# 2022 National Fusarium Head Blight Forum

December 4-6, 2022



# PROCEEDINGS

GRAND HYATT TAMPA BAY TAMPA, FLORIDA

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# **Opening Session Presentations**

# The Story behind the U.S. Wheat & Barley Scab Initiative's Establishment in FY1998 and its Evolution to What it is Today

#### Mike Davis

Founder, Birchwood Farms; Past President, American Malting Barley Association; Past Executive Secretary, National Barley Improvement Committee; Washington Island, WI *Corresponding Author:* Mike Davis, mikedavisbwf@gmail.com

#### Abstract

The scab epidemic of 1993 was devastating to North American barley and wheat crops and the beginning of a long-term threat to production. Limited federal and state resources did not allow for adequately funded and coordinated efforts to address the epidemic. Barley and wheat producers and researchers in Minnesota were the first to organize and advocate, securing state funding of \$800,000/year in 1995 for the Minnesota Scab Initiative. Subsequently, Minnesota and North Dakota grower, researchers, and industry stakeholders joined forces, met and organized, leading to the first federal appropriation of \$500,000 by Congress in FY1998, which led to the founding of the US Wheat & Barley Scab Initiative (USWBSI). The first Steering Committee meeting was subsequently held at Michigan State University (MSU), East Lansing, to allocate funding and continue organizing. In the years that followed, the National Barley and Wheat Improvement Committees, which represent US barley and wheat producers, researchers, and value-added end users, secured Farm Bill authorizations and increasing appropriations, prevented proposed cuts, and elimination of the program. These efforts by dedicated stakeholders brought funding up to its current authorized and appropriated funding of \$15 Million/year. During its twenty-four years, the USWBSI has evolved from the first Steering Committee meeting of key stakeholders, a dinner in the basement of the MSU Kellogg Hotel & Conference Center, to the sophisticated and efficient organization it is today. This is the story of the stakeholder efforts that made it happen.

**Opening Session – Keynote Presentation** 

### Feeding the 10 Billion: Thinking through the Journey

#### Ed Souza

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#### Abstract

We as crop scientists are challenged to increase our effectiveness in delivering products of our research to farmers. Those products will need to increase production, be healthful food for people, and tolerate if not mitigate climate change. Effectiveness is the percentage of our research projects that are realized in a farmgate product that significantly improves one of those three needs. This paper presents a standard, non-novel, but useful methodology for critically evaluating research related to the wheat crop. Scientists within the USWBSI work on projects that fall along a continuum. Some are basic discovery projects that have 20 timelines for product delivery. Others are life-cycle management of current technologies with immediate application. Balancing delivery time and magnitude of impact are crucial for our innovation investment to be effective. Selection of technologies with appropriate probability of success will be discussed.

**FHB Management** 

#### **MGMT Coordinated Project Overview and Kansas Perspective**

Kelsey Andersen Onofre<sup>1</sup>, Erick De Wolf<sup>1</sup>, Wanderson Bucker Moraes<sup>2</sup> and Pierce A. Paul<sup>2</sup> <sup>1</sup>Kansas State University, Department of Plant Pathology, Manhattan, Kansas and <sup>2</sup>The Ohio State University, Department of Plant Pathology, Wooster, Ohio *Corresponding Author:* Kelsey Andersen Onofre, andersenk@ksu.edu

#### Abstract

Fusarium head blight (FHB) management continues to be a challenge for wheat producers in Kansas. Over the past five years, the estimated average losses to FHB in Kansas alone exceeded \$20 million annually. With the expansion of corn acreage in central and western Kansas, millions of acres of wheat are at risk annually. Furthermore, only a small number of hard red winter wheat varieties with intermediate resistance and well adapted to central and eastern Kansas are available, leading to a reliance on fungicide treatment programs. Here we present an analysis of climate and cropping system practices that have influenced FHB development in Kansas along with potential future risks. In recent years, new fungicide products have become labeled for FHB control in the United States, giving Kansas producers and wheat producers throughout the country new options for FHB management. These new products include Miravis Ace® (Propiconazole + Pydiflumetofen), Sphaerex® (Metconazole + Prothioconazole) and Prosaro Pro<sup>®</sup> (Prothioconazole + Tebuconazole + Fluopyram). A synthesis of multi-state trials comparing the efficacy of these products when applied at early flowering (Feekes 10.5.1) indicate that they each provide significant control of FHB and DON when compared to the non-treated check. Additional comparisons with the industry standard fungicide Prosaro<sup>®</sup> (Prothioconazole + Tebuconazole) will be presented. In addition, with the availability of less expensive generic fungicides producers may see two-treatment programs as an economically viable option. Results for two-treatment fungicide programs will be presented, including the application of Miravis Ace at early flowering (Feekes 10.5.1) followed by an application of Folicur<sup>®</sup> (Tebuconazole) or generic equivalent. Results indicate that this two-treatment program significantly reduced by FHB and DON when compared to a single, early flowering (Feekes 10.5.1) application. Additional results will be presented and discussed. These practical results will provide important insights for the wheat industry.

#### Effects of Fungicides and Cultivar Resistance on Fusarium Head Blight of Wheat

Mahnoor Asif<sup>1</sup>, Stephen Wegulo<sup>1</sup>, Julie Stevens<sup>1</sup>, Katherine Frels<sup>2</sup>, Heather Hallen-Adams<sup>3</sup> and Kent Eskridge<sup>4</sup> <sup>1</sup>Department of Plant Pathology, <sup>2</sup>Department of Agronomy and Horticulture, <sup>3</sup>Department of Food Science and Technology, and <sup>4</sup>Department of Statistics, University of Nebraska-Lincoln, Lincoln, Nebraska *Corresponding Author:* Stephen Wegulo, swegulo2@unl.edu

#### Abstract

Fusarium head blight (FHB), caused mainly by Fusarium graminearum, results in considerable yield and economic losses worldwide. Additionally, the pathogen produces the mycotoxin deoxynivalenol (DON), which is harmful to humans and animals. The objective of this study is to evaluate the effects of fungicides and cultivar resistance on FHB. In 2022, a field trial was conducted at an irrigated site at the Havelock Research Farm (Latitude 40.9, Longitude -96.6) in Lincoln, Nebraska. Two cultivars, an FHB moderately resistant cultivar, Zenda, and a susceptible cultivar, Wesley, were subjected to nine fungicide treatments including the untreated check. The treatments consisted of two fungicides, Sphaerex<sup>®</sup> (a triazole) and Aproach<sup>®</sup> (a strobilurin), each applied at different combinations of growth stages (Feekes 6 (Fk6) + Fk10.51; Fk9 + Fk10.51; Fk6+ Fk9 + Fk10.51; and Fk10.51). Due to unusually prolonged dry weather conditions, FHB, DON, and FDK developed only to low levels. FHB index ranged from 3.6% to 8.0% and there were no significant differences (P = 0.05) between cultivars or among fungicide treatments. DON and Fusarium damaged-kernels (FDK) were significantly higher in Wesley (0.90 ppm, 27%) than in Zenda (0.68 ppm, 15%). Among fungicide treatments, DON ranged from 0.25 ppm (Sphaerex Fk9 + Fk10.51 in Zenda) to 1.4 ppm (Aproach Fk6 + Fk10.51 in Wesley). FDK did not differ among treatments in Wesley. In Zenda, FDK differed among treatments and ranged from 10% (Sphaerex Fk9 + Fk10.51) to 29% (Aproach Fk6 + Fk10.51). Overall, treatment with Aproach resulted in 30% higher DON compared to Sphaerex. Yield did not differ among treatments in either cultivar and ranged from 4661 kg/ha (Wesley untreated check) to 7200 kg/ha (Zenda Fk9 + Fk10.51). Test weight was higher in Zenda (68 kg/hL) than in Wesley (64 kg/hL), but did not differ among fungicide treatments in either cultivar. The results from this study indicate that less DON and FDK developed in the moderately resistant cultivar Zenda than in the susceptible cultivar Wesley, and a triazole (Group 3) fungicide (Sphaerex) was more effective in reducing DON and FDK than a strobilurin (Group 11) fungicide (Aproach).

# Response of Barley and Oat Genotypes to *Fusarium* Pathogens and Associated Mycotoxins

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#### Abstract

Fusarium head blight (FHB) is recognized as a major threat to barley and oat production in Canada. The disease is caused by different species belonging to the genus *Fusarium* and leads to the accumulation of mycotoxins in grains that are toxic for humans and animals. Increases of FHB in barley and oat caused by infections with *F. poae* (FP), *F. sporotrichioides* (FS) and *F. avenaceum* (FA) have been observed in recent years. These pathogens produce Nivalenol, T-2/HT-2, and Enniatins, respectively, and are now considered important threats to the barley and oat industry. The impact of FP, FS and FA on barley and oat production has not yet been identified, and little is known about host resistance elements against these pathogens. This study investigated the pathogenicity of four *Fusarium* species, including *F. graminearum* (FG), FP, FS and FA, on ten barley and ten oat genotypes in 2019 and 2020. The levels of *Fusarium* mycotoxins, including deoxynivalenol, nivalenol, H-2/HT-2 and enniatins, in grain samples were analyzed using an LC-MS/MS method. All *Fusarium* species tested caused infection and mycotoxin contamination in different barley and oat genotypes. The resistance in barley and oat genotypes against FG and FA shows a similar pattern but differs from the responses against FP and FS. Overall, the level of DON in barley and oat grain was the highest, ranging from 0.1 to 5.8 ppm for barley and from 0.1 to 11.5 ppm for oat. NIV had the lowest concentrations in 2019 and 2020, ranging from 0.01 to 0.36 ppm in barley and from 0.01 to 0.16 ppm in oat.

### **Fusarium Head Blight Management in Alabama: Observations from 2021 and 2022 Spring** Seasons

#### Kira L. Bowen

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#### Abstract

As part of the multi-state FHB Management Coordinated Project (MGMT CP), integrated FHB and DON management strategies were evaluated on soft red winter wheat grown in Alabama, with emphasis on Miravis Ace® (Adepydin + propiconazole) and Sphaerex®, over two growing seasons. An Integrated Management (IM) trial was done at the Gulf Coast Research and Extension Center (GCREC: south AL, 30.542, -87.882) of the Alabama Agricultural Experiment Station. The factorial set of treatments consisted of two cultivars and six fungicide treatments, in four blocks arranged in a split-plot with cultivar as the main plots. The cultivars were AGS3030 (moderately resistant) and P26R96 (moderately susceptible). Fungicides were applied using a CO2 backpack sprayer at 32 psi with three TX-12 hollow cone nozzles spaced 20 inches apart and 15 inches above the canopy. Miravis Ace, applied at early flower, was applied alone or followed by tebuconazole; Caramba<sup>®</sup>, Sphaerex, and Prosaro<sup>®</sup> were applied alone at early flower. A non-treated control was included. FHB intensity and foliar diseases were rated on 22 April 2021 and 25 April 2022. Two to three weeks after fungicide application, 30 (in 2021) and 20 (in 2022) heads per plot were collected to estimate the FHB index (IND). Grain samples from each plot were obtained after harvest for evaluation of percent Fusarium damaged kernels (FDK) and DON analysis. In 2021, IND was low and no treatments differed from the control; however, all treatments reduced FDK and DON. Greatest reduction in FDK was seen with the two-application program, while greatest reduction in DON was noted with Sphaerex (40% lower than in control) and the two-application program (36%). It must be noted that DON levels were high, with > 5 ppm in 45% of plots. Yield and test weights in 2021 were higher in all treatments compared to the control; Miravis Ace and Miravis Ace followed by tebuconazole had nearly 20% higher yield (14 bu/A) than non-treated controls. In 2022, IND was again low, averaging < 2% in all treatments; only the Sphaerex treatment had lower IND than the control. FDK did not differ among treatments. DON levels were generally low with 61% of plots having < 1 ppm. All treatments reduced DON, and the greatest reductions were seen with Caramba, Sphaerex, and the Miravis Ace followed by tebuconazole programs. In 2022, no significant differences were noted among treatments with test weight or yield.

#### Acknowledgement and Disclaimer

This material is based upon work supported in part by the U.S. Department of Agriculture under agreement Nos. 59-0206-6-008 and 59-0206-0-153. This is a cooperative project with the U.S. Wheat & Barley Scab Initiative. Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the author(s) and do not necessarily reflect the view of the U.S. Department of Agriculture.

#### Baseline Sensitivities of Fusarium Species to Pydiflumetofen in East Coast Malting Barley

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#### Abstract

Fusarium Head Blight (FHB) is the most devastating plant disease affecting barley (Hordeum vulgare) production in the east coast. While most frequently associated with Fusarium graminearum, recent studies in NY have shown multiple species associated with FHB in malting barley. Since 2008, demethylation inhibitors (DMI's, FRAC code 3) have been the major class of fungicide active ingredients used to control FHB but concerns of fungicide resistance developing have prompted the registration of new fungicides with additional modes of action. In 2019, pydiflumetofen, a succinate dehydrogenase inhibitor (SDHI, FRAC code 7), was labeled for control of FHB in small grains. In effort to document baseline fungicide sensitivities in malting barley, isolates were collected in Delaware, Maryland, and New York during the 2021-22 growing seasons. Isolates were identified to species by amplification of Translation Elongation Factor 1a (TEF1). In the preliminary set of 28 isolates, 79% were identified as F. graminearum, 7% F. verticilloides, 7% F. acuminatum, 3.5% F. poae, and 3.5% F. asiaticum. New York isolates were the most diverse containing four of the species recovered. An in vitro poison plate mycelial assay was conducted using yeast bacto agar (YBA) amended with pydiflumetofen to final concentrations of 0, 0.01, 0.05, 0.25, 1.0, and 5.0 µg/mL. A subset of F. graminearum isolates was also grown on PDA amended to the same concentrations for comparison of media types. The effective concentration to reduce mycelial growth by 50% (EC50) was determined for each isolate and averaged by species. Average EC50 values were 1.09 µg/mL for F. graminearum (n=22), 1.07  $\mu$ g/mL for *F. verticilloides* (n=2), 1.51  $\mu$ g/mL for *F. acuminatum* (n=2), 1.09  $\mu$ g/mL for *F. poae* (n=1), and 1.09 µg/mL for *F. asiaticum* (n=1). Averages of *F. graminearum* on PDA were 1.04 μg/mL (n=8). Growth on YBA was more symmetric and this media will be used for all future studies. The baseline fungicide sensitivity levels documented in this preliminary work will be expanded and used to monitor the response of *Fusarium* spp. to SDHI fungicides as use expands.

FHB Management – Poster

### Is Kernza<sup>®</sup> Susceptible to Fusarium Head Blight?

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#### Abstract

The perennial grain crop Kernza<sup>®</sup>, also known as intermediate wheatgrass (*Thinopyrum intermedium*) is a wild relative of wheat grown primarily in the western United States. Wheat is the third most important field crop in the U.S., after corn and soybean. One of the most important diseases affecting wheat throughout all wheat-growing regions is Fusarium head blight (FHB). As Kernza is a relative of wheat, it may serve as a host for FHB, and proximity or planting into the stubble of susceptible wheat varieties could increase disease in the more resistant Kernza crop. The objective of this project is to identify fungal pathogens associated with Kernza and to confirm the presence of *Fusarium graminearum*. To identify these pathogens, head samples were contributed from on-farm and research trial locations. Spikelets were used to isolate the fungi using morphological methods. Later, single-spored fungi were obtained and DNA was extracted. We performed PCR and DNA sequencing of the amplified products using ITS primers. Of 69 samples, 54 matched *Fusarium species*. Again, six *Fusarium verticillioides* pathogen. The conclusion of the presence of *Fusarium graminearum* or not will be made after the sequencing result from all the samples. However, identifying the species and strains of *Fusarium* that colonize Kernza will be very useful for developing disease-resistant cultivars, as well as better disease management.

### **Evaluation of Organic Copper Fungicide Applications plus Cultivar Resistance to Reduce FHB and DON Infection of Barley in Vermont**

#### Heather Darby and Hillary Emick

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#### 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 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. In Vermont during 2022 we observed the disease and yield impact of cultivar susceptibility, inoculation with *Fusarium araminearum*, and treatment with an organic copper fungicide at two timings. 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 susceptible to FHB). The organic fungicide ChampION® was applied at heading (Feekes growth stage, FGS 10.1) and again four days after heading treatment. After the fungicide had dried, plots were sprayinoculated with a conidial suspension of F. graminearum (40,000 conidia/ ml) to augment the development of FHB. Grain yield, test weight, and DON concentrations were measured for each plot. Treatment means were calculated, subjected to analysis of variance, and separated by Fisher's protected LSD test (P =0.05). The moderately susceptible variety had 56% less DON compared to the susceptible variety. The fungicide treatments did not significantly influence DON concentrations compared to the control. The certified organic treatment of two applications of ChampION did not improve DON concentrations compared to one application. The barley yields did increase when ChampION was applied to the barley compared to the control. For organic producers, these results indicate that selection of varieties that have moderate susceptibility to FHB should help growers mitigate some of the risk associated with this disease. The application of the organic fungicides needs to have continued research to understand if they can provide control of FHB in the northeastern U.S.

#### Objective

To evaluate the individual and interactive effects of moderately resistant cultivars and application timings of an organic copper fungicide 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 2022 we observed the disease and yield impact of cultivar susceptibility, inoculation with *Fusarium graminearum*, and treatment with an organic copper fungicide at two timings.

#### **Materials and Methods**

The trial was conducted in Alburgh, VT during 2022. 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 on April 23, 2022. Main plots were sown with barley at 125 lb ac<sup>-1</sup> 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 susceptible 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). After the fungicide had dried, plots were spray-inoculated with a conidial suspension of F. graminearum (40,000 conidia/ ml) to augment the development of FHB. The second fungicide application occurred four days after heading and inoculated with a conidial suspension of F. graminearum (40,000 conidia/ml) after the fungicide had dried. Fungicide and F. graminearum treatments were applied with a CO<sub>2</sub> backpack sprayer with paired TJ-60 8003vs nozzles mounted at an angle (30° from horizontal) forward and backward, 20-in. apart, pressurized at 30 psi, and calibrated to deliver 20 gal/A. Grain was harvested using an Almaco plot combine (Nevada, IA). Grain plot yield and test weight were recorded. Yield and test weight were adjusted to bushels ac<sup>-1</sup> at 13.5% moisture. Analysis of DON concentration in grain was conducted at the University of Vermont Cereal Grain Testing Laboratory located in Burlington, VT. Treatment means were calculated, subjected to analysis of variance, and separated by Fisher's protected LSD test (P = 0.05).

#### **Results and Discussion**

There were no variety by fungicide treatment interactions indicating that the treatments responded similarly regardless of variety (Table 2). As expected, the barley varieties differed significantly in DON concentrations (Table 3). The moderately susceptible variety had 56% less DON compared to the susceptible variety. When results were combined across cultivars, the fungicide treatments did not significantly influence DON concentrations compared to the control (Table 4). The certified organic treatment of two applications of ChampION did not improve DON concentrations compared to one application. The barley yields did increase when ChampION was applied to the barley compared to the control (Table 4).

For organic producers, these results indicate that selection of varieties that have moderate susceptibility to FHB should help growers mitigate some of the risk associated with this disease. The application of the organic fungicide ChampION at heading and 4 days after heading did not reduce DON concentrations compared to the inoculated control. Overall, DON concentrations were low due to hot and dry conditions during flowering. Additional research should be conducted to assess the efficacy of multiple applications of copper-based fungicide on FHB and DON concentrations.

#### Acknowledgement and Disclaimer

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Fungicide treatments	Company	Fungicide active ingredient	Application rates
Control			Water
Fusarium graminearum			40,000 spores/ml
ChampION <sup>++</sup>	NuFarm	Copper hydroxide	1.5 lbs ac <sup>-1</sup>

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

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

DON	Test weight	Yield
* * *	NS	**
NS	NS	*
NS	NS	NS
	DON *** NS NS	DONTest weight***NSNSNSNSNS

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

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

Cultivar	DON	Test weight	Yield
	ppm	lb bu⁻¹	bu ac <sup>-1</sup>
ND Genesis (moderately resistant)	0.53	44.2	84.9
Robust (susceptible)	1.21	43.8	71.2
LSD ( <i>p=0.05</i> )	0.244	NS	8.50

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

Fungicide + timing	DON	Test weight	Yield
	ppm	lb bu⁻¹	bu ac <sup>-1</sup>
Non-sprayed, non-inoculated control	0.66	44.3	70.6
Inoculated FGS 10.1	0.85	43.4	69.6
ChampION (1.5 lbs) at heading	1.10	44.5	89.0
ChampION (1.5 lbs) at heading & 4 days after			
heading	0.87	44.4	83.1
LSD ( <i>p=0.05</i> )	NS	NS	12.0

# Genetic Strategy for Controlling Fusarium Head Blight Disease of Wheat and Associated Deoxynivalenol Contamination of Grain in Subtropical, Humid, Southern Brazil – Five Years Results

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#### Abstract

Fusarium head blight (FHB) and its associated deoxynivalenol (DON) contamination of grains are recurrent wheat production problems in subtropical, humid southern Brazil. Cooperativa Agraria Agroindustrial from Guarapuava, PR, Brazil, developed an integrated management system for FHB and DON by combining preharvest and post-harvest strategies. The pre-harvest strategies include genetic resistance, different sowing dates, chemical control, a regional climate and disease monitoring network, and improved spray technology. The post-harvest strategies include rules and standards for grain reception, including rapid mycotoxin determination in truckloads, proper grain cleaning, drying and storage, grain peeling, and further processing in the wheat mill. Among the pre-harvest strategies, genetics stands out through screening resistance of wheat genotypes against FHB and DON contamination in wheat grains and whole grain flour (WGF). The genetic resistance approach considers resistance type I – resistance against initial infection; type II – resistance to pathogen spreading in spike; type III – resistance to kernel infection; type IV – tolerance against FHB; and type V – resistance to toxins. The main question was how to reduce mycotoxin levels in wheat grains and WGF based on more tolerant wheat genotypes. Eight commercial cultivars and precommercial genotypes were evaluated in a randomized complete block design with three replications from 2017 to 2021. The plot area was 5.1 m<sup>2</sup>, and overhead sprinkler irrigation supplemented the natural moisture needed for infection and disease development. Plants were artificially inoculated at mid-anthesis by spraying a spore suspension with a concentration of  $5x10^4$  conidia/mL. The inoculum was a pool of isolates representing trichothecenes genotypes of the Fusarium graminearum species complex occurring in the southern Paraná state. The analysis of variance of the FHB index and DON revealed significant effects of growing season, cultivar, as well as for the interaction among these factors ( $P \le 0.02$ ). Significant differences (P<0.05) among wheat genotypes were observed in the FHB index and WGF DON. WGF DON reduction provided by genetic resistance over seasons with less severe to more severe epidemics ranged from 377 ppb to 82 ppb (78.2% reduction) and from 5618 ppb to 2750 ppb (51.5% reduction), respectively. This study showed that genetic resistance was more effective in seasons with less severe FHB epidemics and is a valuable control measure for the integrated management of FHB and DON in southern Brazil.

