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Opening Session Presentations

A Field Pathologist's Insights on Fusarium Head Blight: Four Decades and Still Learning

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I have witnessed steady progress in understanding and managing Fusarium head blight (FHB) and mycotoxin contamination since the 1980s. My context is soft winter wheat and malting barley as rotational cash crops in the Northeast. Highly susceptible varieties are no longer planted and varieties with moderate resistance are readily available to growers, yet there is still risk of deoxynivalenol (DON) contamination above purchase limits in our wet, humid climate. Anthesis-timed applications of effective fungicides to suppress FHB, DON, and flag leaf fungal diseases has become routine. We documented the first isolate of *Fusarium graminearum* in North America with resistance to a triazole fungicide, so we are vigilant for resistance development in regional pathogen populations to all fungicides that are being applied. We tested potential biofungicides and other alternatives to synthetic fungicides but never identified a consistently efficacious product for use by the growing number of organic small grain producers in the state. My group has had a special interest in the epidemiology, aerobiology, and population biology of *F. graminearum*. A multi-year, multi-site experiment with several collaborators in the U.S. and Canada provided field-based, quantitative evidence of long-distance atmospheric dispersal of *Fusarium graminearum* ascospores. The results allowed us to estimate that, on average, approximately one-third of spore inoculum for FHB in a wheat field came from within-field sources (i.e., overwintered corn residue) and two-thirds of the inoculum came from atmospheric deposition of spores originating outside of the field. New York is an excellent place to study pathogen populations with overlapping populations NA1, NA2, and NA3 occurring along North-South and East-West clines. We demonstrated the presence of four mycotoxin-producing chemotypes in the state (15 ADON, 3 ADON, NIV, and NX2). Populations in regions under intensive crop cultivation were predominately 15 ADON genotypes; while populations where agriculture was sparse showed a greater proportion of 3 ADON and NX2 genotypes. We postulated that populations on non-cultivated grasses influence the population structure in regions with limited agriculture and showed that *Fusarium* is widely present in the inflorescences and stubble of native grasses and can cross-infect cereal crops. Our current research emphasis is on *Fusarium* spp. and mycotoxin contamination of barley grain for malting. While *F. graminearum* and DON predominate, were have documented great species and toxin diversity across alpha- as well as beta-trichothecene contamination in barley grain, suggesting the need for broader screening of mycotoxins to assure safety of barley-based foods and beverages.

Supply Chain Solutions, Vision for the Future

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The Arthur Companies is a 118-year-old company that services growers through retail agronomy and grain marketing. Managing FHB risk with growers can be challenging, from variety selection to management practices and can complicate the grain channel marketing process. The Arthur Companies is focused on building relationships and utilizing research and development to help provide data driven decisions to growers.

FHB Management

University of Idaho Uniform Fungicide Trial Results for Control of Fusarium Head Blight in 2024

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The trial at the University of Idaho research farm in Aberdeen, ID used soft white spring wheat 'WB6211CLP' (highly FHB-susceptible) to evaluate fungicide efficacy against Fusarium Head Blight (FHB). In order to optimize disease development, experimental plots were set up in a randomized full block design with four replications, planted on May 1, 2024, under irrigation. An additional supplementary sprinkler system was installed across the experimental plot to create an environment conducive for disease infection and development at anthesis. Different fungicide combinations were used as treatments applied at 50% anthesis and 4-6 days later, following the UFT protocol. Treatments 1 (untreated check) and 4 (Miravis® Ace 13.7 oz/A applied with NIS Induce at anthesis) were requested by USWBSI, Bayer and Syngenta. A *Fusarium graminearum* spore suspension (100,000 spores/ml) was used in the afternoon of July 8, the same day as the anthesis fungicide treatments were applied that morning. The second fungicide application occurred 15 July for treatments 8 and 9. The FHB index was calculated as (incidence × severity) / 100. FHB ratings, including incidence and severity, were recorded 23 days after anthesis. There was no evidence of phytotoxicity after fungicide treatments. Plots were harvested on 6 Sept with a small plot combine. Yield and test weight was determined utilizing the HarvestMaster system on the combine. Analysis used PROC GLIMMIX in SAS 9.4 with LSD ($\alpha = 0.05$) for mean comparisons. Even while untreated check plots achieved an FHB score of 47%, the results indicated that cooler, wet spring conditions reduced FHB growth. Fungicide treatments effectively decreased FHB incidence, severity, and index as compared to untreated plots, with significant reductions ($P < 0.01$) observed across disease and yield metrics. The untreated check plot had a lower test weight (55 lbs/bu) and the lowest grain yield (77 bu/A). All fungicide treatments had yields that were statistically comparable, but Treatment 8, which included Miravis Ace and Sphaerex™, produced the highest yield at 100 bu/A. Mean FHB incidence, severity, and index ranged from 15 to 70%, 17 to 68%, and 3 to 47%, respectively, with untreated plots showing the highest values. Test weight ($P < 0.0001$), yield ($P < 0.01$), FHB incidence ($P < 0.01$), FHB severity ($P < 0.0001$), and FHB index ($P = 0.0001$) all showed significant differences. DON results are pending. All fungicide applications successfully decreased FHB infection. Fungicide treatments improved yield and grain quality while effectively controlling FHB, thus supporting their role in FHB management strategies for spring wheat.

Quantifying the Benefits of Two-Row, Naked, and Fall-Sown Barley on Reducing Deoxynivalenol in Grain

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Breeding for Fusarium head blight (FHB) resistance and lower deoxynivalenol (DON) in barley has been challenging due to substantial genotype by environment interactions, lack of QTL with large effects, and associations with agro-morphological traits (e.g. late flowering, tall plant height, and two row spike morphology) which are generally considered avoidance mechanisms rather than true genetic resistance. The dominant classes of barley grown in the U.S. are spring six-row and two-row with the latter becoming more favored by the malting and brewing industries. Increased interest in developing winter barley for regions that have traditionally produced spring barley is spurred by the need to develop more sustainable cropping systems. Similarly, there is increased interest in developing naked (or hulless) barley for human food, animal feed, and malting, particularly for organic production systems. As growers and end-users explore these other barley classes, we recognized their potential to impact DON contamination. For example, two-row barley is often observed to be lower in disease compared to six-row barley and mapping studies often link spike type with FHB resistance. Similarly, DON is known to accumulate in the hull of barley and therefore the loss of the hull in naked barley could also reduce DON. Additionally, fall-sown winter barley matures earlier than spring sown barley and this modified timing of plant development could reduce the risk of DON contamination. While there are many considerations that affect the extent to which two-row, naked, or winter barley, should be deployed on the landscape, it would be desirable to quantify the specific advantage these types of barley have in mitigating DON contamination. We used data from the North American Barley Scab Evaluation Nursery from the years 2007 – 2017 in which multiple breeding programs submitted two-row and six-row elite breeding lines to evaluate for FHB and DON. We developed and tested near isogenic lines for the *nud* locus to quantify the effect of hull loss on DON concentration. Lastly, we compared fall-planted and spring-planted facultative barley lines to determine the effect of phenological development on DON. These studies indicate that altered spike morphology, hull loss, and fall-planting reduce DON levels by 18%, 48%, and 66%, respectively. This work was supported by the USDA-ARS U.S. Wheat Scab Initiative, USDA-NIFA OREI, and the American Malting Barley Association.

