsRNA-GQDs interaction testing by gel shifting assay, we observed that the presence of dsRAS1 and dsMGV1 in PDA media can restrict the growth of *Fg* on plate. In addition, dsMGV1+GQDs and dsRAS1+GQDs spray restricted infection symptoms on intact wheat spikes, evidenced by reductions in percent symptomatic spikelets (PSS). For example, H2O+GQDs control resulted in up to 100% PSS while synthetic dsMGV1+GQDs reduced PSS to ~35%. In a very recent study, involving dsRNA against eight genes including MGV1, RAS1, COT1, FgPp2A (responsible for growth and development of *Fg*), CAK1, TRI5, GMK1, and YCK1 (responsible for pathogenicity of *Fg*), we observed that when all dsRNAs are mixed and applied on spike as cocktail post-inoculation, the PSS significantly reduced to 20% compared with 100% PSS observed in control.

FHB Management

Evaluation of Organic Fungicides Plus Cultivar Resistance to Reduce FHB and DON Infection of Barley in Vermont

Heather Darby [1] and Hillary Emick [1]
1. University of Vermont State and Agricultural College, Burlington, VT
Corresponding Author: Heather Darby, heather.darby@uvm.edu

Abstract
Public interest in sourcing local foods has extended into beverages leading to a rapid expansion of the northeast malting industry. This has provided farmers with new market opportunities and many of these markets are interested in purchasing certified organic barley. However, all farmers are struggling to produce barley that is not infected with Fusarium head blight (FHB) and the mycotoxin deoxynivalenol (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. There has been little to no research conducted to evaluate organic approved fungicides. In Vermont during 2021 we observed the disease and yield impact of cultivar susceptibility, inoculation with *Fusarium graminearum*, and treatment with fungicides (organic and conventional comparison) at two timings. Overall, the growing season was dry and hot leading to low DON concentrations in the trial. The organic fungicides were not as effective as the conventional fungicide applied at heading. However, all fungicides provided enhanced yields compared to the *Fusarium* inoculated control. Additional research should be conducted to assess the efficacy of multiple applications of copper-based fungicide on FHB.

Objective
To evaluate the individual and interactive effects of moderately resistant cultivars and application timings of two organic 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 leading to a rapid expansion of the northeast malting industry. This has provided farmers with new market opportunities and many of these markets are interested in purchasing certified organic barley. However, all 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. There has been little to no research conducted to evaluate organic approved fungicides. 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 2012 we observed the disease and yield impact of cultivar susceptibility, inoculation with *Fusarium graminearum*, and treatment with fungicides (organic and conventional comparison) at two application times.

Materials and Methods
The trial was conducted in Alburgh, VT during 2021. The soil type was a Benson silt loam soil. The plot size was 5 x 20 ft including seven rows with 7-in spacing. Planting occurred April 9, 2021. Main plots were sown with barley at 125 lb ac^{-1} with a Great Plains grain drill (Salinas, KS). 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. The two spring barley varieties were 'Robust' (susceptible to FHB) and 'ND Genesis' (moderately resistant to FHB). Fungicide treatments are shown in Table 1. The first fungicide application (with surfactant at 0.125% V/V) was applied at heading (Feekes growth stage, FGS 10.1) on June 16, 2021. 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 ChampION fungicide was the only treatment applied at heading, 4 days after heading, and both at heading and 4 days after heading. Fungicide and *F. graminearum* treatments were applied with a CO_2 backpack sprayer with paired TJ-60 8003vs nozzles mounted at an angle (30^o from horizontal) forward and backward, 20-in. apart, pressurized at 30 psi, and calibrated to deliver 20 gal/A. Grain was harvested using an Almaco plot combine (Nevada, IA). Grain moisture, plot yield, and test weight were recorded. Yield and test weight were adjusted to bushels ac^{-1} at 13.5% moisture. Deoxynivalenol (DON) concentrations were analyzed at the McMaster lab at Virginia Tech on an Agilent 6890N / 5975 GC/MS. This method has a detection range of from 0.025ppm – 15ppm. Treatment means were calculated, subjected to analysis of variance, and separated by Fisher's protected LSD test (P = 0.05).

Results and Discussion

Interactions
There were no variety by fungicide treatment interactions indicating that the treatments responded similarly regardless of the variety (Table 2).

The growing season was warmer than normal overall, although the month of July was cooler than average. There was a surplus of growing degree days early in the season and a deficit in July, resulting in a season just 36 growing degree days above normal. There was 4.99 inches less precipitation than normal. Low precipitation through heading and flowering stages resulted in low fusarium infection rates and DON concentrations in 2021.

All treatments and timings, including the control and the Fusarium inoculated plots, had average DON concentrations below the 1 ppm threshold recommended by the FDA. However, there were significant differences observed in DON concentrations for the fungicide treatments (Table 2). The highest DON concentrations in the trial were in the Regalia treatment (applied at heading) at 0.925 ppm but statistically similar to the ChampION treatments applied at heading or 4 days after heading. ChampIon applied at heading and again 4 days after heading had lower DON concentrations compared to the other organic fungicide treatments. The conventional fungicide, Miravis had the lowest DON concentration of all fungicide treatments (0.290 ppm) and was the only treatment statistically lower than the *Fusarium* inoculated control. Interestingly, the application of fungicides increased yields significantly compared to the Fusarium inoculated control (Table 4).

The barley varieties performed similarly in DON concentrations but differed significantly in yield and test weight (Table 2). The DON concentrations for both varieties were below the 1 ppm recommendation.

Overall, the growing season was dry and hot leading to low DON concentrations in the trial. The organic fungicides were not as effective as the conventional fungicide applied at heading. However, all fungicides provided enhanced yields compared to the Fusarium inoculated control. Additional research should be conducted to assess the efficacy of multiple applications of copper-based fungicide on FHB.

Acknowledgement and Disclaimer
This material is based upon work supported in part by the U.S. Department of Agriculture under agreement No. 59-0206-0-141. This is a cooperative project with the U.S. Wheat & Barley Scab Initiative. Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the author(s) and do not necessarily reflect the view of the U.S. Department of Agriculture.

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

Fungicide treatments	Company	Fungicide active ingredient	Application rates
Control			Water
Fusarium graminearum			40,000 spores/ml
Champ ION^{++}	NuFarm	Copper hydroxide	1.5 lbs ac^{-1}
Regalia Biofungicide	Morrone Bio Innovations	*Reynoutria sachalinensis*	2.0 qts ac^{-1}
Miravis	Syngenta	*Pydiflumetofen*	13.7 fl oz ac^{-1}

Table 2. Statistical significance of treatment effects on DON, test weight, and yield of barley.

Source of variation	DON	Test weight	Yield
Variety	NS†	***	***
Fungicide + timing	***	NS	*
Variety x fungicide + timing	NS	***	NS

†statistical significance - ***, p=0.001; **, p= 0.01; *, p= 0.05; NS, not significant.

Table 3. Main effect of cultivar on deoxynivalenol (DON) concentration, test weight, and grain yield at Alburgh, VT, 2021.

Variety	DON	Test weight	Yield
	ppm	lb bu^{-1}	bu ac^{-1}
ND Genesis (moderately resistant)	0.670	47.8	89.8
Robust (susceptible)	0.570	44.0	76.5
LSD (p=0.05) †	NS‡	1.28	5.26

†LSD; least significant difference at p-value = 0.05.
‡NS; no significant difference.

Table 4. Main effect of fungicide + timing on deoxynivalenol (DON) contamination and grain yield at Alburgh, VT, 2021.

Fungicide + timing	DON	Test weight	Yield
	ppm	lb bu^{-1}	bu ac^{-1}
Non-sprayed, non-inoculated control	0.165	45.4	80.3
Inoculated FGS at heading	0.650	44.4	72.4
ChampION at heading	0.800	47.4	83.8
ChampION 4 days after heading	0.895	44.7	85.5
ChampION at heading plus 4 days after heading	0.630	45.6	83.8
Regalia Biofungicide at heading	0.925	45.7	90.3
Miravis at heading	0.290	46.5	86.0
LSD (p=0.05) †	0.190	NS‡	9.83

†LSD; least significant difference at p-value = 0.05.
‡NS; no significant difference.

Update on National Efforts to Improve FHB Forecasting with Model Ensembles and Machine Learning Techniques

Erick De Wolf [1]
1. Kansas State University, Department of Plant Pathology, Manhattan, KS
Corresponding Author: Erick De Wolf, dewolf1@ksu.edu

Abstract
The multistate effort to forecast epidemics of Fusarium head blight (FHB) in wheat and barley has made considerable progress in recent years. Collaborative work between the disease FHB Management Coordinated Project has expanded the dataset available for modeling to more than 1,200 cases. These observations incorporate information from additional production environments. This information has enabled the U.S. to expand the forecasting system for use in additional states, and account for advances in variety development. The continued effort to gather new observations also helps ensure the long-term stability of the forecasting models within changing climates. Recent advances in model development focused on building ensembles of predictive models based on Random Forests (RF) machine learning algorithms. This approach resulted in models that improved the overall prediction accuracy of the forecasts relative to previous generations of modeling. The RF modeling approach yielded multiple models with sensitivity and specificity greater than 80%. The RF models also provided useful insights into weather patterns that favor the development of FHB epidemics. For example, variables describing the stability of temperature prior to crop anthesis were the most commonly selected by the RF models. Consistent with previous modeling efforts, variables summarizing atmospheric moisture, such as relative humidity, dew point and vapor pressure deficit, were identified as critical predictors of FHB epidemics. There were also important advances with the web-based tools used to deploy the forecasting models including features that better display regional commentary of disease specialists along with the maps of disease risk. Features that enable users to visualize local weather conditions and trends in the disease risk will be available for the 2024 growing season. Recent user surveys of the forecasting system helped document the value of the information to small grain producers in the United States. These surveys indicated that 89% of the users thought that the information improved the profitability of their farm. Users reported that the forecasting system helped them avoid unnecessary fungicide applications and estimated that the value of the information exceeded $70 million annually.

Screening for FHB-Resistance in Barley Lines Adaptable for South Dakota

Tasneem Fathima [1], Tapish Pawar [1], Sunish K. Sehgal [1], Christopher Graham [1], Jose L. Gonzalez-Hernandez [1], Shaukat Ali [1], Shyam Solanki [1] and Gazala Ameen [1]
1. Department of Agronomy, Horticulture & Plant Science, South Dakota State University, Brookings, SD
Corresponding Author: Gazala Ameen, Gazala.Ameen@sdstate.edu

Abstract
Fusarium Head Blight (FHB) is a globally destructive disease that affects small grains, causing significant yield and quality losses. South Dakota ranks as the 12th barley-producing state in the US. FHB is one of the major diseases that have significantly reduced barley production in the Midwestern United States. However, the recent reports of the Brewers Association suggest a six percent dollar growth in the market share of craft beer, which has escalated the demand for barley within the brewing industry. This shift has placed significant emphasis on developing FHB-resistant barley cultivars with reduced accumulation of mycotoxins, to meet the stringent requirements of the brewing industry. In this comprehensive study, a set of 29 spring barley genotypes, sourced from public and private breeding programs across the United States, underwent screening for FHB resistance. A comparative analysis of key factors, including disease severity, Fusarium Damaged Kernels, and accumulation of mycotoxin content, was conducted across these diverse barley genotypes in two consecutive years, 2022 and 2023. This research revealed substantial variability in disease severity and mycotoxin content. Most genotypes exhibited a positive correlation between the causal fungal pathogen, *Fusarium graminearum* incidence, and the accumulation of mycotoxins, particularly deoxynivalenol. Specifically, statistical tests indicated that the genotype factor significantly influenced disease severity, DON, NIV, FDK, and mycotoxin accumulation in both years. Notably, in both years of field testing 'S2M190' consistently displayed moderate resistance with lower disease severity, fewer FDK, and minimal mycotoxin content. Several other genotypes exhibited low DON levels in either 2022 or 2023. Further, the results can be validated through greenhouse and multi-location trials for effective disease screening. These findings provide valuable insights into genotype performance, with implications for breeding and crop management strategies to mitigate FHB's impact on barley production. The identified superior cultivars, when integrated into appropriate crop rotation practices, hold the potential to significantly contribute to Fusarium head blight (FHB) management.

2022 and 2023 Hard Red Spring Wheat Uniform Fungicide Trials (UFT) in North Dakota

Bryan Hansen [1], Jessica Halvorson [1], Scott Meyer [1], Venkata Chapara [2], Larissa Jennings [2], Amanda Arens [2], and Andrew Friskop [1]

1. Department of Plant Pathology, North Dakota State University, Fargo, ND
2. Langdon Research and Extension Center, North Dakota State University, Langdon, ND

Corresponding Author: Andrew Friskop, andrew.j.friskop@ndsu.edu

Abstract

Fungicide applications are a vital component for the integrated management of Fusarium head blight (FHB) on hard red spring wheat (HRSW) in North Dakota (ND). Updating fungicide data pertaining to FHB efficacy and agronomic response is needed to address the needs of HRSW growers and ag professionals in ND. Four HRSW UFT were conducted to compare the timing and efficacy of fungicide premixes on reducing FHB, reducing deoxynivalenol (DON), protecting yield, and protecting test weight. Research sites were established at the Langdon Research and Extension Center (Langdon; mist irrigated) and North Dakota State University (Fargo; dryland). Field experiments were conducted in a randomized complete block design with four replications. All plots were sown with a susceptible HRSW cultivar that was also grown on wide acreage in ND. Treatments evaluated included a non-treated control (NTC), Prosaro® (prothioconazole + tebuconazole), Caramba® (metconazole), Miravis Ace® (propiconazole + pydiflumetofen), Prosaro Pro® (prothioconazole + tebuconazole + fluopyram), Sphaerex® (metconazole + prothioconazole), and Folicur® (tebuconazole). Fungicide timings included Feekes 10.51 (early-anthesis), and 3 to 7 days after Feekes 10.51, or sequential applications of both timings. Three of the four HRSW trials developed moderate to high levels of disease and data was combined for analysis. Prior to analysis, FHB and DON suppression values were developed (percent reduction to NTC) and yield and test weight response values were developed (percent response compared to NTC). A very high level of FHB (74 to 97%) and DON (52 to 85%) suppression was achieved with the fungicide treatments. Sequential applications tended to provide the greatest reduction in both FHB and DON. However, some single application treatments were similar to sequential applications suggesting growers can rely on one well-timed fungicide. All fungicides protected both yield and test weight. The two recently labeled fungicide premixes of Prosaro Pro and Sphaerex at either early-anthesis or 3 to 7 days after early-anthesis provided similar to sometimes better control than the industry standards of Prosaro, Caramba, and Miravis Ace.

Acknowledgment and Disclaimer

This material is based upon work supported by the U.S. Department of Agriculture, under Agreement No. 59-0206-2-124. 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.

Elevated CO_2 Can Worsen Fusarium Head Blight Disease Severity in Wheat but the *Fhb1* QTL Provides Reliable Disease Resistance

William T. Hay [1], James A. Anderson [2], David F. Garvin [2], Susan P. McCormick [1], Mark Busman [1], and Martha M. Vaughan [1]

1. USDA, Agricultural Research Service, National Center for Agricultural Utilization Research, Mycotoxin Prevention and Applied Microbiology Unit, 1815 N, University Street, Peoria, IL
2. Department of Agronomy & Plant Genetics, University of Minnesota, St. Paul, MN

Corresponding Author: William Hay, william.hay@usda.gov

Abstract

Fusarium head blight (FHB) is a destructive fungal disease of wheat that causes significant economic loss due to lower yields and the contamination of grain with fungal toxins (mycotoxins), particularly deoxynivalenol (DON). FHB disease spread and mycotoxin contamination has been shown to worsen at elevated CO_2, therefore, it is important to identify climate-resilient FHB resistance. This work evaluates whether wheat with the *Fhb1* quantitative trait locus (QTL), the most widely deployed FHB resistance locus in wheat breeding programs, provides reliable disease resistance at elevated CO_2. Near-isogenic wheat lines (NILs) derived from either a highly FHB susceptible or a more FHB resistant genetic background, with or without the *Fhb1* QTL, were grown in growth chambers at ambient (400 ppm) and elevated (1000 ppm) CO_2 conditions. Wheat was inoculated with *Fusarium graminearum* and evaluated for FHB severity. At elevated CO_2, the NILs derived from more FHB-resistant wheat had increased disease spread, greater pathogen biomass and mycotoxin contamination, and lower rates of DON detoxification; this was not observed in wheat from a FHB susceptible genetic background. The *Fhb1* QTL was not associated with increased disease severity in wheat grown at elevated CO_2 and provided reliable disease resistance.

Evaluation of Durum Wheat for FHB Susceptibility Under High and Low-Disease Pressure Environments in North Dakota

Clair Keene [1] and Venkata Chapara [2]
1. North Dakota State University, Department of Plant Sciences, Fargo, ND
2. North Dakota State University, Langdon Research Extension Center, Langdon, ND
Corresponding Author: Clair Keene, clair.keene@ndsu.edu

Abstract
Durum wheat (*Triticum durum*) is an important, high-value grain crop grown on approximately 1 million acres annually in North Dakota. Fusarium Head Blight is the most economically important pathogen in durum wheat production. Durum wheat lacks the genetic sources of resistance that have been found and successfully exploited in bread wheat (*Triticum aestivum*). With little genetic resistance available to exploit, combining the management strategies of variety selection and a post-anthesis fungicide application are currently the recommended best management practices for control of FHB. Studies were conducted in 2020-2023 under misted and dryland conditions at Langdon and Prosper, North Dakota, respectively using an RCB split-plot design with 3 replications. FHB incidence was higher under irrigated than dryland conditions and DON levels were also higher. Yield was not significantly impacted by the use of fungicide in any year of the study in Prosper but was higher with fungicide use than without at Langdon. DON levels in the grain were decreased with the use of fungicide in 1 of 4 years at Prosper and in 2 of 4 years at Langdon. Variety selection decreased DON accumulation in 2 of 4 years at Prosper and 2 of 4 years at Langdon. Of the 8 varieties tested, 'ND Grano' and NDSU experimental line D111068 are showing less DON accumulation than known highly susceptible variety 'Mountrail' and are ranking in the top half of varieties in terms of yield. ND Grano appears to be a good option in higher disease risk locations such as eastern North Dakota while the newer release 'ND Stanley' may be better suited to lower disease risk locations in western North Dakota where its higher yields may be obtained with lower risk of high DON accumulation.