# MSE FindR: An R Shiny App Tool for Recovering Variance in Designed Experiments using Treatment Means and Post-Hoc Test Results

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#### Abstract

Research synthesis methods such as meta-analysis rely on either individual participant data or appropriate summary statistics (e.g., measurement of precision such as standard deviation and standard error) of trial data for implementation. A barrier to study inclusion in research synthesis occurs when no precision metrics are explicitly included in the primary report. Typically, such otherwise credible studies are omitted in the research synthesis leading to potential publication bias and loss of statistical power. We developed a userfriendly R shiny web app to estimate the mean squared error (MSE) from published reports (e.g., Plant Disease Management Reports) considering information on treatment means, significance level, number of replications, and post-hoc test results from balanced and randomized trials analyzed via ANOVA. To achieve this, users upload a csv file containing trial information into the app, then specify the experimental design and the post-hoc test that was applied in the trial analysis. Multiple trials can be processed by specifying a trial identifier column in the csv file. Validation of the procedure using 1,000 individual participant simulated datasets with variable number of treatments and effect sizes demonstrated a strong Lin's concordance correlation (0.503  $\leq \rho_c \leq 0.994$ ) between values of MSE obtained with ANOVA and MSE FindR. Additionally, values of the bias correction factor ranged from 0.875 to 0.999 across all simulation scenarios, indicating an excellent agreement between the best fitted and identity lines. With MSE FindR, researchers can conveniently obtain an estimate of variability from published reports lacking measurement of precision, ultimately enabling the inclusion of such studies in a quantitative review of research data evaluating product efficacy or genotype performance in coordinated Fusarium head scab wheat and barley trials in the US. Inclusion of summary statistics (e.g., means, estimates of precision, significance level, and the number of replications) should still be considered as a standard approach in summary trial reports for the US wheat and barley scab initiative community.

FHB Management – Presentation

#### **Coordinated Fungicide Sensitivity Project**

#### Alyssa Koehler

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#### Abstract

For many years, fungicide control of Fusarium Head Blight (FHB) primarily relied upon the application of demethylation inhibitor (DMI) fungicides. In 2019, the first fungicide containing a succinate dehydrogenase (SDHI) class fungicide was labeled for FHB management in cereal grains. A national effort was initiated in 2020-21 to document baseline fungicide sensitivities of *Fusarium* species causing FHB in United States wheat to the new active ingredient pydiflumetofen. Isolates were submitted from 16 states as part of a United States Wheat and Barley Scab Initiative (USWBSI) FBH Management (MGMT) project. From the total isolate set, 177 F. graminearum isolates were screened for fungicide sensitivity, including 98 collected in 2020, 65 collected in 2021, and 14 historic isolates collected from 1991-2014 that were never exposed to pydiflumetofen. An in vitro assay with fungicide-amended PDA plates at 0, 0.01, 0.05, 0.25, 1.0, and 5.0  $\mu$ g/mL was established to determine the effective concentration to reduce mycelial growth by 50% (EC50) for each isolate and EC50 values were averaged by state. Average EC50 values for 2020-21 isolates grouped by state ranged from 0.18 to 0.73  $\mu$ g/mL, with 2020 and 2021 cumulative averages of 0.40  $\mu$ g/mL and  $0.37 \,\mu\text{g/mL}$ , respectively. The average EC50 value of historic isolates was  $0.35 \,\mu\text{g/mL}$ . This project established preliminary baseline sensitivities to enable monitoring of fungicide sensitivity levels as SDHI fungicide exposure becomes more prevalent across small grain production regions of the U.S. Two testing locations have been established to expand this effort to include isolates from additional states, geographies, and cropping systems while also screening additional SDHI and DMI active ingredients. Midwest and Pacific Northwest regions can submit samples and isolates to Dr. Martin Chilvers at Michigan State University and southern and east coast regions can submit to Dr. Alyssa Koehler at the University of Delaware. This project seeks to facilitate isolate collection, storage, and fungicide characterization to promote collaborative efforts to monitor for changes in sensitivity levels over time.

### Implications of Elevated Carbon Dioxide on Wheat Type I Resistance against Fusarium Head Blight

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#### Abstract

Fusarium head blight (FHB) is a devastating cereal crop disease that can cause significant yield losses and contaminate grain with hazardous mycotoxins. Recent data suggest that elevated carbon dioxide (CO<sub>2</sub>) can increase wheat susceptibility to FHB and mycotoxin contamination. Thus, resilient host genetic resistance to *Fusarium* is key to ensuring the safety and security of our grain supply. There are different types of host resistance mechanisms. Resistance to initial infection (type I) and resistance to fungal spread between florets (type II). Type I resistance is thought to provide protection against fungal infection through plant morphological traits including height and anther extrusion. The Fhb5 qualitative trait loci (QTL) is associated with both type I FHB resistance and anther extrusion. Since elevated  $CO_2$  has been shown to affect wheat floral development, we hypothesized that elevated  $CO_2$  may compromise *Fhb5* associated resistance to initial infection. Four wheat varieties which contain different combinations of the *Fhb1* (predominant marker for type II resistance) and Fhb5 QTL: Alsen (Fhb1 and Fhb5), Bolles (Fhb5), Rollag (Fhb1), and Glenn (neither QTL), were evaluated for differences in initial infection at 400 ppm and 1000 ppm CO<sub>2</sub>. Interestingly, while there was an on average increase in disease for all four varieties evaluated, only Alsen and Bolles which contain Fhb5, had a significant increase in disease at elevated CO<sub>2</sub>. The amount of deoxynivalenol mycotoxin contamination was significantly higher at elevated CO<sub>2</sub> for all four varieties, but the significance of this difference was much greater for Alsen and Bolles. Experiments assessing differences in variety anther extrusion are currently underway.

#### Summary of 2022 Uniform Fungicide Trials (UFT) in North Dakota

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#### Abstract

The use of a well-timed fungicide application can help protect spring barley, durum, and hard red spring wheat (HRSW) from Fusarium head blight (FHB) and deoxynivalenol (DON). With funding from the U.S. Wheat and Barley Scab Initiative, six fungicide trials were conducted in 2022 in North Dakota. The primary objective was to compare the timing and efficacy of fungicide premixes on reducing FHB and protecting yield. Research sites were established at the Langdon Research and Extension Center (Langdon; mist irrigated), Williston Research Extension Center - Nesson Valley (Nesson Valley; linear pivot), North Central Research and Extension Center (Minot; dryland), and North Dakota State University (Davenport and Fargo; dryland). Trial locations were as follows; one barley trial each in Fargo and Davenport, one durum trial each in Nesson Valley and Minot, one HRSW trial each in Langdon and Fargo. Trials were conducted in a randomized complete block design with four to five replications. All plots were sown with a susceptible cultivar relative to the target grain. Fungicides evaluated included Prosaro® (prothioconazole + tebuconazole), Caramba<sup>®</sup> (metconazole), Miravis Ace<sup>®</sup> (propiconazole + pydiflumetofen), Prosaro Pro<sup>®</sup> (prothioconazole + tebuconazole + fluopyram), Sphaerex<sup>®</sup> (metconazole + prothioconazole), and Folicur<sup>®</sup> (tebuconazole). Fungicide timings evaluated in barley included Feekes 10.5 (full-head) and 3 to 7 days after Feekes 10.5. For durum and HRSW, Feekes 10.51 (early-anthesis) and 3 to 7 days after Feekes 10.51. When appropriate, FHB, DON and yield data from each location/grain class was combined for analysis. Disease was low to moderate at five of the six locations. All fungicide treatments provided significant reduction of FHB in spring two-row barley, durum, and hard red spring wheat. The greatest amount of FHB suppression, lowest DON levels, and highest yield responses often included treatments with two fungicide applications with one occurring at FGS 10.51 (durum and HRSW) or FGS 10.5 (spring barley) and a second application occurring 3 to 7 days later. The recently labeled fungicide premixes of Prosaro Pro and Sphaerex provided similar to sometimes better control than the industry standards of Prosaro and Caramba.

#### **Acknowledgement and Disclaimer**

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#### Efficacy of Essential Oils in the Management of Fusarium Head Blight in Spring Wheat

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#### Abstract

Fusarium head blight (FHB), caused by *Fusarium graminearum (Fq)*, is one of the most important diseases of wheat. FHB results in grain yield loss, seed quality reduction, and the accumulation of mycotoxins such as deoxynivalenol (DON). FHB disease management is mainly with the use of partially resistant cultivars and the application of synthetic fungicides to guarantee a higher yield. However, the constant application of synthetic fungicides causes negative impacts on humans and the environment. Additionally, fungicides used to control Fq belong mainly to one class of fungicides, the triazoles, increasing the risk of loss of pathogen sensitivity. Therefore, there is a need to test and implement new eco-friendly alternatives for managing FHB. Essential oils (EOs) are volatile secondary metabolites extracted from plants, composed of a mix of bioactive compounds with known antifungal properties. In this study, we evaluate the efficacy of two pure EOs (tea tree and lemongrass), two commercial EOs (Thymox<sup>®</sup> and Timorex<sup>®</sup>), and two bioactive compounds present in EOs (carvacrol and thymol) in the management of FHB. The study was conducted in vitro using inhibitory disk diffusion to detect the minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC). Additionally, conidia germination inhibition was evaluated, and volatile activity was reported. The EOs and constituents were tested under greenhouse and field conditions. FHB Disease index (DI), yield, Fusarium damaged-kernels (FDK), and DON were evaluated. There was 100% MIC in Fq with lemongrass at 1000 ppm, Thymox at 500 ppm, and carvacrol, and thymol at 200 and 500 ppm, respectively. One hundred percent spore germination inhibition was observed at 1000 ppm of lemongrass, tea tree, and Thymox, and at 500 ppm of carvacrol and thymol. All the EOs concentrations showed a MFC equal to the MIC except for carvacrol, where fungal regrowth was observed at 500 ppm. Volatility fungistatic activity was observed in lemongrass, Thymox, and thymol with 6.25 to 38.44% of mycelial growth inhibition compared with the untreated. For the greenhouse and field studies, Plants treated with lemongrass, carvacrol thymol, and Thymox showed a significantly lower DI (P<0.05) relative to the untreated. In the field trial, the EOs showed a DI reduction of 40% and a decrease of DON concentration between 36 and 59% for all the treatments with EOs compared to the untreated. In vitro and in vivo experiments, we reported the fungicidal and fungistatic potential of the EOs lemongrass, carvacrol, thymol, and Thymox in the control of Fg.

# **Fusarium Head Blight Management Coordinated Project: Integrated Management Trials 2022**

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#### Abstract

The demethylation inhibitors (DMI) prothioconazole, metconazole, and tebuconazole are three of the most effective fungicide active ingredients (AI) for Fusarium head blight (FHB) and deoxynivalenol (DON) control in wheat and barley. Given that Prosaro®, a premix of two of these AIs (tebuconazole + prothioconazole), is one of the most consistently effective fungicides against FHB and DON, a logical question is whether other mixtures of AIs will be just as or more effective than Prosaro. Several new mixtures of DMI AIs or DMI + SDHI (Succinate Dehydrogenase Inhibitor) Als are now being marketed for FHB and DON management. These include Sphaerex<sup>®</sup>, a premix of metconazole and prothioconazole, Prosaro Pro<sup>®</sup>, a premix of tebuconazole, prothioconazole, and the SDHI Fluopyram, and Miravis Ace®, a premix of the DMI Propiconazole and the SDHI Pydiflumetofen. More information is needed on the overall efficacy of these new products when used alone or in combination with genetic resistance. Therefore, in 2022, field experiments were conducted in several US wheat-growing states, representing all major grain market classes and different production regions to evaluate the integrated effects of these new fungicide mixtures and genetic resistance on FHB and DON. 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\_UT). Mean IND and DON in S\_UT ranged from 0 to 61% and 0 to 22 ppm, respectively. Averaged across environments, C for IND and DON were highest when the fungicide treatments were applied to an MR cultivar. Across the tested fungicide programs, C for DON ranged from 87 to 93% on MR and 77 to 87% on MS cultivars, compared to 63 to 74% on S cultivars. Finding 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. The experiments will be repeated in 2023, and all data will be analyzed to formally quantify management combination effects.

#### Acknowledgement 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, 59-0206-0-132; 59-0206-8-210, 59-0206-0-140; 59-0206-8-199, 59-0206-0-122; 59-0206-8-211, 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; 59-0206-0-185; 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 2022

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#### Abstract

Sphaerex<sup>®</sup> and Prosaro Pro<sup>®</sup> were recently registered for use in wheat for Fusarium head blight (FHB) and deoxynivalenol (DON) management. However, it is unclear whether these new fungicides will be just as effective as or more effective than the current industry standards Prosaro® and Caramba®, and Miravis Ace® against FHB and DON. Field experiments were conducted in multiple US wheat-growing states in 2022 to compare the efficacy of Prosaro Pro and Sphaerex to that of Prosaro, Caramba, and Miravis Ace. Separate replicated plots of susceptible cultivars were subjected to the different fungicide programs and 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 programs relative to CK. Mean IND and DON in the checks across environments ranged from 1 to 42% and 1 to 22 ppm, respectively. Across environments and fungicides, efficacy in terms of C ranged from 68 to 95% for IND and 54 to 86% for DON. The double-application treatments (VI, VII, and VIII) were the most effective of all tested fungicide programs. Averaged across environments, C for IND was 95% for VI, 95% for VII, and 93% for VIII. Similarly, C for DON contamination of grain was 83, 86, and 72% for VI, VII and VIII, respectively. Based on these results, there is evidence suggesting that the combination of an anthesis application of Miravis Ace followed by a "late" application of one of the other tested fungicides can be more effective at reducing FHB and DON than an anthesis-only application of any of the tested fungicides. Finding from this study will provide stakeholders with information regarding the efficacy of the new 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.

#### Acknowledgement 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, 59-0206-0-132; 59-0206-8-210, 59-0206-0-140; 59-0206-8-199, 59-0206-0-122; 59-0206-8-211, 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.

### Post-anthesis Rainfall Effects on the Efficacy of Genetic Resistance and Fungicide Application against Fusarium Head Blight and Mycotoxins in Wheat

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#### Abstract

Fusarium head blight (FHB), caused by *Fusarium graminearum*, significantly reduces yield, and leads to grain contamination with mycotoxins under warm, humid conditions during pre- and early post-anthesis periods. Thus, fungicides are most warranted for FHB control when wet, rainy conditions occur during and shortly after anthesis. Here, we investigated the influence of persistent post-anthesis rainfall on the efficacy of tebuconazole+prothioconazole (Prosaro®) in combination with genetic resistance against FHB and mycotoxins in two field experiments conducted during 2018 and 2019 in Wooster, OH. Separate plots of susceptible (S), moderately susceptible (MS), and moderately resistant (MR) soft red winter wheat cultivars were treated at anthesis (TR) or left untreated (UT), inoculated with a spore suspension of Fusarium graminearum, and then subjected to one of three simulated rainfall regimes: R1) rain during the first 10 days after anthesis, R2) rain during the second 10 days after anthesis, and R3) no simulated rainfall. Relative to the susceptible UT check, mean FHB index (IND), deoxynivalenol (DON) and zearalenone (ZEA) contamination of grain were lowest, and mean grain yield (YLD) and test weight (TW) were highest under all rainfall regimes when the fungicide was applied to the MS or MR cultivar. The estimated net cash income (NCI) of integrated management (IM) programs was consistently higher than the NCI of fungicide-only or resistance-only programs across different grain prices and fungicide application costs under all rainfall regimes. Post-anthesis rainfall had a greater effect on the efficacy of TEBU+PROT against DON than IND. Mean DON was considerably higher in UT plots subjected to R1 (UT R1) than R2 or R3 across all cultivars. However, DON levels were similar for R1 and R2 when plots received an anthesis application of Prosaro. Estimated slopes for relationships between IND and DON (on the log scale) were highest for UT\_R1, but not significantly different among the three simulated rainfall regimes for TR, suggesting that the efficacy of Prosaro at reducing the rate of increase in mean DON per unit increase in mean IND was not compromised under R1. Under R2, estimated slopes were not significantly different between UT and TR. Persistent rainfall shortly after anthesis had little effect on the efficacy of Prosaro against DON, but efficacy was severely compromised by persistent late-season rainfall.

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# Rainfastness of Fungicides for Fusarium Head Blight and Deoxynivalenol Reduction in Soft Red Winter Wheat

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#### Abstract

Fungicides are most warranted for FHB control when wet, rainy conditions occur during anthesis and early grain fill. However, rainfall following treatment application may affect efficacy against FHB and DON, and this effect may vary among fungicides. In this study, we determined the rainfastness of the fungicides Prosaro<sup>®</sup> (prothioconazole + tebuconazole), Caramba<sup>®</sup> (metconazole), and Miravis Ace<sup>®</sup> (pydiflumetofen + propiconazole) when applied to wheat spikes at anthesis in three field experiments conducted in 2020, 2021, and 2022. The experimental design was a randomized complete block, with a split-plot arrangement of simulated rainfall treatment (combinations of durations [0, 15, 30, 60, and 120 min] and start times [0, 15, 30, and 60 min] after fungicide application) as whole-plot and fungicide treatment (Prosaro, Caramba, and Miravis Ace) as sub-plot. The three fungicides were applied to separate plots at their respective labelrecommended rates of 6.5 (Prosaro), 13.5 (Caramba), and 13.7 (Miravis Ace) fl. oz./A, with or without the nonionic surfactant Induce (0.125 v/v). Irrigation risers mounted in each whole plot were then used to simulate the desired rainfall treatment at an intensity of 6.5 mm/h. Approximately 24-36 h after the treatments were applied, all plots were spray inoculated with a spore suspension of F. graminearum. Results were fairly consistent across experiments and among fungicide treatments within experiments. On average, when applied with Induce, fungicide treatments reduced FHB index (IND) by 71 to 87% and DON by 62 to 70%, with the performance of the three fungicides being fairly consistent across rainfall treatments. For DON, percent control relative to the nontreated check (C) ranged from 58 to 73% across rainfall durations and 60 to 73% across rainfall start times. However, efficacy was substantially lower (C < 51% for both IND and DON) when the fungicides were applied without the surfactant and plots were subjected to 120 min of simulated rainfall, beginning immediately after the treatments were applied. These results suggested that Prosaro, Caramba, and Miravis Ace are very rainfast once applied with Induce. Data from the three experiments will be pooled and analyzed to formally quantify fungicide and rainfall treatment effects.

#### **Acknowledgement and Disclaimer**

This material is based upon work supported by the U.S. Department of Agriculture, under Agreement Nos. 59-0206-8-187, 59-0206-0-131 and 59-0206-2-132. This is a cooperative project with the U.S. Wheat & Barley Scab Initiative. Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the authors and do not necessarily reflect the view of the U.S. Department of Agriculture.

#### Management of Fusarium Head Blight in Organic Winter Wheat Production in Indiana

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#### Abstract

Fusarium graminearum is the causal agent of the Fusarium head blight (FHB), an important disease of small grains that significantly reduces yield and quality of grains. In addition, it can cause the accumulation of the mycotoxin deoxynivalenol (DON) impacting both human and animal health. In 2021, 1.69% loss in wheat production were reported in the North Central States a value of \$109.8 million (458,000 metric tons). Currently disease management recommendations are limited for organic wheat production on Organic Materials Review Institute (OMRI) approved products. The objective of this study was to evaluate the effects of two winter wheat organic cultivars, Kaskaskia - FHB susceptible, and Harpoon - FHB resistant, and four OMRI products for FHB integrated management in Indiana. Field trials were established in 2020 and 2021. The experiments had a split-plot design with four replications; cultivars were the main plot and fungicide treatments as subplots. The four OMRI products evaluated included Reynoutria sachalinensis 12% (0.9 L ha<sup>-1</sup>); Bacillus pumilus QST 2808 (2.3 L ha<sup>-1</sup>), Streptomyces lydicus WYEC 108 (0.6 L ha<sup>-1</sup>), and copper hydroxide (1.7 kg ha<sup>-1</sup>), and compared to a conventional fungicide (prothioconazole + tebuconazole, 0.8 kg  $ha^{-1}$ ) and a non-treated control. All products were applied at Feekes 10.5.1. All plots were inoculated with a spore suspension of F. graminearum 24 hours after treatment. Disease ratings were assessed at Feekes growth stages 11.2 and data combined across years. No differences were detected in both cultivars. There were no significant interactions between cultivar and treatment, therefore main effects were compared. No differences between products and non-treated were detected for FHB incidence (P = 0.4587). FHB severity (P = 0.0143) was only significantly reduced by copper hydroxide over non-treated control. In addition, FHB index (P = 0.0220) was reduced by copper hydroxide and prothioconazole + tebuconazole. No significant differences between treatments and non-treated control were observed for yield (P = 0.9197) and DON concentrations (P = 0.4172). These preliminary results presented provide evidence that copper hydroxide might be an option for FHB management in organic winter wheat systems. Future work should continue to evaluate OMRI approved products to determine their efficacy and to provide information about disease management tools for organic farmers in Midwest.
## **Biocontrol of FHB: Beyond the Greenhouse**

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### Abstract

In North America, Fusarium head blight (FHB) of wheat is primarily caused by the hemibiotrophic fungal pathogen, Fusarium graminearum. Management of FHB has largely focused on selecting moderately resistant wheat cultivars and the timely application of fungicides. A biocontrol agent (BCA) that can reduce FHB would have economic value, especially for organic grain production, and could help prolong the efficacy of fungicides. However, success has been mostly limited to greenhouse trials. *F. poae*, which is weakly aggressive on wheat, has previously been found to decrease deoxynivalenol (DON) accumulation by more than 50% in spikes of a bread wheat cultivar when the weaker species was applied one day prior to inoculation with F. graminearum in the greenhouse (Tan et al., 2020, doi.org/10.1111/mpp.12996. We tested the ability of two prospective BCAs F. acuminatum and F. poae, to decrease visual symptoms and DON in the soft red winter wheat cultivar Shirley in both greenhouse and field trials when inoculated at the same time as F. graminearum (COI), or two days prior (PRE). As compared to a single F. graminearum inoculation (G), the PRE treatments for both BCAs and the COI treatment for F. acuminatum decreased deoxynivalenol (DON), disease severity, and disease incidence in the field. Results from the greenhouse were mixed. F. acuminatum and F. poae COI treatments decreased fitted DON by 29.6% and 15.2% in relation to G. These values correspond to an 88% and 77% likelihood that mean DON estimates were less for each COI treatment in relation to the G treatment. PRE treatments showed no efficacy for reducing greenhouse DON levels, while greenhouse area under disease progress curve (AUDPC) for the PRE treatments increased with respect to G. In conclusion, COI treatments of F. poae and F. acuminatum, and PRE treatments of F. acuminatum, were able to impede the ability of F. graminearum to produce visual symptoms and toxins in a susceptible cultivar in a field setting. In the future, a lower concentration of F. graminearum inoculum in the greenhouse will be tested to understand the effect of FHB disease pressure on the efficacy of the BCAs.