Identifying Differentially Expressed Genes Influencing FHB Resistance in Hard Red Spring Wheat

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Fusarium Head Blight (FHB) is a significant threat to wheat which impacts global food security. FHB-resistant varieties, such as the Chinese cultivar 'Sumai3', containing the major resistance gene, *Fhb1*, is crucial for controlling this disease. In this study, we examined gene expression in eight hard red spring wheat (HRSW) lines, under three treatments: non-inoculated, mock-inoculated, and Fusarium-inoculated. RNA sequencing using newly annotated Sumai3 genome sequence revealed over 4000 differentially expressed genes (DEGs) across various comparisons with unique expression patterns observed under different conditions, especially on chromosome 3B near the Fhb1 locus. This suggests their potential roles in FHB defense. Although the exact functions of most of these DEGs are yet unknown, the orthologs function of one of the DEGs upregulated in resistant lines, TRAES.SUM.r1.3DG00524960 reveals that it is a putative disease resistance protein gene, suggesting its potential role in defense mechanisms. These insights will assist guide breeding programs by providing new candidate genes for FHB resistance, with future research focusing on validating these markers that signal expression changes across diverse wheat cultivars. This study emphasizes the value of transcriptomic analysis in advancing FHB resistance in wheat breeding efforts.

Evaluating the Influence of Seeding Rates when Integrated with Fungicide Applications and Genetic Resistance on Fusarium Head Blight Control in Kansas

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Fusarium head blight (FHB) is an important disease impacting wheat production globally and has become an increasing concern in Kansas in recent years. Best management practices for FHB include the combination of genetic resistance and fungicide application at early anthesis (Feekes 10.5.1) when conditions are favorable for disease development. Previous work has suggested that higher seeding rates can result in more uniform flowering periods. We hypothesize that higher seeding rates will improve the performance of a well-timed fungicide combined with genetic resistance under high FHB pressure. To test this, field experiments were conducted in Parsons, KS in 2022-2023, and Manhattan, KS and Ottawa, KS in 2023-2024. Treatments were set up as a full-factorial randomized complete block design evaluating two levels of genetic resistance ('Green Hammer' – Susceptible and 'Zenda' – Moderately Resistant), three seeding rates (500,000, 1 million, and 1.5 million seeds/A), and two fungicide treatments (Miravis® Ace 13.7 fl oz/A applied at Feekes 10.5.1 compared with an untreated control). Results indicate that higher seeding rates resulted in lower disease and DON for both moderately resistant and susceptible varieties when compared to the same treatments at the lowest seeding rate. The moderately resistant variety planted at the highest seeding rate and receiving a single fungicide application resulted in the lowest visual disease, lowest DON, and highest yield. The trend of increasing DON and visual severity with decreasing seeding rates was apparent across all treatment combinations. Wheat producers use a range of seeding rates for various reasons. Unraveling the relationship between seeding rate and fungicide efficacy could provide more precise FHB management recommendations for wheat producers.

Evaluating the Impact of Weather Variables on FHB Infection within Virginia Wheat Germplasm

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Breeding for resistant lines to scab or fusarium head blight (FHB) is significant in soft red winter wheat (*Triticum aestivum L.*) production, most especially in southeastern and Mid-Atlantic regions of USA. FHB is a fungal disease caused by *Fusarium graminearum* which accounted for economic losses annually due to devastating effects on yield and grain quality. The species of *Fusarium* pathogen that cause scab produce mycotoxins called deoxynivalenol (DON), which is detrimental to living organisms that consume the infected grains. As the FHB infection takes place at flowering, it may be valuable to consider weather variables relative to this time point on an individual genotype basis. Therefore, this study aims (i) to explore how the environmental covariates prior to flowering and during the flowering influence the infection of fusarium head blight (FHB) or scab, and (ii) to determine genetic relationship between agronomy traits and FHB traits, and their impact on agronomic performance. A linear mixed model was fitted on 3-year scab nursery trials data with 745 lines evaluated across years 2019-2021 for which flowering date data were recorded. We observed variance component of weather covariates similar to main environmental factor in a standard GxE model suggesting relatively few weather variables can capture the trend of G x E across years. The FHB traits typically showed negative effect on agronomic traits indicating breed for FHB resistant wheat lines with minimal negative on yield component is paramount.

Trichothecenes in Europe: An Update

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The overarching principle for regulating contaminants in food and feed in the European Union (EU) is that edibles placed on the market shall be safe. To keep e.g. mycotoxin levels as low as reasonably achievable, the EU food and feed legislation is based on risk analysis, consisting of risk assessment, risk management and risk communication. Risk assessment is based on available scientific evidence and undertaken in an independent, objective and transparent manner, typically by the European Food Safety Authority (EFSA). Risk management, performed by the European Commission (EC), takes into account the results of risk assessment, other legitimate factors (e.g. cost-benefit considerations, health risk-benefit considerations or analytical achievability) and additional precaution where appropriate.
EFSA updated its risk assessment on deoxynivalenol (DON) and also included its acetylated and glucosylated derivatives (DOI:10.2903/j.efsa.2017.4718). As such, a group tolerable daily intake value of 1 µg/kg bodyweight per day has been set for the sum of DON, DON-3-glucoside, 3-Acetyl-DON and 15-Acetyl-DON. As a result, the former guidance level for DON in dog feed was lowered from 5 mg/kg to 2 mg/kg several years ago (EC Recommendation 2016/1319). Last year, EC regulation 2023/915 on maximum levels for certain contaminants in food entered into force. Currently, DON is the sole regulated trichothecene in food with maximum levels reaching from 200-1750 µg/kg depending on the commodity and the intended use. For modified forms of Fusarium mycotoxins, including DON, T-2 and HT-2 toxins, zearalenone and fumonisins a regulatory follow-up is expected, once analytical aspects are properly addressed. Since the start of 2024, a total of 542 notifications regarding mycotoxins have been reported in the European Rapid Alert System for Food and Feed (RASFF). The vast majority of those were regarding aflatoxins (456) or ochratoxin A (68) and only two cases exceeded the maximum level of DON in food or the guidance level for the sum of T-2 and HT-2 toxins in feed, respectively. The natural occurrence of NX-toxins in Europe appears to be very low at the moment.

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

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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. In Vermont during 2024 we observed the disease and yield impact of inoculation with *Fusarium graminearum*, and treatment with two organic fungicides at two timings. The ChampION™ and Double Nickel fungicides were applied at heading. ChampION was also applied both at heading and 4 days after heading. Finally, in combination ChampION was applied at heading with an application of Double Nickel 4 days after heading. The 2024 growing season was conducive to Fusarium infection with mild conditions and above average rainfall throughout the spring and summer. All fungicide treatments and timings, including the control and the Fusarium inoculated plots, had DON concentrations above the 1 ppm threshold recommended by the FDA. However, there were significant differences observed in DON concentrations for the fungicide treatments. The highest DON concentration was in the ChampION treatment (applied at heading) at 3.82 ppm and was significantly higher than all other treatments. The ChampION treatment applied at heading and 4 days after heading had the lowest DON concentration (1.44 ppm) but was statistically similar to ChampION applied at heading combined with Double Nickel applied 4 days after heading, the uninoculated control, and the Fusarium inoculated plots. Overall, the organic treatments did not reduce DON concentrations compared to the control; however, the application of organic fungicides at heading and 4 days after heading provided some control over just one application at heading. Additional research should be conducted to evaluate combinations of organic fungicides and timing of application.

Objective

To evaluate the effects of application timings of two organic fungicides on barley yield and the 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 2024 we observed the disease and yield impact of inoculation with *Fusarium graminearum*, and treatment with two organic fungicides at two timings.