Fusarium Head Blight Management Coordinated Project: Integrated Management Trials 2023

Isaack Kikway[1], Wanderson Bucker Moraes[1], Gary Bergstrom[2], Kaitlyn Bissonnette[22], Kira Bowen[5], Carl Bradley[4], Emmanuel Byamukama[6], Martin Chilvers[11], Alyssa Collins[23], Christina Cowger[8], Heather Darby[13], Erick DeWolf[21], Ruth Dill Macky[12], Paul Esker[7], Andrew Friskop[9], Nathan Kleczewski[3], Alyssa Koehler[14], David Langston Jr.[16], Laurence Madden[1], Juliet Marshall[15], Hillary Mehl[16], Martin NegelKirk[11], Nidhi Rawat[1], Damon Smith[18], Darcy Telenko[19], Stephen Wegulo[24], Heather Young-Kelly[2] and Pierce A Paul[1]

1. The Ohio State University/OARDC, Wooster, OH
2. Cornell University, Ithaca, NY
3. University of Illinois, Urbana, IL
4. University of Kentucky, Princeton, KY
5. Auburn University, Auburn, AL
6. South Dakota State University, Brookings, SD
7. The Pennsylvania State University, University Park, PA
8. North Carolina State University/USDA-ARS, Raleigh, NC
9. North Dakota State University, Fargo, ND
10. University of Maryland, College Park, MD
11. Michigan State University, East Lansing, MI
12. University of Minnesota, St. Paul, MN
13. University of Vermont and State Agricultural College, St. Albans, VT
14. The University of Delaware, Georgetown, DE
15. University of Idaho, Aberdeen, ID
16. Virginia Tech, Suffolk, VA
17. University of Nebraska-Lincoln, Lincoln, NE
18. University of Wisconsin-Madison, Madison, WI
19. Purdue University, West Lafayette, IN
20. The University of Tennessee at Knoxville, Jackson, TN
21. Kansas State University, Manhattan, KS
22. University of Missouri, Columbia, MO
23. The Pennsylvania State University, Manheim, PA
24. University of Nebraska, Lincoln, NE

Corresponding Author: Pierce Paul, paul.661@osu.edu

Abstract

Integrated approaches for managing Fusarium head blight (FHB) and deoxynivalenol (DON) contamination of grain include agronomic practices, resistant cultivars, and chemical control. Prothioconazole, metconazole, and tebuconazole are three of the most effective demethylation inhibitors (DMI) fungicide active ingredients (AIs) for FHB and DON control. While the efficacy of Prosaro® (a premix of the DMI AIs tebuconazole and prothioconazole) and Miravis® Ace (a premix of the DMI Propiconazole and the SDHI Pydiflumetofen), industry standards for FHB and DON management, has been well documented, it is informative to determine whether newly labeled products such as Prosaro® Pro (a premix of the DMI tebuconazole and prothioconazole and the SDHI Fluopyram) and Spaherax® (a premix of metconazole and prothioconazole) will be just as or more effective than the industry standards when used in combination with cultivar resistance. Therefore, the overall objective of the study was to determine the efficacy of these new fungicides when used alone or as part of integrated management programs. To accomplish this objective, field experiments were conducted in several U.S. wheat-growing

states during 2023 wheat growing season. Separate replicated plots of susceptible (S), moderately susceptible (MS), or moderately resistant (MR) cultivars were treated with Prosaro, Miravis Ace, Prosaro Pro, or Sphaerex at Feekes 10.5.1 or left untreated, and subsequently inoculated with spores of *Fusarium graminearum*. Percent control (C) was estimated for FHB index (IND) and DON for each cultivar x fungicide program combination relative to the non-treated susceptible check (S_CK). Mean IND and DON in S_CK ranged from 0 to 74% and 0 to 3.2 ppm, respectively. Averaged across environments, combination of MR cultivars and fungicide treatments showed higher C for IND and DON than treatments applied to S cultivars. For instance, across the tested fungicide programs, C for IND ranged from 88 to 94% on MR and 82 to 92 % on MS cultivars, compared to 82 to 89% on S cultivars. Additionally, C for Miravis Ace, Prosaro, Prosaro Pro and Sphaerex combination with cultivar resistance ranged between 89 to 94% for MR and 82-92% for MS. Preliminary findings from this study will provide stakeholders with useful information regarding the efficacy of the new fungicide mixtures relative to the industry standards when used as part of integrated management programs to control FHB and DON.

Acknowledgment and Disclaimer
This material is based upon work supported by the U.S. Department of Agriculture, under Agreement Nos. 59-0206-8-195, 59-0206-0-126; 59-0206-9-120, 59-0206-0-125; 59-0206-6-008, 59-0206-0-153; 59-0206-5-007, 58-6070-9-019, 59-0206-0-184; 59-0206-8-192, 59-0206-0-115; 59-0206-8-189, 59-0206-0-138; 59-0206-5-005, 59-0206-9-122, 59-0206-0-139; 59-0206-8-190, 59-0206-0-141; 59-0206-6-015, 59-0206-0-155; 59-0206-4-016, 59-0206-9-117, 59-0206-0-132; 59-0206-8-210, 59-0206-0-140; 59-0206-8-199, 59-0206-0-122; 59-0206-8-211, 59-0206-0-144; 59-0206-0-173; 59-0206-0-188; 58-2050-8-013, 59-0206-0-175; 59-0206-6-010; 59-0206-8-189; 59-0206-0-179; 59-0206-6-012, 59-0206-0-189; 59-0206-9-123, 59-0206-0-118; 59-0206-6-014, 59-0206-0-191; 59-0206-9-009, 59-0206-0-185; and 59-0206-8-187, 59-0206-0-131. 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.

Fusarium Head Blight Management Coordinated Project: Uniform Fungicide Trials 2023

Isaack Kikway[1], Wanderson Bucker Moraes[1], Gary Bergstrom[2], Kaitlyn Bissonnette[22], Kira Bowen[5], Carl Bradley[4], Emmanuel Byamukama[6], Martin Chilvers[11], Alyssa Collins[23], Christina Cowger[8], Heather Darby[13], Erick DeWolf[21], Ruth Dill Macky[12], Paul Esker[7], Andrew Friskop[9], Nathan Kleczewski[3], Alyssa Koehler[14], David Langston Jr.[16], Laurence Madden[1], Juliet Marshall[15], Hillary Mehl[16], Martin NegelKirk[11], Nidhi Rawat[1], Damon Smith[18], Darcy Telenko[19], Stephen Wegulo[24], Heather Young-Kelly[2] and Pierce A. Paul[1]

1. The Ohio State University/OARDC, Wooster, OH
2. Cornell University, Ithaca, NY
3. University of Illinois, Urbana, IL
4. University of Kentucky, Princeton, KY
5. Auburn University, Auburn, AL
6. South Dakota State University, Brookings, SD
7. The Pennsylvania State University, University Park, PA
8. North Carolina State University/USDA-ARS, Raleigh, NC
9. North Dakota State University, Fargo, ND
10. University of Maryland, College Park, MD
11. Michigan State University, East Lansing, MI
12. University of Minnesota, St. Paul, MN 55108
13. University of Vermont and State Agricultural College, St. Albans, VT
14. The University of Delaware, Georgetown, DE
15. University of Idaho, Aberdeen, ID
16. Virginia Tech, Suffolk, VA
17. University of Nebraska-Lincoln, Lincoln, NE
18. University of Wisconsin-Madison, Madison, WI
19. Purdue University, West Lafayette, IN
20. The University of Tennessee at Knoxville, Jackson, TN
21. Kansas State University, Manhattan, KS
22. University of Missouri, Columbia, MO
23. The Pennsylvania State University, Manheim, PA
24. University of Nebraska, Lincoln, NE

Corresponding Author: Pierce A. Paul, paul.661@osu.edu

Abstract

Fungicides are essential for Fusarium head blight (FHB) and deoxynivalenol (DON) management in small grain crops. However, for successful FHB control, fungicide application timing, rate, and product are all important. With emphasis on newly registered fungicides, Prosaro® Pro and Sphaerex®, the objective of FHB management coordinated project were to compare the efficacy of Prosaro Pro (a premix of the DMIs tebuconazole and prothioconazole and the SDHI Fluopyram) and Sphaerex (a premix of metconazole and prothioconazole) to that of Prosaro®, Caramba®, and Miravis® Ace, industry standards for FHB and DON management. To accomplish this objective, field experiments were conducted in multiple U.S. wheat-growing states in 2023. Different fungicides programs were applied to replicated plots of susceptible cultivars, then artificially inoculated with spores of *F. graminearum*. The fungicide programs consisted of a non-treated check (CK), or an application of Prosaro (I), Caramba (II), Miravis Ace (III), Prosaro Pro (IV), or Sphaerex (V) at anthesis, or Miravis Ace at anthesis followed by an application of Prosaro Pro (VI), Sphaerex (VII), or Tebuconazole (VIII) at 4-6 days after anthesis. FHB index (IND) was assessed, and grain

samples were tested for DON. Percent control (C) was estimated for IND and DON for each fungicide program relative to CK. Mean FHB IND and DON in the checks across environments ranged from 0 to 20% and 1 to 1.2 ppm, respectively. Generally, the most effective of all tested fungicide programs were combinations of Miravis Ace followed by one of the other tested fungicides. Averaged across environments, C for IND was 99% for III, I, or II, 90% for VI, and 94% for VII. Additionally, C for DON contamination of grain was 98 and 96% for VII and VIII, respectively. The results suggest that the combination of an anthesis application of Miravis Ace followed by a "late" application of \Prosaro, Prosaro Pro, Sphaerax or Tubaconazole can be more effective at reducing FHB and DON than an anthesis-only application of any of the tested fungicides. The outcome from this study will provide stakeholders with information regarding the efficacy of the newly registered fungicides relative to the industry standards, as well as the efficacy of two-treatment fungicide programs against FHB and DON. Further analyses will be conducted to formally quantify efficacy and determine the additivity of AI mixtures and sequentially applied fungicide treatments.

Acknowledgment and Disclaimer
This material is based upon work supported by the U.S. Department of Agriculture, under Agreement Nos. 59-0206-8-195, 59-0206-0-126; 59-0206-9-120, 59-0206-0-125; 59-0206-6-008, 59-0206-0-153; 59-0206-5-007, 58-6070-9-019, 59-0206-0-184; 59-0206-8-192, 59-0206-0-115; 59-0206-8-189, 59-0206-0-138; 59-0206-5-005, 59-0206-9-122, 59-0206-0-139; 59-0206-8-190, 59-0206-0-141; 59-0206-6-015, 59-0206-0-155; 59-0206-4-016, 59-0206-9-117, 59-0206-0-132; 59-0206-8-210, 59-0206-0-140; 59-0206-8-199, 59-0206-0-122; 59-0206-8-211, 59-0206-0-144; 59-0206-0-173; 59-0206-0-188; 58-2050-8-013, 59-0206-0-175; 59-0206-6-010; 59-0206-8-189; 59-0206-0-179; 59-0206-6-012, 59-0206-0-189; 59-0206-9-123, 59-0206-0-118; 59-0206-6-014, 59-0206-0-191; 59-0206-9-009, 59-0206-0-185; and 59-0206-8-187, 59-0206-0-131. This is a cooperative project with the U.S. Wheat & Barley Scab Initiative. Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the authors and do not necessarily reflect the view of the U.S. Department of Agriculture.

A Network Meta-Analysis of Results from Over a Quarter-Century of Uniform Fungicide Trials in the U.S.

L.V. Madden [1], W.B. Moraes [1], and P.A. Paul [1]

1. Ohio State University, Department of Plant Pathology, Wooster, OH

Corresponding Author: Laurence V. Madden, madden.1@osu.edu

Abstract

Early research by Marcia McMullen and colleagues starting in 1995 showed that a single application of a triazole (DMI) fungicide had the potential to reduce FHB index (field severity). This led to the establishment of the Uniform Fungicide Trials (UFTs) by the USWBSI, with field studies commencing in 1998. Early trials focused on propiconazole (trade name Tilt®) and tebuconazole (Folicur®). Metconazole (Caramba®) was first tested in 2000, and tebuconazole+prothioconazole (Prosaro®) in 2002. Starting in 2008, strobilurins were tested in the UFTs, and different application timings for DMIs were evaluated starting in 2009. A series of network meta-analyses of over 300 separate trials from 1995 through 2013 definitively showed the benefits of a well-timed single application of a DMI fungicide at or shortly after anthesis. Prosaro and Caramba were clearly more efficacious than other fungicides, with an average percent control (percentage reduction relative to the untreated check) of 48-54% for disease index and 37-44% for DON. Results gave final verification that an application of a strobilurin-based fungicide between flag leaf stage and anthesis resulted in substantially higher levels of DON than the check, and thus should be avoided. Recommendations for management of FHB with fungicides were generally well established based on the results through 2013. However, with the registration of the new SDHI fungicide pydiflumetofen combined with the DMI propiconazole (Miravis® Ace), there was the opportunity to reassess the best fungicide treatments for FHB. Based on a new network meta-analysis of 68 trials from 2018-2021, Miravis Ace applied at anthesis had higher mean percent control for index (~65%) and DON (~50%) than Prosaro or Caramba applied at anthesis or Miravis Ace applied around heading (~55% for index and ~30% for DON). A late application of Miravis Ace (4-6 days post-anthesis) was equally effective as an application at anthesis for DON control, but was not as effective for control of disease index. Treatments with two sequential fungicide applications had substantially greater percent control for index and DON. By pooling all UFTs from 2000-2021, stability of the efficacy of Caramba and Prosaro was also characterized. Using novel methods in network meta-analysis, heterogeneity of efficacy was characterized through empirical BLUPs, and time trends were represented with natural cubic splines. Although there were significant changes in percent control with Prosaro and Caramba over the years, there was no evidence of a loss of efficacy or change in sensitivity to the fungicides.

Fusarium Graminearum Virus-1 Strain FgV1-SD4 Infection Eliminates Mycotoxin Deoxynivalenol Synthesis by *Fusarium graminearum* in FHB

Bimal Paudel [1], Connor Pedersen [1,2], Yang Yen [1], and Shin-Yi Lee Marzano [1,2]
1. Department of Biology and Microbiology, South Dakota State University, Brookings, SD
2. United States Department of Agriculture, Agricultural Research Service (USDA-ARS), Toledo, OH
Corresponding Author: Shin-Yi Marzano, shinyi.marzano@usda.gov

Abstract
Deoxynivalenol (DON) toxin production during the infection of *F. graminearum* in small grain crops is one of the most harmful virulence factors associated with economic losses. Metatranscriptome sequencing and RT-qPCR traced back that the only mycovirus infecting an *F. graminearum* isolate, designated as Fg-4-2, was a novel strain of Fusarium graminearum virus 1 (FgV1), designated as FgV1-SD4. The isolate Fg-4-2 showed significantly reduced virulence against wheat compared to the virus-free culture, designated as isolate Fg-4-1, which was obtained by deep freezing and single conidial germination. Notably, no DON accumulation was detected in the harvested wheat seeds infected by Fg-4-2, whereas ~18 ppm DON was detected in seeds infected by Fg-4-1. Comparison of the genome sequence of FgV1-SD4 with other identified strains of FgV1, i.e., FgV1-DK21 and FgV1-ch, indicates mutations on ORF-2 and the 3' -UTR in the genome that might be associated with hypovirulence. This mycovirus strain alone and specific genetic components of FgV1-SD4 can be further optimized to be developed as a biocontrol agent to reduce Fusarium head blight and to lower the DON accumulation levels in small grain crops due to this fungal disease.

Optimum Closeup in Wheat FHB Detection with 360-Degree Deep Scanning Method and Using an Efficient Transformer Model

Ali M Nafchi [1], Babak Azad [1], Ahmed Abdalla [1], Karl Glover [1], Sunish Kumar Sehgal [1], Shaukat Ali [1], and Kwanghee Won[2]

1. South Dakota State University, Department of Agronomy, Horticulture, and Plant Science, Brookings, SD
2. South Dakota State University, Department of Electrical Engineering and Computer Science, Brookings, SD

Corresponding Author: Ali M Nafchi, ali.nafchi@sdstste.edu

Abstract

Fusarium head blight (FHB) poses a significant threat to crops, impacting food security and environmental and human health. Traditional FHB management methods are often inefficient and may lead to overuse of chemicals. Utilizing artificial intelligence (AI) for FHB detection enhances disease identification precision, enabling near real-time responses and more calculated interventions. Early detection is crucial in managing crop diseases. While deep convolutional neural networks have been explored, conventional segmentation frameworks like U-Net face challenges in handling FHB variability. We introduce TransDAE, an innovative approach improving the self-attention mechanism to address spatial and channel dimensions, overcoming the limitations of existing frameworks and refining precision through an inter-scale interaction module. For training and testing, we collected 12,000 wheat field images using an advanced rotational deep scanning robot. This robot, equipped with a cutting-edge drone featuring a 360-degree rotational camera, provides a unique aerial perspective, enhancing data collection efficiency. The drone's capabilities, including swift image capture from all angles, counterweight system, and calibrated velocity, ensure high-quality images for FHB detection and segmentation. The 360-degree rotational camera adds unprecedented detail to our dataset, advancing our understanding of wheat fields.

Evaluation of Fungicide Efficacy, Timing and Cultivar Resistance for Management of Fusarium Head Blight of Durum in North Dakota

Audrey Kalil [1], Edson Ncube [1], John Teixeira [1], Taheni Gargouri-Jbir [1], Dimitri Fonseka [1], and Andrew Friskop[2]
1. North Dakota State University, Williston Research Extension Center, Williston, ND
2. North Dakota State University, Department of Plant Pathology, Fargo, ND
Corresponding Author: Audrey Kalil, audrey.kalil@ndsu.edu

Abstract
Fusarium graminearum causes Fusarium Head Blight (FHB) in durum wheat (*Triticum durum* Desf.), resulting in yield loss, low test weight and deoxynivalenol (DON) contamination. FHB is largely managed through the selection of varieties with increased resistance and fungicides. The goal of this study was to evaluate the efficacy of fungicides in single and sequential applications to manage FHB and DON in a susceptible (S) and a moderately susceptible (MS) cultivar. Field experiments were conducted under overhead irrigation to increase disease pressure, and set up in a randomized complete block design in 1.5 x 5.5 m plots, with four replications. Six field experiments were conducted from 2017-2022. The five fungicides treatments: Sphaerex® (Met+Pro), Miravis® Ace (Pro+Pyd), Miravis Ace followed by Tebuconazole (Pro+Pyd fb Teb), Prosaro® (Pro+Teb) and Caramba® (Met) were applied at different stages of crop development: half-head (Feekes 10.3), flowering (10.5.1), post flowering (4-6 days post 10.5.1), and both flowering and post-flowering (10.5.1 fb 4-6 days post-10.5.1) for a total of 8 fungicide x application timing treatments. The crop was assessed for visual FHB symptoms expressed as FHB index at soft dough, and yield and DON post-harvest. Fungicides significantly reduced FHB, reduced DON and protected yield in the experiments. Six fungicide treatments significantly protected durum yield. The sequential treatment of Prop+Pyd fb Teb provided the greatest amount of DON and FHB reduction, while having the highest yield. The MS cultivar exhibited a reduced FHB index and higher yield than the S cultivar. However, there was no significant difference in DON (P = 0.1811). Therefore, the integration of genetic resistance and proper timing of an effective fungicide application is an important strategy in the management of FHB. As new durum cultivars are released, it is essential to update existing and new fungicide performance data to convey messaging on efficacy and agronomic response, especially in growing regions that have sporadic FHB epidemics.

Introducing Synthetic Spike-In Metabarcoding: a Novel, Sensitive, and Quantitative Method for Identifying *Fusarium* Species

Peter Oppenheimer [1], Francesco Tini[2], Briana Whitaker[3], Imane Laraba[3], Rebecca Whetten[4], and Christina Cowger [1,4]

1. North Carolina State University, Department of Entomology and Plant Pathology, Raleigh, NC
2. University of Perugia, Department of Agricultural, Food and Environmental Sciences, 06121 Perugia, Italy
3. USDA-ARS National Center for Agricultural Utilization Research, Mycotoxin Prevention and Applied Microbiology Unit, Peoria, IL
4. USDA Agricultural Research Service, Plant Science Research Unit, Raleigh, NC

Corresponding Author: Christina Cowger, christina.cowger@usda.gov

Abstract

Fusarium is a fungal genus encompassing a variety of species that cause Fusarium head blight (FHB) in small grains. *Fusarium* produces mycotoxins during infection that can be harmful to human and livestock health and vary in type and toxicity by fungal species. In U.S. wheat, the majority of FHB infections are caused by *Fusarium graminearum*; however, recent surveys have shown other species can be more prevalent in certain fields and up to 5 different *Fusarium* species can contaminate an individual wheat spike. Exploring this fusarial diversity means confronting the limited applicability of traditional diagnostic assays that rely on deriving single-spore isolates and Sanger sequencing of the *translation elongation factor 1*-a gene (*TEF1*), since fusaria do not grow at the same rate in culture and Sanger sequencing provides only qualitative results (present/absent). Recognizing this limitation, qPCR and metabarcoding assays were developed that are culture-independent and provide insight into the abundance of different species in a single sample. However, these approaches either (1) are low throughput, (2) are limited to a small subset of *Fusarium*, (3) cannot identify novel *Fusarium*, or (4) are not completely quantitative. To overcome these limitations, we have developed a synthetic spike-in metabarcoding method (SSIM) that can quantify all fusaria in a single assay and is high-throughput with species-level resolution. We compared the accuracy and precision of this assay against a lower-throughput quantitative PCR metabarcoding method (qM) and found that the two methods had near-identical behavior for quantifying *F. graminearum*, *F. acuminatum*, and *F. poae* in the same sample (R^2 ~ 0.99, 0.99, and 0.93 respectively) across a wide range of Fusarium concentrations within samples. Additionally, we determined how to address the bias in estimates of absolute abundance that can be created by common bioinformatic pipelines that use read-quality filtering. This research represents the first reported use of SSIM in plant disease diagnostics and an improved tool for investigating *Fusarium* epidemiology and control.