FHB Management – Presentation

## A Trip across the Pond and a Few New Friends

## Debra Seymour

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## Abstract

In the late 80's Billy Joe Miles, president of Miles Farm Supply was invited to England. While there, he was impressed with 100 bushel per acre yields at a time when the Kentucky state average was 33 bushel per acre. In a major initiative, to bring this agronomic knowledge to the states, Miles brought consultants to the U.S. and exchanged information with farmers, extension agents and local input providers. This was the beginning of intensive wheat cultivation in the U.S. The concept that higher inputs with proper timing can improve yields and quality has had a huge impact on profitability and increasing our food supply. This is the story of how asking "why not" has had a major impact on wheat cultivation in the U.States.

## **Development of an AI and Cloud Based Real-time Wheat FHB Detection Platform**

#### Fengyun Shi and Ce Yang

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### Abstract

Cloud based applications are rapidly developed in recent years and have reshaped models and algorithms. They can offer a user experience like a program installed entirely on a local machine, but with reduced resource needs, more convenient updating, and the ability to access functionality across different devices. A cloud based real-time & wheat spike object and disease detection scheme is being developed to achieve plant object detection and segmentation through a smartphone application. The detection platform are divided into two parts - detection and application. For wheat spike and disease detection, artificial intelligence (AI) models were trained on a wheat dataset collected from the wheat trial field from Saint Paul, MN. Preprocessing of images were conducted including augmentation and resizing. A Yolov5Tiny model with 87.1 mean Average Precision (mAP) was chosen to detect wheat spikes. For application, a user interface was designed, and functions were developed for Android platforms. The android application uploads images obtained from the smartphone camera or stored in the smartphone to the cloud server for detection modeling. Amazon Web Service (AWS) was chosen as the cloud computing platform, and Microsoft Azure was chosen as its backup. By integrating the Yolov5Tiny model and the Android application, we expect to develop a smartphone application for near real-time plant object detection on users' Android smartphones.

## **Evaluation of Integrated Disease Management Options for Conventional and Organic** Wheat Production in Indiana

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### Abstract

Fusarium Head Blight (FHB), caused by *Fusarium graminearum*, is in important disease on wheat in Indiana. In addition, F. graminearum can contaminate grain with deoxynivalenol (DON), which can impact both animal and human health. In favorable disease years high levels of FHB and DON can cause load rejections across Indiana, therefore this disease is of concern to both conventional and organic grain growers. Cultivars with moderate resistance to FHB have not always provided desirable levels of disease control, and fungicides have become an important component in FHB and DON management plans. The overall goal of this research was to use an integrated approach that combines genetic resistance and fungicide application to achieve optimal FHB management. From 2019 to 2022, wheat trials were established at the Purdue Agronomy Center for Research and Education (ACRE) in Tippecanoe County, IN and at the Southwest Purdue Agricultural Center (SWPAC) in Knox County, IN to evaluate both fungicide efficacy and the integration of cultivars and fungicides. In general, the experiments were either a randomized complete block design to evaluate fungicide efficacy or a strip-plot design to evaluate the integration of cultivars with fungicides. Synthetic and OMRI approved products were applied at Feekes 10.3 and/or Feekes 10.51 (early-anthesis) and 3 to 7 days after Feekes 10.51. Data collection included FHB incidence, severity, and index, DON, and yields. A summary of the last four years of research in Indiana in both conventional and organic grain will be shared and will help determine the impact of implementing these FHB management tools in both conventional and organic wheat in Indiana.

### **Acknowledgement and Disclaimer**

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## Management of Fusarium Head Blight with Demethylation Inhibitors and Succinate Dehydrogenase Inhibitors Combinations under High Disease Pressure in Spring Wheat

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## Abstract

Triazoles, the most used fungicides, belong to demethylation inhibitors (DMI) which disrupt the sterol biosynthesis pathway that is responsible for fungal cell membrane integrity. The disruption of the sterol pathway leads to depletion of sterols which results in cell membrane structure and function failure. The cell membrane malfunction is manifested through inhibited fungal cell growth, abnormal morphological development and eventual death. A second group of fungicides in this study is the succinate dehydrogenase inhibitors (SDHI). This group binds to the succinate dehydrogenase complex and hinders energy production in fungal mitochondria resulting in growth inhibition and cell death. The efficacy of different combinations of DMI and DMI+SDHI were evaluated for managing Fusarium head blight (FHB) and the impact of host genotype on fungicide effectiveness under high disease pressure. Prosaro® (Bayer CropScience, Prothioconazole+Tebuconazole), Miravis Ace® (Syngenta, Pydiflumetofen+Propiconazole), Sphaerex® (BASF, Meticonazole+Prothioconazole) and Prosaro Pro® (Bayer CropScience, Prothioconazole+Tebuconazole) and Prosaro Pro® (Bayer CropScience, Prothioconazole+Tebuconazole+Fluopyram) were applied at early flowering to spring wheat cultivars. Spring wheat cultivars namely, Boost (moderately resistant), Brick (resistant), Samson (susceptible), and CP3099A (susceptible) were used in an FHB management field trial conducted at Volga research farm, in South Dakota

during the 2022 summer season. Plots were inoculated with corn spawn infested with Fusarium graminearum at the boot stage (Feekes 10) and intermittently misted overnight to maintain optimal conditions for disease development. The trial was set as a randomized complete block design with a split plot arrangement. Cultivars were considered the main plot factor while fungicides were subplot. Data on disease incidence, severity, index, and *Fusarium* damaged kernels (FDK) were collected and analyzed using the generalized linear model with applicable link functions and reps were random effects. Brick showed the lowest FHB index and FDK followed by Boost. The highest FHB index and FDK were observed in Samson. A spearman correlation coefficient (r) between FHB index and FDK was 0.8, p<.0001. Both DMI combinations and DMI+SDHI combinations produced FHB indices and FDK values that were lower than those observed in untreated plots (p < 0001). However, the differences among various active ingredient combinations, DMIs or DMI+SDHIs, were not statistically significant ( $p \le 0.05$ ). Host genotype, resistant or susceptible, was instrumental to FHB incidence and severity which was consequential to FHB index and FDKs. The DMI and SDHI fungicides used in this study were effective in controlling FHB index and FDK. Fungicides were effective at further lowering FHB indices and FDKs in resistant hosts compared to untreated resistant hosts. The use and availability of fungicides with different modes of action is a positive development to combat pathogen resistance to synthetic fungicides.

# **Evaluation of Sphaerex® for Control of Fusarium Head Blight and DON in Barley in Southeastern Idaho**

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## Abstract

Fusarium head blight (FHB) is a serious disease of wheat and barley that affects both yield and grain quality. Recently a new fungicide, Sphaerex<sup>®</sup>, was registered for the control of FHB and deoxynivalenol (DON) accumulation on wheat. Sphaerex contains two active ingredients: metconazole and prothioconazole. We conducted a trial to evaluate the efficacy of Sphaerex on FHB and DON on barley at the University of Idaho Aberdeen Research and Extension Center during the 2022 growing season. In the first year of the trial, experimental plots (5 × 9.3 ft) were arranged in a randomized complete block design with six replicates. Plots were inoculated with *Fusarium graminearum* infected corn spawn three weeks before heading. They were later inoculated with macroconidial suspensions (100,000 spores/ml) 24 hours after heading application of fungicides. The susceptible variety, LCS Odyssey, was used to compare the efficacy of 1) single application of Sphaerex at full heading to the applications of 2) Priaxor<sup>®</sup> at herbicide timing followed by Sphaerex at full heading, 3) Miravis Ace<sup>®</sup> at half heading, 4) Miravis Ace at full heading, 5) Prosaro<sup>®</sup> at heading 6) Prosaro Pro® at heading, and 7) untreated plots were used as checks. FHB incidence and index (plot severity) were determined from 100 randomly chosen heads per plot at soft dough. Plots were harvested with a small plot combine and yield (bu/A) was determined with the HarvestMaster system. Subsamples from three replications were measured for DON, test weight and protein content at the Illinois Crop Improvement Association lab in Champaign, IL. Data were analyzed using the GLIMMIX procedure in SAS 9.4.

## Disclaimer

Mention of products in this work is not an endorsement of the product.

## Food Safety & Toxicology

## Control of Fusarium and Deoxynivalenol during Malting using Peracetic Acid

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### Abstract

The use of *Fusarium*-infected barley for malting and brewing can lead to mycotoxin production and result in decreased malt quality. Chemical methods for treatment of *Fusarium*-infected barley may be effective in preventing these safety and quality defects and allow use of otherwise good quality barley. In this study, micro-malting experiments were conducted using peracetic acid (PAA) to evaluate effectiveness in reducing *Fusarium* survival and associated production of the mycotoxin deoxynivalenol (DON) while maintaining germinative energy in barley. Addition of PAA to the steep water was effective in reducing both the infection rate and vitality of *Fusarium* on barley. The magnitude of the antifungal effect of PAA was dependent on both concentration and contact time. The treatments were less effective in reducing the rate and viability of *Fusarium* when the pH was modified to 9.0. PAA treatment of the steep water was effective at reducing DON levels in finished malt. Germination characteristics of the grain were significantly impaired by PAA at concentrations as low as 290 ppm when included in the steep water. Reductions in germination were not as severe when pH was modified to 9.0. The results of the study suggest that PAA may have potential for treatment of *Fusarium*-infected malting barley without detrimental effects on germination, especially when used as a short first immersion (rinse).

## Impact of Hop Essential Oil-in-Water Nanoemulsion Treatment to Control the Safety of Malting Barley using Naturally Fusarium Head Blight (FHB) infected Barley Grains

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## Abstract

*Fusarium* mycotoxin contamination of malting barley has been a persistent food safety issue for malting company. Our previous studies indicated that a number of essential oils have the antifungal and mycotoxin inhibitory activities in vitro such as thyme, clove and hop oils by disrupting of cell wall composition formation and cytoplasm membrane permeability. As hop is one of four essential beer brewing ingredients, it would be interest to understand the potential application of hop essential oil (HEO) as a dual-functional ingredient during malting process.

In our study, the effect of hop essential oil (HEO) nanoemulsion to control of fungal biomass and mycotoxin production during malting process was evaluated. In addition, fluorescent dye coupled with advanced microscopy techniques including confocal laser-scanning microscopy (CLSM) and scanning electron microscopy (SEM) were applied to study the localization of fungal hyphae on original barley kernels and the development of fungal hyphae within malted barley tissue as affected by HEO nanoemulsion. Lastly, the impact of HEO nanoemulsion on the final malt flavors was measured. The application of HEO nanoemulsion was able to reduce fungal biomass marker (Tri DNA) and deoxynivalenol (DON) content at each stage of malting process when compared with control malts. With the aid of CLSM and SEM, majority of fungal hyphae were observed in furrow crease (transverse section), distal end (longitudinal section), the surface of husk, followed by between husk and pericarp, testa layers. Steeping process greatly decreased the large amount of fungal hyphae in furrow crease, distal end and on the surface of husk as compared to malt samples. However, an increased fungal colonization between husk and pericarp, testa layers was identified in the final malts because of the ideal environmental conditions during germination stage. Regarding the impact of HEO nanoemulsion on the final malt flavor, the flavor profile of both malts shared similarity. In addition, HEO nanoemulsion could prevent the formation of free aldehydes in final malts. As a result, it might act as antioxidant to prevent beer staling.

## Acknowledgement and Disclaimer

This material is based upon work supported by the United States Department of Agriculture (USDA) Agricultural Research Service (ARS) under agreement Nos. 59-0206-8-212, 59-0206-0-130 and 59-0206-2-131 in cooperation with the U.S. Wheat and Barley Scab Initiative (USWBSI), and the USDA National Institute of Food and Agriculture (FAR0032822). We thank Mr. Pawel Borowicz from NDSU for his assistance with CLSM image. We also thank Dr. Xiang S Yin from RAHR Corporation for his support on the arrangement of naturally FHB- infected barley samples. Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the authors and do not necessarily reflect the view of the U.S. Department of Agriculture.

# The Role of *Fusarium* Damaged Kernels in DON Accumulation during the Malting of FHB Infected Wheat

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## Abstract

The considerable increase of Deoxynivalenol (DON) levels was observed during the malting of FHB infected hard-red spring wheat in my previous research. In this study, the role of *Fusarium* damaged kernels (FDKs) was evaluated by separating the malting of FDKs and healthy wheat kernels. With the naturally FHB infected wheat, DON contents increased from  $0.65\pm0.11 \mu g/g$  to  $2.42\pm0.23 \mu g/g$  following malting. The content of DON increased from  $2.96\pm0.43 \mu g/g$  to  $19.38\pm4.26 \mu g/g$  during the malting of FDKs, but no significant (*p*>0.05) increase of DON contents (i.e. < $0.50 \mu g/g$ ) was found in the malting of healthy kernels. With the severely FHB infected wheat (i.e. wheat inoculated with *F. graminearum* using a grain spawn method in the field), DON contents increased from  $4.22\pm0.55 \mu g/g$  to  $18.55\pm4.07 \mu g/g$  following malting. The content of DON increased from  $8.26\pm1.48 \mu g/g$  to  $41.11\pm6.31 \mu g/g$  during the malting of FDKs, but no significant (*p*>0.05) increase of DON contents (i.e. < $1.00 \mu g/g$ ) was found in the malting of healthy kernels. The fungal hyphae were observed extremely heavy in the wheat and malt FDKs that contained high contents of *Fusarium Tri5* DNA and DON (i.e. up to  $276.72 \mu g/g$ ), versus the relatively healthy kernels. The results indicated that wheat FDKs were responsible for the DON accumulation during malting, which associated with the interior infection of *Fusarium* hyphae within the kernels.

# Mechanisms of Antifungal and Mycotoxin Inhibitory Properties of Thyme Essential Oil and its Major Chemical Constituents in Emulsion-based Delivery System

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## Abstract

Cereals, such as wheat and barley, which are ranking the most produced grains, are consumed as major staple food. However, because of the inseparable contact with soil, cereal crops are extremely prone to soilborne toxigenic fungi infections such as *Fusarium* spp. that causing yield loss and grain quality reduction. In particular, F. graminearum, a causal agent of Fusarium head blight (FHB), causes millions of lost on wheat production in Northern and Central America every year. The current research studied the antifungal, mycotoxin inhibitory activity of emulsions formed with thyme oil and its four major components (thymol, pcymene, y-terpinene, and linalool) on two chemotype of F. graminearum isolates, and their corresponding mode of action (MOA). Among five essential oil (EO) compounds, thymol presented the strongest antifungal and mycotoxin inhibitory efficacy followed by thyme oil emulsion. The 50% inhibition of spore germination  $(EC_{50})$  on two chemotype Fusarium graminearum isolates can be achieved by thymol emulsion at the concentration of 0.38 and 0.48 mg/ml, respectively. According to the SEM and CLSM images, spore morphology changes and cell membrane destruction were the main reasons attributed to the inhibition of spore germination. Significant (p < 0.05) mycotoxin reduction was also observed when five EOs in emulsion based delivery systems were applied. Interestingly, linalool was shown to be a more effective spore germination inhibitor rather than mycotoxin production inhibitor, while p-cymene and y-terpinene behaved the opposite way. We further investigated the MOA of thyme and thymol emulsions on suppression of mycotoxin production on genetic level. We observed that thyme oil significantly (p < 0.05) up-regulated the expression of the genes studied (Tri3, Tri4, and Tri5), while thymol did not up-regulate any gene expression. This study established the potential application of thymol and thyme oil emulsion as a safe and more effective preservative in food. Future research will focus on discovering the potential synergistic effect of thymol with other compounds to reduce the essential oil usage level.

## Acknowledgement and Disclaimer

This material is based upon work supported by the United States Department of Agriculture (USDA) Agricultural Research Service (ARS) under agreement Nos. 59-0206-8-212, 59-0206-0-130 and 59-0206-2-131 in cooperation with the U.S. Wheat and Barley Scab Initiative (USWBSI), and USDA National Institute of Food and Agriculture (FAR0032822). 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.

## **Gene Discovery & Engineering Resistance**

# Host-induced Gene Silencing of the Fungal Gene *FgGCN5* in Barley for Improving Resistance to Fusarium Head Blight

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## Abstract

Fusarium head blight (FHB) caused by the fungal pathogen *Fusarium graminearum* is one of the most devastating diseases in barley. However, effective resistance has not been identified in barley germplasm. To enhance barley resistance to FHB, we used host-induced gene silencing (HIGS) to target the *F. graminearum* histone acetyltransferase gene *FgGCN5* in the present study. In the loss-of-function *Fggcn5* mutant ( $\Delta$ *FgGCN5*), acetylation levels of histone H3 were significantly decreased at several specific lysins, leading to a genome-wide differential expression and impaired metabolic processes affecting pathogenicity of *F. graminearum*. Using *Agrobacterium*-mediated gene transformation, we have generated transgenic plants and selected homozygous transformants in the late generations. Despite demonstrated production of small-interfering RNAs (siRNAs) homologous to *FgGCN5* in the transgenic barley; the disease severity, DON accumulation, and fungal biomass showed no significant difference from wild-type. In line with these observations, quantitative revere transcription PCR (qRT-PCR) analysis showed the expression levels of *FgSCN5* were not affected by the HIGS construct in the transgenic plants, indicating an inefficiency of the generated siRNAs on silencing the target gene. This research allows for more in-depth analysis for the use of HIGS against FHB. Follow-up investigations with more independent transgenic lines are ongoing to address the incompetence of HIGS targeting *FgSCN5* to provide FHB resistance.

# The UDP-Glycosyltransferase UGT13248 is required for Type 2 Resistance to Fusarium Head Blight in Barley

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## Abstract

Fusarium head blight (FHB) of *Hordeum vulgare* (barley) causes reduction in yield and grain quality. Glycosylation of the trichothecene mycotxin deoxynivalenol (DON) to the less toxic DON-3-glucoside (D3G) is catalyzed by UDP-glucosyl transferases (UGTs). Barley *UGT13248*, was previously shown to convert DON to D3G in yeast, *Arabidopsis* and wheat. In wheat, expression of UGT13248 decreased FHB severity. To explore the natural genetic diversity of *UGT13248* in barley, we sequenced *UGT13248* from 26 barley accessions with varying degree of FHB resistance and analyzed the *UGT13248* sequence of 20 accessions of the barley pan genome. We identified five protein variants. A survey of the *UGT13248* sequence from exome capture sequencing data of a diverse panel of barley genotypes identified eight additional protein variants. Accessions carrying eleven of these thirteen *UGT13248* protein variants did not show strongly reduced sensitivity to DON in seedling root growth assays, suggesting that *UGT13248* is functionally conserved.

Barley lines overexpressing UGT13248 were generated and two independent TILLING lines carrying mutations in *UGT13248* were identified. Protein models suggested that these mutations were in close proximity to the UDP-sugar binding site, *UGT13248* (T368I) and *UGT13248* (H369Y). The T368I and H369Y mutants showed hypersensitivity to DON in seedling root growth assays and strongly impaired conjugation of DON to D3G in barley spikes. Constitutive expression of *UGT13248* resulted in increased resistance to DON in seedling root growth assays and increased conjugation of DON to D3G in spikes. Field tests of the TILLING mutants showed increased FHB disease severity, suggesting that DON to D3G conversion contributed to FHB resistance. Point inoculation experiments showed increased FHB disease severity and increased spread of FHB symptoms in the spikes of the TILLING mutants as well as reduced disease severity in plants overexpressing *UGT13248*. The rachis of the H369Y mutant contained more *Fusarium graminearum* and DON compared to wild-type plants. *F. graminearum* was detected within rachis nodes up to four nodes basi- and acropetal of the inoculated spikelet in H369Y mutants but not in wild-type plants. We conclude that *UGT13248* is required for Type 2 resistance in barley.