Materials and Methods

The trial was conducted in Alburgh, VT during 2024. The soil type was a Benson silt loam soil. The plot size was 5 x 20 ft including seven rows with 7-in spacing. Planting occurred April 23, 2024. The plots were sown with 'Robust' (susceptible) barley at 350 seeds per m^2 with a Great Plains grain drill (Salinas, KS). The experiment was set up as a completely randomized block design with fungicide treatments, randomized in four replicated blocks. Fungicide treatments are shown in Table 1. The first fungicide application (with surfactant at 0.125% V/V) was applied at heading (Feekes growth stage, FGS 10.1) on June 13, 2024. After the fungicide had dried, plots were spray-inoculated with a conidial suspension of *F. graminearum* (40,000 conidia/ ml) to augment the development of FHB. The ChampION and Double Nickel fungicide was applied at heading. ChampION was also applied both at heading and 4 days after heading. Finally, in combination ChampION was applied at heading with an application of Double Nickel 4 days after heading. Fungicide and *F. graminearum* treatments were applied with a CO_2 backpack sprayer with paired TJ-60 8003vs nozzles mounted at an angle (30° from horizontal) forward and backward, 20-in. apart, pressurized at 30 psi, and calibrated to deliver 20 gal/A. Grain was harvested using an Almaco plot combine (Nevada, IA). Grain moisture, plot yield, and test weight were recorded. Yield and test weight were adjusted to bushels ac^{-1} at 13.5% moisture. Deoxynivalenol (DON) concentrations were analyzed at the McMaster lab at Virginia Tech on an Agilent 6890N / 5975 GC/MS. This method has a detection range of from 0.025ppm – 15ppm. Treatment means were calculated, subjected to analysis of variance, and separated by Fisher's protected LSD test ($P = 0.05$).

Results and Discussion

Weather conditions in Vermont during the 2024 growing season can be characterized as moderate temperatures with excessive rain. Temperatures were at or slightly above the 30-year average. Rainfall was well above the 30-year average with 7.15 inches of precipitation more than normal from April to August. Overall, weather conditions were conducive for Fusarium infection.

All fungicide treatments and timings, including the control and the Fusarium inoculated plots, had DON concentrations above the 1 ppm threshold recommended by the FDA (Table 2). However, there were significant differences observed in DON concentrations for the fungicide treatments. The highest DON concentration was in the ChampION treatment (applied at heading) at 3.82 ppm and was significantly higher than all other treatments. The ChampION treatment applied at heading and 4 days after heading had the lowest DON concentration (1.44 ppm) but was statistically similar to ChampION applied at heading combined with Double Nickel applied 4 days after heading, the uninoculated control, and the Fusarium inoculated plots. Overall, the organic treatments did not reduce DON concentrations compared to the control; however, the application of organic fungicides at heading and 4 days after heading provided some control over just one application at heading.

Additional research should be conducted to assess the efficacy of multiple applications of organic fungicides on FHB.

Acknowledgement and Disclaimer

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Table 1. Organic fungicide treatments, active ingredients and rates applied.

Fungicide treatments	Company	Fungicide active ingredient	Application rates
Control			Water
Fusarium graminearum			40,000 spores/ml
ChampION^{++}	NuFarm	Copper hydroxide	1.5 lbs ac^{-1}
Double Nickel	Certis Biologicals	*Bacillus amyloliquefaciens strain D747*	6.0 qts ac^{-1}

Table 2. Effect of organic fungicide + timing on deoxynivalenol (DON) contamination and grain yield at Alburgh, VT, 2024.

Fungicide + timing	**DON**	**Test weight**	**Yield**
	ppm	**lb bu^{-1}**	**bu ac^{-1}**
Non-sprayed, non-inoculated control	1.84	44.8	65.4
Inoculated FGS at heading	2.04	44.4	55.4
ChampION at heading	3.82	43.8	60.4
Double Nickel at heading	2.63	45.7	59.7
ChampION at heading plus 4 days after heading	1.44	44.9	66.9
ChampION at heading plus Double Nickel 4 days after heading	1.98	45.7	63.9
LSD (p=0.05) †	1.05	0.89	11.8

†LSD; least significant difference at p-value = 0.05.

Dual Fungicide Applications for Fusarium Head Blight Management in Hard Red Winter Wheat: a Three-Year Summary of Uniform Fungicide Trial Results from Kansas

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Fusarium head blight (FHB), caused by *Fusarium graminearum*, is a globally important wheat disease. A single fungicide application at early flowering (Feekes 10.5.1) is generally recommended when conditions favor disease development. We hypothesize that FHB control can be improved by making a single application at Feekes 10.5.1 followed by a second application 4 days later. Field experiments evaluating the efficacy of dual applications were conducted over three winter wheat seasons across two locations per season. The FHB susceptible variety 'KanMark' was used during 2021-2022 season, and the variety 'Green Hammer' was used during 2022-2023 and 2023-2024 seasons. Four *F. graminearum* isolates collected from Kansas were used to produce infested corn spawn inoculum. Plots were inoculated and received supplemental sprinkler irrigation. Treatments, which were consistent with the USWBSI MGMT-CP uniform fungicide trial (UFT) protocol, consisted of 1) untreated control, 2) Prosaro® 6.5 fl oz/A at flowering; 3) Miravis® Ace 13.7 fl oz/A at flowering; 4) Prosaro Pro 10.3 fl oz/A at flowering; 5) Sphaerex™ 7.3 fl oz/A at flowering, 6) Miravis Ace 13.7 fl oz/A followed by Prosaro Pro 10.3 fl oz/A; 7) Miravis Ace 13.7 fl oz/A followed by Sphaerex 7.3 fl oz/A; and 8) Miravis Ace 13.7 fl oz/A followed by TebuStar 4 fl oz/A. Response variables were subjected to mixed model ANOVA and means were compared using Tukey's HSD test. Additionally, the influence of disease level on treatment responses was modeled by including FHB index as a continuous covariate in a mixed model ANOVA for yield and DON. All fungicide treatments reduced FHB index and DON relative to the untreated control. Treatments including one Miravis Ace application had the lowest disease and DON levels with FHB index values between 7-12% and DON concentrations between 9-12 ppm. In comparison, the other fungicide treatments had FHB index values between 16-18% and DON levels ranging from 14-16 ppm. These differences in disease control also influenced grain yields with treatments including Miravis Ace yielding between 62-65 bu/A and the other fungicide treatments yielding 52-56 bu/A. The covariate analysis with FHB index demonstrated that the efficacy of Miravis Ace was more pronounced at high levels of disease. Overall, the application of Miravis Ace alone or in combination with a second fungicide applied 4 days after Feekes 10.5.1 provided enhanced FHB control, particularly under high disease pressure.

Summary of Hard Red Spring Wheat Uniform Fungicide Trials in North Dakota from 2022 to 2024

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Fungicides are routinely applied at early-anthesis in North Dakota (ND) to reduce damage from Fusarium head blight (FHB) and protect yield. Given the variability in weather among growing seasons, it is important to continually update data pertaining to the use of fungicides in variable FHB environments. Seven hard red spring wheat (HRSW) uniform fungicide trials (UFT) were conducted to compare the timing and efficacy of fungicide premixes on reducing FHB incidence, severity, and index, while protecting yield and test weight. Research sites were established at the Langdon Research Extension Center (Langdon; mist irrigated; 2022-2024), North Dakota State University (Fargo; dryland; 2022-2024), and North Central Research Extension Center (Minot; dryland; 2024). Field experiments were conducted in a randomized complete block design with four replications. All plots were sown with a susceptible HRSW cultivar that is also grown on wide acreage in ND. Treatments evaluated included a non-treated control (NTC), Prosaro®® (prothioconazole + tebuconazole), Miravis® Ace (propiconazole + pydiflumetofen), Prosaro Pro (prothioconazole + tebuconazole + fluopyram), Sphaerex™ (metconazole + prothioconazole), and Folicur® (tebuconazole). Fungicide timings included Feekes 10.51 (early-anthesis), and 3 to 7 days after Feekes 10.51, or sequential applications of both timings. Six of the seven field experiments developed moderate to high levels FHB and were combined for analysis using PROC GLIMMIX. Results indicated that all fungicide treatments reduced incidence, severity and index. Single applications of Miravis Ace, Sphaerex, and Prosaro Pro had similar FHB index values to sequential application treatments. All fungicide treatments had significantly higher yield and test weight when compared to the NTC. The application of Sphaerex at 3 to 7 days after Feekes 10.51 had similar disease suppression to Sphaerex at Feekes 10.51, yet higher yield. This suggests that there may be an agronomic benefit associated with a "later" application of a fungicide to manage FHB. Results have been summarized and are available on a 360 interactive tool/website. Summarized data sets will also be presented at numerous Extension events and influence fungicide input decisions on potentially 5 to 6 million acres of HRSW.