Fungicide Application Regimen for Reducing Fusarium Head Blight and Deoxynivalenol Accumulation in Wheat Under High Disease Pressure

Dalitso Yabwalo [1], Shaukat Ali [1], Karl Glover [1], Connie Tande [1], and Madalyn Shires [1]
1. South Dakota State University, Agronomy, Horticultural & Plant Science Department, Brookings, SD
Corresponding Author: Dalitso Yabwalo, dalitso.yabwalo@sdstate.edu

Abstract
Fusarium graminearum is Fusarium head blight's (FHB) or scab disease main causal agent. Apart from reducing yield and kernel quality, one of FHB's critical impacts is accumulation of mycotoxins such as deoxynivalenol (DON). This study assessed the efficacies of newer fungicides on FHB and DON management under different application regimens. The fungicide application regimen comprised three schemes; a single application of Prosaro® (6.5 fl oz/ac), Caramba® (13.5 fl oz/ac), Miravis® Ace (13.7 fl oz/ac), Prosaro® Pro (10.3 fl oz/ac) and Sphaerex® (7.3 fl oz/ac) at early anthesis (Feekes 10.5.1), a sequential application of two different products starting at Feekes 10.5.1 followed by an application at completion of flowering (Feekes 10.5.3), and a single application of Prosaro Pro, Sphaerex, and Miravis Ace at the completion of flowering. 'CP3099A', an awnless spring wheat variety was used as a model susceptible host. The study was set up as a randomized complete block design with four blocks and all experimental units were inoculated at boot stage with *F. graminearum* infected corn spawn at 42g m^{-2}. Data on FHB index, FDK and DON were analyzed using the generalized linear model with applicable functionalities. The sequential fungicide application and the Feekes 10.5.3 single application regimes had lower FHB index, FDK and DON accumulation relative to the widely used single applications. Miravis Ace applied at Feekes 10.5.1 followed by Prosaro Pro at Feekes 10.5.3 had the lowest FHB index, FDK and DON accumulation. No statistically significant differences in DON accumulation were observed among treated plots regardless of application regimen. However, plots subjected to sequential and late applications had distinctly lower DON than plots with a single fungicide application at Feekes 10.5.1. Although none of the samples analyzed in this study met the FDA restrictions of 1ppm for safe human consumption, results suggest that a sequential scheme is the most effective for managing FHB index, FDK, and DON accumulation. The single fungicide application scheme at Feekes 10.5.3 provided better FHB index and DON protection compared to single application at Feekes 10.5.1. These preliminary observations defy the current recommendation to apply fungicide at Feekes 10.5.1 for effective FHB management. Perhaps, there is an association between application times and host genotype, disease pressure and environmental conditions.

Acknowledgment and Disclaimer
This is based upon work supported by the USDA Agreement No 59-0206-2-090. 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.

Validation of the Fusarium Head Blight Risk Tool in Pennsylvania

Olanrewaju Shittu [1], Mladen Cucak[2], Felipe Dalla Lana[3], Wanderson Bucker Moraes[4], Pierce A. Paul[5], Denis A. Shah[6], Erick D. De Wolf[6], and Paul D. Esker [1]

1. Penn State University, Department of Plant Pathology and Environmental Microbiology, State College, PA
2. Syngenta Crop Protection, Crop Protection Development, Basel, Switzerland
3. Louisiana State University, LSU Ag Center, Baton Rouge, LA
4. Corteva Agriscience, Crop Protection Operation Center, Indianapolis, IN
5. The Ohio State University, Department of Plant Pathology, Wooster, OH
6. Kansas State University, Department of Plant Pathology, Manhattan, KS

Corresponding Author: Olanrewaju Shittu, oms5169@psu.edu

Abstract

Fusarium head blight (FHB) threatens Pennsylvania's wheat industry, causing economic losses due to reduced grain quality, yield, and mycotoxin contamination. The yield loss caused by the disease in Pennsylvania is estimated to be $19 million between 2018 and 2022. Managing this devastating disease requires accurate risk assessment that can aid farmers in making informed decisions regarding disease prevention and control strategies. In this study, we present the validation of the Fusarium Head Blight Weather-Based Risk Tool in the context of Pennsylvania, a region with a notable wheat industry. The FHB Risk Tool is a web-based forecasting system that predicts FHB epidemic risk (>10% FHB index) and guides in-season fungicide application decisions. The FHB Risk Tool uses weather variables such as temperature and humidity, which are known to impact FHB development, along with different levels of wheat resistance to FHB. This research aims to validate the FHB Risk Tool's predictive accuracy in Pennsylvania for the years 2018, 2020 - 2023. Flowering dates, FHB incidence, and severity data were collected from various research trials at the Russell E. Larson Agricultural Research Center at Rock Springs (40.710208, -77.950024) and the Southeast Agricultural Research and Extension Center at Manheim (40.118769, -76.427366). We hypothesized that there is no significant difference between the forecasted FHB risk and the actual FHB incidence and severity in these locations for the years 2018 and 2020 - 2023. Preliminary results show that the FHB tool forecasted risk for 2021 and 2023 aligned with the observed FHB index at both locations. However, the tool predicted high FHB risk for 2018 and 2020, but the observed FHB disease index was low at both locations, below the 10% threshold. Meanwhile, in 2022, the tool predicted a high risk, but this was observed only in Manheim. The difference between the FHB risk forecasted and the FHB index observed in the field may be due to factors not considered by the tool, such as rotation history, residue management, and the three small-grain production zones in Pennsylvania. These results are important to guiding extension educational programs focused on the integrated management of wheat diseases. We expect this research will encourage stakeholders to adopt the FHB Risk Tool as a reliable resource for making informed, in-season decisions in their wheat production strategies.

Survey of *Fusarium* and Mycotoxin Diversity in Illinois Winter Wheat

Briana K. Whitaker [1], Imane Laraba[2], Christina Cowger[3], Pete Oppenheimer[4], Susan McCormick [1], Mark Busman [1], and Martha Vaughan [1]

1. USDA-Agricultural Research Service, National Center for Agricultural Utilization Research, Mycotoxin Prevention & Applied Microbiology Unit, Peoria, IL
2. Agriculture and Agri-Food Canada, Ottawa, ON, Canada
3. USDA-Agricultural Research Service, Plant Science Research Unit, Raleigh, NC
4. North Carolina State University, Department of Entomology and Plant Pathology, Raleigh, NC

Corresponding Author: Briana Whitaker, Briana.whitaker@usda.gov

Abstract

Fusarium head blight (FHB) is an economically important disease of small grains globally and is primarily caused by species in the genus *Fusarium*. Although the deoxynivalenol- (DON) producing *F. graminearum* is often considered to be the primary causal agent of concern in N. America, other species or chemotypes can be more frequent in certain geographic regions or during certain years. In particular, little is known about *Fusarium* diversity in scabby U.S. wheat and barley spikes, nor about the environmental factors that cause local outbreaks of "emerging mycotoxin" producers (e.g., the *F. tricinctum* species complex). Thus, we are conducting surveys of *Fusarium* and mycotoxin diversity in FHB symptomatic wheat and barley to better understand the environmental factors driving higher frequencies of minority species, and emerging mycotoxins of concern. In 2022, we sampled 19 Illinois hard red winter wheat farms, isolated *Fusarium* from symptomatic heads, and quantified mycotoxin concentrations of scabby heads. In total, we isolated 1192 strains, which ranged from 10-82 per field. Most of the successfully isolated strains came from heads with low disease (<20% infected spikelets) and only a single visible point of infection. From TEF-1α partial sequencing, most isolates belonged to the *Fusarium sambucinum* species complex (>95% of isolates), with the remaining belonging to the *Fusarium tricinctum*, *fujikuroi*, and *incarnatum-equiseti* species complexes. Mycotoxin analyses revealed greater than expected concentrations of nivalenol (NIV) and culmorin (CUL) present in scabby wheat heads from southern Illinois. In particular, we found up to 14 µg of NIV per gram of grain material and up to 20 µg of CUL per gram of grain material in some fields. By contrast, we only found up to 2 µg of DON per gram of grain material across all 19 fields. A strong correlation was detected between NIV and CUL and is suggestive of the presence of *Fusarium* strains capable of producing both CUL and NIV. Ultimately, this indicates that there may be a significant proportion of NIV-producing *F. graminearum* in Illinois.

Food Safety and Toxicology (FST)

Photosensitization Effect of Curcumin for Controlling *Fusarium graminearum* Growth and Deoxynivalenol Production

Anil Kunapareddy [1], Xiaoxi Qi [1], Shaobin Zhong[2], Bingcan Chen [1], and Jiajia Rao [1]
1. Department of Plant Sciences, North Dakota State University, Fargo, ND
2. Department of Plant Pathology, North Dakota State University, Fargo, ND
Corresponding Author: Jiajia Rao, jiajia.rao@ndsu.edu

Abstract
The Fusarium head blight (FHB) species complex generates mycotoxins, leading to not only reductions in crop quality and yield but also posing significant health risks to both humans and livestock. Therefore, it is of great urgency to develop a green strategy to inhibit *Fusarium* mycotoxin production that can be applied in sprouted grains. Recent studies have shown that photosensitization, an emerging technology, effectively combats various microorganism and fungi through the interaction between photosensitizers (PS) and visible light in the presence of oxygen. The main limitation in this technology is the use of PS as they have weak antifungal activity in natural light, have low solubility, specificity, and high cost. This research seeks to understand the effectiveness of antifungal and mycotoxin inhibitory activity of natural PS (curcumin) *in vitro* and their antifungal mechanism of action. Initially, the water dispersible physically stable curcumin nanoemulsion was formed by preparing 3mM concentration of curcumin in 10 wt% propylene glycol, 10 wt% of Tween 80 and 80 wt% of water mixture. With regard to inhibition of spore germination of two chemotypes of *Fusarium graminearum* isolates (10-124-1 and 10-125-1), the concentration of curcumin nanoemulsions on 50% spore inhibition rates (EC_{50}) were 2.32 mM/mL and 2.56 mM/mL, respectively. As revealed by scanning electron microscope (SEM), shrunken spores with rough and corrugated surfaces were observed in spores treated with curcumin nanoemulsions due to the massive loss of cytoplasm matrix in spores. Moreover, significant ($p< 0.05$) suppression on mycotoxin inhibition were achieved by treated with 3mM of curcumin nanoemulsion with light exposure. We further investigated the mechanism of action of curcumin nanoemulsion by studying Tri gene cluster (*Tri3, Tri4*, and *Tri5*) at genetic levels. Results indicated that the curcumin nanoemulsion significantly ($p < 0.05$) up-regulated all the treatment samples than control (*Tri3, Tri4*, and *Tri5*) in isolate 10-124-1, whereas in isolate 10-125-1, all the *Tri* gene cluster in treatment conditions were down-regulated significantly ($p < 0.05$) than control. Overall, this study established the potential application of natural PS as a safe preservative in grains.

Acknowledgment and Disclaimer
This material is based upon work supported by the U.S. Wheat and Barley Scab Initiative under Agreement No. 59-0206-2-131. 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

Move Over DON - Multitoxin Testing Is Here

Carrie Maune[1]
1. Trilogy Analytical Laboratory, Washington, MO
Corresponding Author: Carrie Maune, carrie@trilogylab.com

Abstract
Mycotoxins are one of those topics that many would prefer just "goes away." For years, it has been researched, discussed, legislated, evaluated and tested by everyone from seed experts to end users. But it has most definitely not gone away. In the past 5 to 10 years, external factors such as extreme weather events and increasing international regulations are also factors for mycotoxins in wheat and barley. What's next? Enter - Multi-mycotoxin testing. In the past decade the use of LC-MS/MS for mycotoxins has increased dramatically. This multi-toxin panel has changed the way data is reviewed for many matrices. In the past, samples analyzed by HPLC or GC were specific for one, or perhaps up to three mycotoxins. The contamination levels for a specific crop year were generalized on those toxins, and samples were rarely submitted for much else besides DON, or perhaps the Acetyl versions of DON. The tandem mass spectroscopy methods have changed that focus. Most samples that enter our facility at Trilogy are analyzed for multiple toxins with every run. Our methods quantify each of the toxins we report, however other methods in the industry will analyze for 200+ mycotoxins, perhaps not with quotation, but with simply a reportable level detected. This changes the playing field for international trade, and for the general parameters of the products. Toxins that were never a concern prior to LC-MS/MS are now being found and reported with multi-toxin methods. Are all the reported toxins found at a level of concern for food and feed safety? Is more data "better data"? What mycotoxins are found in areas and levels that pose new concerns? Many of these questions are now front and center of discussions, trade regulations, and quality topics. Let's explore what these methods have found. What mycotoxins of concern may truly be, and what a good take away from this new technology and testing can give us as an industry.

Arabidopsis Callus Culture as a Conduit for Quantification of Trichothecene Mycotoxins from *Fusarium graminearum*

Lola O. McMullan[1], Shae E. Forwood[1], Hope Gruszewski[1], Nicole McMaster[1], Bastiaan O. R. Bargmann[1], and David G. Schmale III[1]
1. Virginia Tech University, School of Plant and Environmental Sciences, Blacksburg, VA
Corresponding Author: Lola McMullan, lolamcmullan@vt.edu

Abstract
New and improved strategies are needed to detect plant pathogens before symptom onset. The fungus *Fusarium graminearum* produces trichothecene mycotoxins that pose a threat to the health of domestic animals and humans. This research aims to address knowledge gaps pertaining to early detection of pathogens in agricultural settings and the feasibility of using callus culture as a conduit for pathogen detection and mycotoxin characterization. Calli and whole plants of the Landsberg *erecta* accession of *Arabidopsis thaliana* were infected with two strains of *F. graminearum* (3-ADON and 15-ADON genotypes), and mycotoxins were quantified using Gas Chromatography Mass Spectrometry (GC-MS). It is expected that concentrations will be congruent between whole plants and calli, with the greatest concentration of trichothecene mycotoxins being produced in the 15-ADON strain due to the greater prevalence in this genotype. If successful, our work has the potential to demonstrate that callus culture is a viable approach to studying the pathosystem in plants, paving the way for a faster method for estimating *F. graminearum* trichothecene production *in planta*.

Matrix Effect in Quantitative Analysis of DON and DON-3-glucoside in Wheat, Barley, and Malt Using Liquid Chromatography-Mass Spectrometry

Yejune Moon [1], James Gillespie [1], Thomas Studzinski [1], and Zhao Jin [1]
1. North Dakota State University, Department of Plant Sciences, Fargo, ND
Corresponding Author: Zhao Jin, zhao.jin@ndsu.edu

Abstract
Deoxynivalenol (DON) and its conjugate, DON-3-glucoside (D3G), are mycotoxins of significant concern in various cereal-based foods and beverages. The transformation of DON into D3G occurs from the field through food processing and is closely linked to Type II resistance of host plants against *Fusarium* infection. Accurate and precise quantification of DON and D3G is essential not only for food safety and regulatory compliance, but also for evaluating the efficacy of crop resistance strategies. Liquid Chromatography-Mass Spectrometry (LC-MS) emerged as a powerful analytical tool for the quantification of DON and D3G, especially since D3G cannot be detected by GC. However, in LC-MS analysis, the surrounding matrix, which comprises the complex mixture of compounds from grain extracts, exerts a significant influence on the accuracy and reliability of quantification. This matrix effect can lead to systematic biases in measurement; therefore, requires careful evaluation. In this study, we explored the critical issue of the matrix effect for the quantification of DON and D3G in wheat, barley, and malt using LC-MS. The goal of our study was to evaluate the matrix effect for the quantification of DON and D3G on LC-MS and to analyze the D3G transformation. The matrix effect (ME) was calculated with slopes of calibration curves from acetonitrile/water (84/16, v/v), barley and wheat extract, and malt extract. The MEs of wheat, barley and malt for DON were 86% and the MEs of barley/wheat and malt for D3G were 90 and 94% respectively. These results indicate significant ion-suppression for DON and D3G in these matrixes. Five samples of hard-red spring wheat and ten samples of two-row spring barley samples were analyzed for D3G transformation. The production of DON and the transformation of D3G varied between barley and wheat and varieties. We observed the molar ratios of D3G/DON increased after germination which significantly varied between samples; this underlines its significance in evaluating the resistance of barley and wheat to Fusarium infection, particularly in the context of type II resistance.

Acknowledgment
U.S. Department of Agriculture under Agreement No. 59-0206-9-064 (cooperative project with USWBSI). James Gillespie, Thomas Studzinski, and Dr. Zhao Jin for assistance with this work. Dr. Gazala Ameen, and Dr. Shyam Solanki from SDSU for providing barley samples. Dr. Xuehui Li from NDSU for providing wheat samples.

Gene Discovery & Engineering Resistance (GDER)

Quantitative Trait Loci Mapping for Fusarium Head Blight Resistance in a Wheat Ems Mutant from Jagger

Ruolin Bian [1], Amy Bernardo [2], Paul St. Amand [2], Allan Fritz [1], Jessica Rupp [3], and Guihua Bai [2,1]
1. Department of Agronomy, Kansas State University, Manhattan, KS
2. USDA-ARS, Hard Winter Wheat Genetics Research Unit, Manhattan, KS
3. Department of Plant Pathology, Kansas State University, Manhattan, KS
Corresponding Author: Guihua Bai, guihua.bai@ars.usda.gov

Abstract
Wheat Fusarium head blight (FHB) is one of the most destructive diseases of wheat and causes significant yield and quality losses in wheat worldwide. Using resistant cultivars is one of the most effective approaches to control FHB. Although more than 600 quantitative trait loci (QTLs) have been reported, most of them showed minor effects and not consistent across environments. To explore new sources of resistance, an EMS-induced mutant population derived from an FHB moderately susceptible hard winter wheat cultivar 'Jagger' was screened for FHB resistance. One mutant line (JagMut1095) showed significantly higher FHB resistance than Jagger ($P < 0.001$), with mean percentage of symptomatic spikelets (PSS) in a spike of 32% for JagMut1095 and 69% for Jagger. A population of 154 recombinant inbred lines (RILs) was developed from the cross of JagMut1095 x Jagger and was used for QTL analysis on FHB resistance in four greenhouse environments. A total of 3,757 high quality GBS-SNPs were mapped on 21 wheat chromosomes using recombinant inbred lines (RILs), and 1,003 unique SNPs were mapped to 22 linkage groups corresponding to the 21 chromosomes after binning the SNPs in the same positions. The map is 3,106 cM in genetic length with a marker density of 3.1 cM per marker. Composite interval mapping identified 12 QTLs for Type II FHB resistance on chromosomes 1B, 2B, 3A, 4B, 4D, 5A, 5B, 5D, 6A, 6B, 7A and 7B. Among them, four QTLs on chromosomes 3A (*QFhb.hwwg-3AS*), 4B (*QFhb.hwwg-4BS*), 5A (*QFhb.hwwg-5AL*) and 7A (*QFhb.hwwg-7AS*) were repeatable in at least two environments. *QFhb.hwwg-5AL* was repeatable in all environments and for the best linear unbiased estimated (BLUE) values, and *QFhb.hwwg-3AS* was repeatable in three of the four environments (2019S, 2019F and 2020S). These two QTLs had the resistance alleles from the mutant and explained up to 11.3% and 14.5% of the phenotypic variation, respectively. *QFhb.hwwg-4BS* and *QFhb.hwwg-7AS* were both detected in three environments (2018F, 2019S and 2020S) and by the BLUE values, and Jagger contributed resistance alleles at both loci. *QFhb.hwwg-4BS* showed the largest effect on FHB resistance and explained up to 32.8% of the phenotypic variation, therefore, was chosen for fine mapping. This QTL was delimited to a 1.5 Mb interval on 4BS carrying 17 high confidence genes including *Rht1*, a 'Green Revolution' reduced height gene. *QFhb.hwwg-4BS* overlaps with the major QTL *QPh.hwwg-4BS* for plant height, thus, *QPh.hwwg-4BS* is most likely *Rht-1B*. Map-based cloning and molecular characterization of the resistance mechanisms underlying *QFhb.hwwg-4BS* will facilitate determination of the gene identity and effective deployment of the resistance QTL in breeding.

Acknowledgment 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. Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the US Department of Agriculture. USDA is an equal opportunity provider and employer.