### **Acknowledgement and Disclaimer**

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## Develop a Transgene-Free CRISPR/Cas Genome Editing System for Improvement of FHB Resistance in Wheat

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## Abstract

Epidemics of Fusarium head blight (FHB) cause a serious reduction in wheat grain yield and quality, which results in significant economic losses to wheat producers. Growing resistant varieties is the most sustainable and effective approach to minimize FHB damage. Manipulating susceptibility genes (S-genes) in crops has become one of the promising breeding strategies to create new sources of resistance. CRISPR/Cas genome editing system can precisely knock out S-genes, therefore, is a powerful tool to generate resistant mutants for functional validation of candidate genes and the creation of new resistant sources for breeding. However, conventional genome editing requires transgenic process to deliver gRNA and Cas gene, which raises GMO regulatory concerns, and a new method is urgently needed to produce transgene-free edited cultivars for commercial production. Transient expression of CRISPR/Cas RNP (ribonucleoprotein) complex is the most effective way to avoid transgene integration and off-target editing and nanoparticles (NPs) has proven to be a very promising material for gene delivery in numerous biotechnological applications. Previously, we used NPs to deliver CRISPR/Cas9 DNA constructs into wheat cells through the floral dip method, which generated several inheritable mutations in the targeted regions of a histidine rich calcium binding protein (TaHRC), the causal gene for Fhb1. In this study, we evaluated the possible application of NPs to deliver RNP complexes to the meristematic cells that grown directly from imbibed wheat seeds, therefore, the edited mutants derived from the regrown plants are transgene-free. Our preliminary results showed that the GFP and GUS report genes were successfully delivered into wheat tissues by NPs and successfully expressed in both wheat leaves and shoot. We evaluated the binding capacity and ratio of NPs to RNP complexes and validated the RNP activity to edit the targeted genes by *in vitro* DNA cleavage assay. We delivered specific RNP complexes targeting *Fhb1* susceptible allele into embryos of imbibed dry 'Bobwhite' seeds and will screen the regrown seedlings for *Fbh1* mutants. This new genome editing system will provide a new tool for routine gene editing to create transgene-free, new sources of FHB resistance for wheat breeding programs after further optimization.

### **Acknowledgement and Disclaimer**

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Gene Discovery & Engineering Resistance - Poster

## Discovery of a Susceptibility Factor for Fusarium Head Blight on Chromosome 7A of Wheat

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## Abstract

Fusarium head blight (FHB), primarily caused by a hemi-biotrophic fungus *Fusarium graminearum*, is a key disease of small grains. Diseased spikes show symptoms of premature bleaching shortly after infection and have aborted or shriveled seeds, resulting in reduced yields. The fungus also deteriorates quality and safety of the grain due to the production of mycotoxins, especially deoxynivalenol (DON), which can result in grain being rejected at the point of sale. Manipulation of susceptibility factors, the plant genes facilitating disease development, offers a novel and alternative strategy for enhancing FHB resistance in plants. In this study, a major effect susceptibility gene for FHB was identified on the short arm of chromosome 7A (7AS). Nullisomic-tetrasomic lines for homoeologous group-7 of wheat revealed the dosage effect of the gene, with tetrasomic 7A being more susceptible than control Chinese Spring wheat, qualifying it as a genuine susceptibility factor. The gene locus was found to be conserved across chromosome 7A substitution lines of five wheat varieties of diverse origins and a tetraploid Triticum dicoccoides genotype. The susceptibility factor was named as Sf-Fhb-7AS and mapped on chromosome 7AS to a 50 Mb peri-centromeric region between del7AS-3 and del7AS-8. Our results show that deletion of Sf-Fhb-7AS imparts 50-60% type-2 FHB resistance. Seed and pollen irradiation panels have been developed in Chinese Spring and Jagger background to further narrow down the region. Identification and manipulation of *Sf-Fhb-7AS* will be useful for enhancing genetic resistance against FHB in wheat.

## **Genetic Engineering of Barley to Improve Fusarium Head Blight Resistance**

#### Alison Dineen, Michael A. Lawton and Rong Di

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## Abstract

Fusarium head blight (FHB) caused by *Fusarium graminearum* (Fg) is major disease in barley, causing significant yield losses in the United States and other parts of the world. Most cultivars in the US are susceptible to Fq infection. Transgenic and gene editing technologies allow us to study underlying molecular mechanisms of host FHB resistance, uncover host FHB susceptibility genes, and produce barley cultivars that are resistant to FHB. All these approaches rely on developing efficient protocols for tissue culture, transformation and regeneration. Cereals are notoriously recalcitrant to in vitro culture, and the protocols established for barley are genotype-dependent. To address this problem and assist the USWBSI community of barley researchers, we have established a barley genetic engineering facility to develop tissue culture and transformation protocols for a wide range of agronomically important barley cultivars. Our improved plant tissue culture protocols permit the regeneration of multiple barley plantlets from a single scutellum explant from the two-rowed cultivar Genesis and the six-rowed cultivar Morex. Transgenic barley plants have been produced using these cultivars using both biolistic and Agrobacterium-mediated transformation methods. Additionally, we have improved our CRISPR-gene editing platform to generate knock-outs (KO) for several host genes involved in conditioning Fq susceptibility. We have adopted the polycistronic tRNA-gRNA (PTG) platform, which enhances gRNA production and mutation efficiency in transformed plants by creating double cuts at targets in the barley genome. Our studies with the model plant Arabidopsis have shown that CRISPR-mediated knock-outs of the AtEIN2 (ethylene insensitive 2) gene result in an augmented Fq resistance. Additionally, the HvUGT (uridine diphosphate qlycosyltranferase) promoter has been shown to play a vital role in FHB susceptibility. We have constructed transient and integrating gene editing vectors based on the PTG platform to disrupt HvEIN2 and modulate regulation of the promoter of barley HvUGT. Results using barley protoplasts and calli confirm that this modified CRISPR approach reliably produces site-specific mutations within the *HvEIN2* coding region and the *HvUGT* promoter.

### **Acknowledgement and Disclaimer**

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## **Trichothecenes Impact Chloroplast Protein Homeostasis and Stress Responses**

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Abstract

The molecular mechanisms underlying trichothecene toxicity in plants are poorly understood and this places limits on our ability to engineer resistance to trichothecenes and ultimately Fusarium graminearum (F.g.). Previously we found that deoxynivalenol (DON) induces the chloroplast unfolded protein response (cpUPR) in Chlamydomonas and that mutants that play a role in the cpUPR were identified as highly sensitive to trichothecenes. In addition, several other mutants in proteins which function to resolve proteotoxic stress within the chloroplast were found to have substantially reduced fitness compared to the wild type. We have since identified that DON rapidly induces the VESICLE-INDUCING PROTEIN IN PLASTIDS 2 (VIPP2) gene which plays a role in managing chloroplast membrane stress. This gene was previously found to be upregulated under high light and oxidative stress conditions. Induction of VIPP2 is part of a retrograde stress response which upregulates small heat shock proteins (sHSPs) like HSP22E/F, sHSPs which function to return the chloroplast to homeostasis following periods of stress. If trichothecenes similarly target higher plant chloroplasts, we predict that the resulting chloroplast stress would contribute to F.g. virulence. To begin this investigation, we are testing the impact of trichothecenes on VIPP2 orthologs in Arabidopsis, wheat, and barley. The goal is to identify if chloroplasts from higher plants are impacted by trichothecenes, measure the magnitude of impact, and understand the potential role of the chloroplast protein quality control (cpPQC) system in response to trichothecene-induced damage. This study may provide unique methods to increase resistance to trichothecenes and F.g.

### Acknowledgement

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# Preliminary Study on RNAi-Mediated Resistance to Fusarium Head Blight in Barley (*Hordeum vulgare*)

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### Abstract

Fusarium head blight (FHB), mainly caused by the fungus *Fusarium graminearum*, is one of the most destructive diseases of barley, wheat and other small cereals in the world. Intensive efforts on screening FHB resistance in barley have not been fruitful yet. The RNA interference (RNAi) approach has been successfully used to control various plant pathogens. However, the application of RNAi to enhance barley FHB resistance is limited. We constructed vectors containing the fragmented *Tri6* gene, a transcriptional regulator within tricothecene gene cluster of *Fusarium graminearum* and transferred the vectors into a malting barley variety Germcraft using the meristem transformation method. We generated 15 T0 transgenic plants including five generated on hygromycin selection and 10 on G418 selection. The T1 seeds were planted and grown for PCR analysis. Expected bands were amplified from 10 T1 lines. Droplet digital PCR (ddPCR) with the T1 transgenic plants identified lines with various copies of the transgene. Nearly 200 T2-generation plants were analyzed by PCR and ddPCR, and five homozygous T2 lines with single copy transgene were identified. Some of the T2 plants were phenotyped through DIP inoculation with a most virulent *Fusarium graminearum* strain PH1. Significant variations in both disease severity and deoxynivalenol (DON) accumulations were observed among the T2 transgenic plants. We plan on conducting large-scale FHB phenotyping and expression of small RNAs of *Tri6* gene with T3 stable transgenic lines.

## Application of dsRNA coated with Graphene Quantum Dots Offers a Promising Strategy for Silencing Fungi and Plants Genes

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## Abstract

Spray-induced gene silencing (SIGS) is a non-transgenic RNA interference (RNAi) strategy used for the control of various pathogen and pest. In this system, naked or nanomaterial-coated dsRNA is sprayed on plants to cause silencing of selective genes. Our aim is to use this strategy for the silencing of genes responsible for the growth and pathogenicity in *Fusarium graminearum* (Fg). The hypothesis is that the nanoparticle coating offers stability and slow-release to siRNAs or dsRNAs with continuous inhibition of Fg during the critical time of infection facilitating on-farm RNAi success. When treated with 20 mM siRNA oligo of Fg genes *MGV1* and *COT1* coated with chitosan nanoparticles, conidia of Fg failed to germinate. However, the blank controls with chitosan nanoparticles alone also displayed inhibitory effects on conidium germination in Fg. For MGV1, conidium germination rate was 2.1% at 6 h when siRNA was coated with Graphene quantum dots (GQDs). When blank GQDs without siRNA were used, the germination rate was 81.7%. For COT1, treatments with 25% siRNA oligo reduced over 60% germination rate at 6 h but treatments with 50% siRNA oligo blocked germination. Treatments with siRNA oligoes coated with GQDs targeting the *PMK1* gene significantly reduced appressorium formation and virulence in the rice blast fungus, indicating that coating with GQDs may be suitable for other fungal pathogens. For wheat, we designed native and synthetic RNAi construct (200-300) bp partial coding region of the selected genes, including wheat phytoene desaturase (PDS), by using the pssRNAit web server. The dsRNA for PDS was produced using the double T7 plasmid L4440 harboring the native and synthetic RNA segments and transformed into HT115 (DE3) strain of E. *coli* which was induced to produce dsRNA using Isopropyl  $\beta$ -D-1thiogalactopyranoside (IPTG). Preliminary results showed that GQDs-coated dsRNAs targeting PDS gene resulted in photobleaching phenotype, indicating the effects of siRNA on degrading the complementary mRNA sequences of the PDS gene. This gives the clues to move forward with the use of wide range of GQD concentrations for the improvement of dsRNA effectiveness and targeting Fg genes for managing FHB disease in wheat.

Gene Discovery & Engineering Resistance – Presentation

## **Fungal Elicitor-triggered Immune Responses in Wheat and Barley**

### Guixia Hao

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## Abstract

Reactive oxygen species (ROS) burst is one of the earliest plant defense responses during plant and pathogen interactions. However, the complex roles of ROS during FHB development remain unclear. Fungal cell walls consist of two main components, chitin, and  $\beta$ -glucans, which play important roles in elicitor triggered-immunity. Using luminol-based chemiluminescent assays, we investigated ROS burst in different wheat and barley tissues triggered by chitin and laminarin (a  $\beta$ -1,3-glucan polymer with degree of polymerization). We discovered: 1) chitin triggers tissue-specific ROS burst in wheat tissues, primarily rachis and rachis nodes, whereas chitin triggers high ROS in barley leaves but low ROS in lemmas and paleae; 2) laminarin induces high and broad ROS in wheat and barley head tissues; and 3) ROS induction is enhanced in both wheat and barley head tissues treated with the combination of chitin and laminarin. In addition, we found that the expression of plant defense genes was upregulated in wheat heads treated with chitin, laminarin or both. Furthermore, we showed that chitin treatments reduce Fusarium head blight spread in wheat and mycotoxin contamination.

## Acknowledgement and Disclaimer

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

## *Fhb1* Disease Resistance QTL does not Exacerbate Wheat Grain Protein Loss at Elevated CO<sub>2</sub>

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### Abstract

Fusarium head blight, a devastating cereal crop disease, can cause significant yield losses and contaminate grain with hazardous fungal toxins. Concerningly, recent evidence indicates that substantial grain protein content loss is likely to occur in wheat that is moderately resistant to head blight when it is grown at elevated CO<sub>2</sub>. Although wheat breeders in North America utilize a number of resistance sources and genes to reduce pathogen damage, the *Fhb1* gene is widely deployed. To determine whether *Fhb1* is associated with the protein content loss at elevated CO<sub>2</sub>, twelve near-isogenic spring wheat lines from either a susceptible or moderately susceptible genetic background, and with, or without the *Fhb1* QTL, were grown at ambient and elevated CO<sub>2</sub> conditions. The near-isogenic lines were evaluated for differences in physiology, productivity, and grain protein content. Our results showed that the *Fhb1* QTL did not have any significant effect on plant growth, development, yield, or grain protein content at ambient or elevated CO<sub>2</sub>. Therefore, other factors in the moderately susceptible wheat genetic background are likely responsible for the more severe grain protein loss at elevated CO<sub>2</sub>.

# Microscopy and RNA-Seq Analysis of Fusarium Head Blight Infection in a Barley Mutant Deficient in Deoxynivalenol Detoxification

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## Abstract

Fusarium graminearum causes head blight in wheat and barley. Disease symptoms can spread to neighboring spikelets through the rachis in wheat heads facilitated by the type B mycotoxin deoxynivalenol (DON). In contrast, barley spikes exhibit native resistance to disease spread and this resistance is associated with the activity of a UDP-glycosyltransferase (UGT) gene, HvUGT13248. Utilizing a TILLING mutant of UGT13248 (H369Y) and a Fusarium reporter strain with TRI5prom::GFP and gdpAprom::dsRed, we performed confocal microscopy to monitor trichothecence biosynthesis (using GFP expression) and fungal growth in planta (using dsRed expression). Fungal infection progressed faster and DON production was consistently higher at 2, 4, and 6 days post inoculation (dpi) in the H369Y mutant compared to its wildtype sibling-line. At 4 dpi, GFP expression was more extensive in the rachis node in the mutant spike. At 6 dpi, the GFP signal was much reduced in the rachis node in the mutant compared to the wildtype. In wildtype, both the GFP and dsRed signal were confined in the rachis node while in the mutant the fungus progressed through rachis node and GFP and dsRed signals can be observed in the vascular vessels of the internode. Taken together, F. graminearum infection in the mutant progressed at a faster pace and spread through rachis node into vascular vessels. Trichothecene production was strongly induced at 4 dpi in rachis node and at 6 dpi in internode as the fungus spread over the spike. To understand the transcriptomic changes that underlie these infection differences, we performed an RNA-Seq experiment on the infected floret and rachis tissues at 2, 4 and 6 dpi in the mutant and wildtype. Standard bioinformatics workflow was applied to the raw data to remove sequencing adaptors, align to the MorexV3 genome and count reads. Principal component analysis (PCA) at the sample level revealed that tissue type explained variation on PC1 (38.7%) and treatment on PC2 (29.3%). Differentially expressed genes (DEGs) were identified using DESeg2. In the floret, more DEGs were identified at 2 and 4 dpi in H369Y than in wildtype but it was reversed for 6 dpi. In the rachis, more DEGs were identified in H369Y through all timepoints. The list of DEGs contained transcripts that have been shown to respond to FHB infection, including UGT13248, ABC transporters, GSTs, receptorlike kinases, as well as novel transcripts (e.g., Fusarium resistance orphan protein). Over-representation and pathway analyses of the DEGs are being conducted to reveal enrichment of gene sets and pathways and to construct a gene regulatory network.

## Acknowledgement and Disclaimer

This material is based upon work supported by the U.S. Department of Agriculture, under Agreement 59-0206-0-135. 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.

## **Engineering Gene-for-Gene Resistance to Fusarium Head Blight in Wheat and Barley**

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### Abstract

'Decoy' engineering is an emerging strategy that introduces novel, new-to-nature disease resistance traits against crop plant pathogens. In this approach, a protease recognition sequence within a host protein, PBS1, is replaced with a protease cleavage sequence recognized by other pathogen-secreted proteases, thereby generating a PBS1 decoy variant. Proteolytic cleavage of the PBS1 decoy protein by the pathogen-secreted protease activates a disease resistance protein, thereby conferring resistance to the pathogen. This strategy has been successfully deployed in Arabidopsis and soybean to confer resistance against both bacterial and viral pathogens. Similar to Arabidopsis and soybean, wheat and barley contain multiple PBS1-like proteins that, when cleaved, activate the disease resistance protein PBR1. Therefore, we predict the decoy engineering strategy can be extended to these crop plants with the goal of introducing novel disease resistance traits to *Fusarium graminearum* based on the recognition of *F. graminearum* effector proteases. To the end, we have identified an effector protease from *F. graminearum*, which we have termed FgTPP1, that is expressed during early symptomless stages of disease development. Subcellular localization analyses showed that this effector protease localizes to the chloroplast stroma, suggesting that this protease is translocated into wheat cells during infection. Importantly, knockout of FgTPP1 significantly reduced F. graminearum virulence in wheat spikes, indicating FgTPP1 plays an important role in fungal virulence. Future work will involve identifying preferred cleavage site specificities of FgTPP1 and testing whether cleavage of the wheat and barley decoy variants by FgTPP1 activate PBR1-dependent immune responses.

### Acknowledgements

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## Editing Wheat Genome through Wide Hybridization with Maize Expressing Cas9 and Guide RNA

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## Abstract

CRISPR/Cas9-mediated genome editing technology is a powerful tool for targeted gene mutagenesis in various crops. In wheat, most of the genome editing studies so far have directly or indirectly relied on callus induction and plant regeneration from the explants receiving gene construct or preassembled Cas9/guide RNA (gRNA) ribonucleoprotein complex delivered by particle bombardment or Agrobacterium-mediated methods. Unfortunately, the efficiency of callus induction and plant regeneration is generally very low for most wheat genotypes, limiting the application of genome editing in commercially grown wheat varieties. Recently, a new approach named "Haploid Inducer-Mediated Genome Editing" or "HI-Edit" has been developed, which combines haploid induction with genome editing to generate transgene-free doubled haploid plants with the target genes edited. However, few studies have been reported in wheat using this approach. In this study, we aimed to use HI-Edit to mutate two wheat genes, TaHRC and Tsn1, which confer susceptibility to Fusarium head blight (FHB) and three foliar diseases (tan spot, Septoria nodorum blotch and spot blotch), respectively. For each gene, two target sites were designed to make a guide RNA, which was cloned into a CRISPR/Cas9-mediated genome editing plasmid. The gene constructs were then transformed into the hybrid maize variety Hi-II by Agrobacterium-mediated transformation. The resulting  $T_0$  and  $T_1$  maize plants with high expression of Cas9 and gRNA were used to pollinate emasculated florets of wheat variety Dayn. Haploid wheat plants were generated from the tissue-culture rescued embryos derived from the wide crosses. PCR amplification and sequencing indicated that 15-33% of the haploid plants contained mutations at the target sites of the targeted gene. Doubled haploid plants with mutations at the target genes were generated and phenotype data on FHB inoculation or ToxA infiltration in the greenhouse will be presented. This wheat × maize hybridization combined with genome editing approach provides a very useful tool for targeting wheat genes of interest for functional characterization and trait improvement without regulatory issues.

### **Acknowledgement and Disclaimer**

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## Transfer Fhb7 to Barley through CRISPR-mediated Targeted Gene Insertion

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## Abstract

Fusarium head blight (FHB; scab) is a devastating disease in barley and wheat caused by the same pathogen. While significant progress has been made in understanding and improving host resistance in wheat with molecular cloning of the major QTL *Fhb1* and *Fhb7*, similar research with barley has lagged behind due to the lack of highly resistant genotypes, which makes it difficult to effectively control FHB and DON contamination. Thus, there is an urgent need for a breakthrough in gene discovery and germplasm development to achieve higher levels of FHB resistance and a greater capacity to detoxify DON in barley using transformative approaches.

The use of wheat genes to breed barley FHB resistance is the road not taken because of strong reproductive barriers. Considering that *Fhb7* detoxifies DON, we hypothesize that *Fhb7* can also greatly contribute to FHB resistance in barley. Taking advantage of our ongoing work on *Fhb7* and CRISPR-based genome editing, we propose to continue our effort with an overall goal to transfer *Fhb7* to barley through CRISPR-mediated targeted gene insertion as a proof of concept. The proposed research includes three objectives:

- 1) Generate transgenic barley expressing both CRISPR/Cas9 and Fhb7 donor.
- 2) Evaluate the *Fhb7* function in transgenic barley.
- 3) Screen the transgenic plants for targeted *Fhb7* insertion events.

Supported by the USWBSI-TRSC program, we are establishing a CRISPR-mediated targeted gene insertion system in barley: developed an all-in-one construct to express CRISPR/Cas9 and the *Fhb7* donor DNA and 39 transgenic plants by *Agrobacterium* mediation. Detached leaf assay analysis of the transgenic plants together with the non-transgenic control showed that *Fhb7* functions in barley in suppressing the growth of *Fusarium graminearum*. While screening the T1 population for targeted insertion events, we have transformed thousands of embryos of Gold Promise (GP) and Excelsior Gold (EG; an elite two-row barley cultivar from the Cornell University) embryos by Biolistic-bombardment of an optimized CRIPSR/Cas construct together with phosphorylated and phosphorothioate linkage-protected PCR product of the *Fhb7* gene. After optimizing the parameters, both EG and GP transformation starts to regenerate transgenic plants.

Results from the proposed research will have a positive impact on barley production and the malting (feed and food) industry, benefiting barley growers and end-users.

## Measuring the Impact of Barley Apoplastic Exosomes on FHB

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### Abstract

Plants and fungi produce exosomes that contain sRNAs and proteins that play a role in modulating plant-fungal interactions. However, it is not well understood if barley uses exosomes to transfer extracellular proteins and sRNAs to F. graminearum and how those exosomes may impact fungal growth. The primary goal of this research is to isolate exosomes from mock inoculated and F. q. infected barley seedlings and characterize the contents by high-throughput analysis of exosomal proteins and sRNAs. Global analysis of exosome cargo will provide a catalog of candidate genes that can be quickly tested for their role in pathogenicity. This work will provide novel insights into how barley controls Fusarium infection and identify new proteins and sRNAs that can be used to improve resistance to Fusarium head blight (FHB). Apoplastic barley exosomes (50-100 nM vesicles) are isolated using ultracentrifugation and density gradient purification from barley leaf tissue. We identified an Arabidopsis TET8 antibody, a marker for exosomes, that binds a barley TET8-orthologous protein isolated from our barley exosome total protein preparations. In addition, proteomic analysis (LC-MS/MS by spectral count) revealed enrichment of specific exosome proteins, including the syntaxin, PENETRATION 1 (PEN1) in the purified apoplastic preparations compared to the raw exosome preparations (not purified via density gradient methods). Recent research from Arabidopsis suggests that exosomes inhibit fungal penetration. Our current work is exploring which fractions of the barley exosome preparations reduce fungal spread when tested directly on barley leaves. Additionally, we are exploring the use of tangential flow filtration (TFF) along with polyethylene glycol (PEG) precipitation to isolate the highest quality exosomes from the apoplast. This method has advantages over the ultracentrifugation methods, which may impact both the exosome content cataloging and fungal bioassays.