Acknowledgement and Disclaimer

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Fusarium Head Blight Management Coordinated Project: Integrated Management Trials 2024

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Integrated management approaches such as good agronomic practices, resistant cultivars, and chemical control are being employed for managing Fusarium head blight (FHB) and deoxynivalenol (DON) contamination of wheat grain. Demethylation inhibitor (DMI) fungicide active ingredients (AI) such as prothioconazole, metconazole, and tebuconazole have historically been the most effective for FHB and DON control. The current industry standards for management of FHB and DON are Prosaro, a premix of two DMI AIs (tebuconazole and prothioconazole) and Miravis® Ace, a premix of the DMI Propiconazole and the SDHI Pydiflumetofen. Questions are being as whether newly registered products such as Prosaro® Pro (a premix of the DMI tebuconazole and prothioconazole and the SDHI Fluopyram) and Sphaerex™ (a premix of the metconazole and prothioconazole) will be just as or more effective than the industry standards in integrated management programs. During the 2024 growing season, separate replicated plots of susceptible (S), moderately susceptible (MS), or moderately resistant (MR) cultivars were treated with Prosaro, Miravis Ace, Prosaro Pro, or Sphaerex at Feekes 10.5.1 or left untreated, and subsequently inoculated with spores of *Fusarium graminearum*. Mean FHB index (IND) and DON in S_CK ranged from 0 to 74% and 0 to 3.2 ppm, respectively. Relative to the non-treated susceptible check (S_CK), all fungicide x cultivar treatment combinations resulted in significantly lower for mean IND and DON. Averaged across the environments, the combination of a MR cultivar and a fungicide treatment

showed significantly lower mean IND and DON than all treatments applied to S cultivars. Percent control (C) was estimated for IND and DON for each cultivar x fungicide program combination relative to S_CK. Averaged across environments, the combination of an MR cultivar and a fungicide treatment resulted in the higher C values for IND and DON than treatments applied to an S cultivar. For instance, across the tested fungicide programs, C for IND and DON ranged from 85.7 to 90% on MR cultivars and 69 to 82% on MS cultivars, compared to 56 to 70% on S cultivars. Findings from this study conducted under different agronomic conditions provide useful information on 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.

Acknowledgement and Disclaimer

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Fusarium Head Blight Management Coordinated Project: Uniform Fungicide Trials 2024

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Prosaro® Pro, a premix of the demethylation inhibitor (DMI) fungicide active ingredients (AI) tebuconazole and prothioconazole and the succinate dehydrogenase (SDHI) AI Fluopyram, and Sphaerex™, a premix of the DMIs metconazole and prothioconazole, are new fungicides marketed as possible replacements for industry standards such as Prosaro and Caramba® for Fusarium head blight (FHB) and deoxynivalenol (DON) control. In addition, Miravis® Era, a new premix of Pydiflumetofen, a relatively new member of the SDHI family of compounds, and the DMI prothioconazole, is being tested for efficacy against FHB and DON. In 2024, uniform fungicide trials were conducted in 24 US states to compare existing and new fungicides for FHB and DON management in single- and double-application treatment programs, consisting of: Prosaro (I), Caramba (II), Miravis Ace (III), Prosaro Pro (IV), or Sphaerex (V) applied at anthesis, Miravis Ace at anthesis followed by an application of Prosaro Pro (VI), Sphaerex (VII), or Tebuconazole (VIII) at 4-6 days after anthesis, Miravis Era at anthesis, plus a non-treated check (CK). FHB index (IND) was assessed, and grain samples were collected and assayed for DON. Across environments, mean IND and DON in the checks ranged from 0 to 47% and 0 to 9.4 ppm, respectively. All fungicide treatments resulted in significantly ($P < 0.001$) lower mean IND and DON than CK. Although differences among fungicide programs were not always significantly significant for IND, treatment VII, VII and Miravis Era resulted in the lowest mean IND values. Treatment VII had significantly ($P < 0.001$) lower mean DON than treatments I and III. Miravis Era was not significantly different from

treatment I, II, III, IV and VII for FHB index (*P* > 0.122). However, mean DON were significantly lower for V, VI, and VII than Miravis Era. Based on these results, all treatments were of similar efficacy against IND, but the double-application treatments VI and VII were the most effective against DON contamination of grain.

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Exogenous dsRNA Coupled with Graphene Quantum Dots Targeting *Fusarium graminearum* Genes Attenuates Fusarium Head Blight Disease in Wheat

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Fusarium head blight (FHB) is a devastating fungal disease of wheat caused by the fungus *Fusarium graminearum* that results in the substantial yield loss and grain contamination with mycotoxins. Currently, chemical fungicides remain the major component of FHB disease control, which carries risks to human and environmental health. Use of exogenous dsRNA to induce RNAi against pathogen's genes, is an effective and sustainable approach for the control of FHB. In this study, we investigated the in-vitro and in-vivo effects of double-stranded RNA (dsRNA) application on growth and pathogenicity of *Fusarium graminearum* (*Fg*). We selected eight genes *FgMGV1*, *FgRAS1*, *FgCOT1*, *FgPp2A*, *FgCAK1*, *FgTRI5*, *FgGMK1*, and *FgYCK1*, that have previously been identified to have role in growth or pathogenicity of *Fg*. For the application, we produced and purified the dsRNA in the laboratory using in-vivo methods along with graphene quantum dots (GQDs) as a nanocarrier. For the Fusarium assay studies, we collected *Fg* isolate from Indiana wheat fields in 2022. Growing *Fg* isolate in 1X synthetic low nutrient poor media without agar (SNA) media under different concentrations of dsRNA i.e., 1, 5, 10, 25 and 50 µg/mL showed a significant reduction in optical density evidenced at concentrations of 25 µg/mL and higher. These reductions in optical density were further validated by the regrowth experiments using fresh growth media. The performance of ds*TRI5*, ds*MGV1*, ds*YCK1* and ds*COT1* in reducing fungal biomass in SNA medium was better than dsRNA of other genes, evidenced by lowering fungal biomass by almost half. The inhibitory effect of dsRNA on fungal growth in dsRNA incorporated Potato dextrose Agar (PDA) media was observed, showing distinct inhibition zones. When dsRNA was sprayed in the two wheat genotypes i.e., 'AL105' and 'Gilat' already inoculated with *Fg*, the percentage of symptomatic spikelets significantly reduced to 20-25% in both genotypes compared to 100% in controls. In addition, we also evaluated the effect of applied dsRNA on *Fg* infected spikes for lowering the accumulation of mycotoxin deoxynivalenol (DON) using gas chromatography–mass spectrometry (GC/MS) method. The results revealed lower accumulation and spread of DON in the neighboring spikelets of plants treated with dsRNA compared to no dsRNA treatment group. Furthermore, analysis of transcript abundance for two genes *FgRAS1* and *FgPP2A* after the application of dsRNA in the liquid culture showed the gene expression reduction in treated *Fg* cultures by one-third as compared to untreated cultures.