Genetic Determinants of Lipopeptide Sensing Promote Plant Susceptibility to *Fusarium graminearum*

Elizabeth K. Brauer [1,2], Whynn Bosnich [1], Kirsten Holy [1], Indira Thapa [1], Srinivasan Krishnan [3], Moatter Syed[1,2], Melissa Bredow [4,a], Amanda Sproule [1], Monique Power [1,2], Anne Johnston [1], Michel Cloutier [1], Naveen Haribabu[5], Izhar U.H. Khan [1], Jean-Simon Diallo[5], Jacqueline Monaghan[4], Denise Chabot [1], David P. Overy[1], Rajagopal Subramaniam [1], Miguel Piñeros[3,6], Barbara Blackwell [1] and Linda J. Harris [1]

1. Ottawa Research and Development Centre, Agriculture and Agri-Food Canada, Ottawa, ON, Canada
2. Department of Biology, University of Ottawa, Ottawa, ON, Canada
3. Boyce Thompson Institute for Plant Research, Cornell University, Ithaca, NY
4. Biology Department, Queen's University, Biological Sciences Complex, 116 Barrie St, Kingston, ON, Canada
5. Ottawa Hospital Research Institute, 501 Smyth Road, Ottawa, Ontario K1H 8L6, Canada
6. Robert W. Holley Center for Agriculture and Health, United States Department of Agriculture–Agricultural Research Service, Cornell University, Ithaca, NY

a. Present address: Department of Plant Pathology and Microbiology, Iowa State University, Ames, IA

Corresponding Author: Elizabeth Brauer, elizabeth.brauer@agr.gc.ca

Abstract

Fusarium graminearum produces secondary metabolite virulence factors during infection to overcome host defenses and survive in a nutrient-poor environment. We recently identified a cyclic lipopeptide produced by *Fusarium graminearum* called gramillin which is a virulence factor in maize silks but not in wheat spikes. Gramillin is toxic to maize but not to wheat cells, though the mechanism for gramillin toxicity is unknown. Our work indicates that gramillin targets plant membranes which it disrupts ion homeostasis causing necrosis and cell death. In artificial membranes, gramillin functions as a cation-conducting ionophore, and in leaf mesophyll cells, gramillin causes plasma membrane depolarization and K^+ leakage. Gramillin's toxic effect on plant membranes extends across monocots and dicots where it also induces cellular stress responses including a ROS burst. Its virulence function extends to promoting infection in barley spikes and Arabidopsis seedlings where it suppresses callose formation and promotes expression of secondary metabolite biosynthetic genes. Gramillin-induced ROS bursts in the plant are dependent on the stress response genes *ILK1* and *RBOHD*. During infection, *RBOHD* and *ILK1* suppress callose production and enhance fungal virulence gene expression, leading to plant susceptibility. We conclude that gramillin's ionophore activity is detected by plants and that gramillin targets plant membrane responses to promote susceptibility to the *F. graminearum* fungal pathogen.

Barley Genetic Engineering Facility for FHB Research Community

Alison Dineen [1], Michael A. Lawton [1], and Rong Di [1]
1. Rutgers, the State University of New Jersey, Department of Plant Biology, New Brunswick, NJ
Corresponding Author: Rong Di, rongdi@sebs.rutgers.edu

Abstract
Genetic engineering technology has emerged as a powerful tool to elucidate mechanisms of plant disease resistance and develop disease resistant plants. This technology has potential in the battle against *Fusarium graminearum* (*Fg*) in barley, especially as no resistant barley cultivars are currently available. Nevertheless, the potential of this technology is constrained by the ability to transform and regenerate gene-edited plants *in vitro*, with barley proving to be particularly challenging. The established protocols for producing genetically engineered barley are genotype dependent, exhibit low efficiency, and are labor-intensive. To address this issue, we established the Barley Genetic Engineering Facility for FHB Research Community since 2022. This facility, supported by the USWBSI, provides services to develop tissue culture protocols for customer-based barley cultivars and for optimizing transformation efficiency to produce transgenic barley plants with customer-provided constructs. Plant tissue culture protocols using immature scutellum explant have been developed for several barley cultivars, including the two-rowed spring barley 'Genesis' and 'Lightning,' the winter cultivar 'Thunder,' and the six-rowed barley 'Morex'. We are currently developing barley tissue culture protocols using seedling-derived leaf tissues as explants. To optimize barley transformation efficiency, we have utilized the pUBI:RUBY vector to assist screening for transformation events. We have also implemented CNQX pretreatment to improve callus induction and plant regeneration from scutellum tissues. Additionally, we have developed constructs that incorporate morphogenic genes, such as *Babyboom* and *Plethora,* which may enhance barley transformation and regeneration efficiencies. Our optimized, genotype-independent barley tissue culture and transformation protocols will aid in the production of transgenic and gene-edited barley plants for the FHB Research Community.

Acknowledgment and Disclaimer
This material is based upon work partially supported by the U.S. Department of Agriculture, under Project ID #FY22-GD-003. This is a cooperative project with the U.S. Wheat & Barley Scab Initiative. Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the authors and do not necessarily reflect the view of the U.S. Department of Agriculture.

Molecular Investigations into Fusarium Head Blight of Barley and Wheat

Lovepreet Singh [1], Yadong Huang [1], Gerit Bethke [1], Erin Schwister [2], Gary Muehlbauer [1], and Mitch Elmore[2]
1. University of Minnesota, Department of Agronomy and Plant Genetics, St. Paul, MN
2. USDA-ARS Cereal Disease Lab, St. Paul, MN
Corresponding Author: Mitch Elmore, mitch.elmore@usda.gov

Abstract
Plant-pathogen interactions are shaped by complex, multi-level signaling networks that ultimately control disease outcomes. By reconstructing the topology of these networks, we can begin to understand how signals are propagated and predict the genes that have the largest influence on plant phenotypes. To this end, we are using integrative multi-omics coupled with network biology to dissect the molecular basis for Fusarium Head Blight (FHB) disease of barley and wheat. We performed time course infection experiments in barley leaves and spikes to analyze host and pathogen signaling during disease progression. Tissue was collected and multi-omics profiling revealed differentially regulated transcripts, proteins, and phosphorylated peptides at specific stages of infection. Preliminary proteomics experiments quantified a total of 12,000 protein groups and 14,000 phospho-peptides, including 3,100 proteins and 4,100 phospho-peptides from *F. graminearum*. We use a systems-biology approach to integrate these orthogonal datasets into multi-level models of cellular signaling. Gene regulatory networks predict the transcription factors that regulate modules of differentially expressed transcripts. Phospho-signaling networks identify activated kinases and predict their phosphorylated targets. Proteins with high centrality in the reconstructed networks will be prioritized for functional validation. We are especially interested in host genes that contribute to susceptibility, as well as genes in *Fusarium* that regulate pathogenicity. This dual-organism, multi-omics strategy represents a powerful approach to predict and test the genes that regulate FHB and serves as a foundation for understanding and engineering disease resistance in cereal crops.

Reduction of Fusarium Head Blight and Trichothecene in Transgenic Wheat Expressing *Fusarium graminearum* Trichothecene 3-O-acetyltransferase

Guixia Hao [1], Gabdiel Yulfo-Soto [1,2], Hui Chen[3], Guihua Bai[3,4], Harold N. Trick[5], and Susan McCormick [1]

1. USDA, Agricultural Research Service, National Center for Agricultural Utilization Research, Mycotoxin Prevention and Applied Microbiology Research Unit., Peoria, IL
2. Oak Ridge Institute for Science and Education, USDA, Agricultural Research Service, National Center for Agricultural Utilization Research, Mycotoxin Prevention and Applied Microbiology Research Unit, Peoria, IL
3. Department of Agronomy, Kansas State University, Manhattan, KS
4. USDA-ARS, Hard Winter Wheat Genetics Research Unit, Manhattan, KS
5. Department of Plant Pathology, Kansas State University, Manhattan, KS

Corresponding Author: Guixia Hao, guixia.hao@usda.gov

Abstract

Fusarium graminearum, the causal agent of Fusarium head blight (FHB), produces various mycotoxins that contaminate grains and cause profound health problems in humans and animals. Deoxynivalenol (DON) is the most common trichothecene mycotoxin occurring in contaminated grains. Our previous study showed that Arabidopsis expressing *F. graminearum* trichothecene 3-*O*-acetyltransferase (*FgTRI101*) can convert DON to 3-acetyldeoxynivalenol (3-ADON) and excrete it outside of Arabidopsis cells. To investigate whether wheat could similarly convert and excrete 3-ADON, potentially reducing the incidence of FHB and mycotoxin contamination, *FgTRI101* was cloned and introduced into wheat cv Bobwhite, driven by the maize ubiquitin promoter (Ubi-1). Four independent transgenic lines containing *FgTRI101* were identified. Gene expression studies revealed high expression levels of *FgTRI101* in both wheat leaf and spike tissues in one of the transgenic lines (Tri101-1606). We compared the phytotoxic effects of purified DON on the root growth of transgenic wheat expressing *FgTRI101*. The seedlings from two FgTri101 transgenic wheat lines displayed significantly longer root lengths on media containing DON than the controls. However, the conversion of DON to 3-ADON in the FgTri101 transgenic wheat seedlings was only detected in the Tri101-1606 line inconsistently. FHB evaluation assays showed that transgenic wheat plants expressing FgTri101 enhanced FHB resistance. Significantly less DON accumulation was observed in FgTri101 expressing lines, but no 3-ADON was detected in infected wheat spikes, suggesting that 3-ADON is unstable in wheat. Our study suggests that utilizing the fungal self-protection mechanism is a promising method to control FHB and mycotoxin contamination.

Acknowledgment 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.

Mutations in *WhNPR3* and *WhNPR4* Increase Resistance Against *Fusarium graminearum* in Arabidopsis and Wheat

Md Ashraful Islam [1], Isha Mittal [1], Elena Shulaev [1], Anil Girija [1], Steve Scofield [2] and Jyoti Shah [1]
1. Department of Biological Sciences and BioDiscovery Institute, University of North Texas, Denton, TX
2. USDA-Agricultural Research Service, West Lafayette, IN
Corresponding Author: Jyoti Shah; Ph 940 565-4494; Email: Jyoti.Shah@unt.edu

Abstract
In Arabidopsis, NPR1 and its role in SA signaling has been very well studied. NPR1-like proteins NPR3 and NPR4 bind to the same TGA factors that bind NPR1, to inhibit their ability to activate defense gene expression. NPR3 and NPR4 function counteract the ability of NPR1 to activate defenses. Knockdown of *NPR3* and *NPR4* results in enhanced resistance to some pathogens, confirming that they are 'susceptibility factors. *WhNPR3* and *WhNPR4* are expressed in *Fg* infected wheat spikes. In comparison to the Arabidopsis wild-type plants, *npr3*/*npr4* mutant exhibited HR-like symptoms and elevated levels of ROS accumulation, along with reduced fungal biomass in the infected leaves. Besides, *npr3*/*npr4* mutant showed enhanced resistance to *Fg* in Arabidopsis inflorescence compared to wild-type plants. We further demonstrated that non-GMO knockdown of alleles of *WhNPR3* and *WhNPR4* in Kronos TILLING lines resulted in enhanced resistance against *Fg* compared to Kronos wild-type plants. Therefore, natural variants with missense mutation that knockdown activity of *WhNPR3*/*WhNPR4* could provide novel genetic material for integration into FHB resistance breeding programs.

Identification and Functional Characterization of a Candidate Effector Protease from *Fusarium graminearum*

Namrata Jaiswal [1], Martin Darino [2], Erika Kroll [2], Youhuang Xiang[3], Martin Urban [2], Ariana Myers [1], Steven Scofield [1], Roger Innes[3], Kim Hammond-Kosack [2], and Matthew Helm [1]
1. Crop Production and Pest Control Research Unit, USDA-ARS, West Lafayette, IN
2. Rothamsted Research, Harpenden
3. Indiana University Bloomington, Department of Biology, IN
Corresponding Author: Namrata Jaiswal, namrata.jaiswal@usda.gov

Abstract
Most fungal pathogens secrete effector proteins inside host cells to circumvent host immune responses, thereby promoting pathogen virulence. One such fungal pathogen is *Fusarium graminearum*, which causes Fusarium Head Blight (FHB) disease on wheat and barley. Recent transcriptomic analyses revealed that *F. graminearum* likely secretes nearly six hundred effector proteins during the early phases of the infection process, some of which are annotated as proteases. However, the function of *F. graminearum* effector proteases in plant pathogenesis remains unknown. Here, we identified a *F. graminearum* endopeptidase, FgTPP1 (FGSG_11164), that is highly upregulated during early stages of wheat spikelet infection. To elucidate the potential role of FgTPP1 in *F. graminearum* virulence, we generated FgTPP1 deletion mutants (ΔFgTPP1) and performed FHB virulence assays. While the ΔFgTPP1 mutant was able to colonize the inoculated spikelet, the disease symptoms were consistently reduced when compared to wild-type *F. graminearum* strain PH-1. To further elucidate the potential mechanisms involved, we transiently expressed FgTPP1 in *Nicotiana benthamiana* using agroinfiltration. Our analyses revealed that FgTPP1 localizes to the chloroplast stroma and suppresses both chitin-dependent reactive oxygen species (ROS) production as well as NLR-mediated defense responses. Our results provide new insights into the functions of a *F. graminearum* effector protease in plant pathogenesis.

Apoplast and Exosome Content Changes in Barley Leaves in Response to *Fusarium graminearum* Infection

John E. McLaughlin [1], Aysha Ponna [1], Silvia Rojas Juarez [1], and Nilgun E. Tumer [1]
1. Rutgers, The State University of New Jersey, Department of Plant Biology and Pathology, New Brunswick, NJ
Corresponding Author: John E. McLaughlin, mclaughj@sebs.rutgers.edu

Abstract
Host-induced gene silencing (HIGS) and spray-induced gene silencing (SIGS) have been shown to be effective in controlling plant pathogens. Understanding the role of both natural and engineered trans-kingdom RNAi in plant immunity is recognized as important for reducing FHB. *F.g*. grows within the apoplast prior to infecting cells so understand that growth environment is critical to developing a better understanding of the plant-pathogen interaction. Previous work in the lab has shown that exosomes isolated from barley apoplastic fluid from *F.g*. infected plants contain increased amounts of small heat shock proteins, annexins, GAPDH and GSH enzymes, among other induced proteins. To better understand the apoplast proteome in response to *F.g*. infection, a time-course was set up using the barley variety Conlon and leaf tissue was sampled at: 0, 24, 48, and 72 hours post-infection (HPI). Apoplast fluid was sampled, and label-free proteomic analysis was performed via spectral scanning. The results reveal that barley leaf apoplastic fluid (48 HPI) becomes enriched with pathogenesis-related (PR) proteins such as chitinases, nsLTPs, thaumatin-like proteins, Bowman–Birk-type trypsin inhibitors, and leucine-rich repeat proteins, among other PR-related proteins. The development of an efficient apoplastic isolation method using barley allowed us to reveal these differences between *F.g*. infected plants and mock treatment. The proteins identified suggest potential resistance mechanisms specific to the apoplast which may impact the severity of FHB which develops in small grain cereals.

Dual RNA-Sequencing Analysis of *Lpx3* Conferred Resistance in Wheat During *Fusarium graminearum* Infection

Isha Mittal [1], Syeda Alam [1], Katherine Berg[2], Yanhong Dong[3], Harold N. Trick[4], Michael Kolomiets[2], Steve Scofield[5], and Jyoti Shah [1]

1. University of North Texas, Department of Biological Sciences and BioDiscovery Institute, Denton, TX
2. Texas A&M University, Department of Plant Pathology and Microbiology, College Station, TX
3. University of Minnesota, Department of Plant Pathology, St. Paul, MN
4. Kansas State University, Department of Plant Pathology, Manhattan, KS
5. USDA-Agricultural Research Service, West Lafayette, IN

Corresponding Author: Jyoti Shah, Jyoti.Shah@unt.edu

Abstract

Fusarium head blight (FHB), which is caused by *Fusarium graminearum* (*Fg*), is a highly significant and destructive disease affecting wheat. It results in decreased grain yield and contamination with mycotoxins. Understanding the molecular mechanisms of wheat resistance to FHB is crucial to develop effective strategies for managing this disease. Our research has identified a susceptibility gene, *Lpx3*, which is encoded by the 9-lipoxygenase (9-LOX) gene and is involved in the biosynthesis of oxylipins, which might be involved in fungal development and inter-kingdom communication. Fungal infection of wheat cells trigger dynamic changes of gene expression in both *Fg* and wheat, resulting in intricate interactions between the pathogen and the host. Dual RNA-seq for wheat and fungal transcripts was done to gain insights on the mechanism of *Lpx3*-knockdown-conferred resistance to FHB. In this study, we conducted a comparative analysis of the wheat transcriptome and *Fg* transcriptome in planta during the infection of susceptible (Bobwhite BW) and resistant (*Lpx3* RNAi in BW background) wheat lines after 72 h of infection. Our analysis revealed a regulation of significant number of genes in both wheat and *Fg* that are involved in processes such as plant hormone signal transduction, fatty acid metabolism, cellulose metabolism, cell wall degradation, fungal virulence, and pathogenicity during the infection. In summary, we generated databases of wheat genes and in planta-expressed genes of *Fg* during FHB infection which can provide valuable insight into the interactions between wheat and *Fg* and the mechanism underlying *Lpx3*-knockdown-conferred enhanced resistance to FHB.

Fg-DUMP Cleaves Wheat TaCRR1 and Induces Plant Defenses

Todd A. Naumann [1], Guixia Hao [1], Patrick F. Dowd [1], Eric T. Johnson [1], Michael J. Naldrett[2], Neil P.J. Price [1], and Michael J. Bowman[1]
1. USDA-ARS National Center for Agricultural Utilization Research, Peoria, IL
2. Nebraska Center for Biotechnology, Beadle Center, University of Nebraska-Lincoln, Lincoln NE
Corresponding Author: Todd A. Naumann, todd.naumann@usda.gov

Abstract
We recently reported a fungal subtilase-type protease from *Verticillium dahliae* that cleaves a secreted defense protein called CRR1 in cotton. CRR1 contains two non-catalytic protein domains called DUF26 (domain of unknown function 26) and is implicated in defense against Verticillium wilt. DUF26 domains are associated with diverse proteins involved in defense. The fungal protease, which we called Vd-DUMP for *V. dahliae* DUF26 modifying protein, induces plant cell death when infiltrated into plant leaves. Here we report that *Fusarium graminearum* and *Fusarium verticillioides*, mycotoxin producing pathogens of wheat and corn, also secrete DUMPs. We also identify wheat protein TaCRR1 as a DUMP target.

Unraveling the Biochemical and Molecular Role of the UDP-Glucosyltransferase UGT13248 During Fusarium Head Blight

Gerit Bethke [1], Sean P. O'Mara [1], Yadong Huang [1], Franz Berthiller[2], Gerhard Adam[3], and Gary J. Muehlbauer [1]

1. University of Minnesota, Department of Agronomy and Plant Genetics, St. Paul, MN
2. University of Natural Resources and Life Sciences, Department of Agrobiotechnology, Vienna, Austria
3. University of Natural Resources and Life Sciences, Institute of Microbial Genetics, Vienna, Austria

Corresponding Author: Gary Muehlbauer, muehl003@umn.edu

Abstract

Barley, *Hordeum vulgare*, has been shown to have broad type-2 resistance to the disease Fusarium Head Blight caused by *Fusarium graminearum*. The UDP-Glucosyltransferase UGT13248 detoxifies the major *F. graminearum* mycotoxin Deoxynivalenol (DON) by conversion to the glucoside Deoxynivalenol-3-O-glucoside (D3G). UGT13248 confers type-2 resistance to *F. graminearum* by limiting fungal spread within the rachis. Wildtype barley plants inoculated with a wildtype strain of *F. graminearum*, PH1, show similar FHB symptoms as barley plants inoculated with trichothecene-deficient strain of *F. graminearum*, *Δtri5*. Barley plants lacking a functional UGT13248 (H369Y) are highly susceptible to wildtype PH1, but are indistinguishable from wildtype plants when inoculated with a *Δtri5* strain. This suggests that type-2 resistance in barley is dependent on UGT13248 conversion of trichothecenes to their glucoside derivatives. Various strains of *F. graminearum*, including PH1, 00-500, 02-15, 06-205, are capable of producing different chemically related trichothecenes: 15-acetyl-deoxynivalenol (15ADON), 3-acetyl-deoxynivalenol (3ADON), Nivalenol (NIV) and NX2, respectively. Previously, it was shown that UGT13248 converts NIV to NIV-3G. Here, we show that UGT13248 converts the remaining mycotoxins to their glucoside derivative. Further, plants lacking UGT13248 function (H369Y) show increased susceptibility to each *F. graminearum* chemotype. This suggests that UGT13248 confers type-2 resistance to a broad range of *F. graminearum* chemotypes and their associated mycotoxins. RNA-seq analysis is ongoing and attempts to determine the molecular pathways involved in type-2 resistance in barley. Wildtype barley plants are being compared to H369Y plants at 2, 4, and 6 days post inoculation with *F. graminearum*.