### **Acknowledgement and Disclaimer**

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## **Complex Interactions between FHB Resistance, Plant Height, and Anther Retention in Wheat Analyzed by Genomic-estimated Breeding Values**

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## Abstract

Achieving high Fusarium head blight (FHB) resistance is an ultimate goal in any wheat breeding program to secure grain yield and avoid mycotoxin contamination of the harvest. Despite great efforts by breeding companies, the gain from selection was negligible in the last 20 years in Central Europe. Quantitatively resistant cultivars are available, but there are only few of them, they have a low acreage, and are often unfavourable for agronomic characters. Besides a complex inheritance, the main cause for this is the complex interactions between FHB resistance, plant height, and anther retention. In the high-input wheat production in Central Europe, semi-dwarf genotypes are indispensable, accounting for >70% of all varieties. It is known that FHB resistance shares pleiotropic loci with plant height (PH) and anther retention (AR). The dwarfing gene Rht-D1b (Rht1) increase FHB susceptibility, whereas Rht24 is FHB neutral (Miedaner et al. 2022). Also, plant height per se has an effect with taller genotypes being more resistant (r = -0.42, P<0.001). The main objective of this study was to dissect the effect of morphological traits on FHB resistance. We combined correlation and path analyses, genome-wide association studies (GWAS), and genomic prediction (GP, Akohoue et al. 2022). High genotypic correlation ( $r_g$ =0.74) and direct path effect (0.57) were detected between FHB severity and anther retention (AR). A moderate correlation ( $r_{e}$  = -0.55) was found between FHB severity and plant height (PH) with a high indirect path via AR (-0.31). Single-trait GWAS identified 25 quantitative trait loci (QTL) for FHB severity, PH and AR, while multi-trait GWAS detected six QTL across chromosomes 2A, 4D, 5A, 6B and 7B conveying pleiotropic effects on the traits. *Rht-D1b* was associated with high AR and FHB susceptibility with a negatively pleiotropic effect. The use of GP for selecting the genomic background was more effective than selection based on GWAS-detected markers. Strategies for the selection of semi-dwarf varieties with higher FHB resistance include (1) selection of cleistogamous wheat genotypes (AR >96%), (2) use of several FHB-neutral Rht genes, or (3) use of genomic-estimated breeding values for the selection of semi-dwarf genotypes with a high FHB resistance background.

Gene Discovery & Engineering Resistance - Poster

## **Targeting Susceptibility Genes in Wheat to Enhance Resistance against Fusarium Head Blight**

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### Abstract

Host genes and mechanisms that promote *Fusarium graminearum* virulence and/or facilitate infection provide novel targets for controlling Fusarium head blight. The goal of this project is to identify these susceptibility genes/mechanisms and to assess if knockdown of these 'susceptibility genes' in wheat can promote resistance against FHB. When combined with wheat variants that contain missense or nonsense mutations that lower the activity of susceptibility genes, this approach provides an inheritable non-GMO approach for enhancing FHB resistance and reducing DON accumulation. Two sets of susceptibility genes have been targeted for knockdown in wheat: (1) A 9-lipoxygenase (9-LOX) encoded by *Lpx3*, which is involved in the biosynthesis of oxidized lipids (oxylipins); and (2) Genes encoding the defense repressors NPR3 and NPR4, which repress activity of the SA receptor NPR1 that contributes to resistance against *Fusarium graminearum*. Progress on these efforts will be presented.

### Acknowledgement and Disclaimer

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## Spherical Nucleic Acids for Fusarium graminearum Gene Regulation

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### Abstract

Plants and fungi natively regulate expression of genes through the RNA interreference (RNAi) pathway, a mechanism where small interfering RNAs (siRNAs; 19-21 base pairs in length) bind to target mRNAs preventing their expression. The use of siRNAs to limit fungal virulence and decrease plant susceptibility to infections is promising pathways to realizing green fungicides due to the high specificity of this process. However, efficient delivery of siRNA remains challenging for many applications due to their susceptibility to nucleases and poor entry. Here, we will discuss approaches for developing non-toxic and biocompatible liposomal nanoparticles that use natural products for the delivery of siRNA to plants. Specifically, we focus on the development of spherical nucleic acids, nanoparticle consisting of a spherical core with a dense and highly oriented nucleic acid shell, as potential fungicidal vehicles. We have demonstrated the uptake of LNP formulation consisting of micelles and liposomal templates in *Fusarium graminearum* at short incubation times <20 mins, highlighting the promise of this approach. In addition, we have found that our LNPs lead to knockdown of potential target genes.

## A Wheat Practical Haplotype Graph to Facilitate Low-Cost Genotyping for FHB Resistance Mapping

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### Abstract

Next-generation sequencing (NGS) technologies enable high-throughput, low-cost genotyping in wheat. However, these methods generate large numbers of missing sites and several imputation methods have been developed to predict these missing data with accuracies dependent on reference selection, sequencing coverage, minor allele frequencies, etc. A graph-based computational framework called Practical Haplotype Graph (PHG) was recently introduced to efficiently store sequence based genotyping data and infer highdensity genotypes by imputing low-coverage skim genotype sequences. The PHG serves as a database to store large-scale genomic variation in the form of pangenome haplotype and enables imputation of highdensity genotypes from low-density genotyping platforms. In this study, we used whole exome capture sequencing dataset to develop a wheat PHG database. The objectives of this study were to estimate the accuracies of imputing whole exome capture genotypes from simulated skim-sequencing, genotyping-bysequencing, and Illumina array data. We show that with a wheat PHG built to store WGS information for 95 diverse wheat genotypes, the PHG SNP-calling accuracy was minimally affected by sequencing coverage and imputation accuracies for low-coverage sequencing data ranged between 83% (0.01x) and 87% (1x). Beagle 5.2 on the other hand could accurately impute low-coverage skim sequencing data with 81% accuracy (1x). The imputation with GBS was, surprisingly, higher with an average accuracy of 96%, and could prove to be very useful to achieve genomic selection goals at lower sequencing cost. It is yet to be determined how the genotypes imputed using PHG would help identify SNPs with a broader range of FHB resistance traits in genetic mapping experiments.

## Wheat Pore-forming Toxin-like Protein Confers a Broad Spectrum Resistance against Multiple Fungal Pathogens in *Arabidopsis*

Lovepreet Singh<sup>1</sup>, Arunima Sinha<sup>1</sup>, Megha Gupta<sup>1</sup>, Shunyuan Xiao<sup>1,2</sup> and Rosemarie Hammond<sup>3</sup> <sup>1</sup>Department of Plant Science and Landscape Architecture, University of Maryland College Park, Maryland, 20742, USA; <sup>2</sup>Institute for Bioscience and Biotechnology Research, Rockville, Maryland, 20850, USA; and <sup>3</sup>Molecular Plant Pathology Laboratory, U.S. Department of Agriculture, Agricultural Research Service, Beltsville, Maryland, 20705, USA

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## Abstract

Fusarium head blight is a devastating disease of wheat. Fhb1 is the most widely used quantitative trait locus in wheat cultivars globally providing type-2 resistance against *Fusarium graminearum*. Previously, using mutation analysis, gene silencing, and overexpression, a Pore-forming toxin-like (PFT) protein was shown to underlie the Fhb1-mediated resistance in wheat by Rawat et al (2016). PFT is a chimeric plant lectin with two agglutinin domains of Amaranthus caudatus and one bacterial toxin domain of the aerolysin pore-forming toxin family of bacterial toxins. In the present work, heterologous expression of wheat-PFT was done in Arabidopsis ecotype Landsberg erecta. The transgenic Arabidopsis plants were challenged with multiple fungal pathogens including F. graminearum, Colletotrichum higginsianum, Sclerotinia sclerotiorum, and *Botrytis cinerea*. The wheat PFT was found to provide a broad-spectrum resistance against all the tested fungal pathogens in the T<sub>2</sub> and T<sub>3</sub> generation of the transgenic Arabidopsis plants. However, the transgenic plants were not resistant to bacterial and oomycete pathogens *Pseudomonas syringae* and *Phytophthora* capsici, respectively. To investigate the underlying cause of the specific resistance of wheat PFT to fungal pathogens and not bacterial and oomycete pathogens, PFT protein was expressed in Nicotiana followed by its extraction and purification. The purified PFT protein wash was hybridized to an array of 300 different carbohydrate monomers and oligomers. Interestingly, glycan microarray assay revealed that PFT specifically binds to chitin monomer, N-Acetyl glucosamine (GlcNAc), which is an exclusive component of fungal cell walls and is not present in bacteria or Oomycete. Together, these results suggest that PFT is an atypical broad-spectrum disease-resistance protein that may contribute to resistance against a wide range of fungal pathogens in multiple crop plants.

## **Constitutive Expression of** *SbCCoAOMT* **in the Phenylpropanoid Pathway can Improve Resistance to Fusarium Head Blight of Wheat**

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### Abstract

Fusarium head blight (FHB) of wheat is caused by the devastating pathogen Fusarium graminearum, which can contaminate grain with the mycotoxin deoxynivalenol. To sustainably manage FHB, it is important to identify novel mechanisms of resistance to F. graminearum. Lignin, a product of monolignol biosynthesis in phenylpropanoid metabolism, rigidifies cell walls and may be a barrier to pathogen infection and spread. In sorghum, altered expression of some monolignol biosynthesis genes was shown to improve resistance to some Fusarium spp. Our goal was to determine if constitutive expression (CE) of the sorghum genes SbC3'H (coumaroyl shikimate 3-hydroxylase) and SbCCoAOMT (caffeoyl coenzyme A 3-O-methyltransferase) in the moderately susceptible spring wheat line CB037 improves resistance to FHB. In the greenhouse, Type II resistance (to pathogen spread) was determined on two CE lines for each gene and CB037, using the area under the disease progress curve (AUDPC) and Fusarium-damaged kernels (FDK). The CE line CCoAOMT413 had the lowest AUDPC and FDK as compared with the CE line, CCoAOMT421, both C3'H CE lines and CB037. RNA sequencing of F. graminearum or mock point-inoculated heads of CB037 and both SbCCoAOMT CE lines at 12 and 72 hours post inoculation (hpi) was performed. Principal component analysis showed that expression varied the most according to time-point then according to treatment and line. At 12 hpi, no mutually-expressed genes were significantly (p-value > 0.05) differentially regulated in the SbCcoAOMT CE lines as compared with CB037 (the recipient line). At 72 hpi, 474 mutually-expressed genes were significantly upregulated (*p*-value  $\leq$  0.05) in the SbCCoAOMT CE lines. Gene ontology (GO) enrichment included GO terms for chitin metabolism, regulation of jasmonic acid pathway, and defense response that might be associated with the observed moderate resistance in CCoAOMT413. Phloroglucinol staining of F. graminearum-inoculated rachis samples at 72 hpi showed that CCoAOMT413 was more darkly stained compared to CB037 and CCoAOMT421, indicating increased lignin accumulation in CCoAOMT413. We are examining targeted secondary metabolite accumulation in SbCcoAOMT CE and CB037 lines. Results from this research can be used to identify targets for development of novel resistance to FHB.

### Acknowledgement and Disclaimer

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## **Pathogen Biology & Genetics**

# Searching for *F. graminearum* Effectors Controlling Fusarium Head Blight: A Core Arsenal Targeting Conserved Susceptibility Drivers in Bread Wheat?

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#### Abstract

Over the past two decades, the multiple evidences of plant genes required for pathogen infection have opened new opportunities to improve immunity in crops providing alternative sources of resistance for designing original epidemic control strategies. These genes, the so-called susceptibility (S) genes, are involved in a wide range of plant fundamental processes that are hijacked by the pathogen through the delivery of pathogen effectors. In the wheat - F. graminearum pathosystem, the role of such S genes in the interaction outcome has already been demonstrated in several studies but the identification of their diversity and the characterization of their precise contribution to the disease development remain a major challenge. Improving our knowledge of effectors and of their targets is therefore a particularly promising lever for tracing original S genes. In this framework, we designed original dual-omics approaches to decipher the intricate interplay of F. graminearum effectors with wheat responses during the early stages of the infection progress. Taking advantage of strains of contrasting aggressiveness facing wheat cultivars of different susceptibility, we profiled fungal genes coding for secreted proteins and exhibiting significant expression changes along the infection progress to refine the secretome gene sets and to identify further the most reliable effector's gene candidates. This approach shed light on a highly conserved and complex infection program based on a core-effectome sequentially expressed during the infection progress. Among the predicted sub-cellular localization of these putative effectors, the plant cell nucleus emerged as a central target and was further validated using heterologous expression in tobacco leaves. The dual-integration of wheat's responses with the expression data of the F. graminearum core-effector genes led to the identification of putative susceptibility factors mostly involved in the inhibition of plant defense mechanisms of which, several genes corresponded to highly connected master regulators orchestrating wheat responses to FHB. Taken together, our results lead to the identification of reliable key fungal genes involved in the wheat susceptibility to Fusarium graminearum and brought valuable information for further researches to identify their targets.

## Fusarium Species Composition and Fungicide Sensitivity in Michigan

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## Abstract

Over 560 isolates of *Fusarium* were collected from 121 wheat and corn fields in Michigan and identified to species level. While *F. graminearum* comprised 82% of recovered isolates, members of the *Fusarium tricinctum* complex were also identified in nine fields of wheat. In corn, members of the *Fusarium fujikuroi* species complex comprised 50% of isolates recovered. The largest proportion were *F. subglutinans* (33.3%). *F. awaxy*, a species not yet reported in corn in the U.S., was identified in six fields, comprising 4.6% of the collection. Isolates of *F. graminearum* collected were also genotyped to determine the type of trichothecene mycotoxins they produce. The large majority, 413 isolates (92%) were the 15-acetyldeoxynivalenol (15-ADON) type and twenty-six (6%) were 3-acetyl-deoxynivalenol (3-ADON). In addition, seven isolates (1.5%) were classified as the NX-2 chemotype. Interestingly, most of the NX-2 and 3-ADON isolates were found in the same region, from five fields in the far norther eastern part of the state with less intensive agricultural land use.

*In vitro* sensitivity to triazole chemistries registered in the United States (metconazole, tebuconazole, and prothioconazole) were assessed with mycelial growth assays. Isolates were most sensitive to metconazole, and less sensitive to prothioconazole and tebuconazole. Species-specific differences in sensitivity were uncovered, with FTSC members significantly less sensitive than *F. graminearum*, and FFSC significantly more sensitive. All EC<sub>50</sub> values were below 4 µg/mL for *F. graminearum*, and sensitivity between the three chemistries was highly correlated. A small portion of isolates within *F. graminearum* had EC50 values 10-fold greater than sensitive isolates. A field trial was established to investigate sensitivity *in vivo* with eight isolates of differing *in vitro* sensitivities. No differences in fungicide efficacy were observed. While there may not be practical resistance in Michigan currently, monitoring should continue as there is variation in *in vitro* sensitivity. Mycelial growth EC50 values ranged from 0.008 to 0.263 µg/mL, with a mean of 0.060 µg/mL. EC50 values for spore germination assays ranged from 0.167 to 0.538 µg/mL, with a mean of 0.321 µg/mL Source: Management and characterization of fungal diseases in Michigan wheat. PhD Thesis. M Breunig
# Genetic Basis of Variation in DMI Fungicide Sensitivity in U.S. *Fusarium* graminearum Isolates

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# Abstract

Increased FHB pressure due to rotation with corn, no-till farming practices, and conducive weather conditions has increased the threat of FHB epidemics, and coupled with the recent high value of wheat has led to a surge in the use of fungicides. Fusarium graminearum (Fq) isolates collected after 2000, after the beginning of widespread fungicide use on wheat, show significantly lower sensitivity to tebuconazole than isolates collected before. With sufficient exposure to demethylation inhibiting (DMI) fungicides, Fa strains resistant to DMI fungicides will develop, complicating if not crippling current chemical control strategies for FHB. Our aim is to identify genes harboring functional variation that contributes to variation in DMI fungicide sensitivity to inform pathogen management decisions. Specifically, we have performed genomewide association studies (GWAS) to find the genetic basis of variation in fungicide sensitivity by scanning single nucleotide polymorphisms (SNPs) throughout the Fq genome generated by genotyping-by-sequencing (GBS). We collected fungicide sensitivity data for over 150 Fg isolates, primarily from the NA1 population or carrying an NX-2 TRI locus genotype, using an efficient, high-throughput 96-well microtiter plate in vitro assay. Fungicide sensitivity, as measured by estimates of EC<sub>50</sub>, varies significantly across isolates within populations, and sensitivity to propiconazole is highly correlated to sensitivity to tebuconazole. Isolates carrying genotypes consistent with the NX-2 trichothecene show a clear trend of greater DMI resistance compared to isolates from the NA1 population, which usually carry 15-ADON genotypes. GWAS scans that screened for statistical associations between SNPs and EC<sub>50</sub> estimates for each fungicide implemented various models that could account for potential population structure as well as cryptic relationships between isolates from the same populations. A GWAS scan for tebuconazole sensitivity in a sample that included NA1 and NX-2 isolates identified several associated loci, including the TRI gene cluster. Separate scans for sensitivity to propiconazole and tebuconazole in the NA1 population detect a few candidates, including one SNP common to the two fungicides that is located in the fusarin C gene cluster.

# **Acknowledgement and Disclaimer**

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# Metagenomic and Amplicon Investigations of FHB and Microbial Interactions in Mature Wheat Heads

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# Abstract

Plant-associated microbes (collectively the microbiome) are important contributors to plant health. They are known to play roles in increasing yield via improving stress tolerance, promoting growth, and suppressing the activity of plant pathogens. Fusarium head blight (FHB), caused by Fusarium graminearum (sensu stricto), is a devastating disease of wheat and barley in the US. Managing FHB is challenging because FHB impacts on plant health and agronomic properties are strongly integrated and genetic resistance is incomplete. Here we report recent investigations of amplicon and metagenomic based microbial responses to the presence of FHB among wheat varieties with varying levels of genetic resistance, sensitive (S), moderate resistance (MR), and resistant (R). Using field trials across three locations, we identified changes in bacterial community (16S rRNA gene amplicons) composition that were associated with environment and host-resistance level, but not maturity group or variety. In addition, we have identified important taxonomic groups that are differentially abundant in the presence of the pathogen. In our preliminary analysis of the full community analysis (metagenomic sequencing) we have found that bacteria in this system comprise 85% of the most abundant reads. Among the fungal taxa present Fusarium spp. and Cercospora spp. are among the most abundant. Predictably, among the Fusarium spp. present F. graminearum shifts in abundance from 54% to > 90% corresponding with visual FHB ratings. Surprisingly, however, in fields with little to no visual FHB, F. graminearum is 1 of 8 Fusarium spp. present. Exploring the communities present we find that these communities are relatively simple, with just 289 bacteria identified (thus far) across samples. Of these, notable shifts in these communities occur among host resistance genotype (R, MR, S), even grown in the same field, with taxa like Sphingomonas exhibiting substantially reduced relative abundance in MR and R lines in the presence of *Fusarium*. Conversely, members of the genus *Methylobacterium* do not show a change in relative abundance across R, MR, or S hosts. Finally, we find shifts in particular members of these genera, which have previously been identified with suppression of FHB, across host genotype. Future investigations will add additional metagenomic sequences and fungal amplicon data to unravel pathogen-microbial community interactions associated with infection.

# Development of an Endophytic Fungal RNAi Delivery Platform to Control Fusarium Head Blight and Mycotoxin Contamination

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# Abstract

Fusarium head blight (FHB) caused by *Fusarium* species is a major threat to food safety and security by reducing crop yields and contaminating grains. RNA interference (RNAi) technology has been widely applied in plant protection. Host-induced gene silencing (HIGS) and spray-induced gene silencing (SIGS) have been shown to be effective at controlling plant diseases and pests. However, HIGS application has been limited by availability of efficient plant transformation systems and public acceptance of genetically modified organisms. SIGS is limited by the cost of dsRNA synthesis and its short life span. Therefore, it is critical to develop alternative RNAi production and delivery systems. Endophytes form intrinsic relationships with their hosts and inhabit the hosts without causing damage. Delivery of RNAi using endophytes is cost-effective and sustainable. Sarocladium zeae is an ideal candidate since it has been demonstrated to provide biocontrol function towards *Fusarium* in corn and wheat. To reduce FHB and mycotoxin contamination, our goal was to produce and deliver RNAi using an endophytic fungal strain S. zeae 34560 (Sz34560). First, we generated a Sz34560 RNAi strain expressing a GFP hpRNA construct and examined the GFP-dsRNA and -siRNA production. We confirmed GFP-dsRNA production in the Sz34560 GFP-RNAi strain. Second, we generated a F. graminearum strain expressing an RNAi construct targeting the trichothecene biosynthesis gene TRI5, which is essential for trichothecene production. We showed that F. graminearum TRI5-RNAi strain significantly reduced TRI5 expression and trichothecene production in liquid agmatine media. Then we introduced the TRI5-RNAi construct to Sz34560 and generated multiple Sz34560 TRI5-RNAi strains. We demonstrated that the Sz34560 TRI5-RNAi strain produced TRI5-dsRNA and reduced trichothecene produced by F. graminearum when co-cultured in vitro. Preliminary results showed FHB and mycotoxin reduction in wheat heads treated with Sz34560 TRI5-RNAi strains followed by F. graminearum inoculations. Further investigations are underway to determine the effectiveness of reducing FHB and toxin contamination using different treatment methods using Sz34560 RNAi strains.