USWBSI Uniform Fungicide Efficacy Trial for Management of Fusarium Head Blight in Irrigated Durum in North Dakota

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Durum wheat (*Triticum durum* Desf.) production in western North Dakota can be severely impacted by Fusarium Head Blight (FHB) due to lack of host resistance. Episodic infections and environmental conditions that are not always conducive for disease make it difficult to justify fungicide applications as an annual input. The goal of this study was to evaluate the effect of timed single and sequential fungicide applications on FHB index, yield, test weight (TW), and protein on an FHB-susceptible durum variety. Trials were conducted from 2022 to 2024 under overhead irrigation and set up in a randomized complete block design in 75 ft^2 plots with five replications. Fungicide treatments evaluated included non-treated check, Prosaro® (prothioconazole + tebuconazole), Miravis® Ace (pydiflumetofen + propiconazole), Prosaro Pro (prothioconazole + tebuconazole + fluopyram), Sphaerex™ (prothioconazole + metconazole), and a Syngenta confidential product. Fungicide timing included single applications at early flowering (FKS 10.5.1), 4-6 days after FKS 10.5.1, or sequential applications at FKS 10.5.1 followed by an additional application 4-6 days later. Durum was assessed for visual FHB symptoms at soft dough while yield, TW, and protein data were collected post-harvest. Data were analyzed using the PROC GLIMMIX procedure in SAS, with fungicide and year as fixed effects, and replicate as a random effect. Results showed that all fungicides protected yield and TW in 2022 and 2024. Levels of FHB varied across the three study years; however, it was consistently negatively correlated with yield. In 2022, the highest yield was in the treatments which received sequential fungicide applications, which were also those that had significantly reduced FHB index. In that year, single fungicide applications did not reduce FHB index compared to the untreated check. The sequential applications as well as single applications of Prosaro Pro, Miravis Ace and Sphaerex (FKS 10.5.1) suppressed FHB in 2023 while all fungicide treatments suppressed FHB in 2024. The delayed Sphaerex application timing (4-6 days post FKS 10.5.1) did not perform as well as the FKS 10.5.1 in FHB suppression in 2024 and yield in both 2022 and 2024. Grain protein levels showed no response to fungicides. This study suggests that under typical environmental conditions a single application timed at FKS 10.5.1 is sufficient to manage Fusarium Head Blight in western North Dakota.

Spray Application Technology for Fusarium Head Blight Suppression with Fungicides

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Spray application technology research conducted at North Dakota State University (NDSU) from the mid- to late-2000s informed NDSU Extension recommendations for the suppression of Fusarium head blight (FHB) with fungicides applied by air or ground. Despite the increased prevalence of large, high-clearance, self-propelled sprayers in North Dakota since that time, and concurrent advancements in spray application technology, NDSU Extension recommendations had not been updated since 2008. In the absence of contemporary NDSU research, we surveyed the literature to update recommendations for fungicide applications by ground. In this presentation, I will summarize the updated recommendations and highlight changes from the historical NDSU Extension recommendations. The most significant changes center around nozzle angle, spray quality, and water volume (i.e. droplet size). While historical recommendations were to use a single forward-facing nozzle applying a fine to medium spray quality at a water volume of 10 gallons per acre (gpa), the new recommendations are to use an asymmetrical backward- and forward-facing spray at a coarse spray quality and a water volume of 10 to 20 gpa. Changes in recommended practices are primarily due to differences in spray droplet aerodynamics when ground spraying operations are conducted at 6 miles per hour in calm wind conditions, as was the case in the 2000s era research, as opposed to the breezy conditions and elevated ground speeds common during present-day ground spraying operations in North Dakota. To close the presentation, I will discuss the lessons learned while revising the recommendations in relation to contemporary applied research on drone spraying.

Assessing Fusarium Head Blight Risk and Disease Impact: Comparative Analysis of On-Farm and Research Station Trials on FHB Index and DON

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Fusarium Head Blight (FHB) is a kernel disease affecting winter wheat production in the Northeast United States. The causal agent, *Fusarium graminearum*, reduces yield and produces deoxynivalenol (DON), which poses food safety risks. FHB is managed using an integrated disease management (IDM) approach, which includes moderately resistant varieties, rotating with non-host plants, monitoring disease risk, and applying fungicides only when predicted FHB risk is high. Trials for FHB management are conducted with support of the U.S. Wheat & Barley Scab Initiative. Trials focus on testing wheat varieties with different levels of resistance and fungicides applied at flowering. These trials provide valuable data for quantifying the long-term effect of FHB. Locations are monitored for FHB risk using the Fusarium risk tool (https://www.wheatscab.psu.edu/). Translating results from coordinated trials is valuable, but improving the quantification of FHB risk at the field scale is important. Nonetheless, on-farm fungicide efficacy for FHB management is rarely documented, and the Fusarium risk tool is not validated. Our field study aimed to compare fungicide efficacy in the management of FHB in small plot research trials with on-farm strip trials. We also aimed to validate the FHB risk tool at these locations in Pennsylvania. Trials were planted at two Penn State research farms (Russell E. Larson Agricultural Research Centre, Rock Springs (RS) and Southeast Agricultural Research and Extension Centre (SEAREC)) in October 2023 using susceptible wheat variety. Five on-farm trial locations were identified in the spring of 2024, and the cooperating farmers were provided with a fungicide of their choice. Farmer cooperators left a section of their field untreated during the fungicide application at early anthesis. Disease assessments were made for all trials at the soft-dough growth. At harvest, grain samples were obtained from treated and untreated plots and sent for DON analysis. Results indicated that FHB risk predictions were high at all locations during their respective flowering periods. Disease assessments indicated a high FHB-index (above 10%) at SEAREC (20.4%) and two on-farm locations (17.8% and 21.7%) in the untreated plots. In untreated samples, DON levels were greater than (or equal to) 1 ppm at SEAREC (3.2 ppm) and three on-farm sites (1.0, 3.2, 4.0 ppm). Fungicides significantly reduced FHB index and DON levels compared to the untreated checks at all locations except RS. These results indicate deviations from the predicted FHB risk at some locations, and the fungicides effectively controlled the FHB and DON at all locations.

Efficacy of Fungicide Applications for Fusarium Head Blight Control Across Barley Varieties

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Malting barley is a specialized variety of barley (*Hordeum vulgare*) expanding as an important crop in southern Delaware following continued growth of the craft brewing industry and the opening of a local malting facility. Malting and brewing industries require grain to meet strict quality standards that can be reduced due to disease. One of the most important diseases impacting malting barley is Fusarium head blight (FHB) primarily caused by the fungus *Fusarium graminearum*. FHB results in infected grain with deteriorated quality. Grain quality is further impacted by the production of mycotoxins like deoxynivalenol (DON), which may result in dockage or total rejection of harvested grain when present above threshold levels. Management of FHB relies on partial resistance, which is limited in current malting varieties, and the application of properly timed fungicides. As new varieties and fungicide products enter the market, testing is needed to evaluate their efficacy for management of FHB. Violetta was the most widespread variety planted in DE, but growers have been shifting to a newly released variety, Avalon. In this project, these two varieties were assessed in combination with four fungicide treatments, Prosaro®, Prosaro Pro, Miravis® Ace, and Sphaerex™ and two non-treated controls (one inoculated, one non-inoculated) to evaluate response of FHB. With the exception of plots for the non-treated non-inoculated control treatment, all plots were inoculated with colonized corn spawn. Plots were evaluated for FHB incidence, FHB severity, Fusarium damaged kernels (FDKs), yield, and for DON. Comparing the non-treated inoculated controls of both varieties, the %FDK and DON levels in Violetta were significantly higher than in Avalon. For Avalon, plots treated with Prosaro, Prosaro Pro, and Miravis Ace had significantly lower FHB incidence than the non-treated inoculated plots. Additionally, for Avalon, DON was significantly lower in plots treated with Prosaro Pro and Miravis Ace-compared to the non-treated inoculated plots. In Violetta, plots treated with Miravis Ace had significantly reduced FDK, and plots treated with Prosaro Pro, Miravis Ace, and Sphaerex had significantly reduced DON levels compared to the non-treated inoculated plots. Results indicate that reduced FHB and DON levels in Avalon compared to Violetta support the transition to this variety. Additionally, the use of Prosaro Pro and Miravis Ace applied at anthesis can improve crop quality by reducing FHB and DON levels for both varieties. This study provides practical insights for barley growers in Delaware that guide informed integrated approaches to manage FHB.