Application of Wheat Resistance Genes for FHB Control in Barley

Mitchell Ritzinger [1], Shaobin Zhong [1], and Shengming Yang [1,2,3]
1. North Dakota State University, Department of Plant Pathology, Fargo, ND
2. North Dakota State University, Department of Plant Sciences, Fargo, ND
3. USDA-ARS Cereals Research Unit, Edward T. Schafer Agriculture Research Center, Fargo, ND
Corresponding Author: Shengming Yang, shengming.yang@usda.gov

Abstract
Fusarium head blight (FHB) caused by the fungal pathogen *Fusarium graminearum* is one of the most devastating diseases in barley (*Hordeum vulgare*). However, sources of effective resistance to FHB are very limited in barley germplasm. In the present study, we manipulated and employed host genes from wheat (*Triticum aestivum*), including *Fhb1* and *Fhb7*, to enhance barley resistance to FHB. BLAST searches revealed that the susceptibility allele (*TaHRC*) of *Fhb1*, which encodes a putative histidine-rich calcium-binding protein, is widely conserved in cereal species. Using CRISPR-mediated mutagenesis, we have generated loss-of-function mutations in *HvHRC*, the barley ortholog of *TaHRC*. Additionally, we also transferred *Fhb7*, driven by either the native promoter or a constitutive promoter, into barley cv. 'Bowman' through *Agrobacterium*-mediated transformation. However, the *Fhb7* gene was silenced in most of the transgenic plants carrying when it is constitutively expressed. Homozygous loss-of-function *HvHRC* mutants and *Fhb7*-transformants were identified using Sanger sequencing and real-time PCR (q-PCR), respectively, which were challenged with the pathogen and phenotyped under field conditions. Our preliminary results suggest that both the disruption of *HvHRC* and transformation of *Fhb7* into barley improved resistance to FHB. Therefore, bioengineering cloned genes involved in FHB resistance in wheat may provide novel strategies to reduce losses to this destructive disease in barley.

Molecular Mapping of Fusarium Head Blight (FHB) Resistance in a Spring Wheat Mapping Population

Shahed Safar [1], Yueqiang Leng [1], Alireza Poursafar [1], Joe Mullins [1], Amna Riasat [1], Olawumi Amusan [1], Amy Bernado[2], Yuzhou Xu[3], Paul St. Amand[2], Chenggen Chu[4], Guihua Bai[2], Steven Xu[5], and Shaobin Zhong [1]

1. Department of Plant Pathology, North Dakota State University, Fargo, ND
2. USDA-ARS, Hard Winter Wheat Genetics Research Unit, Manhattan, KS
3. Department of Agronomy, Kansas State University, Manhattan, KS
4. USDA-ARS, Sugarbeet and Potato Research Unit, Fargo, ND
5. USDA-ARS Western Regional Research Center, Albany, CA

Corresponding Author: Shaobin Zhong, shaobin.zhong@ndsu.edu

Abstract

Fusarium head blight (FHB), mainly caused by *Fusarium graminearum, is* a devastating disease in wheat worldwide. The disease causes significant yield losses and reduces grain quality. Use of host resistance is one of the major components in the integrated approach to manage the disease. However, resistance to FHB is a complex trait controlled by quantitative trait loci (QTL) and affected by environmental conditions. Identification of QTL for FHB resistance and development of DNA markers associated with them in the resistance sources are critical for their effective deployment in wheat breeding programs. In this study, we aimed to map and validate QTL for FHB resistance in a bi-parental population consisting of 115 recombinant inbred lines (RILs) derived from the cross between a spring wheat cultivar 'Glenn' and a spring wheat breeding line 'GP112'. Glenn was developed by NDSU spring wheat breeding program from a cross between 'ND2831' and 'Steele-ND', and it exhibits moderate resistance to FHB. GP112 is an FHB-resistant RIL derived from a cross between 'Grandin' (an FHB-susceptible wheat cultivar) and 'PI 277012' (a wheat line conferring a high level of FHB resistance). The RIL population was evaluated for FHB resistance in three greenhouse experiments and three field disease nurseries located in Fargo and Langdon in 2021 and 2022, respectively. Genotyping-by-sequencing (GBS) of RILs and their parents (Glenn and GP112) was conducted to generate SNP markers and a genetic map with 806 unique SNP markers covering 1723.73 cM was constructed. QTL analysis identified one major FHB resistance QTL on chromosome 3A, which explained 16% of the phenotypic variation in the greenhouse experiments. Three minor QTL for FHB resistance were detected on chromosomes 1A, 2D and 4B in only one or two field experiments. Our findings provide insights on genetics of FHB resistance and may facilitate FHB resistance improvement in wheat breeding programs.

Acknowledgment and Disclaimer

This material is based upon work partially supported by North Dakota Wheat Commission and the U.S. Department of Agriculture under Agreement No. 59-0206-2-162 (USWBSI). This is a cooperative project with the U.S. Wheat & Barley Scab Initiative. Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the authors and do not necessarily reflect the view of the U.S. Department of Agriculture.

A *Fusarium graminearum* Effector FgTPP1 Targets Chloroplasts and Suppresses Plant Immunity

Youhuang Xiang[1] and Roger W. Innes[1]
1. Department of Biology, Indiana University, Bloomington, Indiana
Corresponding Author: Roger Innes, rinnes@indiana.edu

Abstract
Fusarium head blight (FHB), caused mainly by *Fusarium graminearum*, is a common and destructive fungal disease that frequently occurs worldwide, leading to immense economic cost due to yield loss and grain contamination. However, sustainable genetic resources for FHB are still limited yet. Therefore, engineering resistance in wheat to FHB by creating decoy substrates for FHB effector proteases may be considered as one of promising coping strategies. Here we characterized one putative *F. graminearum* effector FgTPP1, which contains chloroplast transit peptide, and demonstrated it partially accumulates in chloroplasts and harbors two major forms when transiently expressed in *Nicotiana benthamiana*. Deletion of the N-terminal propeptide section of FgTPP1 results in translocation of FgTPP1 from cytosol to chloroplast. Transient expression of FgTPP1 inhibits RPS5-mediated HR response in tobacco leaves. Wheat LSD1-type zinc finger protein (TaLSD1) was identified as a likely interacting partner of FgTPP1 by yeast two-hybrid screening, and the protein interaction was verified by co-immunoprecipitation. Overall, our results show that FgTPP1 is translocated into chloroplasts once its propeptide is removed, and it may avoid triggering cell death by targeting to TaLSD1 and possibly interfere with plant immunity.

Effects of a Glutathione S-transferase Gene (*Fhb7*) on FHB Resistance in Wheat

Lanfei Zhao[1,2], Amy Bernardo[3], Fanmei Kong[1,2], Wei Zhao[1,2], Yanhong Dong[4], Harold N. Trick[5], Hyeonju Lee[5], Jessica Rupp[5], and Guihua Bai[1,3]

1. Department of Agronomy, Kansas State University, Manhattan, KS
2. National Key Laboratory of Wheat Improvement, Shandong Key Laboratory of Wheat Improvement, College of Agronomy, Shandong Agriculture University, Taian, Shandong, China
3. USDA-ARS Hard Winter Wheat Genetics Research Unit, 4008 Throckmorton Hall, Manhattan, KS
4. Department of Plant Pathology, University of Minnesota, St. Paul, MN
5. Department of Plant Pathology, Kansas State University, Manhattan, KS

Corresponding Author: Guihua Bai, guihua.bai@usda.gov

Abstract

Fusarium head blight (FHB), mainly incited by *Fusarium graminearum* Schwabe, has caused great losses in grain yield and quality of wheat and barley globally. *Fhb7*, a major gene for FHB resistance, has been cloned and found to encode a glutathione S-transferase (*GST*). *Fhb7* originated from 7E chromosome of *Thinopyrum ponticum* and confers broad resistance to *Fusarium* species. However, some recent reports raised doubt about whether *GST* is the causal gene of *Fhb7*. To validate the gene function of *GST* in wheat, we phenotyped *Fhb7* near-isogenic lines (Jimai22-*Fhb7* vs Jimai22) and *GST* over-expression lines for FHB resistance. The *Fhb7* NILs were planted in the Kansas State University FHB nursery in 2018 and 2020. Jimai22-*Fhb7* showed significantly higher FHB resistance with a lower PSS, FDK and DON concentration than susceptible Jimai22. In the *GST* transgenic lines, *GST* was highly overexpressed in almost all the positive transgenic lines driven by either the maize ubiquitin promoter (MubiP) or its native promoter (NP) in the wheat cultivar 'Fielder' and those transgenic T_2 plants showed high FHB resistance. Only one MubiP-driven transgenic line showed low *GST* expression and similar susceptibility as Fielder, suggesting high *GST* expression confers *Fhb7* FHB resistance. We also knocked out *GST* in Jimai22-*Fhb7* line using the CRISPR-Cas9-mediated gene-editing. The *GST*-knockout lines showed significantly higher FHB susceptibility compared with the non-edited control plants. Therefore, *GST* is the causal gene of *Fhb7* FHB resistance and its gene expression level significantly infects the gene function in FHB resistance.

Pathogen Biology & Genetics (PBG)

Experimentally Tractable Systems for Investigating Fusarium Head Blight-Microbiome Interactions on Barley: A Pilot Study

Joan Acaso[1], Brooke Benz[2], Thomas Baldwin[2], Briana Whitaker[3], and Barney Geddes[1]
1. North Dakota State University, Department of Microbiology, Fargo, ND
2. North Dakota State University, Department of Plant Pathology, Fargo, ND
3. USDA-ARS, Mycotoxin Prevention & Applied Microbiology Unity, NCAUR
Corresponding Author: Joan Acaso, joan.acaso@ndsu.edu

Abstract
Fusarium graminearum causes the catastrophic Fusarium head blight (FHB), affecting wheat and barley and negatively impacting yields and quality. Currently, there is no effective strategy for suppressing FHB utilizing the barley phyllosphere microbiome. This pilot study aims to establish a stable microbial community with reproducible assembly in the barley phyllosphere to investigate plant-microbe interactions that suppress pathogen proliferation leading to plant protection in barley. In this case, the microbes utilized are members of the barley spike phyllosphere core microbiome as predicted from a large amplicon sequencing study that profiled the composition of 800 diseased and non-diseased barley heads of ten genotypes collected from four misted nurseries across the U.S. A trial *Hordeum vulgare* phyllosphere Synthetic Community *(Hv*-PSC-Fargo) constructed from a cultured microbiome collection derived from diseased and non-diseased Barley spikes collected in Fargo, ND. A number of experiments were carried out to investigate approaches to establish the *Hv*-PSC-Fargo community in barley plants grown in growth chamber settings. These involved optimizing the sterilization of barley seeds to remove the background seed microbiome, cultivating strains, and determining the initial community member ratios to preserve community diversity. Using these approaches, one trial involved inoculating sterilized seeds with *Hv*-PSC-Fargo, heat-killed *Hv*-PSC-Fargo, and PBS only to assess successful recruitment to the spikes using ND Genesis barley. In another experiment, short barley genotypes were grown in inert soil substitutes (sand: vermiculite) and Promix: Osmocote on Lennard jars to explore the transition to more gnotobiotic systems for growth. In the long term, this study will improve our knowledge of some of the many variables influencing the assembly of microbial communities, allowing us to tackle the shortcomings of the inconsistent efficacy of biocontrol strains and gain a deeper understanding of key drivers of microbial community assembly in the context of disease, and its impact on disease outcomes.

Host Genotype and Fusarium Head Blight Status Impact Microbiome Assembly of a Barley Breeding Population Across Four Locations

Brooke R. Benz[1], Joan Acaso[2], Abbeah R. Navasca[1], Diel D. Velasco[1], Eglantina Lopez Echartea[2], Briana Whitaker[3], Thomas Baldwin[1], and Barney Geddes[2]

1. North Dakota State University, Department of Plant Pathology, Fargo, ND
2. North Dakota State University, Department of Microbiology, Fargo, ND
3. USDA Agricultural Research Service, National Center for Agricultural Utilization Research, Mycotoxin Prevention and Applied Microbiology Research Unit, Peoria, IL

Corresponding Author: Thomas Baldwin, thomas.t.baldwin@ndsu.edu

Abstract

Understanding plant microbiomes may offer new insights into plant disease management. Fusarium head blight (FHB), caused by *Fusarium graminearum*, is a serious disease of wheat and barley that impacts both grain yield and quality via mycotoxin contamination. We hypothesized that differential enrichment in the barley spike in response to disease could identify plant-recruited microbiota with the potential to inhibit FHB. Selective breeding of barley lines may then be used to harness the barley spike microbiome in a beneficial manner. We analyzed the composition of the barley spike microbiome during FHB disease onset from ten genotypes of a breeding population grown in four FHB nurseries (ID, MN, ND, and NY). Bacteria and fungi were identified using amplicon sequencing and analyzed for their responsiveness to disease and genotype. Barley genotype significantly affected bacterial community composition at each location, while disease had a significant effect only in MN, where disease pressure was highest. There was also a significant genotype by disease effect at three locations. The microbiome at each location contained plant-beneficial taxa including: *Alternaria, Crytococcus, Pantoea, Pseudomonas, Pseudozyma,* and *Sphingomonas*, among others. High-throughput bacterial culturing on 2022 samples resulted in the collection of ~2,000 isolates. These will be used to generate synthetic communities to test microbiome drivers by host genotype and the ability to protect against FHB.

Genetic Basis of Variation in Ascospore and Mycotoxin Production in U.S. *Fusarium graminearum* Isolates

Upasana Dhakal [1], John F. Leslie [1], and Christopher Toomajian [1]
1. Kansas State University, Department of Plant Pathology, Manhattan, Kansas
Corresponding Author: Christopher Toomajian, toomajia@ksu.edu

Abstract
Fusarium graminearum causes Fusarium head blight, a devastating disease of wheat and barley. The pathogen also contaminates infected grains with the mycotoxin deoxynivalenol (DON), making them unsuitable for food and feed. The objective of this study is to identify the genetic basis of variation in ascospore and mycotoxin production using genome-wide association (GWAS). *Fusarium graminearum* overwinters on crop debris, and under favorable conditions produces perithecia, the pathogen's sexual fruiting bodies. The perithecia produce ascospores, which are the primary source of infection in the field. DON, besides having health consequences, is also a virulence factor. Genes responsible for the biosynthesis of DON and its derivatives cluster in three *TRI* loci, but genes outside the *TRI* loci may affect the amount of DON produced by different isolates. We measured ascospores and mycotoxins (DON and 15ADON) produced by 152 isolates in controlled laboratory experiments. Ascospores were collected from cultures grown on carrot agar plates and counted. The amounts of DON and 15ADON produced from isolates grown on rice cultures were measured using GC-MS. GWAS was conducted using the models implemented in two R packages. We identified 14 SNPs significantly associated with ascospore production, six of which were identified by more than one model. SNPs associated with ascospore production are located in, or in close proximity to, a secondary metabolite biosynthesis cluster and genes that code for various proteins, including: a peroxisomal-coenzyme A synthetase, a copper transporter, and a permease. Similarly, eight SNPs were significantly associated with DON, and three with 15ADON production. One associated SNP is common to DON and 15ADON production and falls in a polyketide biosynthesis gene cluster. Other associated SNPs are found in, or are in linkage disequilibrium with, SNPs in: an efflux family gene or genes that code for a non-ribosomal peptide synthetase and an MFS transporter. Many associations we identified are novel and provide new candidates for functional studies. Once verified, they can be used as markers to monitor field populations for their ascospore and DON production potential, which can further inform management decisions.

Acknowledgment and Disclaimer
This material is based upon work supported in part by the U.S. Department of Agriculture under Agreement No. 59-0206-2-162. 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.

Integrative Genome Analysis of *Fusarium graminearum* Isolated from Diverse Small Grain Hosts

Hugo Conde [2], Tasneem Fathima [1], Rachel C. Hall[3], Jose Gonzalez [1], Shaukat Ali [1], Gazala Ameen [1], and Shyam Solanki [1]

1. Department of Agronomy, Horticulture & Plant Science, South Dakota State University, Brookings, SD
2. Department of Sciences and Mathematics, Belmont University, Nashville, TN
3. Department of Plant, Soil and Microbial Sciences, Michigan State University, East Lansing, MI

Corresponding Author: Gazala Ameen, Gazala.Ameen@sdstate.edu

Abstract

Fusarium head blight (FHB) caused by *Fusarium graminearum* (*Fg*), an ascomycete fungi, is a persistent disease of cereal crops in world food baskets including South Dakota, and a serious threat to the quality and quantity of grain production in wheat, barley, triticale, and oats. *Fusarium* secretes various mycotoxins that are harmful to humans and animals making infected grain unsuitable for food and feed. Being a broad host range pathogen, its genome related to virulence, mycotoxin production, and yet to be characterized plant-pathogen-interaction loci are continuously incorporating nucleotide changes responding to host immunity. These changes and their maintenance in the genome correspond to their potential utility in pathogen survival fitness, and phenotypes related to host adaptations resulting in fluidic spatio-temporal pathogen genome diversity. Thus, our goal is to study temporal pathogen genome diversity in relation to the diverse small grain hosts. To do so, we sequenced 23 *Fg* isolates collected from barley, wheat, and oat fields in South Dakota from 2012 to 2022 using the Illumina Nextseq500 platform. For genome analysis, we used both denovo assembly as well as reference-based reads mapping to both genome and the mycotoxin-producing *Tri* gene cluster. In our approach, we used 5 different assembly tools (for short and paired-end reads) and found SPAdes works best for our data. Ongoing work includes whole genome comparison and diversity analysis focusing on mycotoxins related well-studied genes *Tri5* and *Tri3*. We aim to characterize putative genetic shift in the pathogen genome accounting for the additional layer of complexity of its evolution on multiple hosts affecting strategies of resistance deployment.

Role of Chemotype in Aggressiveness and Toxigenicity of *Fusarium graminearum* on Wheat

Simran Goyal [1], Gabdiel E. Yulfo-Soto [1,2], Etta M. Nuckles [1], Robert H. Proctor[3] and Lisa Vaillancourt [1]
1. University of Kentucky, Department of Plant Pathology, Lexington, KY
2. Current Affiliation: USDA-ARS Beltsville Agricultural Research Center, Sustainable Perennial Crops Laboratory, Beltsville, MD
3. USDA-ARS National Center for Agricultural Utilization Research, Mycotoxin Prevention and Applied Microbiology Research, Peoria, IL

Corresponding Author: Lisa Vaillancourt, vaillan@uky.edu

Abstract

Managing Fusarium Head Blight (FHB) caused by *Fusarium graminearum* sensu stricto and other members of the *F. graminearum* species complex (FGSC), is challenging due in part to the diversity of genetic factors impacting disease severity and toxin accumulation. In North America, FHB is primarily caused by three populations of *F. graminearum* ss. The NA1 population mainly produces the trichothecene toxins DON and 15ADON, while NA2 mostly produces 3ADON instead of 15ADON. The NA2 population has been advancing across the Eastern U.S. and Canada, and it has been hypothesized that 3ADON confers higher aggressiveness, toxigenicity, and competitiveness than 15ADON. The present study has three objectives (i) investigate whether toxin chemotype and other genetic markers segregate according to Mendelian principles among progeny resulting from crosses between different populations of *F. graminearum*, (ii) assess differences in aggressiveness, toxigenicity, and competitiveness among individuals and mixtures of progeny resulting from outcrosses in susceptible and moderately resistant wheat, with or without fungicides, (iii) identify DNA markers associated with aggressiveness and toxigenicity by analyzing whole genome sequence data derived from pools of progeny exhibiting variation in these traits. A self-sterile mating tester strain of *F. graminearum*, generated by deleting the *MAT1-1-1* locus in the NA1-15ADON strain PH-1, was crossed with NA2-3ADON, NA3-NX2, and NIV strains. The parents were selected from 19 strains collected from different locations in the United States based on morphology, fertility, and ascospore viability. The NA2-3ADON and NA1-15ADON parents were more aggressive on wheat than the NA3-NX2 and NIV parents. The NA2-3ADON parent produced more mycotoxin than the other parental strains. Ninety-three progeny were randomly chosen from the NA1-15ADON:NA2-3ADON cross, and tested using primers for the *MAT1-1-1* and chemotype loci. The progeny displayed anticipated Mendelian segregation patterns for both primer sets. Subsequently, 80 of the 93 were chosen for additional assays, including disease severity and mycotoxin production on 'Wheaton' and 'Alsen' wheat varieties. This study will provide new insights into the complex genetics of pathogenicity and aggressiveness of *F. graminearum* and the role of chemotype and generate tools for further research aimed at improving management of this important pathogen.

Acknowledgment 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.