# **Acknowledgement and Disclaimer**

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

# Genome-wide Identification of *Fusarium* Effectors and 3D Protein Structure Prediction with AlphaFold Analysis

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# Abstract

Plant pathogenic fungi secrete small proteins known as effectors that allow them to overcome plant defenses and cause disease. Effectors that are conserved in multiple species of fungi are known as core effectors. Advances in genome sequencing technologies and Artificial Intelligence/Machine Learning tools have markedly improved the identification of protein families and the understanding of their distribution across phylogenetically diverse species. Using these technologies and tools, we identified potential core effectors in the Fusarium head blight (FHB) fungus F. graminearum by analyzing the genome sequences of 199 species that represent all 23 Fusarium species complexes. Using the program EffectorP, we identified 2,916 genes encoding putative core effectors from the 199 genome sequences. Subsequently, using the insilico secreted protein prediction program SecretSanta, we found that 462 of the putative effector proteins are likely to be secreted. We also examined the putative effectors using functional annotation tools and the program AlphaFold to assess the diversity of their sequences and to determine whether their 3D structures are similar to known effectors, respectively. Based on these analyses, we selected 21 putative core effector genes that occur in F. graminearum and determined that seven were highly expressed during early stages of wheat head infection. Gene deletion experiments are underway to assess the role of the seven effector genes in pathogenesis of F. graminearum on wheat. Understanding the structural diversity and function of F. graminearum effectors has potential to aid development of FHB control strategies

# Selection Due to Host Resistance in Fusarium graminearum

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#### Abstract

The aggressiveness of a pathogen is the quantitative variation of disease signs and symptoms on a susceptible host. Effective use of disease resistance relies on understanding the pathogen's potential to evolve and overcome resistance. Increased pathogen aggressiveness is less likely to diminish the effectiveness of quantitative resistance compared to qualitative resistance. There is also more understanding on the evolution of pathogen races in gene-for-gene systems of resistance. It is not fully understood how quantitative host resistance affects pathogen aggressiveness creating a need to better comprehend the population biology of plant pathogens. One theory is that resistance is selecting for aggressiveness. The pathogen must be more aggressive to overcome resistance while aggressiveness compensates for lowered reproduction. Another theory is that a susceptible host allows for higher pathogen genetic variance, leading to rapidly increasing aggressiveness through selection and competition. Fusarium graminearum causes Fusarium Head Blight (FHB) in wheat, producing significant yield losses and mycotoxin contamination. We hypothesized that the wheat's level of resistance is selecting on the F. graminearum populations for isolate aggressiveness. Thirty-one isolates from highly susceptible and 26 isolates from moderately resistant wheat lines were used. The isolate's aggressiveness was measured by AUDPC that was calculated from three disease severity ratings, visually damaged kernels, and deoxynivalenol contamination. DNA was extracted and sequenced to create a SNP data set that was used to perform a whole-genome scan for selection and a phylogenetic analysis. The results of this research found that the population of *F. graminearum* from susceptible wheat had more aggressive isolates. This led to the conclusion that resistant wheat varieties have not selected for a population with primarily aggressive isolates but mixed levels of aggressiveness. The population of isolates from highly susceptible wheat were primarily aggressive suggesting that on a susceptible host aggressive isolates might be outcompeting weaker isolates. The level of wheat resistance added selection pressure to the F. graminearum isolates as both populations had unique regions of the genome and genes under selection. The fixed level of isolate aggressiveness and the increased number of genes under selection suggests that there is stronger selection pressure occurring in the population from highly susceptible wheat. These results indicate that host resistance will be a durable and long-term solution to FHB on wheat.

# Insight into the Species and Mycotoxin Diversity within Fusarium

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#### Abstract

In North America, Fusarium head blight is caused predominantly by Fusarium graminearum, a member of the F. sambucinum species complex (FSAMSC). However, recent largescale surveys revealed that other species in the FSAMSC and the F. tricinctum species complex (FTSC) have emerged as pathogens of wheat and barley in some settings. Among toxins produced by the FSAMSC, trichothecenes pose the most significant threat to public health. However, some fusaria in the FTSC can produce other mycotoxins, including enniatins (ENNs) and moniliformin (MON) which are also concern with respect to food and feed safety. Herein we conducted large surveys of FSAMSC and FTSC strains hosted in culture collections to evaluate their species diversity, mycotoxin potential, and pathogenicity towards wheat. Maximum likelihood (ML) analyses of a multilocus dataset revealed that the FSAMSC encompasses indeed 74 genealogically exclusive species, including 33 novel, distributed among six strongly supported clades: Sporotrichioides, Graminearum, Novel, Sambucinum, Longipes, and Brachygibbosum. Members of these clades produced type A, type B, or both types of trichothecenes. Surprisingly, two novel species within the Sambucinum Clade synthesized two new type A trichothecenes, 15-keto NX-2 and 15-keto NX-3. These two species, with all members of the Graminearum Clade, were aggressive toward heads of the susceptible cultivar Apogee. Other taxa within the FSAMSC were nonpathogenic or infected only the inoculated floret. Altogether, these data highlight that aggressiveness toward wheat appears to be toxin-dependent and that it is not limited to F. graminearum and its close relatives. We also elucidate that the phylogenetic, mycotoxigenic, and pathogenic diversity of the FSAMSC and FTSC was underestimated, and their members uniformly produce several emerging and neglected mycotoxins. Evolutionary relationships inferred from a 5-locus sequence dataset resolved FTSC strains into 24 genealogically phylogenetically distinct species, of which nine were novel. Nineteen of the species were toxin-producing lineages that shared the same mycotoxin profile characterized by a production of ENNs, MON, aurofusarin (AUR), and 2-amino-14,16-dimethyloctadecan-3-ol (AOD). Interestingly, five species in the most basal clade of the FTSC phylogeny synthesized ENNs and/or AUR but did not produce MON or AOD.

# Impact of Elevated Carbon Dioxide on Fusarium graminearum Mycotoxin Production

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# Abstract

Mycotoxins are toxic secondary metabolites produced by fungal pathogens that infect cereal crops such as wheat and barley. Crop susceptibility to fungal infection and mycotoxin contamination is dependent on the environment, and climate change is predicted to increase the frequency and severity of conditions that favor contamination. However, it is unclear how rising atmospheric carbon dioxide (CO2) will impact mycotoxin production. We hypothesized that atmospheric CO2 levels would influence mycotoxin production. To test this hypothesis, we inoculated grain of two barley cultivars with three diverse strains of *Fusarium graminearum*, a devastating mycotoxigenic pathogen of cereals. The inoculated grain was placed into growth chambers controlled at 400 ppm (ambient) or 1,000 ppm (elevated) CO2 conditions. The fungus was allowed to grow, colonize the grain, and produce mycotoxins for 14 days. The grain was then collected, lyophilized, pulverized, and separated for molecular and metabolic analyses. Fungal biomass was estimated by quantifying DNA using *Fusarium* species-specific primers. Mycotoxin profiles were determined using gas chromatography mass spectroscopy and liquid chromatography mass spectroscopy. Results suggest that *F. graminearum* mycotoxin production was influenced by elevated CO2 in a manner that was dependent on barley cultivar and *F. graminearum* strain. This study provides valuable information needed to assess future food safety risk and will be of interest to farmers and regulatory agencies.

# Effect of Pigmentation on Structure and Function of Perithecia Produced by *Fusarium* graminearum

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# Abstract

Meiotic spores (ascospores) produced by *Fusarium graminearum* are an important primary inoculum for Fusarium head blight. The spores are formed within darkly pigmented fruiting bodies (perithecia) from which they are forcibly expelled by a buildup of turgor pressure. Although the structure of the perithecial pigment(s) is not known, it is a product of the biosynthetic pathway that forms the mycelial pigments fusarubins. We hypothesized that pigmentation affects the structure of perithecia and dispersal of ascospores. To test this hypothesis, we are examining the effects of deletion of *F. graminearum PGL1*, the gene encoding the polyketide synthase that catalyzes the first committed step in fusarubin biosynthesis. As expected, perithecia produced by *pg/1* deletion mutants lacked dark pigmentation. Although perithecia of the mutants tended to be less resistant to compression than wild type, the differences in resistance were not consistent. In scanning electron microscopy, perithecial walls of *pg/1* mutants had indentations that did not occur in perithecial walls of the wild type. There were no differences in the numbers of perithecia produced or ascospores dispersed by the *pg/1* mutants and wild type. However, in preliminary analyses, ascospores of *pg/1* mutants were dispersed farther than those of the wild type. These results indicate that pigmentation affects both structure and function of perithecia, but it remains to be determined whether perithecial pigmentation affects the ability of *F. graminearum* to cause head blight epidemics.

# **Biofilms Have Roles in FHB Disease**

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#### Abstract

While much is known about how *Fusarium graminearum* initiates disease on host plants, there are still knowledge gaps related to mechanisms that increase success in infection. To better understand early infection, we focused on two aspects of the plant-pathogen interaction. Biofilms are three-dimensional formations important to many microbes for protection from adverse environmental conditions. Biofilms have primarily been studied in single-celled organisms, but there is a growing body of work on filamentous fungal biofilms. Biofilm formation is initiated *in vitro* with the adhesion of propagules to a surface, followed by growth of the structures and development of an extracellular matrix, then dispersal of propagules and senescence of biofilms. We profiled the transcriptome of biofilm formation over time, and characterized genes significant to this process. Aquaporins are channel proteins that bring molecules dissolved in water into cells. In *F. graminearum*, aquaporins are important in growth, development, and spore formation. Knockout mutants of these genes and others identified by transcriptomics, produced altered biofilms, especially in matrix composition. We have identified novel early infection characteristics in *F. graminearum* through characterization of the biofilm *in vitro* and *in planta*.

# **Bacterial Endophyte Biocontrols Indirectly Impact Fusarium Head Blight Disease in Wheat**

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# Abstract

Fusarium head blight (FHB) is an economically important disease of small grains globally and is primarily caused by Fusarium graminearum in North America. Recently, microbial biocontrols have risen in importance as sustainable agents of disease control. However, the path to implementation of microbial biocontrols in agriculture will require an understanding of how microbiota impact both plant performance overall and vary with inherent host disease resistance. Using a full-factorial, controlled greenhouse experiment, we tested how bacterial endophyte treatments impacted both plant physiology prior to disease onset and FHB disease progression in Triticum aestivum (wheat). Seven bacterial endophytes were tested as seed soaks on two wheat varieties varying in FHB resistance. Leaf-level physiology and morphology measurements were made prior to inoculation with F. graminearum, while F. graminearum toxin accumulation (deoxynivalenol or DON) was quantified post-harvest. Bacterial endophyte treatments strongly impacted the light dependent reactions of photosynthesis, with changes in plant traits regulating light energy allocation and the build-up of electrochemical energy storage across the thylakoid membrane. Physiological responses were contingent on host variety. The direct effects of bacterial endophytes on wheat response to infection were weak and dependent on the inherent disease resistance of the host variety. However, disease outcomes were indirectly mediated by bacterial impacts on plant traits, with proton motive force traits emerging as common predictors of disease response across both host varieties and other traits indicating potential trade-offs in host response to bacterial inoculants and F. graminearum infection. Our results provide an alternate mechanism for microbial biocontrol efficacy other than direct antagonism with the pathogen inside the host. Furthermore, the chlorophyll-fluorescence and absorbance-based markers assessed here may have translational potential as a phenotyping tool for FHB susceptibility in wheat and other small grains.

# Decoding Adaptive Traits in Fusarium graminearum using Integrated Omics

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#### Abstract

Fusarium graminearum, the predominant causal agent of Fusarium head blight (FHB) in cereal crops, impacts the North American wheat industry mainly through the contamination of infected grains with mycotoxins. Understanding the molecular mechanisms underlying adaptive traits in field populations can accelerate the improvement of durably resistant cultivars and effective fungicides. Previously, a Nested Association Mapping Population of F. graminearum (FgNAM) was developed via sexual crosses using Nit-5 (a Nit isolate derived from isolate PH-1) as a recurrent parent and eight other isolates from North America representing phenotypic variation in some adaptive traits, including aggressiveness toward wheat species, DON chemotypes (3ADON vs. 15ADON), and sensitivity to triazole fungicides. This project aims to identify genes associated with these adaptive traits among F. graminearum populations in North America. A pangenome reflecting diversity in the populations occurring in North America was constructed using nanopore sequencing from the nine isolates as funders for FgNAM. The pan-genome analyses provided additional chromosome-scale reference genomes and annotation based on RNA-seq data obtained from FgNAM founders. Several candidate effectors co-located with hypervariable regions were detected in 3ADON isolate SK1797. Besides, two candidate fungicide insensitivity genes, YC79 (a transcription factor) and MFS1 (a multi-drug transporter) were identified in Metconazole insensitivity isolate 106Eb1 based on fungicide sensitivity phenotyping. This study provides a pan-genome based on the nine FgNAM founders' genomes combined with transcriptome analysis, which will facilitate the identification of novel genes related to fungicide insensitivity, aggressiveness toward wheat and mycotoxin production in *F. graminearum*.

# **Variety Development & Host Resistance**

# FHB Stage Detection, Deep Scanning Robot

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#### Abstract

Fusarium Head Blight (FHB) is a wheat and barley disease, that results in huge yield and quality loss which reflect direct in the whole economy because it is a strategic importance. Many *Fusarium* species produce mycotoxins fungal chemicals that are harmful to human and animals.

Rank the level of infection on develop lines by breeders needs expertise and the process is a time consuming and labour intensive. Chemical controls in a late stage, provide partial control of FHB and associated mycotoxin contamination. Several foliar fungicides have been used to manage FHB in some areas and are applied around the period of wheat flowering. To solve this issue, there are two ways: first by developing resistive lines by breeders for contamination control. Secondly, early detection will make the use of chemicals to be more effective and feasible.

The advancements in both Artificial Intelligence (AI) and image processing now (literally) will change the picture for farmers. The amount of image processing applications in precise agriculture is growing steadily with the availability of higher-quality measurements coupled with modern algorithms and increased possibility to fuse multiple sources of information from satellite imagery and sensors positioned in fields. Utilising such technology for early detecting disease like the FHB in wheat and barley will make a revolution change.

The Objectives was to create and develop an intelligent 360° Deep Scanning Capturing System, then calibrate the scanner system to capture images at specific angle with a synchronised traveling speed, then collect the photos that have been captured to create a Deep Convolutional Neural Network (DCNN) data set to train the AI robot for detecting the FHB early stages. As a result, a scanning cart has been designed and constructed in SDSU fabrication shop by Precision Ag team, and the Photos have been captured to create the data set. Then DCNN is being used in the training stage to detect the targeted locations in the infected areas.

# Acknowledgement and Disclaimer

Confidential- please do not share the content of the submission publicly before the 2022 National FHB Forum. This material is based upon work supported by the U.S. Department of Agriculture, under Agreement, 59-0206-2-143 and59-0206-2-153. This is a cooperative project with the U.S. Wheat & Barley Scab Initiative. Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the author(s) and do not necessarily reflect the view of the U.S. Department of Agriculture.

# Assay Validation through Dip Inoculation of Fusarium Head Blight in Spring Barley Training Population

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# Abstract

The level of deoxynivalenol (DON) in Fusarium head blight (FHB) infected barley grains is a key outcome of FHB resistance breeding because DON diminishes quality. However, the financial and resources required for DON measurements limits most early breeding programs to rely on visual severity score for selection. Because visual disease severity scores may not correlate well with DON levels post-harvest and only one cycle can occur in a field season, breeders would benefit from a greenhouse screening method. A dip inoculation method was developed as a high-throughput assay that would estimate FHB response under controlled conditions. This method is an attempt to create an evaluation of FHB resistance in the greenhouse with better correlation to DON than field severity. The objectives of this study were to: 1) examine the sources of variation within this controlled environment assay; 2) determine the correlation between severity and DON; and 3) determine the correlation between fungal biomass estimates and DON. In this study, 6 two-row barley cultivars from the Aberdeen ID training population were evaluated after inoculation with the FHB isolate PH1 at two concentrations (10<sup>5</sup> and 2 x 10<sup>5</sup> conidia per mL). Ten heads of each line were evaluated at each inoculum level for severity, fungal biomass, and DON (ppm). DON level was influenced by line (P=0.0054), but severity score and fungal biomass were not (P>0.05). This suggests a role for the host in DON accumulation that may be independent of fungal growth. Inoculum concentration had no effect on DON, severity score, or fungal biomass in this study (p>0.05). A strong correlation was observed between fungal biomass and DON (r=0.9, P<0001), while the correlation between severity score and DON was lower (r=0.4, P<0001). This suggests that fungal biomass may be a more accurate predictor of DON accumulation than a visual severity score, at least under controlled environmental conditions.

# Large-scale Wheat-FHB Disease Analysis with Deep Neural Networks

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#### Abstract

Fusarium head blight (FHB) is a serious disease that affects wheat and barley production. South Dakota alone suffers \$20 million in losses annually from FHB. The early detection of FHB disease will improve the efficiency, accuracy, and capability of FHB resistance screening in wheat, durum, and barley breeding. Such diseases can be automatically or semi-automatically diagnosed using supervised machine learning algorithms and image processing techniques. Several methods have been proposed to plant disease detection. Among these methods, Convolutional Neural Networks (CNNs) are an effective method to automatically identify these diseases. Recently, different approaches have been proposed to boost CNNs' performance. Most of these methods, however, suffer from a lack of a mechanism that allows them to integrate global and local contextual information adaptively to extract hidden patterns inside the input images. In this regard, we propose the multi-scale attention U-Net. Our main goal is to improve U-net by introducing an attention mechanism at the network bottleneck. In the proposed method, to adaptively emphasize the importance of both spatial and channel dimensions we propose to include the dual attention mechanism. To this end, using the global information of each channel we learn the scaling coefficient to improve the object learning process. In addition, by learning the self-attention map we impose the spatial attention map on the feature space to adaptively emphasize the important regions. We apply the suggested dual attention in a multi-scale fashion to encourage a multi-scale learning process. To train the proposed network, we captured 12,000 images in an SDSU wheat field in Volga, SD. In addition to covering healthy and diseased plants, these images include different stages of the disease. A group of plant disease experts annotated the prepared images to be used for training the deep model. The model was trained to separate the diseased areas from the healthy areas by receiving the input images using the annotated images. For the first time to the best of our knowledge, we determined the stage of disease based on the segmentation results of the model. As a result, we could detect the diseased areas of the plants, as well as determine the disease level of infection. In the test phase, an automatic plant scanning robot was developed and used to evaluate the performance of the proposed model. The experimental results demonstrate that our approach accurately estimates crop contamination.

# Acknowledgement and Disclaimer

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# **Genotype and Fusarium Head Blight Selection for Microbiomes across Barley Spikes**

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#### Abstract

Understanding the ability of the plant microbiome and its ability to aid in biotic and abiotic stress response is an emerging topic of research. This approach may offer new insight on plant disease management. Fusarium head blight (FHB) is a disease caused by *Fusarium graminearum* that affects wheat and barley worldwide. This disease reduces grain quality by contamination with the mycotoxin deoxynivalenol (DON). We considered that members of the microbiome with the potential to inhibit FHB could be identified based on differential enrichment in the barley spike during disease. Moreover, if the same taxa respond to plant genotype, that would indicate the potential for using breeding to manipulate the barley spike microbiome in a beneficial manner. We analyzed the composition of the barley spike microbiome during FHB disease onset roughly three weeks after heading in ten genotypes from a breeding population at FHB misted nurseries across four locations in the United States: ID, MN, ND and NY. Bacterial groups were identified using 16S amplicon sequencing and were analyzed for their responsiveness to both FHB disease and barley genotype. Barley genotype had a significant effect on bacterial community composition at each location, while disease had a significant effect only in the St. Paul, MN which had the highest disease severity. There was also a significant genotype by disease effect at 3 locations: ND, ID and MN. Each location also had several taxa present that are known to have members that are plant-beneficial organisms including Pantoea, Pseudomonas, and Sphingomonas, among others. The results of these experiments will help highlight the potential for barley genotypes to recruit beneficial microorganisms in response to diseases like FHB.

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# High-Throughput Deoxynivalenol Concentration Detection and Prediction in *Fusarium*-Damaged Wheat Kernels using Handheld Hyperspectral Imaging Platform

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# Abstract

Deoxynivalenol (DON) accumulation in wheat due to Fusarium Head Blight (FHB) negatively affects grain quality and subsequently reduces grain yield. Evaluation of DON is an integral part in breeding FHB-resistant wheat varieties. Here we explored the potential of hyperspectral imaging to indirectly detect and predict DON concentration. A total of 172 wheat genotypes evaluated for DON concentration using GC/MS (Liquid Chromatography – Mass Spectrometry). Hyperspectral imaging of *Fusarium*-damaged wheat kernels (FDKs) for each genotype was carried out using a handheld hyperspectral imaging camera, Specim IQ (Specim Ltd., Oulo, Finland). Processing of hyperspectral images was carried out using QGIS 3.10.2 and spectral reflectance values were carried out using Raster package in R. Of the 204 wavebands (397 nm – 1004 nm) generated, genotypes showed significant variation (p-value < 0.05) in 196 wavebands. However, only the first 130 wavebands (397 nm – 778 nm) were used for further analysis due to obvious noise in the remaining wavebands. Pearson's Correlation revealed significant correlation (p-value < 0.05) between DON concentration and reflectance values in all the 130 wavebands (r=0.32 to r=62). All 130 wavebands were used in a simple Linear Regression Model generating an r<sup>2</sup> value of 0.95. A cross validation accuracy of actual vs. predicted value yielded an r and r<sup>2</sup> value of 0.73 and 0.53, respectively. Similarly, Ridge Regression Best Linear Unbiased Prediction (rrBLUP) yielded a cross validation accuracy of r=0.75. Five wavebands: 622, 619, 628, 613, and 616 were identified through feature selection to have the most contribution to observed variation. Taking the results into account, this study has demonstrated the potential use of hyperspectral imaging in detecting and predicting DON Concentration in Fusarium-damaged wheat kernels.