2024 Fusarium Head Blight Management: Efficacy of Various Application Regimen in South Dakota Spring and Winter Wheats

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Scab or Fusarium head blight (FHB) is a commercially important disease of wheat. The major causative pathogen of the disease is *Fusarium graminearum*, a fungus that can reduce kernels to shriveled and light tombstones thereby lowering yield and commodity quality. In addition, the tombstone kernels are loaded with mycotoxins such as deoxynivalenol (DON). The efficacies of several fungicides for managing scab were assessed under different application regimes. The treatments or application regimens included (i) a single application of Prosaro® (6.5 fl oz/ac), ME (10.3 fl oz/ac), Miravis® Ace (13.7 fl oz/ac), Prosaro Pro (10.3 fl oz/ac) and Sphaerex™(7.3 fl oz/ac) at early anthesis (Feekes 10.5.1), (ii) an application of two products in sequence, starting with an application at Feekes 10.5.1 and a second application at Feekes 10.5.3, finally (iii) a single application at Feekes 10.5.3 (complete flowering). A spring wheat variety, 'CP3188', and a winter wheat variety, 'Draper', were the susceptible hosts for this endeavor. The study comprised four blocks with each treatment randomly allocated to each of the blocks as a plot. Plots were inoculated at Feekes 10.1 (visible spikelets) with *F. graminearum* infected corn spawn at 42g m^{-2}. The generalized linear model was used to analyze FHB incidence, FHB severity and FHB index on beta distribution while yield and test weight were on gaussian. Observations showed the highest FHB incidence, severity and index in untreated plots and lowest yield as well as overall kernel quality (test weight) in both wheat classes. Although scab prevalence was over 20% higher in spring wheat than in winter wheat, efficacy results revealed congruency regardless of wheat class. The sequential application scheme involving Miravis Ace at Feekes 10.5.1 and Prosaro Pro at Feekes 10.5.3 had the lowest FHB index in both wheat classes. However, statistically significant ($p \leq 0.05$) differences between the highest performing application regimen and the rest of the treatments were not observed. Negative Spearman correlations coefficients between FHB index and yield (-0.42, $p<0.05$) and overall kernel quality (-0.66, $p<0.05$) were observed in winter wheat. Similarly, the associations between FHB index and yield as well as FHB index and kernel quality were inverse, -0.53 ($p<0.05$) and -0.63 ($p<0.05$), respectively. These observations encourage wheat producers to still apply fungicides at Feekes 10.5.1 up to Feekes 10.5.3 to reduce the potential impact of scab. Further analyses will be conducted to establish mycotoxins trends once DON data becomes available.

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Food Safety and Toxicology

Prevention, Mitigation and Detection of Mycotoxins in Wheat and Barley

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Fusarium graminearum fungal pathogens pose significant food safety concerns because they infect cereal crops and contaminate grain with harmful mycotoxins. Most *F. graminearum* strains in the U.S. produce the FDA regulated mycotoxin deoxynivalenol (DON), but an emerging NA3 population of *F. graminearum* produces the mycotoxin called NX with a slightly different chemical structure. Since little was known about the aggressiveness of NA3 strains and the function of NX during plant disease, ARS researchers in the Mycotoxin Prevention and Applied Microbiology Research Unit, Peoria, Illinois, compared disease development of the NA3 population to other North American *F. graminearum* populations. While NA3 strains typically spread more slowly in wheat, during initial infection they produced more mycotoxins in comparison to other populations. Furthermore, both NX and DON promote disease spread, but NX also has a unique function in enhancing pathogen initial infection. This work provides insights into the potential threat of emerging pathogen populations and mycotoxin types, but also identifies potential population specific control strategies.

Controlling *Fusarium* Contamination of Malting Barley with *Brassicaceae* Seed Meal Volatiles

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Mycotoxin accumulation during the malting of wheat and barley grain causes millions of dollars in annual losses for the U.S. malting and brewing industry. This research investigates the use of biofumigant treatments from plant derived metabolites to reduce grain *Fusarium* contamination and mycotoxin production during malting. Defatted seed meals from five glucosinolate-containing members of the *Brassicaceae* Family: *Brassica juncea, Brassica carinata, Lepidium sativum, Sinapis alba,* and *Thlaspi arvense* were used to fumigate inoculated wheat and barley. We found that naturally produced gaseous allyl isothiocyanate was able to control *Fusarium graminearum* growth and mycotoxin contamination without harming wheat or barley germination. Treatment of *Fusarium* contaminated barley with volatiles from defatted *Brassica juncea,* in simulated malting conditions, completely prevented mycotoxin accumulation. No residual isothiocyanates were detected on the malted barley post kilning. This research demonstrates an alternative valorization of *Brassicaceae* seed meal byproducts as potent inhibitors of fungal contamination during malting.

Photosensitization for the Inactivation of *Fusarium* spp. In Vitro and in Wheat

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Fusarium spp. is one of the most dangerous fungal pathogens, causing substantial economic losses worldwide by reducing crop yield and producing mycotoxins. Photosensitization is a novel, non-thermal, and effective technology for inactivating fungi. However, there were not many studies on controlling *Fusarium* growth and mycotoxin production using photosensitization. In this study, we investigated the effects of three natural photosensitizers, including menadione sodium bisulfite (MSB), coumarin and curcumin, on inhibiting the growth of *Fusarium graminearum* both in vitro and in wheat in the field. In general, photosensitization effectively inhibited spore germination and mycelial growth of the two tested *F. graminearum* isolates (10-124-1 and 10-125-1) in a dose-dependent manner, with MSB showing a more pronounced effect. For example, 50% spore germination inhibition rates (EC_{50}) were observed at 3.07 mM/mL and 1.61 mM/mL, respectively, after treating with MSB and coumarin, along with UV light exposure at 233 J/cm^2 for 30 mins in isolate 10-124-1. According to results from scanning electron microscopy (SEM), fluorescence spectroscopy, and confocal laser scanning microscopy (CLSM), the generated intracellular reactive oxygen species (ROS) oxidized lipid and/or protein in cell membranes. This led to the observation of shrunken spores and mycelial with rough, corrugated surfaces due to significant loss of the cytoplasmic matrix. Furthermore, the impact of PS on inhibiting *Fusarium spp.* growth and mycotoxin production in wheat in the field was investigated. The susceptible wheaton cultivars were planted in 20 rows following a randomized complete block design (RCBD) with five blocks. During the flowering stage, the wheaton cultivars were point inoculated with a mixture of *Fusarium spp.* and the application of photosensitizers (curcumin, MSB, and coumarin) was applied at two different timings of a day after one day of point inoculation. The FHB scoring data was recorded after 17 days and 21 days of infection. Although the results of FHB scoring didn't show statistically significant reductions in Fusarium infection at $P < 0.05$ by the applications of three PS. However, some data points of FHB scoring have shown promising results due to the application of curcumin and MSB. In addition, FHB scoring data was correlated with the mycotoxin data. For example, DON has shown significant lower level at $p < 0.05$ for curcumin treated seeds as compared to control. In addition, spraying time also plays an important role on mycotoxin production. NIV demonstrated significant differences at $p < 0.05$ for three different PSs compared to control samples in morning-sprayed samples, as opposed to those in the evening.