Variation in Genome Sequence, Mycotoxin Production and Aggressiveness of *Fusarium* Isolates Used in Barley Screening Nurseries in Six States

Hye-Seon Kim [1], Ieva Tolkaciovaite [1], Robert H. Proctor [1], Martha Vaughan [1], Susan McCormick [1], Mark Busman [1], and Kirk Broders [1]

1. USDA Agricultural Research Service, National Center for Agricultural Utilization Research, Mycotoxin Prevention and Applied Microbiology Research Unit, 1815 N. University, Peoria, IL

Corresponding Author: Hye-Seon Kim, hyeseon.kim@usda.gov

Abstract

Fusarium head blight (FHB) is one of the most economically important diseases of wheat and barley because it occurs widely, reduces grain yield and quality, and contaminates grains with mycotoxins. Although efforts to improve FHB resistance in barley are underway at multiple state screening nurseries, it is unclear whether variation in *Fusarium* strains used by different nurseries impacts efforts to enhance resistance. To address this knowledge gap, we are examining variation in genome sequences, mycotoxin production and aggressiveness of 97 *Fusarium* isolates from barley screening nurseries in six states. Phylogenetic analyses of DNA sequences from three housekeeping genes retrieved from genome sequences of a subset of 66 isolates confirmed that the isolates were all *Fusarium graminearum*. Chemical analysis for trichothecene mycotoxin production indicated that nurseries in two states (Idaho and Minnesota) included both 3-acetyldeoxynivalenol (3-ADON)-producing and 15-acetyldeoxynivalenol (15-ADON)-producing isolates, while the nurseries in the other four states included only 15-ADON-producing isolates. One isolate from the Minnesota nursery did not produce any trichothecene mycotoxins in culture. Assessment of disease induced by a subset of 16 isolates on two barley varieties indicated that some isolates differ markedly in aggressiveness. However, most isolates tested caused high levels of FHB. Chemical analysis of both barley varieties following inoculation indicate that DON levels did not always align with FHB severity. Understanding and incorporating information on genetic and phenotypic diversity of FHB isolates used in screening programs should facilitate development of barley varieties that are resistant to FHB under diverse environmental conditions.

Trichothecene Structural Diversity, Detoxification Enzymes, and Management of Fusarium Head Blight

Robert H. Proctor [1], Susan P. McCormick [1], Guixia Hao [1], Todd A. Naumann [1], Santiago Gutierrez [2] and Mark Busman [1]

1. USDA-ARS, National Center for Agricultural Utilization Research, Mycotoxin Prevention and Applied Microbiology Research Unit, Peoria, IL
2. University of León-Ponferrada, Area of Microbiology, Ponferrada, Spain

Corresponding Author: Robert H. Proctor, robert.proctor@usda.gov

Abstract

Glucosyl transferases and other trichothecene detoxification enzymes are promising tools in efforts to control Fusarium head blight (FHB) because they can reduce both the crop disease and contamination of grain with the mycotoxin deoxynivalenol (DON). Knowledge of DON and structurally related trichothecene analogs has potential to enhance utilization of the enzymes. Trichothecenes are a family of toxic secondary metabolites that are produced by species of at least 10 fungal genera with diverse ecologies. Some of the fungi, such as the FHB fungus *F. graminearum*, are plant pathogens, others are insect pathogens, and still others are saprotrophs. Thus, trichothecenes are produced in varied habitats where they likely function in interactions between diverse organisms. Although approximately 200 trichothecene analogs have been described, individual fungal strains tend to produce only one or a few analogs as final products of trichothecene biosynthesis. All trichothecene analogs share a core chemical structure but differ from one another by the pattern of chemical groups attached to various positions of the core structure. The existence of 200 trichothecene analogs raises the question, what drives trichothecene structural diversity. The role of DON in pathogenesis of *F. graminearum* combined with activities of trichothecene detoxification enzymes provide a potential answer. That is, substrate specificity of trichothecene detoxification enzymes likely drives trichothecene structural diversity. This idea is supported by findings of multiple studies. For example, Michlmayr et al. (2018 Toxins 10:111) found marked variation in activities of glucosyl transferases from barley, rice and *Brachypodium* against the trichothecene analogs DON, nivalenol and T-2 toxin. These and other findings suggest that when a trichothecene-producing fungus encounters a trichothecene detoxification enzyme, adaptations that result in production of structurally novel trichothecene analogs allow the fungus to elude the detoxifying effects of the enzyme. This scenario is reminiscent of gene-for-gene relationships that occur in some plant-parasite interactions. Therefore, when deploying trichothecene detoxification enzymes to combat FHB, incorporation of broad substrate specificity has potential to improve resilience of the enzymes to adaptations in *Fusarium*.

Investigating the Mechanism of Trichothecene Suppression by a Fungal Endophyte

Nicholas Rhoades[1,2], Susan McCormick[1], Mark Busman[1], HyeSeon Kim[1], Robert Proctor[1], Briana Whitaker[1], Martha Vaughan[1], and Guixia Hao[1]

1. USDA-Agricultural Research Service, National Center for Agricultural Utilization Research, Mycotoxin Prevention and Applied Microbiology Research Unit., Peoria, IL
2. Oak Ridge Institute for Science and Education, USDA-Agricultural Research Service, National Center for Agricultural Utilization Research, Mycotoxin Prevention and Applied Microbiology Research Unit, Peoria, IL

Corresponding Author: Guixia Hao, guixia.hao@usda.gov

Abstract

Fusarium head blight (FHB), caused by *Fusarium graminearum*, is a major threat to food safety and security, because it reduces crop yields and contaminates grains with mycotoxins which negatively impact the health of both humans and livestock. Production of the mycotoxin deoxynivalenol (DON) is not required for initial *F. graminearum* infection of wheat heads but is essential for the spread of the fungus within wheat heads. Therefore, suppression of DON production is an attractive FHB control strategy. Endophytes, bacteria or fungi, form intrinsic relationships with their hosts and can inhabit host tissue without causing damage. Previous studies have shown that the corn and wheat endophyte, *Sarocladium zeae*, produce pyrrocidines that suppress production of fumonisin, a mycotoxin produced by *F. verticillioides.* In this study, we discovered that *S. zeae* also suppressed production of 15-acetylDON (15-ADON) in a trichothecene induction medium when co-cultured with *F. graminearum.* The suppression of 15-ADON production was not due to growth inhibition of *F. graminearum* or degradation of 15-ADON by *S. zeae.* Additionally, the suppression was neither caused by pyrrocidines, nor correlated with presence of pyrrocidine synthesis genes in *S. zeae*. This suggests a novel mechanism of suppression of trichothecene production. Therefore, we used transcriptomic and metabolomic approaches to investigate the mechanism by which *S. zeae* suppresses 15-ADON production. Our preliminary analyses showed that the expression of trichothecene biosynthetic genes was down regulated in *F. graminearum* and *S. zeae* co-cultures compared to *F. graminearum* mono-cultures. Furthermore, we tested over 30 *S. zeae* strains and found that 20 of them inhibited 15-ADON production in co-cultures. Interestingly, we found different secondary metabolite profiles in strains that suppressed toxin production compared to those that did not suppress toxin production in cocultures of *S. zeae* and *F. graminearum*. This study will improve our understanding of how *S. zeae* interacts with *F. graminearum* to inhibit toxin production and could lead to a novel strategy to control FHB and mycotoxin contamination.

Development of a CRISPR/Cas9-mediated Genome Editing Protocol for Gene Knockout in *Fusarium graminearum*

Amna Riasat[1], Alireza Poursafar[1],Yueqiang Leng[1], and Shaobin Zhong[1]
1. Department of Plant Pathology, North Dakota State University, Fargo, ND
Corresponding Author: Shaobin Zhong, shaobin.zhong@ndsu.edu

Abstract
Fusarium graminearum Schwabe [teleomorph *Gibberella zeae* (Schweinitz) Petch] is the fungal pathogen that causes Fusarium head blight (FHB), which is the most devastating disease in cereal crops worldwide. FHB significantly reduces yield and grain quality under favorable environmental conditions. Understanding the molecular mechanism underlying the virulence of the pathogen is critical for designing novel strategies to manage the disease. In this study, we aimed to develop a CRISPR/Cas9-mediated genome editing protocol for efficiently knocking out genes in *F. graminearum*. The *Tri5* gene required for DON production in the fungus was chosen as the target and two sites of the gene were selected for designing single guide RNAs (sgRNAs) for the genome editing experiments. The hygromycin resistance gene (HygR) was amplified by PCR using primer pairs containing 50 bp or 60 bp sequences flanking the target sites of *Tri5* and used as donor DNA. PEG-mediated transformation of *F. graminearum* protoplasts was conducted using the donor DNA with or without the Cas9/sgRNA complex added. The results showed that more transformants with the target gene disrupted were generated with the donor DNA combined with the Cas9/sgRNA complex than with the donor DNA only, suggesting that the Cas9/sgRNA complex increased the gene knockout efficiency. Our experiments also indicated that more gene knockout mutants were generated with the donor DNAs containing the 60 bp flanking sequences than those with the 50 bp flanking sequences. The Cas9/sgRNA-mediated gene knock strategy developed in this study will be helpful in advancing functional genomics studies in *F. graminearum*.

Acknowledgment and Disclaimer
This material is based upon work partially supported by North Dakota Wheat Commission and the U.S. Department of Agriculture under Agreement No. 59-0206-2-162 (USWBSI). 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.

High Resolution Melting (HRM) Assay for Rapid Identification and Differentiation of the *Fusarium graminearum* NX-2 Chemotype

Lovepreet Singh [1], Milton T. Drott [2,3], and J. Mitch Elmore [2,3]
1. University of Minnesota, Department of Agronomy and Plant Genetics, St. Paul, MN
2. USDA-ARS Cereal Disease Laboratory, St. Paul, MN
3. University of Minnesota, Department of Plant Pathology, St. Paul, MN
Corresponding Author: J. Mitch Elmore, mitch.elmore@usda.gov

Abstract
Fusarium graminearum isolates are classified into different chemotypes depending on the type of mycotoxin produced, including the type B trichothecenes 3-acetyl deoxynivalenol (3-ADON), 15-acetyl deoxynivalenol (15-ADON), nivalenol (NIV), and the recently identified type A trichothecene NX-2. Molecular tools to differentiate NX-2 producers from other chemotypes have remained relatively laborious and time consuming. In this study, we developed and validated a high-resolution melting (HRM) assay for identification and differentiation of NX-2 chemotype. By analyzing *TRI1* coding sequences from 183 geographically diverse isolates representing all four *F. graminearum* chemotypes, we selected a 75 base pair region containing four non-synonymous single nucleotide polymorphisms (SNPs) that are specific to NX-2 genotypes. The amplicon generated two HRM profiles, one of which was specific for only NX-2. We validated the assay using a panel of 72 diverse pure-culture isolates collected from North America and it unambiguously differentiates NX-2 from other chemotypes. The HRM assay was also successful in identifying NX-2 producers directly from DNA extracted from infected wheat spikes with varying levels of disease severity and fungal biomass. The assay is very sensitive and can detect as little as 0.01 ng of fungal DNA in a background of 50 ng of plant DNA. This new diagnostic tool can be used for high-throughput molecular surveillance of the NX-2 chemotype of *F. graminearum* from plant samples and culture collections.

Acknowledgment and Disclaimer
We are thankful to Dr. H. Corby Kistler and Karen Broz for their very helpful discussions. We would like to thank Karen Broz for excellent technical support. Funding was provided by United States Department of 370 Agriculture-Agricultural Research Service project 5062-21220-024-000D. 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.

Comparative Transcriptomics Provides Insights into Fungal-Plant Interactions of a Pathogen and an Endophyte with Barley

Soumya Moonjely [1], Rebecca Shay [1], and Frances Trail [1,2]
1. Michigan State University, Department of Plant Biology, East Lansing, MI
2. Michigan State University, Department of Plant, Soil and Microbial Sciences, East Lansing, MI
Corresponding Author: Frances Trail, trail@msu.edu

Abstract
Comparative transcriptomics provides a powerful tool to identify the genes related to developmental differences in closely related organisms. In this study, we examined transcriptional similarities and differences during the stages of spore germination/initial infection stages of *Fusarium graminearum* and *Metarhizium anisopliae,* two related fungal species with distinct lifestyles. *F. graminearum* is a plant pathogen and the causal agent of Fusarium head blight on cereal crops, whereas *M. anisopliae* is an insect pathogen and root endophyte that forms beneficial associations with barley. Four conidial germination stages were selected for transcriptome analysis on the host, and on a common growth medium: fresh conidia, polar growth, doubling of the long axis, and first hyphal branching. A substantial difference was shown in the transcriptome of spore germination stages between *F. graminearum* and *M. anisopliae* when colonizing barley. *F. graminearum* expressed a higher number of genes encoding CAZymes and specialized metabolites during host colonization, whereas *M. anisopliae* did not. Both fungal species showed upregulated putative effector genes, particularly proteins with the LysM domain during early colonization events with more effector putative effectors upregulated in *F. graminearum*. Moreover, candidate genes associated with indole-3-acetic acid synthesis were upregulated during the appressorial stage in *M. anisopliae*, but not *F. graminearum*. We have also characterized the formation of biofilms in *F. graminearum*, which provides a basis for understanding the functionality of biofilms in the pathogen disease cycle. Collectively, these studies provide valuable insights into the network of genes necessary for spore germination, pathogenesis/symbiosis and significantly broadens our transcriptome data resources on fungal-plant interactions.

Acknowledgment and Disclaimer
Funding was provided by U.S. Department of Agriculture, under agreement no. 59-0206-0-152 to F.T. This is a cooperative project with the U.S. Wheat and Barley Scab Initiative. This material is based upon work supported by the US Department of Agriculture National Institute of Food and Agriculture under Award No. 2020-67013-31185 to FT. We also acknowledge the support of Michigan State University AgBioResearch.

Barley Microbiome Communities Contain Possible Fusarium Biocontrol Endophytes

Nathan Tyler [1] and Briana K. Whitaker [2]
1. Oak Ridge Institute for Science and Education Intern, USDA-Agricultural Research Service Mycotoxin Prevention and Applied Microbiology Research Unit, National Center for Agricultural Utilization Research, Peoria, IL
2. USDA-Agricultural Research Service, National Center for Agricultural Utilization Research, Mycotoxin Prevention & Applied Microbiology Unit, 1815 N University St., Peoria, IL

Corresponding Author: Briana K. Whitaker, briana.whitaker@usda.gov

Abstract

Fusarium head blight (FHB) is an economically and physiologically damaging disease in barley due to revenue loss and the production of harmful mycotoxins. For example, revenue losses due to FHB can total in the hundreds of millions of dollars per year for U.S. barley growers. Breeding for resistance in barley has faced substantial challenges, thus management of FHB is often achieved by applying chemical fungicides. However, pathogens are developing resistance to common fungicides, which is leading to calls for more sustainable solutions. Biological controls are a possible solution that could be used to control FHB pathogens. One potential source of biocontrols is the microbial communities of asymptomatic barley heads, which may be naturally effective against FHB. This study aimed to answer two questions: how the microbiomes of FHB symptomatic and asymptomatic barley heads differ and if the barley microbiome differs between farm locations. Both FHB symptomatic and asymptomatic barley heads were collected from four fields in central and northeastern North Dakota and fungal endophytes were cultured from asymptomatic spikelets to compare differences in microbiome communities. Load (as estimated by fungal isolations) is higher in symptomatic heads, across all four locations. Genetic sequencing is ongoing, but we expect to identify fungal endophyte taxa unique to, or enriched in, the asymptomatic heads relative to the symptomatic heads, and vice versa. In addition, microbiome composition is expected to differ between different field sites, based on previous results in barley and wheat microbiomes. After sequencing the fungal endophytes and analyzing their differences across symptomatic and asymptomatic barley head microbiomes, we hope to test fungal endophytes for their antagonism against *Fusarium* pathogens *in vitro*, as well as their ability to serve as biocontrol agents for FHB in barley plants.

Wheat and Barley Variety and *Fusarium graminearum* Population Affect Trichothecene Mycotoxin Accumulation

Martha M. Vaughan [1], Shoshana Martinez [1], William Hay[1], Susan P. McCormick [1], and Mark Busman [1]
1. USDA Agricultural Research Service, National Center for Agricultural Utilization Research, Mycotoxin Prevention and Applied Microbiology Research Unit. 1815 N. University, Peoria, IL
Corresponding Author: Martha M. Vaughan, martha.vaughan@usda.gov

Abstract
Trichothecene mycotoxins are toxic metabolites produced by fungal pathogens, such as *Fusarium graminearum* (*Fg*), that infect cereal crops reducing grain quality and safety. The severity of trichothecene contamination is strongly dependent on the environment, and meteorological factors associated with climate change are predicted to increase the frequency and severity of conditions that favor disease outbreaks and contamination. However, it is unclear if the impact of meteorological factors directly affects *Fg* aggressiveness or indirectly affects disease outbreaks through stress induced changes in host metabolism. Rising atmospheric carbon dioxide (CO_2) directly influences grain chemistry - particularly protein and carbohydrate content - which can indirectly impact mycotoxin production. Recently, we demonstrated that certain *Fg* strains produced more trichothecenes, on wheat grown at elevated CO_2. To elucidate the direct effects of elevated CO_2 on *Fg* trichothecene production, we inoculated grain of two barley varieties and two wheat varieties of different protein content with 15 *Fg* strains (5 strains from each North American population (NA1, NA2 and NA3)). The inoculated grain was placed into dark chambers controlled at 20°C, 50% relative humidity, and 400 ppm (ambient) or 1000 ppm (elevated) CO_2. The fungus was allowed to grow and colonize the grain for 14 days, at which point the samples were collected for quantification of fungal biomass and trichothecene contamination. Results revealed that only fungal growth on wheat was affected by elevated CO_2. Variety and *Fg* population and the interaction between the two significantly contributed to differences in trichothecene accumulation. Both wheat and barley varieties with relatively lower protein content accumulated more trichothecenes, and grains inoculated with NA3 population strains accumulated significantly higher amounts of trichothecenes in comparison to other populations.

Fusarium Incidence, Severity, and Diversity in Canada

Sean Walkowiak [1]
1. Canadian Grain Commission, Grain Research Laboratory, Winnipeg, Manitoba, Canada
Corresponding Author: Sean Walkowiak, sean.walkowiak@grainscanada.gc.ca

Abstract
Many biotic factors can negatively affect cereal grain quality and safety, including fungal damage due to Fusarium Head Blight (FHB). FHB can be caused by many different species of *Fusarium*, some of which produce trichothecenes, such as deoxynivalenol or DON. Trichothecenes are of particular concern due to their regulation and toxicity to humans and livestock. However, Canada has a robust grain grading and monitoring system to ensure fungal and toxin contamination remains within acceptable levels and that Canadian wheat meets the quality and safety requirements of domestic and export markets. Since 1995, our harvest monitoring program has assessed the occurrence and severity of *Fusarium* Damaged Kernels (FDK) in wheat for over 200,000 harvest samples. The results of our monitoring identified regional and temporal differences in the fungi, with the occurrence of FDK having increased over time, particularly within the last decade. Traditional methods of *Fusarium* species identification involved culturing and manual inspection through microscopy, which is low throughput, laborious, and can not provide information on the toxin potential of the fungi. Using high-throughput DNA testing, we have assessed the *Fusarium* species and toxin chemotypes for fungi in over 40,000 FDK, allowing us to increase our testing capacity and provide robust data on shifts in the pathogen populations and risks for trichothecene occurrence. To complement our DNA testing, we have also developed new biochemical fingerprinting tools and databases, Matrix-Assisted Laser Desorption/Ionization Time of Flight Mass Spectrometry (MALDI-TOF MS), that can be used for fungal identification and biotyping. Our monitoring dataset is one of the largest of its kind, and provides valuable information on trends on FDK spanning the last 28 years.