# **Diversification of FHB Resistance QTL in Winter Wheat Germplasm**

#### Bhanu Dangi and Francois Marais

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#### Abstract

Fusarium Head Blight (FHB) is a devastating disease of small grains which infects the florets and reduce grain yield and quality. Effective integrated control strategies for combating FHB are those that also incorporate genetic resistance. Genetic resistance of wheat to FHB is due to the collective effects of numerous quantitative trait loci (QTL). Some resistance QTL have comparatively larger effects (such as the Sumai-3 derived germplasm); are well characterized and are widely used in breeding. Numerous other lesser resistance QTL with smaller individual effects are also known to occur in wheat; many of these are not well studied. The lesser QTL may nonetheless contribute useful levels of background resistance to QTL pyramids. This study aims to expand and diversify all available genetic variability for FHB resistance in advanced NDSU winter wheat (WW) breeding stock. The first study objective is to introgress the most recently available, "larger effect" resistance QTL, Fhb7 (derived from Thinopyrum elongatum by Cai and co-authors, 2022) through marker-aided, modified backcrosses. Two backcrosses to WW have been completed and B<sub>2</sub>F<sub>1</sub> are currently being grown for doing marker and agrotype selection before completing the third set of backcrosses to WW. The second study objective is to search for useful and diverse sources of background genetic variability for FHB resistance in native winter wheat germplasm. A testcross analysis is being done that aims to measure and compare the general and specific combining abilities of 14 advanced WW lines that were used in testcrosses with four testers. One tester appears (marker data) to have both Fhb1 and Qfhs.ifa-5A; another has Fhb1 but only intermediate resistance, the third tester has intermediate background resistance, and the fourth tester is susceptible to FHB. The 56 F<sub>1</sub> hybrids and 18 parents will be point-inoculated in a greenhouse with mixed Fusarium graminearum isolates to measure FHB Type II resistance (disease spread). The magnitude and significance of combining ability effects will be calculated to determine which lines have the highest levels of background resistance and best complement the known resistance. Remnant F<sub>1</sub> seeds from superior hybrid combinations will be included in future breeding program crossing blocks. F<sub>2</sub> plants that were derived from the superior combinations will be reevaluated for type II resistance and the best plants used to initiate single seed descent inbreeding.

# **DON and FHB Resistance in North American Winter Barleys**

#### Ben Eggers and Eric J Stockinger

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# Abstract

Increasing Fusarium Head Blight (FHB) disease on barley caused by changes in climate and maize cultivation practices is placing the Nation's high-quality malt supply at risk of being inadequate. At the same time, the Independent Craft Brewing Movement, which is undergoing exponential growth, is seeking locally-produced barley. These trends are upending traditional systems. Therefore, breeders across North America must develop portfolios of locally-adapted barley varieties to meet the needs of different end users, each variety resistant to FHB. The goal of this project are to enhance and increase the number of winter barley varieties possessing resistance to Fusarium head blight developed by U.S. public breeding programs. Key objectives of this project are to: 1) coordinate a North American Barley Scab Evaluation Nursery (NABSEN) for winter barley, in which North American winter barley breeders submit their best lines for testing; 2) identify lines in the Ohio breeding program exhibiting FHB resistance in the forms of low DON accumulation and low disease incidence; and 3) utilize modern breeding and molecular marker technologies to efficiently and rapidly introgress those resistances into elite lines for varietal release to farmers.

# **Acknowledgements and Disclaimer**

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# Screening for FHB-Resistance in Barley in South Dakota

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#### Abstract

South Dakota ranks among the top ten barley-producing states in the US with 49,000 acres under cultivation, up 63% from 2021 (NASS 2022). While most of the cultivated barley feeds livestock, some of it goes to the brewing industries. The major threat to barley cultivation comes from Fusarium head blight disease (FHB, Scab), caused by the fungal pathogen Fusarium graminearum. FHB reduces the yield and quality by contaminating the produce with mycotoxins like deoxynivalenol (DON), negatively affecting the consumption of both Wheat and Barley worldwide. The most efficient way of controlling FHB is through imparting host resistance. Therefore, the first objective of our research is to screen spring barley cultivars against FHB disease. Winter hardiness in barley is a desirable trait that can provide South Dakota growers with an alternative to include barley in the crop rotation scheme. Barley, when included in the cornsoybean-small grain-cover crop rotation during winters reaps good returns to the growers while improving soil health and reducing the need for herbicide application. The second objective of our research is to identify winter hardy and FHB-resistant barley lines, which will provide both yield and sustainability benefits to the barley producers. We conducted field trials to test the FHB resistance of 23 and 29 winter barley lines and spring barley lines respectively. Out of the 23 winter barley lines received from different states of the United States, only 6 lines survived the winters of south Dakota. The 29 spring barley lines were planted in four replicates in a randomized complete block design and were spray inoculated at 50% anthesis stage with a virulent strain of *Fusarium graminearum*. The disease severity was rated by scoring the visible symptoms on heads at 21 days and 28 days after inoculation. At 21 days, the mean disease severity for the majority of the lines was below 40%, whereas at 28 days, two spring barley lines S2M196 and S2M197 performed significantly better exhibiting less than 40% severity in each block. The spring and winter barley samples will be proceeded for DON and FDK analysis. Our results show that variability for FHB resistance exists in the germplasm. Growers can make sound decisions by selecting moderately resistant to resistant varieties along with fungicide application against FHB as a disease management strategy.

# Selecting for Fusarium Resistance in the Great Plains

Katherine Frels<sup>1</sup>, Fang Wang<sup>1</sup>, Stephen Wegulo<sup>2</sup>, Xiwen Cai<sup>3</sup>, P. Stephen Baenziger<sup>1</sup> and Vikas Belamkar<sup>4</sup> <sup>1</sup>Department of Agronomy and Horticulture, University of Nebraska-Lincoln; <sup>2</sup>Department of Plant Pathology, University of Nebraska-Lincoln; <sup>3</sup>USDA-ARS Wheat, Sorghum and Forage Research, Lincoln, NE; and <sup>4</sup>Corteva Agriscience

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# Abstract

The University of Nebraska- Lincoln Small Grains breeding program has been addressing the need for FHB resistant varieties in wheat for over 20 years and has recently begun targeting improved resistance in our winter-feed barley program. The disease can often be found in Nebraska small grain production fields, but usually at a low level of severity. Often, our dry climate prevents significant epidemics, but we have seen severe crop damage in 2007, 2008, 2015, and 2019. Our breeding program primarily relies on native resistance identified through phenotypic screening for both wheat and barley with selection optimized through genomic prediction. However, as interest in small grains production increases in FHB-susceptible southeastern Nebraska we are working with colleagues throughout USWBSI to increase major gene resistance in our germplasm.

# **Genomic Prediction to Improve FHB Resistance in Winter Wheat**

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# Abstract

Fusarium head blight (FHB), also known as scab, is a devastating fungal disease of wheat that causes significant losses in grain yield and quality. Quantitative inheritance and cumbersome phenotyping make FHB resistance a challenging trait for direct selection in wheat breeding. Genomic selection to predict FHB resistance traits has shown promise in several studies. Here, we used univariate and multivariate genomic prediction models to evaluate the prediction accuracy (PA) for different FHB traits using 457 elite and advanced breeding lines developed by South Dakota State University hard winter wheat breeding program. These breeding lines were assessed for FHB disease index (DIS), and percentage of Fusarium damaged kernels (FDK) in two FHB nurseries in 2019, and 2020 (TP18, TP19, and TP20) and were evaluated as training populations (TP) for genomic prediction (GP) of FHB traits. We observed a moderate PA using univariate models for DIS (0.39 and 0.35) and FDK (0.35 and 0.37) using TP19 and TP20, respectively, while slightly higher PA was observed (0.41 for DIS and 0.38 for FDK) when TP19 and TP20 (TP19 + 20) were combined to leverage the advantage of a large training population. Although GP with multivariate approach including plant height and days to heading as covariates did not significantly improve PA for DIS and FDK over univariate models, PA for DON increased by 20% using DIS, FDK, DTH as covariates using multi-trait model in 2020. Finally, we used TP19, TP20, and TP19 + 20 in forward prediction to calculate genomic-estimated breeding values (GEBVs) for DIS and FDK in preliminary breeding lines at an early stage of the breeding program. We observed moderate PA of up to 0.59 for DIS and 0.54 for FDK, demonstrating the promise in genomic prediction for FHB resistance in earlier stages using advanced lines. Our results suggest GP for expensive FHB traits like DON and FDK can facilitate the rejection of highly susceptible materials at an early stage in a breeding program.

# Acknowledgement and Disclaimer

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# Releasing Lines Pyramided with Fhb1 and Fhb7

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# Abstract

Fusarium graminearum is the causative agent of Fusarium head blight (FHB) in hexaploidy wheat (Triticum aestivum) which substantially reduces the crop yields and quality. Yield loss is mainly caused by the kernel size reduction and sterility of infected spikelets while the quality loss is caused by the presence of mycotoxins primarily deoxynivalenol (DON). Development of disease resistant crops by deploying host resistance genes is one of the effective approaches for the FHB management. Many genetic loci were reported to contribute to resistance to FHB resistance, including Fhb1. Fhb7, a major QTL for FHB resistance in wheat, has been introgressed into soft red winter wheat (SRWW) at Purdue University. Being an exotic QTL, Fhb7 is associated with the linkage drag impacting agronomic and end-use quality performances. This poster explains the breeding scheme for introducing and pyramiding *Fhb7* into breeding materials that already had *Fhb1*. In the final step of product development, we tested 57 SRWW lines which were advanced through the breeding programs to homozygosity by using PCR diagnosis markers, which revealed that 9 out of 57 showed the presence of both *Fhb7* and *Fhb1*. The expectation is that the stack of *Fhb1-Fhb7* offers additional genetic protection to SRWW against FHB disease. Fhb7-positive lines showed average agronomic and superior FHB performance. In particular, they expressed low severity and DON content. The release of selected wheat breeding lines with the information of multiple genetic markers linked FHB resistant QTL will be a useful resource for our national U.S. Wheat and Barley Scab Initiative (USWBSI) breeding community for incorporation of this novel genetic resistance into their breeding materials.

# Fusarium Head Blight Resistant Durum Wheat in Montana, Development and Testing

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#### Abstract

Montana averages 600,000 acres of durum production and ranks second in the US as a durum producer. Late season rainfall can cause high deoxynivalenol (DON) levels caused by Fusarium head blight (FHB) in harvested grain resulting in it being rejected at the grain elevator. To address FHB concern in Montana grain we have acquired durum lines from our collaborator, Dr. Xiwen Cai, with *Fhb1*, *Fhb5*, and non-FHB sources of resistance integrated from hexaploid wheat that we will test for FHB resistance. Additionally, we are working with Dr. Cai to move *Fhb7* resistance sources into Montana FHB resistant lines. The introgression of *Fhb7* into bread wheat and durum wheat from *Thinopyrom ponticum* has been shown to greatly reduce disease severity and DON accumulation. We will conduct field-based screening for FHB resistance at the MSU Eastern Agricultural Research Center (EARC, Sidney, MT) which is in the center of the most important region for durum production in Montana. In fact, the northeast region of Montana where EARC is located is on the border of North Dakota and their most important durum production area and thus is the most relevant region for durum FHB resistance screening. The development and release of FHB resistant durum lines will substantially decrease durum yield losses due to FHB and reduce DON levels in MT grown durum.

Variety Development & Host Resistance - Poster

# NorGrains and BIG6 Genomic Selection Pipeline at Purdue

#### Mohsen Mohammadi

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# Abstract

In this poster, I outline how I chose to be on only some of the bandwagons (Rex Bernardo 2016) to rebuild Purdue's soft red winter wheat breeding program since 2015. Wheat breeding programs in KY, OH, IL, MI, NY, and IN created a genomic selection (GS) consortium in 2020. In this project, we increased the capacity of stage-1 testing outside of the state of origin, referred to as sparse testing. We also use genomic prediction besides phenotypic selection to increase the confidence of selection from the single-replicated stage-1 trials. Genotypic data were produced after planting of 600 stage-1 Purdue lines. After harvest, genomic estimated breeding values (GEBVs) based on current season "raw yield" and "relative yield" data were predicted. Across 5-fold cross-validation with 20 iterations, the averages and ranges of accuracies, in terms of correlation of true phenotypes and GEBVs, were 0.53 (0.37-0.63) for "raw yield" and 0.57 (0.38-0.69) for "relative yield". The stage-1 lines used in 2020-21 were progeny of 2015 crosses and breeding populations that were developed in large field plots to allow natural and breeder selection for multiple years. I avoided speed breeding because it is associated with the risk of small size breeding populations, lack of natural infield, and lack of breeder selection during the early generations. In our line production pipeline, lines are extracted from F4 bulk generation plots, head rows are planted as F5, and stage-1 testing are planted as F6 generation. For incorporating genomic prediction, I used a combination of phenotype and GEBVs as selection criteria to advance from stage-1 testing. Those that showed highest phenotypes and highest GEBVs received highest priority for advancing. We are now able to produce much more lines than our capacity to place them in stage-1 testing. Maybe genomic prediction of untested lines can be used as a strategy to preselect what should go for testing. We collaboratively continued NorGrains under a new brand of BIG6. The key change is massively increasing the number of advanced lines testing in multiple locations.

# Improving Wheat Breeding Process Efficiency, Utilizing AI and Deep Scanning Model

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#### Abstract

Fusarium head blight (FHB) is a global wheat and barley disease that causes severe and devastating yield and quality losses. Deoxynivalenol produced by the FHB-associated pathogen is injurious to human and animal health. Hard red winter and spring wheat is a primary cereal crop in the Northern Great Plains (ND, SD, MN, NE), grown on nearly 10 million acres. FHB causes significant losses estimated at \$20 million just in the South Dakota during a single year. Therefore, developing high-yielding wheat varieties with resistance to FHB will increase crop productivity to wheat producers in the region. However, breeding for FHB resistant cultivars requires evaluating many genotypes in the field and research plots each year. Therefore, the main objective of this project was, to implement an intelligent deep scanning system that captures images from an optimum close-up to improve the FHB detection rates; Second, to develop a model using artificial intelligence and deep scanning data to enhance the Unmanned Aerial Vehicles reliability on FHB detection; Then, evaluate, calibrate, and validate the developed model and improve the aerial-based phenotyping efficiency. Convolutional neural networks (CNNs) are an effective method to automatically identify these diseases. Recently, different approaches have been proposed to boost CNNs' performance. In this regard, we propose the multi-scale attention U-Net. Our main goal is to improve U-net by introducing an attention mechanism at the network bottleneck. In the proposed method, we propose to include the dual attention mechanism to adaptively emphasize the importance of both spatial and channel dimensions. To this end, using the global information of each channel, we learn the scaling coefficient to improve the object learning process. By learning the self-attention map we impose the spatial attention map, on the feature space to adaptively emphasize the important regions. We apply the suggested dual attention in a multi-scale fashion to encourage a multi-scale learning process. We prepared 12,000 images from the SDSU spring wheat breeding FHB screening nursery near Volga, SD, to train the proposed network. In this project, we will evaluate the developed model. This algorithm will be calibrated and be further validated for accuracy and precision. We will use the phenotyping data and the developed algorithm to improve the quality of the aerial-based phenotyping data process. This study will introduce a new generation of remote sensingenabled information products based on a solid foundation of well-calibrated sensors, new field calibration methodologies, and innovative algorithmic techniques.

#### Acknowledgement and Disclaimer

Confidential- please do not share the content of the submission publicly before the 2022 National FHB Forum. This material is based upon work supported by the U.S. Department of Agriculture, under Agreement, 59-0206-2-143 and 59-0206-2-153. This is a cooperative project with the U.S. Wheat & Barley Scab Initiative. Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the author(s) and do not necessarily reflect the view of the U.S. Department of Agriculture.

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

# **Bipin Neupane and Francois Marais**

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# Abstract

Fusarium head blight (FHB; caused primarily by *Fusarium graminearum*) can severely reduce wheat yield and quality. Improving wheat's inherent ability to withstand FHB is the most effective, economic, and environmentally friendly control strategy. Resistance is inherited through numerous small effect QTL that is strongly affected by GXE interaction. Many resistance QTL have been discovered but only a few of those with comparatively larger effects are well characterized and widely pursued in breeding. Many other, less well-studied resistance QTL, contribute to 'background' or 'native' FHB resistance variation in wheat germplasm. This study aimed to discover useful genetic background FHB resistance in NDSU elite hard winter wheat (Triticum aestivum L.) germplasm. Eleven advanced lines were crossed to produce 55 partial diallel F<sub>1</sub> hybrid combinations. The parents and F<sub>1</sub> were evaluated for FHB type II resistance in a replicated greenhouse trial. ANOVA indicated significant differences in disease severity among entries. Interpretation according to Griffing showed that the parents differed significantly in general combining ability (GCA) and the F<sub>1</sub> differed significantly for specific combining ability (SCA). The overall ratio of GCA:SCA genetic components was 2.88, suggesting that additive QTL effects were of primary importance and that pure line selection was an appropriate breeding strategy for improving resistance. The best general combiners (and most resistant among the parents) were ND Noreen, 18Nord-107, and 19Nord-129. 18Nord-107 and ND Noreen do not have known, well-characterized larger effect resistance QTL but did show significant genetic background resistance.19Nord-129 may also have only background resistance but needs confirmation. Five F<sub>1</sub> combinations showed strong, significant SCA effects for increased resistance over the parents. The Vr–Wr graph (Jinks and Hayman's interpretation) suggested an absence of epistatic gene action and the presence of incomplete dominance of the resistance QTL. It appeared that 18Nord-107 and ND Noreen had mostly dominant resistance QTL, whereas 19Nord-129 had mostly recessive resistance QTL. The material presents an opportunity to accumulate favorable alleles through selection in specific cross combinations. Eight F<sub>2</sub> families will be tested in a second greenhouse trial to confirm the earlier results; identify plants showing possible transgressive resistance and initiate single seed descent (SSD) inbreeding.

# A MAGIC Touch: Developing a Population to Enhance FHB Resistance in Barley

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#### Abstract

Fusarium head blight (FHB), a devastating disease of barley caused primarily by the fungus Fusarium graminearum, can cause significant yield losses and grain contamination with mycotoxins. Enhancing genetic resistance to FHB and the resultant accumulation of mycotoxins is one of the most effective and economical methods of reducing losses caused by FHB. Our future objective is to develop a multi-parent advanced generation intercross (MAGIC) population using the most resistant two-rowed barley accessions possessing complementary haplotypes at characterized FHB/DON loci with the goal of identifying progeny with resistance enhanced beyond the level of the founder parents. A panel of 247 diverse barley accessions from world-wide collections previously observed to exhibit promising levels of FHB resistance was evaluated in up to 10 environments over the past six years at two locations in Minnesota. The panel was genotyped using the 50k Infinium iSelect genotyping array for barley and association mapping was performed to identify quantitative trait loci (QTL) controlling FHB severity and deoxynivalenol (DON) concentration. Multiple FHB and DON QTL were detected, notably independent of QTL influencing heading date, plant height, and row type. These significant marker-trait associations were used to generate multi-marker haplotypes in relevant genomic regions. Haplotype-trait associations were then tested using the haplo.stats package in R. A group of 21 two-rowed candidate parents was selected based on FHB and DON phenotypes, as well as key agromorphological traits including heading date and plant height. The R package PopVar was used to predict genetic variance and superior progeny means for simulated biparental populations for all parental combinations of the 21 selected lines. Utilizing the predicted cross means and variances, as well as the haplotype information for the selected lines, eight parents were selected to serve as the founders for the multi-parent advanced generation intercross (MAGIC) population. Three of the eight founder parents are agronomically advanced lines, which will increase the chances of recovering progeny with desirable agronomic and quality traits beyond resistance to FHB and the accumulation of DON. Over the next three years, additional intercrosses and selfing generations will be performed to generate 800-1000 recombinant inbred lines (RILs) that will be phenotyped for disease reaction and mycotoxin concentration in multiple environments. Progeny carrying novel combinations of alleles from multiple founder parents at key haplotype loci are expected to have enhanced resistance to FHB and DON accumulation.

Variety Development & Host Resistance - Poster

# Artificial Intelligence-based Detection and Sorting of *Fusarium* Damaged Kernels in Wheat and Implications for FHB Resistance Breeding

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# Abstract

Fusarium Head Blight (FHB) or head scab (caused by Fusarium graminearum), is one of the most devastating diseases affecting small grain cereals including wheat; and causes severe reduction in the quality and quantity of grain yield. The traditional screening of large number of breeding lines for FHB resistance is still dependent on low-throughput visual inspections in the FHB inoculated field nursery followed by visual assessment of percent Fusarium Damaged Kernels (FDKs). Further, the visual assessments are a laborintensive and time-consuming process and is biased due to the subjectivity and may have lower reproducibility. Some earlier studies have evaluated the effectiveness and preciseness of image analysis approaches to predict FDKs in wheat grain samples; however, only a few focused at the final application, taking into consideration the association between cost and benefit, resolution, and accuracy. In this study, for the first time, we worked with QualySense on a feasibility study aimed at using their proprietary QSorter<sup>®</sup> Explorer robotic system for the automatic prediction, sorting and final quality inspection assessment of Fusarium damaged wheat kernels. QSorter Explorer is powered by advanced mechatronics and artificial intelligence and equipped with a 3D camera and a high resolution Near Infrared Spectrometer active in the 900-1700 nm spectral range. More than 7,200 single kernel images and NIR spectra were collected from healthy and Fusarium damaged kernels to develop machine learning models to predict FDKs and sort samples at 30 kernels/sec. Independent validations of the FDKs module was conducted using 200 advanced breeding lines (100 winter wheat and 100 spring wheat genotypes) that were visually rated by three persons separately. The same samples were analyzed by the QSorter Explorer by mean of two classification models FDK-Vision and FDK-NIR which are based respectively on the images and NIR spectra, to predict and sort the FDKs. While the visual evaluations of FDKs by the individuals varied significantly, the QSorter Explorer consistently produced better results in a time efficient manner. Additionally, compared to visual assessments, QSorter predictions were more accurate. This automation will certainly make screening for breeding lines more accurate and less prone to personal bias while delivering results faster and streamlining the laboratory efficiency.