Acknowledgement and Disclaimer

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Fusarium and Barley Interaction: Impact on Malt Quality and Soluble Arabinoxylans Characteristics

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Fusarium infection in barley remains a persistent challenge for the brewing industry, primarily due to the pathogen's ability to re-grow and proliferate during the malting process. This study investigated the multifaceted impacts of *Fusarium* infection on malt quality and the characteristics of soluble arabinoxylan (AX), including the content of ferulic acid bound to AX, since the oxidative crosslinking of ferulic acid within AX is associated with the resistance mechanisms of host plant against pathogen infection. Three barley varieties, 'AAC Synergy', 'Colon', and 'S2M184', cultivated in yield trials (Control) and *Fusarium* head blight (FHB) nurseries (Treatment) in St. Paul and Crookston, MN, were used as raw materials. The barley samples were processed into malt and subsequently into wort under standardized conditions. In the control samples, the deoxynivalenol (DON) content in both barley and malt were below 0.6 µg/g. However, in FHB-infected barley and malt, the DON content ranged from 1.7 to 17.5 µg/g. The analysis of wort demonstrated that *Fusarium* infection led to a significant increase in the molecular weight of AX, with an average increase of 46.8%, and values ranging from 8.2% to 127.9%. Conversely, the content of bound monomeric ferulic acid showed a marked decrease, ranging from 13.2% to 50.6%, depending on the barley variety and cultivation plot. This reduction is likely associated with the oxidative crosslinking within AX post-infection, driven by the potential formation of ferulic acid dimers, which may have also contributed to the observed increase in the molecular weight of AX. Notably, the variations in AX molecular weight and bound ferulic acid content in wort correlated closely with their DON levels in barley. Further, *Fusarium* infection resulted in the marked reductions in β-glucan content (average reduction of 75.51%) and α-amylase activity (average decrease of 23.77%), alongside a significant increase in wort color. These findings underscore the complex biochemical consequences of *Fusarium* infection on brewing quality and highlight the necessity for effective management practices to control infection in barley production.

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Calli in Focus: A Novel Lens for Mycotoxin Detection

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This research project aims to address the need for improved early pathogen detection technology by obtaining foundational data for the design of a plant tissue culture-based reporting system to be utilized in an obligate phytopathogen aerosol sampling device. We investigated the feasibility of using calli (masses of undifferentiated plant tissue) as a conduit for mycotoxin quantification. *Arabidopsis thaliana* exhibits similar *Fusarium* disease progression and symptomology to typical cereal grain hosts, and its rapid growth and smaller size were cost-effective for this study. In this experiment, deoxynivalenol (DON) was isolated from plant tissues and calli of resistant, intermediate, and susceptible accessions of *Arabidopsis* after 7, 14, and 21 days-post inoculation (DPI) with *Fusarium.* Fusarium-Arabidopsis Disease (FAD) ratings were recorded to assess visual symptoms in the plants. We hypothesized that higher DON concentrations would be detected in tissues of the susceptible *Arabidopsis* accession and at 21 DPI. From the first biological replicate, we observed general trends of higher DON concentrations in the susceptible plant tissues, and increased DON concentrations with elapsed time. In seeds, the susceptible accession of Arabidopsis had the highest level of DON, and the intermediate accession had the lowest across all three time points. The same trend was observed in flowers at 7 and 14 DPI, but the resistant accession had the highest DON at 21 DPI. FAD ratings generally increased with elapsed time after inoculation and susceptibility. The levels of DON extracted from calli in this first replicate appear inconsistent. These data reflect the first replicate of this experiment, and more trials will be conducted in the future. During additional replicates, we plan to quantify fungal biomass as an indicator of disease severity in calli. In the future, we will take advantage of incorporating RUBY, as a pathogen-inducible reporter, into the customizable system for detecting pathogens.

Transformation of Deoxynivalenol to Deoxynivalenol-3-Glucoside and Gene Expression during Malting

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The widespread occurrence of Fusarium head blight (FHB) has raised considerable concerns about the risk of mycotoxins in grain-based food. Deoxynivalenol (DON) is the most common trichothecene, and its production in North America is primarily associated with *Fusarium graminearum*. DON can be conjugated in different forms and the conjugated forms are less toxic than DON in general. Recent surveys of commercial beers have shown that DON and its conjugate, DON-3-glucoside (D3G) were the frequent contaminants and the main sources of this contamination were brewing grains, including malt and cereal adjuncts. The transformation of DON to D3G occurs from the field through food processing and is closely linked to Type II resistance of host plants against *Fusarium* infection. That is why investigating the transformation of DON to D3G is essential not only for food safety and regulatory compliance but also for evaluating the efficacy of crop resistance strategies. The goal of our study was to explore the transformation during malting by quantifying DON and D3G and measuring the expression of *Fusarium Tri5* gene and barley *HvUGT13248* gene, which are related to the transformation. The *Fusarium Tri5* gene is responsible for the production of DON by encoding a crucial enzyme in the trichothecene biosynthesis pathway and the *HvUGT13248* gene in barley is responsible for the transformation by encoding a barley UDP-glycosyltransferase (UGT) specifically catalyzing the conjugation of DON to D3G. FHB-infected barley samples with three varieties, ‘AAC Synergy,’ ‘Conlon,’ and ‘S2M184’ (St. Paul, and Crookston MN) were used. In all samples, DON and D3G were considerably washed off during steeping but reproduced during germination. The DON and D3G level decreased significantly after steeping by an average of 80% and 58%, respectively; however, after germination, it increased significantly by an average of 39% and 50%, respectively. Compared with the germinated barley without FHB infection (control), the *HvUGT13248* gene expression was not significantly regulated by steeping, but it was significantly upregulated by germination. *Fusarium Tri5* gene expression was upregulated by either steeping or germination varied by varieties.

Challenges and Future State of Mycotoxin Analysis from a Regulatory Perspective

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Mycotoxins are naturally occurring toxins produced by certain fungi. Exposure to mycotoxins may occur through the consumption of contaminated foods or from animals that are fed contaminated feed. To safeguard the nation's food supply, the U.S. Food and Drug Administration (FDA) regulates and monitors mycotoxins in food and conducts research on how best to detect and prevent mycotoxin contamination. Mycotoxin analysis is at the center of the mycotoxin program – concentration data are needed for determining the occurrence of regulated and toxic mycotoxins in food and feed products, estimating dietary exposure, the establishment of guidelines for non-regulated mycotoxins, and accumulating historical data to explore the distribution of mycotoxins as a function of agricultural and food processing practices, geographic location, and environmental conditions. This presentation will discuss the FDA's continuous efforts to develop and incorporate fit-for-purpose analytical tools for mycotoxin analysis with a focus on the relationship between analytical methodologies and scientific assessments. The presentation will further highlight the Agency's proactive approaches to address challenges in sampling and analytical method development. Lastly, as environmental factors (e.g., weather, humidity, climate change) and anthropogenic activities (e.g., agricultural and food processing practices) continue to change, affecting the distribution of mycotoxins in foods, the presentation will explore future possibilities to develop new analytical tools to meet emerging regulatory needs.