Variety Development and Host Resistance (VDHR)

Comparative Evaluation of Fusarium Head Blight Inoculation Methods in Barley Under Controlled Conditions: A Comprehensive Analysis

Sidrat Abdullah[1], Dongying Gao[2], Thomas Baldwin[3], Juliet Marshall [1], and Kathy Esvelt Klos [2]
1. University of Idaho, College of Agricultural and Life Sciences, Aberdeen Research and Extension Center, Aberdeen, ID
2. USDA-ARS, Small Grains and Potato Germplasm Research, Aberdeen, ID
3. North Dakota State University, Department of Plant Pathology, Fargo ND

Corresponding Author: Sidrat Abdullah, sabdullah@uidaho.edu

Abstract

This study explores the impact of two inoculation methods, dip and spray, on FHB severity in transgenic barley lines under controlled conditions. The dip method occasionally resulted in unfilled or non-infected grains, prompting an investigation whether DON is consistently associated only with visibly infected grains or whether additional variables are at play in influencing DON levels. Results revealed a highly significant difference in FHB severity between the dip and spray methods, emphasizing the critical role of the inoculation technique. The 95% confidence interval indicated severity ranges of 61.5% to 71.5% for the spray method and 52.11% to 57.40% for the dip method. Notably, the dip method presented a 7% chance of reaching 100% severity would give a potential method for specific study purpose. *Fusarium*-Damaged Kernels (FDK) percentages varied considerably among spikes within genotypes for both methods, with the dip method exhibiting greater sample severity variation. Neither inoculation method significantly affected deoxynivalenol (DON) levels. However, both methods revealed significant variation in DON levels across multiple spikes within each line. Further analysis focused on DON levels and kernel properties from the dip inoculation study. Regression analysis indicated significant influences of kernel types on DON levels, with the proportion of uninfected and infected kernels serving as highly significant predictors. To ascertain the contribution of asymptomatic and/or unfilled kernels to DON levels, experiments on individual kernels are recommended. A thorough understanding of DON contamination dynamics can be obtained by analyzing DON for each uninfected or unfilled grain separately. This research aims to contribute valuable insights into the complex dynamics influencing DON levels in barley, paving the way for more effective FHB resistance strategies.

Fusarium Head Blight Resistance in Southern Soft Red Winter Wheat: Reflection on Past Progress to Guide Future Improvement

Richard E. Boyles[1], Carolina Ballén-Taborda[1], Gina Brown-Guedira[2], Jose Costa[3], Christina Cowger[2], Noah DeWitt[4], Carl A. Griffey[5], Stephen A. Harrison[4], Amir Ibrahim[6], Jerry Johnson[7], Jeanette Lyerly[8], David S. Marshall[2], R. Esten Mason[9], Mohamed Mergoum[7], J. Paul Murphy[8], Nicholas Santantonio[5], Gautam Saripalli[3], Russell Sutton[6], Vijay Tiwari[3], David van Sanford[10], and Zachary J. Winn[9]

1. Department of Plant and Environmental Sciences, Clemson University, Florence, SC
2. Plant Science Research Unit, USDA-ARS SEA, Raleigh, NC
3. Department of Plant Science and Landscape Architecture, University of Maryland, College Park, MD
4. School of Plant, Environmental and Soil Sciences, Louisiana State University, Baton Rouge, LA
5. Department of Crop and Soil Environmental Sciences, Virginia Polytechnic Institute and State University, Blacksburg, VA
6. AgriLife Research, Texas A&M University, College Station, 77843, TX
7. Department of Crop and Soil Sciences, University of Georgia, Griffin, GA
8. Department of Crop and Soil Sciences, North Carolina State University, Raleigh, NC
9. Soil and Crop Sciences Department, Colorado State University, Fort Collins, CO
10. Department of Plant and Soil Sciences, University of Kentucky, Lexington, KY

Corresponding Author: Richard Boyles, rboyles@clemson.edu

Abstract

Tremendous progress has been made in variety development and host plant resistance to mitigate the impact of *Fusarium* head blight (FHB) since the disease manifested in the southeastern United States in the early 2000s. Much of this improvement was made possible through the establishment of and recurring support from the U.S. Wheat & Barley Scab Initiative (USWBSI). Through the USWBSI-sponsored Variety Development and Host Resistance Coordinated Project, an extensive field phenotyping effort for annual germplasm screening has become a staple tool for selection in public and private soft red winter wheat (SRWW) breeding programs. Dedicated efforts of many SRWW breeders to identify and utilize resistance genes from both native and exotic sources provided a strong foundation for improvement. In recent years, implementation of genomics-enabled breeding has further accelerated genetic gains in FHB resistance. This presentation reflects on the improvement of FHB resistance in southern SRWW and contextualizes the monumental progress made by collaborative, persistent, and good old-fashioned cultivar development. Finally, we will explore opportunities for continued progress in the 21st century based on knowledge accrued and shared by collaborative southern SRWW breeding programs. This expansive knowledge along with scientific advances, germplasm sharing, and cultivar development creates a solid foundation for future improvement of FHB resistance.

Genomic Prediction for Fusarium Head Blight Resistance in the Hard Red Spring Wheat Uniform Regional Scab Nursery

Charlotte Brault[1], Emily Conley[1], Jason Fiedler[2], and James Anderson[1]
1. Department of Agronomy and Plant Genetics, University of Minnesota, Saint Paul, MN
2. Biosciences Research Laboratory, USDA-ARS Genotyping Laboratory, Fargo, ND
Corresponding Author: Charlotte Brault, cbrault@umn.edu

Abstract
Fusarium Head Blight (FHB) is one of the major threatening diseases for wheat in the United States. Genetic progress through breeding has been observed but the phenotypic evaluation of FHB in the field remains tedious. Genomic studies suggest a rather complex genetic architecture with many genes with minor effects involved. For almost 20 years, the Uniform Regional Scab Nursery evaluated breeding lines, mainly from Montana, South Dakota, North Dakota State University, as well as the University of Minnesota. FHB resistance and other related traits were evaluated in 5 locations and for 30 lines each year on average with 9 locations in total. The experimental design was highly unbalanced since almost all lines are evaluated for one year (across all locations for that year). A subset of these lines has recently been genotyped by two genotypic arrays of 90k and 3k markers. Genomic prediction is a statistical method relying on all available genetic markers to predict the trait of interest and we implemented it to assess its efficiency in predicting FHB susceptibility. Filters were applied to discard lines and SNPs with too much missing data, imputation was done with Beagle and the minor allele frequency threshold was 5%, leading to 288 lines genotyped for 53k SNPs. Traits were incidence, severity, disease, visual scabby kernel, DON concentration, and heading date, and were analyzed in a linear mixed model. Genomic prediction was fitted by cross-validation and predictive ability was measured as the Pearson correlation between predicted and observed value in the validation set. The best two methods among the seven tested were rrBLUP (ridge-regression Best Linear Unbiased Predictor) and RKHS (Reproducing Kernel Hilbert Space) across all traits, with an average predictive ability of 0.61 and 0.62, respectively. Highest accuracy was obtained for VSK with a predictive ability of 0.75. Then, we compared the impact of marker density on the predictive ability. We found that predictive ability plateaued with 500 to 3k SNPs, depending on the trait. Finally, we used successively each breeding population (from the different breeding programs) as the validation set, to test the impact of decreasing genetic relatedness. In almost all cases, predictive ability decreased when predicted within a specific breeding program, but various patterns were observed. These results demonstrate a role for genomic prediction in improving Fusarium head blight resistance traits and the density of markers needed is small, thus allowing use of lower cost genotypic arrays.

Molecular Mapping and Epistatic Analysis of Hexaploid-Derived Fusarium Head Blight Resistance Genes in Tetraploid Wheat

Ahmed Charif [1,2], Fang Wang [1,2], Tatiana Danilova [2], Katherine Frels [1], Raja Sekhar Nandety[3], Jason Fiedler[3], Deanna Funnell-Harris[2], Jeffrey Boehm Jr. [2,1], and Xiwen Cai [2,1]

1. Department of Agronomy and Horticulture, University of Nebraska, Lincoln, NE
2. USDA-ARS, Wheat, Sorghum & Forage Research Unit, Lincoln, NE
3. USDA-ARS, Cereal Crops Research Unit, Edward T. Schafer Agricultural Research Center, Fargo, ND

Corresponding Author: PH: 402-326-4055; Email: xiwen.cai@usda.gov

Abstract

Hexaploid wheat (*Triticum aestivum* L.)-derived Fusarium head blight (FHB) resistance genes do not express normally in the tetraploid durum wheat (*T. turgidum* L. ssp. *durum*) backgrounds probably because of epistatic effects. This study aimed to map hexaploid wheat-derived FHB resistance QTL in the recombinant inbred lines (RILs) derived from the cross of durum cultivar 'Divide' by hexaploid wheat accession PI 277012. Divide exhibits moderate resistance to FHB and PI 277012 exhibits high levels of FHB resistance comparable to 'Sumai 3'. In previous studies, two major FHB resistance QTL were identified in PI 277012 and mapped to the short and long arms of chromosome 5A. The RIL population (n=203) was phenotyped for FHB resistance under controlled greenhouse conditions and genotyped using Illumina's wheat 90K SNP arrays. No D-genome chromosomes were detected in the RILs using D genome-specific markers. Substantial phenotypic variation for FHB severity was observed in the RIL population. A linkage map including all 14 A- and B-genome chromosomes was construct using the RIL population. Seven FHB resistance QTL with 3.7% to 10.6% phenotypic variation were identified in the RIL population. Three of them mapped to chromosome 5A, two to 4A, one to 2B, and one to 5B. Out of the seven resistance QTL, four were contributed by PI 277012 and three by Divide. In addition, epistasis was investigated within this RIL population. A total of 25 minor epistatic interactions between loci of the A and B genomes with a LOD score greater than six were detected. The additive-by-additive effects of epistatic QTL were comparatively higher than the additive effect at any corresponding loci. A second season of screening for FHB resistance is in progress to validate these results. Integrative QTL analysis will also be performed using this RIL population and others with shared parents to further examine epistasis of FHB resistance QTL in the tetraploid wheat backgrounds. The exploration of epistatic effects among FHB resistance QTL will enhance our understanding of the interactions between FHB resistance genes, and identify the combinations that enhance resistance.

Infrared Thermal Imaging-Based Selection and Genome Wide Association for Fusarium Head Blight Resistance in Soft Winter Wheat

Jonathan S. Concepcion[1], Amanda D. Noble[1], and Eric L. Olson[1]
1. Department of Plant, Soil, and Microbial Science, Michigan State University, East Lansing, MI
Corresponding Author: Jonathan Concepcion, concep10@msu.edu

Abstract
Continuous development of high yielding FHB-resistant wheat varieties is a top priority for wheat breeding programs. Since selection for FHB-resistance under field conditions remains a challenge due to the scale of manual phenotyping required and potential subjectivity in selection criteria, an on-ground plot-level infrared thermal imaging was carried out during the on-set of FHB-infection in 2022 and 2023. Plot Level infrared thermal readings showed significant correlation with FHB-Severity, FHB-Incidence and FHB-Index. K-Means clustering grouped a larger portion of the evaluated genotypes with susceptible genotype Ambassador in both years. Since Infrared Thermal Imaging was able to delineate FHB-resistance among breeding lines, we conducted genome wide association for FHB-related traits and infrared thermal readings. Significant SNPs associated with FHB Severity were mapped in chromosomes 2A, 3A, 3B, 4A, 5A, 7A, 7B, and 7D, while significant SNPs in chromosomes 2A, 5A, 6A, and 7A were mapped for FHB Index. We found a significant SNP coming from chromosome 2B associated with field level infrared thermal reading co-localized with a previously reported QTL for FHB resistance. Based on the results, infrared thermal imaging showed higher capacity to identify susceptible genotypes over FHB-Index. Further, incorporating infrared thermal imaging increases efficiency and intensity in selecting putative FHB-resistant wheat genotypes. Lastly, the study showed the potential integration of infrared thermal imaging with genome wide association studies in identifying putative SNPs associated with FHB Resistance.

An RGB Based Deep Neural Network Approach for Field-Based High Throughput Phenotyping of Fusarium Head Blight in Wheat

Julian Cooper[1], Chuan Du[2], Zach Beaver[2], Ming Zheng[2], Rae Page[1], Joseph R. Wodarek[3], Oadi Matny[1], Tamas Szinyei[1], Alejandra Quiñones[1], James A. Anderson[4], Ce Yang[5], Brian J. Steffenson[1], and Cory D. Hirsch[1]

1. Department of Plant Pathology, University of Minnesota, Saint Paul, MN
2. Mineral Earth Sciences LLC., Mountain View, CA
3. Northwest Research and Outreach Center, University of Minnesota, Crookston, MN
4. Department of Agronomy and Plant Genetics, University of Minnesota, Saint Paul, MN
5. Department of Bioproducts and Biosystems Engineering, University of Minnesota, Saint Paul, MN

Corresponding Author: Cory Hirsch, cdhirsch@umn.edu

Abstract

Fusarium head blight (FHB) is an economically damaging fungal pathogen of wheat that can cause yield losses over 50%. Host resistance is one of the most effective approaches for disease control, however time, labor requirements, and human subjectivity limit phenotyping efforts. In this study, a novel, high-throughput phenotyping rover was deployed to capture in-field RGB images of inoculated wheat spikes throughout the 2021 and 2022 growing seasons. A deep neural network pipeline was developed to classify wheat spikes in the complex images, segment healthy and diseased tissue, and calculate the FHB disease percentage as the region of intersection between the spike and disease masks. To validate the pipeline's accuracy, inferences generated by the model at both the plot and spike levels were compared with disease scoring performed by five human raters in the field and manual image analysis. Using the phenotyping rover and our developed FHB quantification pipeline surpassed conventional rating methods in both precision and throughput. Aggregate plot-level disease scores derived from pipeline outputs strongly correlated with disease scores assigned by raters in the field and from image analysis. The pipeline disease annotations on single spike images correlated well to manual image annotations by raters, however there was a locational bias for some images. The FHB pipeline displayed generalizability as it performed well across environments, camera angles, and disease progression. These results represent a significant advancement in FHB phenotyping, affording precise and efficient quantification of disease severity on a spike and plot level at a scale unachievable using current rating methods.

DON in an Ohio Winter Malting Barley Panel

Madison Dahn[1], Ben Eggers[1], Pierce Paul[2], and Eric Stockinger[1]
1. The Ohio State University, Department of Horticulture and Crop Science, Wooster, OH
2. The Ohio State University, Department of Plant Pathology, Wooster, OH
Corresponding Author: Madison Dahn, dahn.17@osu.edu

Abstract
Fusarium head blight (FHB), and its subsequent mycotoxin deoxynivalenol (DON), pose constant issues to cereal grains productions. They also pose problems in the beer brewing process, where DON filtrates into the final product and causes a multitude of problems. The study presented here arises from the Ohio State University's FHB nursery, where winter malting barley lines are assessed for their response to FHB in the Ohio climate. The panel analyzed is part of a bigger project assessing a wide range of barley cultivars for several agronomic and malting quality traits. Our results indicate that there are interesting ties to heading date for disease and mycotoxin presence, and a link between climate moisture and mycotoxin levels.

Use of Modified Backcrosses for the Incorporation of *Fhb7* in Winter Wheat Germplasm

Bhanu Dangi[1] and Francois Marais[1]
1. North Dakota State University, The Department of Plant Sciences, Fargo, ND
Corresponding Author: Bhanu Dangi, bhanu.dangi@ndsu.edu

Abstract
Fusarium head blight (FHB) is a floral disease of cereal crops that decreases grain yield and quality and it is best controlled with the use of host resistance. However, wheat resistance to FHB has complex quantitative inheritance and no genotypes have been found to be absolutely resistant. Consequently, discovery, introgression, and pyramiding of FHB resistance genes have been the focus of wheat breeding programs worldwide. Recently, the FHB resistance gene, *Fhb7*, has been transferred via a 7BS.7BL-7E translocation from *Thinopyrum elongatum* to common wheat cultivar 'Chinese Spring'. This study utilized the F1 between 'ND Allison' and a resistant accession, XWC14-255-3-1, to initiate transfer of *Fhb7* through modified backcrosses to diverse hard red winter wheat (HRWW) genotypes. The first backcross was made to four different HRWW cultivars. To confirm the presence of the translocation and the utility of its dominant STS marker (WGC2315), 81 B_1F_1 genotypes and parental controls were genotyped with the Illumina 90K SNP array and marker WGC2315. WGC2315 was detected in 36 B_1F_1 plants and co-segregated with three SNP loci mapped to the reported region at the distal end of chromosome 7B, thus validating both the location and effectiveness of the STS marker. A single B_1F_1 heterozygote for *Fhb7* with superior phenotype was selected for a second backcross to four HRWW parents, one of which was an *Fhb1* homozygote. A third and final backcross was made to another five diverse HRWW parents to produce B_3F_1. Two final backcross parents had no known FHB resistance QTL, while the remaining three likely had "native" FHB resistance from 'ND Noreen'. The latter three parents were each homozygous for markers of both *Qfhb.rwg-5A.1* and *Qfhb.rwg-5A.2*. The B_3F_1 were tested for the presence of *Fhb7* using marker WGC2315. B_3F_2 segregating for the *Fhb7* marker were planted for a final marker analysis. A recently published codominant STS marker set will be used to test all backcross parents and 322 B_3F_2 individuals for the presence of *Fhb7*. In addition, the segregates and controls will be evaluated for FHB type II resistance (greenhouse) and tested with additional markers for *Fhb1*, *Qfhb.rwg-5A.1*, *Qfhb.rwg-5A.2* and certain leaf, stem, and stripe rust resistance genes. The combined data will be used to identify the promising B_3F_2 plants from which potentially useful resistance gene pyramids can be developed. Promising families will be used for ongoing early-generation inbreeding and breeding parents will be selected for use in upcoming routine breeding crossing blocks.

Investigating the Potential of Weighted Genomic Relationship Matrix in Optimizing Prediction Accuracy of Deoxynivalenol Accumulation in Barley

Adenike D. Ige[1] and Kevin P. Smith[1]
1. University of Minnesota, Department of Agronomy and Plant Genetics, St. Paul, MN
Corresponding Author: Kevin P. Smith, smith276@umn.edu

Abstract
Fusarium head blight (FHB) is a devastating fungal disease of barley that negatively affects yield and grain quality. Barley grains infected with *Fusarium graminearum* accumulate the mycotoxin; Deoxynivalenol (DON), and if concentrations exceed the detectable levels, it can cause food poisoning and drastically reduce global market value. Growing resistant genotypes is the most environmentally friendly and effective strategy to manage the disease. Genomic selection is an important genome-based breeding approach that can identify resistant genotypes by predicting their genetic merits with high accuracy. It can save costs associated with DON quantification and the establishment of specialized irrigated nurseries for FHB disease screening. Despite these potential advantages, it is important to optimize the genomic prediction models to accurately predict DON accumulation. GBLUP is one of the preferred prediction models for routine genomic evaluations of genotypes because it exploits information from relatives for predictions and has low computational demand. However, its assumption of the equal contribution of all loci to the genetic variance is violated for traits such as DON, which is controlled by a defined number of quantitative trat loci. Weighted GBLUP (WGBLUP), which incorporates a trait-specific relationship matrix in place of the conventional G-matrix, is a modified approach for predicting breeding values and can account for deviation from the GBLUP assumption. Therefore, the objectives of this study were to (i) identify the optimal weighting method for WGBLUP using results from genomic prediction models (RRBLUP and BayesC), and (ii) compare the predictive ability of WGBLUP models and other models in the training and validation populations of barley. Existing phenotype and genotype data from the University of Minnesota barley breeding populations were used. This study provides useful information on the performance of WGBLUP and other models, as well as the appropriate weighting strategy for the optimal performance of WGBLUP.

FHB Resistance in Canadian Hard Red Spring Wheat

Santosh Kumar[1]
1. Brandon Research and Development Centre, Agriculture and Agri-Food Canada, 2701 Grand Valley Road, Brandon, MB, Canada
Corresponding Author: Santosh Kumar, santosh.kumar@agr.gc.ca

Abstract
Canada Western Red Spring (CWRS) wheat is highly desired globally for a wide variety of end-use in the wheat industry. Canada ranks fifth in the world for hard red spring wheat production, vast majority of which is produced in western Canada. Canada produces approximately 30 million metric tonnes of wheat, of which 20 million metric tonnes (67%) is exported, contributing about $8 billion dollars to the Canadian economy annually. Majority of the spring wheat is grown in the prairie provinces where disease infestation is widespread. *Fusarium* head blight (FHB), caused by *Fusarium graminearum*, is one of the most devastating diseases of wheat causing major yield losses, grade reduction, and grain toxicity. Currently, losses to the wheat industry in Canada due to FHB is up to $300 millions annually. Severe outbreaks happen frequently in the Canadian Prairies necessitating the need for management of FHB disease. Cultivation of wheat varieties with sub-optimum levels of resistance, which also act as an overwintering source of inoculum, is considered one of the main reasons of FHB disease outbreaks. Currently, a spring wheat AAC Tenacious and a winter wheat Emerson, are the only two varieties rated resistant to FHB. Majority of the cultivars currently grown are rated moderately-resistant or intermediate resistant. Breeding programs are currently accessing germplasm in the CWRS class with better agronomics, end use quality and high levels of FHB resistance. The presentation will discuss the development of farm-ready FHB tolerant wheat varieties using maker assisted selection and field phenotyping, which will mitigate disease outbreaks, and reduce economic losses. The new varieties are incorporated into the breeding programs as crossing parents to pyramid additional sources of FHB resistance for sustainable management of the disease.