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This material is based upon work supported by the U.S. Department of Agriculture, under Agreement 59-0206-2-153. This is a cooperative project with the U.S. Wheat & Barley Scab Initiative. Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the author(s) and do not necessarily reflect the view of the U.S. Department of Agriculture

Variety Development & Host Resistance - Poster

# Prediction of DON Content in Wheat Flour Using Close-Range Hyperspectral Imaging Coupled with Machine and Deep Learning Approaches

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# Abstract

Fusarium head blight (FHB), caused by a necrotrophic pathogen Fusarium graminearum Schwabe is one of the most destructive fungal diseases of wheat which is known to produce a harmful mycotoxin called deoxynivalenol (DON). DON contamination of wheat grains and/or flours is serious problem for food safety globally. Currently, DON levels in grain or flour samples can be measured using a variety of methods, including enzyme-linked immunosorbent assay (ELISA) and gas chromatography-mass spectrometry (GC-MS) but these methods are time-consuming, costly, and destructive. Therefore, a limited number of samples were analyzed for DON content in the elite and advanced breeding nurseries and majority of the selections in early and preliminary trials were made based on visual score for FHB or *Fusarium* Damaged Kernels (FDK). Development of rapid and non-destructive and economical method to estimate DON content can help to better facilitate breeding for lower DON. In the present study, GC-MS was used to estimate the DON content in 250 advanced breeding lines from SDSU wheat breeding program that were evaluated in the 2020-21 FHB nursery. The DON content of the 250 selected lines ranged from 0 to 77.6 ppm. The flour samples (3 grams) were analyzed for moisture and protein content on NIR and subjected to close-range hyperspectral imaging (400–1000 nm). Four different machine learning models (PLS regression, Xgboost, RF, and SVM) and one deep learning model (1D CNN) were evaluated on the training (70%) and test (30%) sets to ascertain the most efficient model based on accuracy with the coefficient of determination in prediction (R<sup>2</sup>) and root mean squared error (RMSE). Overall, advanced hyperspectral imaging coupled with machine and deep learning approaches shows great potential in high-throughput estimation of DON content and will enable breeding for lower DON content.

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# **Reducing DON Concentration with Naked Barley**

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# Abstract

A major concern with barley infected with *Fusarium graminearum* is the accumulation of the mycotoxin deoxynivalenol (DON). The hull of barley can account for a significant amount of the DON in *Fusarium* infected grain. Nearly all the barley in the U.S. is covered barley used for animal feed or by the malting and brewing industries. Loss of the hull during harvest as occurs with naked barley could be a way to mitigate the risk of DON contamination in the food chain. To explore this potential benefit, we developed near-isogenic lines (NILs) for the hulless trait. A total of 30 NILs for each class (naked and covered) were evaluated in an inoculated Fusarium head blight (FHB) nursery in 2021. The average concentration of the naked lines compared to the covered lines was 2.4 and 5.9, respectively. This constitutes a 59 % reduction in the DON concentration of the harvested grain. This reduction in DON is comparable to what is possible using a protective fungicide or by using a moderately resistant cultivar versus a susceptible one. It may also be possible to develop selection strategies that increase the amount of DON that accumulates in the hull relative to the kernel further reducing DON levels in the harvested naked grain. Breeding naked barley varieties with reduced DON and that can have multiple end uses could have a substantial impact on reducing the risk of DON contamination.

# Introduction

A major concern with barley infected with *Fusarium graminearum* is the accumulation of the mycotoxin deoxynivalenol (DON). The hull of barley can account for a significant amount of the DON in *Fusarium* infected grain (Clear et al., 1997). Nearly all the barley in the U.S. is covered barley used for animal feed or by the malting and brewing industries. However, naked (or hulless) barley could have value for these uses in addition to direct use as human food (Meints et al, 2021). Loss of the hull during harvest as occurs with naked barley could be a way to mitigate the risk of DON contamination in the food chain. We used near-isogenic lines for the naked caryopsis phenotype to characterize DON concentration in naked and covered harvested grain.

# **Materials and Methods**

We generated a bi-parental mapping population using the FHB susceptible parent Lacey and the moderately resistant and naked parent Hor211 (Sallam, 2005). The population consisted of 495 F6:8 lines that were phenotyped for the naked caryopsis trait, FHB severity, and DON. Five lines in the population were scored as segregating for *nud* and one (HLM-280) was selected to develop NILs. We selected 50 covered and 50 naked kernels from the segregating F6:8 seed lot of the line HLM-280 and grew them up to verify their caryopsis status and increase seed for screening in our FHB nursery (Figure 1). Thirty plants were selected as covered and thirty more were selected as naked and seed produced from these plants were planted in single row plots 1.2 M in length in our FHB nursery in Crookston, MN in 2021. These 60 entries along with the checks Lacey and Quest (replicated twice) were planted in a completely randomized design. Grain spawn inoculum and mist irrigating were used to promote disease. Plots were harvested at maturity, threshed, cleaned, and ground for DON analysis (Mirocha et al., 1998).



**Figure 1. A.** Spike of a naked barley line, B. grain spawn *Fusarium* inoculum, and C. mist irrigated Fusarium Head Blight nursery.

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#### Results

The range of DON concentration for the covered NILs was 2.9 to 12.3 ppm and for the naked NILs was 1.0 to 4.4 ppm. The average DON concentration of the 30 covered and 30 naked barley NILs was 5.9 and 2.4 ppm, respectively (Figure 2). This represents an average reduction of DON concentration of 59%.



# Previous studies to determine the amount of DON that accumulates in the hull have based their estimates on mechanical removal of the hull through pearling (Clear et al., 1997). In this study we used segregating lines derived from a single F6 individual from a bi-parental mapping population of 495 lines. The probability of an F6 line being heterozygous at any one locus is 1.6% which in a population of 495 individual should be about eight. In our study we identified five. The F6 individual that we identified should be homozygous at 98.5% of the genome. This would suggest that the naked and covered progeny lines from this individual are indeed near-isogenic. We are genotyping these lines with the Barley 50K SNP to confirm that the lines differ at the *nud* locus and identify other loci that were heterozygous in the original F6 parent and would be segregating in its progeny.

Reducing DON concentration by 60% would have a substantial effect on reducing the risk to farmers. This advantage is already realized in wheat which threshes clean without hulls. Typical reduction of DON concentration in barley using fungicides is 30-50% (Cowger et al., 2019). Similarly, the amount of reduction

in DON concentration between a susceptible and moderately susceptible variety is about 50 %. In another project we are using a naked barley diversity panel to characterize genetic variation for DON concentration in the kernel and hull. Using this panel for genome-wide association studies we have identified loci for these traits (Hawkins, 2022). Thus, it may be possible to breed to select for lines that accumulate more of the DON in the hull than in the kernel and improve the advantage of naked barley relative to covered barley.

The impact of this work will only be realized if naked barley varieties are developed that are adapted to production regions and have the properties of desired by end users (Massman et al., 2022; Meints and Hayes, 2019). A national project to develop mult-purpose naked barley for organic productions systems is exploring food, feed, and malting uses of naked barley (https://eorganic.info/node/23562). Vertical Malt, a craft malt company in Northern Minnesota has produced small amounts of malt from an advanced naked barley line from our breeding program for distribution to brewers for testing and evaluation. Increasing the footprint of naked barley production and use could be one way to reduce the entrance of DON into our food system.

# Acknowledgements

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Variety Development & Host Resistance - Poster

# **Genomic Selection: Marker Set Optimization Improves Prediction Accuracy**

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# Abstract

Molecular markers increase the efficiency of selection in plant breeding programs. Genomic selection (GS) is one of the latest applications of molecular markers for speeding up the process of plant breeding and genetic gains. GS involves the prediction of unobserved phenotypes from the genotype of the populations. In GS, a 'training population' for which the genotype (marker) and phenotype data are known is used to train a model by which to predict unobserved phenotype, as 'Genomic Estimated Breeding Value' (GEBV), for a 'test population' for which only the genotype data is known. Training a model from the training population is simply estimating the vector of marker effects. The accuracy of GEBV is paramount for the utility of GS, which in turn relies on the selection of the markers and accuracy of their effect estimates. Marker set optimization is critical to achieve higher prediction accuracies. In my poster, I present a method for 'marker-set optimization' by selectively removing a subset of markers (hereby referred to as trivial markers) from the genotype data whose effect size do not seem to be significantly different from zero, thereby increasing the prediction accuracy. This method was demonstrated in the poster by a dataset containing 392 breeding lines and 14,432 genome-wide markers. The 392 breeding lines were randomly divided into two equal subsets, #1 and #2, that were used reciprocally to predict each other. Marker effects sizes were estimated by bootstrapped Ridge-Regression BLUP. Genetic markers were identified as trivial and removed from the dataset if the 95% ranked-based confidence interval of the estimated marker effect overlaps with zero. By applying this methodology, 69.6% and 67.3% of the marker data were detected to be trivial for 'days to heading' and 'grain yield' respectively. These trivial markers were subsequently removed from the GS pipeline. This removal increased prediction accuracy, measured and expressed as Pearson correlation of GEBVs with real phenotype. For example, we observed an improvement from 0.315 to 0.688 (up 218%) for 'days to heading'. We augmented this method to Fusarium resistance traits by 100 iterations of bootstrap resampling (300 training: 92 test individuals) cross validation. The mean prediction accuracies across 100 iterations before marker set optimization were 0.304, 0.235, 0.404, 0.261, and 0.416 for INC, SEV, FDK, FHBdx, and DON respectively, which increased to 0.557, 0.491, 0.610, 0.508, and 0.598, respectively, after marker set optimization.

# Identification and Mapping of Quantitative Trait Loci for Fusarium Head Blight Resistance in Synthetic Hexaploid and Spring Wheat

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# Abstract

Fusarium head blight (FHB), caused by Fusarium graminearum Schwabe, is one of the most devastating diseases of wheat (Triticum aestivum L.). A synthetic hexaploid wheat line Largo, developed from a cross between the durum wheat (T. turgidum L. ssp. durum (Desf.) Husn.] variety 'Langdon' and the Aegilops tauschii Cosson accession PI 268210, was previously found to have a high level of resistance. This study was conducted to identify resistance quantitative trait loci (QTL) using a population of 188 recombinant inbred lines (RILs) from a cross between Largo and the susceptible wheat line ND495. The RILs were evaluated for Type II resistance in two greenhouse seasons and two field environments. The disease severity and existing 90K SNP marker data were used for QTL analysis, which led to the identification of six QTL on chromosomes 1D, 2D, 5B, and 7D. Four QTL (QFhb.rwg-1D, -5B, -7D.1, and -7D.3) with minor effects were derived from Largo, whereas two (QFhb.rwg-2D and -7D.2) with larger effects were from ND495. Five QTL collocated or overlapped with previously reported QTL, whereas QFhb.rwg-1D located in a region with no known QTL, suggesting that it is a novel QTL. QFhb.rwq-2D was the only QTL detected in all four environments and it is likely the same QTL as the major and stable 2D QTL present in many wheat varieties. Because QFhb.rwg-2D was not previously identified in adapted hard red spring wheat germplasm, its detection in ND495 will facilitate its utilization in hard red spring breeding programs. The QTL detected from the resistant and susceptible parents enhance our understanding of FHB resistance expression and provide new resources for improving FHB resistance in wheat, further suggesting that an effective strategy to improve FHB resistance is to pyramid multiple resistance QTL while also eliminating susceptibility genes.

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# Estimating FHB Infection Level in Winter Wheat Spikes using High-Resolution Imaging and Deep Transfer Learning

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# Abstract

Fusarium head blight (FHB), caused primarily by *Fusarium graminearum* Schwabe, is one of the most common and devastating fungal diseases, causing significant losses in wheat grain productivity and quality by producing harmful mycotoxins. Traditionally, wheat breeders mainly focus on visual selection for FHB resistant lines during FHB resistance breeding in wheat. Advanced techniques that enable high-precision and quick identification are required to improve its detection, because the current method i.e., visual assessment is labour- and time-intensive and subject to subjectivity biases. As a result, methods with a higher degree of objectivity have been investigated, and special emphasis has been laid on the use of highresolution imaging (e.g., RGB and thermal) coupled with advanced deep learning methods as the foundation for more reliable detection strategies. In this study, first, infected spikes from the wheat genotypes grown in FHB nursery were visually rated and grouped into four different classes including resistant (0-25%), moderately resistant (26-50%), moderately susceptible (51-70%), and susceptible (71-100%) based on the level of FHB infections. Following this, high-resolution RGB images of FHB infected wheat spikes were collected from the same visually rated FHB inoculated genotypes. Commonly used Convolutional Neural Network (CNN) architectures such as VGG and ResNet etc.) were employed to develop FHB infection level detection models. Additionally, to achieve enhanced model performance in the context of relatively small sample size circumstances, deep transfer learning strategy was adopted as well. To examine and compare the accuracies of different modelling strategies and CNN architectures, independent testing data was used for model assessment. The confusion/error matrix, along with the overall accuracy, Kappa coefficient and F1 score matrix were used for model evaluation. This study shows that high-resolution RGB imaging combined advanced deep learning approach can be used to predict FHB infection in seeds within the spikelet's and to identify very small differences in spike architecture (associated with FHB infection), which can better help breeders predict FHB infection at field level.

# Acknowledgement and Disclaimer

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## Haplotype-Informed Prediction of Fusarium Head Blight Resistance in USA Wheat Breeding Programs

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#### Abstract

Fusarium Head Blight (FHB), a major pathogenic fungal disease in wheat and barley caused by *Fusarium graminearum* (*Gibberella zeae*) has a major economic impact on wheat and barley production due to the production of mycotoxins. Breeding for resistance in adapted wheat varieties is not trivial due to several minor effect alleles identified in QTL studies. To successfully develop resistance, diagnostic markers for QTLs across the wheat genome need to be identified to help pyramid promising resistance genes into adapted wheat varieties in breeding programs. We are developing a Practical Haplotype Graph (PHG) database coupling phenotypic and genotypic data from a panel of 270 wheat cultivars from across the US. The objective of identifying novel QTLs and building a genomic selection (GS) model is underway with one year of field data evaluated. Preliminary results show significant variability in phenotypic scab severity, the normalized area under disease progressive curve (AUDPC), and the severity of *Fusarium* damaged kernels (FDK) (*p-value* > 0.05) 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 genome for association analyses and model building. Preliminary results will be presented and discussed.

Variety Development & Host Resistance – Presentation

# Developing Genetic and Germplasm Resources to Enhance FHB Resistance Winter Wheat Cultivars

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#### Abstract

Enhancing genetic diversity for FHB resistance is critical for addressing the need for FHB-resistant wheat cultivars. Although many resistant QTL have been identified and deployed in US wheat cultivars, effective resistance against FHB is still not fully realized. It emphasizes the need for some innovative approaches to tackling this important disease. We have recently screened wild wheat germplasm from its D-genome as well as A-genome progenitor species to identify resistant novel genes and alleles. We have critically evaluated a large diverse triticale collection against FHB. In addition, we have applied chemical and physical mutagens to develop mutant populations in diploid (*Aegilops tauschii* and *Triticum monococcum*) and polyploid wheat germplasm (including Shirley, Jagger, Corrigin, Chinese Spring, Hilliard, and MD131). We have also developed mutant populations of two winter barley cultivars adapted to MD and the broader mid-Atlantic region. Screening of some of these mutant populations in wheat allowed us to identify mutants with moderate to high resistance against FHB both for disease severity and DON content. Work is in progress on using MutMap and sequence-tagged high-resolution deletion mapping approaches to identify physical regions and associated genetic markers for the utilization of novel FHB resistance in breeding programs.

#### Intermediate Wheatgrass Chromosomal Regions Associated with Disease Resistance

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#### Abstract

Intermediate wheatgrass (*Thinopyrum intermedium* (Host) Barkworth & D. R. Dewey subsp. intermedium] (IWG), Kernza<sup>®</sup>) is a promising perennial grain candidate that has the potential to provide food for humans as well as environmental services. In the long-term vision of growing IWG commercially, reliability of the plants to survive and persist against disease and pathogens is essential for its feasible use. Genomics may be used to efficiently increase disease resistant traits. We evaluated selections from The Land Institute's (TLI) Cycle 7 breeding program from 2018-2020 with each cycle containing 460 Cycle 7 genets and 140 wheat and wheatgrass replicated checks to identify chromosomal regions associated with resistance to disease. Using genotyping-by-sequencing markers, quantitative trait loci (QTL) were mapped for 18 different disease and agronomic traits using genome-wide association analysis. Data was evaluated by-year which revealed 169 unique marker-trait associations (MTA) across 10 different traits. Two loci associated with Fusarium head blight (FHB) resistance were significant across multiple years (p < 0.05). FHB and the mycotoxins produced are devastating to wheat production around the world. Significant loci for FHB related traits were also used to do a candidate gene search which provided potential resistance genes in this new grain. The identification of loci associated with disease resistance or tolerance may be useful in selecting more resistant IWG varieties for future development.

#### Molecular Marker-Assisted Fhb7 Introgression in Common and Durum Wheat

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#### Abstract

A novel allele of the FHB resistance gene *Fhb7*, designated *Fhb7*<sup>The2</sup>, was identified on chromosome 7E of the diploid tall wheatgrass Thinopyrum elongatum (2n=2x=14, EE), and integrated into the wheat B genome through a small 7B-7E translocation (7BS·7BL-7EL) involving the terminal regions of the long arms. Fhb7<sup>The2</sup> conditions Type II FHB resistance and may detoxify deoxynivalenol (DON) according to a previous report. In addition, the FHB-resistant 7B-7E introgression line does not contain the gene for yellow flour pigment, which is closely linked to the *Fhb7* alleles from other sources. The integration of *Fhb7*<sup>The2</sup> into wheat chromosome 7B and the absence of yellow flour pigment gene in the introgression line make *Fhb7<sup>The2</sup>* immediately usable in both common wheat and durum wheat breeding without obvious linkage drag. Both STS and SNP-based PACE markers were developed specifically for *Fhb7<sup>The2</sup>*. They are highly diagnostic for the selection of *Fhb7*<sup>The2</sup> and effective for the introgression of *Fhb7*<sup>The2</sup> into different classes of wheats. They have been used to deploy *Fhb7*<sup>*The2*</sup> in the adapted hard red winter/spring wheat and spring/winter durum varieties through a marker-assisted backcrossing breeding scheme. Starting from the  $BC_1F_1$  generation, the *Fhb7<sup>The2</sup>*-specific PACE marker has been utilized to select *Fhb7<sup>The2</sup>*-positive individuals for further backcrosses. The  $Fhb7^{The2}$ -positive backcross progeny with the highest phenotypic recovery of the respective recurrent parent has been selected for advanced backcrossing. The 7BS 7BL-7EL translocation has been found to transmit in a typical Mendelian fashion over the backcross generations. After 3-4 backcrosses, *Fhb7*<sup>The2</sup> will be deployed in the adapted common and durum wheat varieties, leading to the development of the new FHB-resistant varieties. Meanwhile, we have been transferring  $Fhb7^{The2}$  to the major FHB resistance sources 'Sumai 3', PI 277012, 'Wangshuibai', and 'Frontana' following the similar Fhb7<sup>The2</sup>marker-assisted backcrossing breeding scheme. Stacking Fhb7<sup>The2</sup> with the major FHB resistance genes may enhance FHB resistance and generate new sources with improved FHB resistance.

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#### **Recurrent Selection for Fusarium Head Blight Resistance in a Durum Wheat Population**

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#### Abstract

Fusarium Head Blight (FHB) is a devastating disease that can cause severe loss of grain yield and quality of durum wheat in the northern Great Plain of the US. FHB resistance in wheat is a complex trait controlled by many genes. Recurrent selection is an effective way to increase frequencies of favorable resistant alleles and to develop improved germplasm. In this study, four cycles of recurrent phenotypic selection were conducted for reducing FHB severity from 2019 to 2022 in a durum wheat population derived from intercrossing of 15 elite cultivars and breeding lines. The FHB severity was reduced 34.5% from Cycle 0 to Cycle 3 population. Significant negative correlations were found between FHB severity and both plant height and days to flowering in Cycle 0, Cycle 3 populations. Genomic selection can speed up selection and increase genetic gain in terms of time and cost. A total of 284 S0 parents from the Cycle 2 and Cycle 3 populations were genotyped using 90K SNP array and obtained 2,706 SNP markers. Using ridge regression best linear unbiased prediction (rrBLUP) model, the prediction accuracy for FHB severity was 0.53 with cross-validation. Our results indicate that recurrent phenotypic selection can improve FHB resistance in durum wheat. Implementing genomic-assisted selection in the recurrent selection is practical to accelerate genetic improvement.

### Utilization of a Publicly Available Diversity Panel in Genomic Prediction of Fusarium Head Blight Resistance Traits in Wheat

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#### Abstract

Fusarium head blight (FHB), also known as head scab, is an economically and environmentally concerning disease of wheat (*Triticum aestivum* L). A two-pronged approach of marker-assisted selection coupled with genomic selection has been suggested when breeding for FHB resistance. In the present study, an historical dataset comprised of entries in the Southern Uniform Winter Wheat Nursery (SUWWSN) ranging from 2011-2021 was partitioned and used for cross validation within the dataset from the years 2011-2019 and forward validation on the 2020-2021 environments in the dataset. Two traits were curated:

percent Fusarium damaged kernels (FDK) and Deoxynivalenol (DON) content in parts per million. Two methods of data partitioning were entertained to establish a training population for each trait: 1) subsetting environments by genomic heritability and 2) sub-setting environments based on like performance of checks. Heritability was estimated for each trait-by-environment combination and data were partitioned on environments with greater heritability than 0.10, 0.25, 0.50, 0.75, and 0.90. For each trait assessed, a consistent set of check lines were drawn from each environment in the SUWWSN, and K-means clustering was performed to establish clusters with like-check performance; possible number of clusters from 2 to 15 were entertained and the optimal clustering was selected by multiple clustering criterion. By majority rule among the clustering criterion, two clusters were identified for FDK and three for DON. When using the combined data from 2011-2019 for training, forward validation for FDK on the SUWWSN 2020 and 2021 indicated a predictive accuracy R=0.58 and R=0.53, respectively. In comparison, forward validation using only the environments in the first cluster of like-check-performance environments for FDK indicated a predictive accuracy of R= 0.65 and R=0.60. Forward validation for DON using the total data available indicated a predictive accuracy of R=0.57 and R=0.45. Likewise, forward validation using only the like-check performance environments in cluster one for DON indicated a predictive accuracy of R=0.67 and R=0.60. These results indicate that selecting environments based on like-check performance may produce substantially higher forward prediction accuracies than using the total available data. This work may be used as a model to create a public resource for genomic prediction of FHB resistance traits across public wheat breeding programs.



# U.S. Wheat & Barley Scab Initiative (USWBSI)

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