Recurrent Selection to Improve Fusarium Head Blight Resistance in Durum Wheat

Harika Pothula[1], Jason Axtman[1], Yueqiang Leng[2], Evan Salsman[1], Justin Hegstad[1], Jason Fiedler[3], Steven Xu[4], Shaobin Zhong[2], Elias Elias[1], and Xuehui Li[1]

1. North Dakota State University, Department of Plant Sciences, Fargo, ND
2. North Dakota State University, Department of Plant Pathology, Fargo, ND
3. USDA-ARS, Cereal Crops Research Unit, Edward T. Schafer Agricultural Research Center, Fargo, ND
4. USDA-ARS, Crop Improvement and Genetics Research, Western Regional Research Center, Albany, CA

Corresponding Author: Xuehui Li, xuehui.li@ndsu.edu

Abstract

Fusarium Head Blight (FHB) is a major disease that can cause severe loss of grain yield and quality of durum wheat in the Northern Great Plain of US. Developing FHB resistant germplasm and cultivars is the key to minimize the loss. Hundreds of QTL have been identified for FHB resistance in wheat. A few of resistant alleles were found in elite durum wheat lines, but majority were found in bread wheat and wild relatives. Recurrent selection is an effective way to increase frequencies of resistant alleles and to develop pre-breeding germplasm with improved FHB resistance. A base population was developed using 15 founders including five elite durum wheat breeding lines and 10 FHB resistant durum lines, where resistant alleles were introgressed from wild tetraploid wheat relatives or bread wheat. Four cycles of recurrent phenotypic selection were conducted from 2019 to 2023, one cycle per year. The FHB index was reduced from 82.3% in Cycle 0 to 48.5% in Cycle 4, proving that recurrent phenotypic selection can improve FHB resistance in the durum wheat population. Genomic selection (GS) can speed up selection and increase genetic gain in term of time and cost. We evaluated genomic prediction for FHB index using 318 elite durum wheat breeding lines (including old cultivars, modern cultivars, and breeding lines from the NDSU durum wheat program) and 256 $S_{0:1}$ lines from the recurrent selection Cycle 3 and Cycle 4 populations. The total 574 lines were genotyped using 40K SNP array, and a total of 4,048 SNP markers were obtained with missing values less than 10% and minor allele frequency greater than 5%. The marker data indicated little decrease in genetic diversity from the 15 founders to Cycle 4 population. The 318 elite durum wheat lines were evaluated in the FHB field nurseries in 2019, 2021, and 2023. Using rrBLUP model with cross-validation, the prediction accuracies for FHB index, plant height, and days-to-flowering were 0.51, 0.53, 0.79, respectively. Using the 318 elite breeding lines and 122 $S_{0:1}$ lines from the Cycle 3 population as training population to predict the Cycle 4 population, prediction accuracies were 0.27, 0.39, and 0.63, respectively. Our results indicated that implementing GS in the recurrent selection can potentially accelerate the genetic improvement.

Pyramiding FHB Resistance Genes/QTL Using Marker-assisted Selection and Doubled Haploid in Hard Red Winter Wheat

Shuyu Liu[1], Zhen Wang[1,2], Yahya Rauf[1], Kyle Parker[1,2], Li Paetzold[1], Qingwu Xue[1], Jackie C. Rudd[1], Amir M. H. Ibrahim[2], Russell Sutton[2], Jessica Rupp[3], Sunish Sehgal[4], Katherine Frels[5], Xiwen Cai[6], Gideon Marais[7], and Mary Guttieri[8]

1. Texas A&M AgriLife Research and Extension Center, Amarillo, TX
2. Department of Soil and Crop Sciences, Texas A&M University, College Station, TX
3. Department of Plant Pathology, Kansas State University, Manhattan, KS
4. Agronomy, Horticulture & Plant Science, Brookings, SD
5. Dept. of Agronomy and Horticulture, University of Nebraska–Lincoln, NE
6. USDA-ARS, Lincoln, NE
7. Plant Science, Fargo, ND
8. USDA-ARS, Manhattan, KS

Corresponding Author: Shuyu Liu, Shuyu.Liu@ag.tamu.edu

Abstract

Several major Fusarium head blight (FHB) resistance genes, *Fhb1*, *Fhb6* and *Fhb7* have been identified and utilized in breeding programs. To improve FHB resistance in hard winter wheat (HWW) region, a coordinated project for introgressing these major genes into HWW adapted germplasm was funded by US Wheat and Barley Scab Initiative for 2 years. We have the first-year ongoing project cultured 2291 embryos from haploid plants, after vernalization, more than 700 seedlings were grown in the growth chamber to 3-5 tillers. A set of 707 plants were treated by colchicine at seedling stage then transplanted into big pot in the GH. So far 215 DHLs were harvested. We are expecting to harvest around 500 DHLs. For the 2nd year, we have requested more than 500 F1 and BC1F1 seeds from the eight collaborators. With the experiences from last few years, we are targeting to develop more than 1000 DHLs in this round. All DHLs are available to all HWW collaborators to conduct FHB research in genetic and breeding.

High-Throughput Quantification of *Fusarium graminearum* Biomass in Barley Spikes and Grains Using Taq-Man Multiplex Real-Time PCR

Abbeah Mae Navasca[1], Sandesh Dangi[1], Suzette Arcibal Baldwin[1], Zhao Jin[2], and Thomas Baldwin[1]
1. North Dakota State University, Department of Plant Pathology, Fargo, ND
2. North Dakota State University, Department of Plant Science, Fargo, ND
Corresponding Author: Thomas Baldwin, thomas.t.baldwin@ndsu.edu

Abstract
Fusarium Head Blight (FHB) is a devastating disease commonly affecting North American small grains, primarily caused by *Fusarium graminearum*. Although the disease causes visible symptoms, the more pressing concern is the contamination of grain with trichothecene toxins, especially deoxynivalenol (DON), which can cause toxicity in humans and animals by inhibiting protein biosynthesis. The DON levels are more critical when breeding for resistance, but it does not account for pathogen infection and disease severity. To link DON and *Fusarium* infection, we optimized and validated a real-time multiplex quantitative PCR (qPCR) protocol for high-throughput quantification of *Fusarium* biomass based on the *Tri5* gene, which encodes trichodiene synthase, the first enzyme involved in trichothecene toxin production. The primers and probes were designed to measure the abundance of *Tri5* and normalized to the barley *actin* gene. The assay is repeatable, robust, and sensitive, up to 0.003 ng/ul of *F. graminearum* and 0.3 ng/ul of barley DNA. The method is cost-effective and has the potential to quantify *Fusarium* biomass, monitor disease progression, and determine DON levels in barley spikes and harvested grains.

A Diallel Study to Detect Genetic Background Variation for FHB Resistance in Winter Wheat

Bipin Neupane[1,2] and Francois Marais[1]
1. North Dakota State University, Department of Plant Science, Fargo, ND
2. Washington State University, Department of Plant Pathology, Pullman, WA
Corresponding Author: Bipin Neupane, bipin.neupane@wsu.edu

Abstract
Fusarium head blight (FHB) poses a significant threat to winter wheat cultivation. Resistance breeding programs rely heavily on a limited number of larger-effect FHB resistance QTL that have been identified, mapped, associated, and well-characterized with nearby markers. In addition, smaller-effect (background) resistance QTL may contribute moderate levels of "native" resistance, yet these QTL are generally poorly characterized. The overall resistance of a genotype is determined by the combined action of characterized as well as uncharacterized resistance QTL. FHB resistance can be improved by integrating, known larger-effect QTL through marker-aided introgression into genotypes with significant background resistance. This study aimed to identify and utilize well-adapted, advanced hard red winter wheat breeding lines with useful background FHB resistance QTL. A diallel trial consisting of 11 parents and 55 non-reciprocal F_1 hybrids was tested for Type II FHB resistance in a replicated greenhouse experiment. Data were first analyzed following the Griffing analysis. Significant differences were detected among entries for disease severity (DS), general combining ability (GCA), and specific combining ability (SCA). The ratio of GCA:SCA effects suggested that additive QTL effects were of primary importance. The Hayman analysis provided additional information on the genetic nature of the resistance QTL. Eight F_1 hybrids with the lowest DS resulted from crosses among the best general combiners. F_2 of the latter crosses were compared in a second-greenhouse FHB trial to identify possible transgressive segregates. An arbitrary threshold DS of 14% was used to select a manageable number of the most promising F_2 plants for continued line development.

Identification and Mapping of QTL for Resistance to Fusarium Head Blight in a Durum Wheat Mapping Population

Alireza Poursafar[1], Yueqiang Leng[1], Joseph Mullins[1], Amna Riasat[1], Shahed Safar[1], Olawumi Amusan[1], Amy Bernado[2], Yuzhou Xu[3], Paul St. Amand [2], Chenggen Chu[4], Guihua Bai [2], Steven Xu[5], and Shaobin Zhong[1]

1. Department of Plant Pathology, North Dakota State University, Fargo, ND
2. USDA-ARS, Hard Winter Wheat Genetics Research Unit, Manhattan, KS
3. Department of Agronomy, Kansas State University, Manhattan, KS
4. USDA-ARS, Sugarbeet, and Potato Research Unit, Fargo, ND
5. USDA-ARS Western Regional Research Center, Albany, CA

Corresponding Author: Shaobin Zhong shaobin.zhong@ndsu.edu

Abstract

Fusarium head blight (FHB) is a serious threat to durum wheat production due to its huge impact on crop yield and quality. Most cultivated durum varieties are susceptible to the disease and durum breeding programs have been challenged by limited sources of FHB resistance. Efforts have been devoted to identify and introgress FHB resistance from tetraploid relatives of durum, such as emmer wheat, into adapted durum varieties. However, the quantitative trait loci (QTL) controlling the FHB resistance in emmer wheat and introgression lines have not been well characterized. In this study, we aimed to identify and map FHB resistance QTL in a population of 186 recombinant inbred lines (RILs) derived from a cross between Joppa, a durum wheat cultivar, and LPA-4, a durum introgression line with FHB resistance derived from the Lebsock/PI 254188//Alkabo crosses. The population was genotyped using a genotyping-by-sequencing (GBS) approach for discovery of single nucleotide polymorphism (SNP) markers and phenotyped for FHB disease severity in greenhouse and field, and flowering date and plant height in field. A genetic map with a total length of 1125.61 cM was generated with 757 unique SNP markers. QTL analysis identified five LPA-4 derived QTLs for FHB resistance on chromosomes 1B, 3A (QTL3A-1 and 3A-2), 5B and 6B, which explained up to 14.1, 19.8, 10.7, 13.9 and 10.6% of the phenotypic variation for FHB disease severity, respectively. No QTL were identified for flowering date. However, a major QTL for plant height was detected on chromosome 3A, which was mapped close to but not overlapped with the QTL for FHB resistance. This research will facilitate introgression of the identified FHB-resistance QTL into durum wheat breeding programs.

Acknowledgment and Disclaimer

This material is based upon work partially supported by North Dakota Wheat Commission and the U.S. Department of Agriculture under Agreement No. 59-0206-2-162 (USWBSI). 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.

HSD2-32 a Novel Source of Type-II Resistance to FHB in Wheat

Rajendran Sathishraj[1], Dal-Hoe Koo[1], Moses Nyine[1], Anusha Dahal[2], Myron Bruce[2], Jessica Rupp[2], Eduard Akhunov[1], and Bernd Friebe[1]

1. Wheat Genetics Resource Center, Department of Plant Pathology, Kansas State University, Manhattan, KS
2. Rupp Lab, Department of Plant Pathology, Kansas State University, Manhattan, KS

Corresponding Author: Rajendran Sathishraj, rajendransathishraj@ksu.edu

Abstract

Globally, Fusarium head blight (FHB) is one of the most devastating fungal diseases of wheat. The use of resistant cultivars is the most effective way to reduce the adverse effects of FHB on yield and quality of wheat. However, the development of resistant cultivars has been a challenge for breeders since FHB resistance is a complex trait and only a few resistance sources are available. Hence, there is a constant need to identify new sources of resistance and we found HSD2-32 (2n=42) as a novel source of type II resistance to FHB in wheat. However, the complete information of the genetic markers linked with the genomic loci conferring FHB resistance in HSD2-32 is not available. Here we developed the F_2 bi-parental population of HSD2-32 x Chinese Spring for QTL mapping. The F_2 population was phenotyped for FHB resistance by point inoculation. Genotype-by-sequencing was used for genotyping the F_2 population. Inclusive Composite Interval Mapping (ICIM) was done with Kosambi mapping function using QTL IciMapping Version 4.2 and linkage was considered to be established if a LOD score exceeds 3.0. We found putative QTLs in Chromosomes 2D, 4A, 4B,4D and 7A. A total of 54 markers were designed covering all the positions, out of which 8 chromosome specific markers were selected in 2D and 4A chromosomes that are linked with the QTL regions and these markers were validated using F_3 contrast population. Only one SNP marker in Chr2D (Chr2D_627128588) segregate in 3:1 ratio. The experiment is progressing to develop more markers linked with these QTL regions having polymorphism across different genetic background. Also, the population is being forwarded to subsequent generations to develop recombinant inbreed lines (RILs) by single seed descent method.

Breeding for Lower Deoxynivalenol in Barley

Kevin P. Smith[1], Brian Steffenson[2], Ahmad Sallam[2], Yanhong Dong[2], Karen Beaubien[1], and Adenike Ige[1]
1. University of Minnesota, Dept. of Agronomy and Plant Genetics, St. Paul, MN
2. University of Minnesota, Dept. of Plant Pathology. St. Paul, MN
Corresponding Author: Kevin P. Smith, smith376@umn.edu

Abstract
Genetic resistance to Fusarium head blight (FHB) in barley is controlled by numerous quantitative trait loci (QTL) of relatively small effects that are often associated with agro-morphological traits such as spike morphology (two-row/six-row), heading date and plant height. Breeding for FHB resistance at the University of Minnesota has focused on genomic predictions to select complementary parent combinations, genomic selection of progeny in early generations, followed by field screening in inoculated and mist-irrigated nurseries. Breeding to manipulate other traits in addition to resistance offers the opportunity to further reduce levels of deoxynivalenol (DON). Our program has already changed our focus from six-row to two-row barley in response to industry demand. Hulless or naked barley can reduce DON contamination in the grain by leaving the hull in the field. Winter barley matures earlier potentially avoiding exposure to Fusarium. This presentation will examine the potential of implementing these diverse strategies to reach the overall goal of minimizing the risk of DON contamination in barley grain.

Acknowledgment and Disclaimer
This work was supported by grants from the USDA-NIFA OREI #2020-51300-32179 and USDA-ARS U.S. Wheat Scab Initiative #59-0206-2-150. We are grateful for the assistance of Ed Schiefelbein, Guillermo Velasquez, and John Hawkins for carrying out field trials. 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 USDA or USWBSI.

Identification of Fusarium Head Blight Resistance (FHB) in USA Wheat Breeding Programs

Lawrence Tidakbi[1], Guihua Bai[2], Jessica L. Rupp Noller[1], and Katherine W. Jordan[2]
1. Kansas State University Department of Plant Pathology and Applied Wheat Pathology Lab, Manhattan, KS
2. United States Department of Agriculture-Agriculture Research Services (USDA-ARS) Hard Winter Wheat Genetics Research Unit (HWWGRU), Manhattan, KS

Corresponding Email: katherine.jordan@usda.gov; Email: ltidakbi@ksu.edu

Abstract

Fusarium Head Blight (FHB) is a major pathogenic fungal disease affecting wheat in the USA caused by *Fusarium graminearum* (*Gibberella zeae*), with a major economic impact on wheat production and human health due to the production of mycotoxins. Breeding for FHB resistance in adapted wheat varieties is not trivial due to the complexity of the wheat genome and several minor effect alleles identified in QTL studies. To successfully develop resistance wheat to FHB disease, diagnostic markers for quantitative traits loci (QTLs) across the wheat genome need to be identified to help pyramid promising resistance genes into adapted wheat varieties in breeding programs. We are associating phenotypic and genotypic data from a panel of 270 wheat cultivars from across the US covering all market classes. The objective is to identify novel QTLs for wheat resistance to *Fusarium* and build a genomic selection (GS) model. Preliminary results show significant variability in phenotypic scab severity, the normalized area under the disease progressive curve (AUDPC), and fusarium-damaged kernels (FDK) across breeding lines, including some more resistant than the moderately resistant control. Additionally, ~15x sequencing data from these lines produced nearly 600,000 variants across the wheat genome for association analyses and model building. Using best linear unbiased predictors (BLUP) values of our phenotypic traits (AUDPC & FDK); including DON of the first year, we mapped QTLs conferring resistance and susceptibility alleles for FHB using this panel of wheat lines using mixed linear model genome-wide association studies (GWAS). Markers closely linked to these resistance or susceptibility QTLs will be developed into diagnostic markers for marker-assisted selection of FHB-resistant wheat lines in breeding programs in Kansas and across the USA.

Marker-Assisted Introgression of the FHB Resistance Gene *Fhb7*^{The2} into Hard Red Spring Wheat

Fang Wang[1,2], Ahmed Charif[1,2], Tatiana Danilova[2], Shaobin Zhong[3], Andrew Green[4], Katherine Frels[1], Stephen Wegulo[5], Jeffrey Boehm Jr.[2,1], and Xiwen Cai[2,1]

1. Department of Agronomy and Horticulture, University of Nebraska, Lincoln, NE
2. USDA-ARS, Wheat, Sorghum & Forage Research Unit, Lincoln, NE
3. Department of Plant Pathology, North Dakota State University, Fargo, ND
4. Department of Plant Sciences, North Dakota State University, Fargo, ND
5. Department of Plant Pathology, University of Nebraska, Lincoln, NE

Corresponding Author: PH: 402-326-4055; Email: xiwen.cai@usda.gov

Abstract

Fusarium head blight (FHB) may cause significant yield and quality losses of wheat, including Fusarium-damaged kernels (FDK) and accumulation of deoxynivalenol (DON) in seeds. Enhancing host resistance by exploring new resistance genes is the most cost-effective approach for FHB management. Here we report the marker-assisted introgression of *Thinopyrum elongatum* (2n=2x=14, EE)-derived FHB resistance gene *Fhb7*^{The2} into adapted hard red spring wheat (HRSW) breeding lines for germplasm/variety development. *Fhb7*^{The2} is a novel FHB resistance allele of *Fhb7* originating from *Thinopyrum* species and has been transferred to 'Chinese Spring' wheat through a wheat-*Th. elongatum* 7B-7E translocation (7BS·7BL-7EL), which was released as 'WGC002'. *Fhb7*^{The2} confers Type II FHB resistance and may reduce DON accumulation, as previously reported. In addition, *Fhb7*^{The2} is not associated with the linkage drag negatively impacting flour color from the *Th. ponticum*-derived *Fhb7* allele, making it more desirable for breeding programs. Highly diagnostic sequence-tagged site (STS) and SNP-specific PCR allelic competitive extension (PACE) markers were developed to assist selection of *Fhb7*^{The2} in FHB resistance breeding efforts and have been efficiently applied in the development of adapted FHB-resistant wheat breeding lines. Three HRSW breeding lines with favorable agronomic and end-use quality traits were selected as recurrent parents to cross with WGC002 for *Fhb7*^{The2} introgression using a marker-assisted backcrossing (MABC) breeding pipeline. Four backcrosses utilizing the PACE markers were conducted to develop *Fhb7*^{The2} introgression lines. The *Fhb7*^{The2} introgression lines and their recurrent parents were evaluated in the FHB nurseries at Lincoln, NE, and Fargo, ND in 2023 and rated for FHB severity (SEV), incidence (INC), and FDK. Disease evaluation results indicated that the HRSW introgression lines with *Fhb7*^{The2} had significantly lower ($P < 0.05$) SEV and FDK than their recurrent parents. In addition, kernels of the introgression lines and their recurrent parents harvested from the FHB nurseries are presently being tested for DON accumulation. Thus, *Fhb7*^{The2} enhances and diversifies FHB resistance in wheat. Moreover, the *Th. elongatum* 7EL segment containing *Fhb7*^{The2} is inherited as a single locus, which makes the MABC breeding highly effective for *Fhb7*^{The2} introgression into other wheat backgrounds.