Gene Discovery & Engineering Resistance

Understanding Substrate Specificity and Function of the Barley UDP-Glucosyltransferase UGT13248 During Fusarium Head Blight

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Fusarium Head Blight of wheat and barley is caused by the fungal pathogen *Fusarium graminearum*. Various *F. graminearum* isolates produce trichothecene mycotoxins such as Deoxynivalenol (DON), Nivalenol (NIV) and NX-2. Barley has been shown to have broad type-2 resistance to *F. graminearum*. The barley UDP-Glucosyltransferase UGT13248 detoxifies the major *F. graminearum* mycotoxin DON by conversion to the glucoside Deoxynivalenol-3-O-glucoside (D3G). UGT13248 confers type-2 resistance to *F. graminearum* (PH-1) by limiting fungal spread within the barley spike after point inoculation. Dip inoculation of barley spikes with *F. graminearum* (PH-1) resulted in equal numbers of diseased plants and initial infection points between UGT13248 mutants and wild-type sister lines, while the total number of diseased spikelets was strongly increased in UGT13248 mutants. This suggests that UGT13248 confers only type-2, not type-1, resistance to *F. graminearum.* RNAseq experiments showed that expression of plant immune signaling genes were induced earlier after treatment with *F. graminearum* in UGT13248 mutant plants compared to wild-type sister lines. We are currently using confocal laser scanning microscopy to study the spread of *F. graminearum*, as well as induction of the trichothecene production pathway in wild-type and UGT13248 mutant plants. Further, different *F. graminearum* isolates produce specific trichothecene mycotoxins. UGT13248 glucosylated the trichothecenes DON, NX-2 and NIV. UGT13248 mutant plants were significantly more susceptible to *F. graminearum* isolates producing 15-Acetyl-DON (15-ADON), 3-ADON, and NX-2 compared to wild-type sister lines. This suggests multiple trichothecene mycotoxins act as virulence factors in *F. graminearum* and can be detoxified by UGT13248 resulting in increased barley resistance to these different *F. graminearum* isolates.

Discovery and Mapping of FHB-resistant Mutations in a Susceptible Wheat Variety 'Jagger'

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Genetic resistance against Fusarium Head Blight (FHB) in wheat is quantitative in nature, with over 550 quantitative trait loci (QTLs) identified for FHB severity and DON content. Despite extensive breeding efforts, achieving high levels of FHB resistance remains a significant challenge due to the impact of numerous small-effect QTLs. This study investigates alternative strategies for improving FHB resistance in wheat. We focused on 'Jagger', a hard red winter wheat cultivar, known for its high yield, and better quality but, inherent susceptibility to FHB. A forward genetic screen was performed on 840 M4 mutagenized lines derived from an EMS-mutagenized Jagger population across multiple years in field conditions, followed by confirmation under controlled environment conditions. Ten mutant lines consistently exhibited significantly lower FHB severity, with seven of these lines also showing significantly reduced DON levels. The identified resistant mutants were crossed with FHB-susceptible Jagger wild type to develop MutMap populations. Screening of the F2 population derived from one of the mutants (2020GH-271) identified an 8-10 Mb interval on the short arm of chromosome 2D associated with FHB resistance Future work is underway to fine-map the candidate region to support breeding efforts for improved FHB resistance.

Barley Genetic Engineering Facility for FHB Research Community

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Genetic engineering technology has emerged as a powerful tool to elucidate mechanisms of plant disease resistance and develop disease resistant plants. This technology has potential in the battle against *Fusarium graminearum* (*Fg*) in barley, especially as no resistant barley cultivars are currently available. Nevertheless, the potential of this technology is constrained by the ability to transform and regenerate gene-edited plants *in vitro*, with barley proving to be particularly challenging. The established protocols for producing genetically engineered barley are genotype dependent, exhibit low efficiency, and are labor-intensive. To address this issue, we established the Barley Genetic Engineering Facility for FHB Research Community since 2022. This facility, supported by the USWBSI, provides services to develop tissue culture protocols for customer-based barley cultivars and for optimizing transformation efficiency to produce transgenic barley plants with customer-provided constructs. After testing different explants such as mature barley seeds to save the time to grow up plants with immature embryos, or germinated seedlings to provide the meristematic tissues, we demonstrate that immature scutellum explant proves to be the best material for the production of stable transgenic barely plants. We have shown that different barley cultivars require different hormone regimes at the initial stage to produce regenerable embyogenic calli. We have developed the tissue culture protocols for several barley cultivars, including the two-rowed spring barley “Genesis”, the winter cultivar “Thunder”, and the six-rowed barley “Morex”. We have been able to generate barley embryogenic calli in 3 weeks to be transformed either by gene gun bombardment or by *Agrobacterium*. In 8 weeks, transformed barley calli can be selected and transgenic plantlets can be regenerated. Aided with our improved barley transformation and regeneration protocols, we have been able to transform Morex embryogenic calli with our integrating CRISPR-gene editing vector and produce transgenic Morex plants that are phenotypically normal and producing seeds. Our optimized barley tissue culture and transformation protocols will aid in the production of transgenic and gene-edited barley plants for the FHB Research Community.

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Characterization of a Mycotoxin Trichothecene Transporter in *Arabidopsis thaliana*

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Fusarium graminearum causes Fusarium head blight (FHB) on wheat and produces the trichothecene mycotoxin deoxynivalenol (DON) that promotes FHB spread and contaminates grains. Approaches that detoxify DON can lead to reduced FHB and mycotoxin contamination. Detoxification and excretion of toxic compounds are important mechanisms for organism self-protection. Our previous study showed that transgenic *Arabidopsis thaliana* expressing a fungal self-protection gene, *F. graminearum* 3-O-acetyltransferase (*FgTRI101*) converted mycotoxin DON to the less toxic 3-acetyl-DON (3-ADON) and excreted 3-ADON out of plant cells to protect plant growth and development. In this study, our goal is to identify the transporters for 3-ADON excretion and utilize them to enhance wheat FHB resistance. Transcriptomic studies were conducted in FgTri101 transgenic *A. thaliana* seedlings treated with DON (50 mg/L, 24 h) and untreated controls. Three transporter genes, including two *A. thaliana* detoxification genes (*AtDTX1* and *AtDTX3*) and one ABC transporter (*ABCB4*), were upregulated by DON in RNA seq analyses and confirmed by RT-qPCR as well. *AtDTX1* has been identified as an efflux carrier for alkaloid, antibiotics and toxic compounds. Therefore, an *A. thaliana* mutant line with *AtDTX1* disrupted was obtained. Toxicity analysis showed *Atdtx1* mutant seedlings were sensitive to DON, suggesting the *Atdtx1* mutant line lacks self-protection. Further, *FgTRI101* was introduced into the *Atdtx1* mutant line by floral dip transformation. Transgenic *A. thaliana* plants expressing *FgTR1101* in the *Atdtx1* mutant background were obtained and used for further analysis. Our data showed that *AtDTX1* and at least one additional transporter are involved in 3-ADON excretion. Further identification of the second potential transporter for 3-ADON are underway.

Acknowledgement and Disclaimer

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Loss of *NPR3* and *NPR4* Function Enhances Resistance to the Fungal Pathogen *Fusarium graminearum* in *Arabidopsis* and Wheat

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Salicylic acid (SA) is best known for orchestrating plant immune responses. SA signaling invokes the SA receptor NPR1 (Non-expresser of Pathogenesis-Related genes 1), which has an important role in plant defense against pathogens. SA contributes to resistance against *Fusarium graminearum* (*Fg*), which is the causal agent of Fusarium head blight (FHB) disease in wheat and barley, that can also infect leaf and floral tissues of *Arabidopsis thaliana* under laboratory conditions. NPR3 and NPR4 which are structurally related to NPR1, counteract the activation of NPR1-mediated defenses, by targeting NPR1 protein turnover and the suppression of transcription factors involved in defense gene expression. Knockdown of *NPR3* and *NPR4* results in enhanced resistance to some pathogens, confirming that they are susceptibility factors. To test whether similarly, *NPR3* and *NPR4* function as susceptibility factors for *Fg* infection in *Arabidopsis* and wheat, we tested the response of *Arabidopsis* and wheat plants containing mutations in the corresponding *NPR3* and *NPR4* genes for their response to *Fg*. We observed that the *Arabidopsis npr3/npr4* mutants exhibited a hypersensitive-response (HR)-like phenotype that was accompanied by reduced fungal biomass accumulation and an increase in reactive oxygen species levels, expression of the SA-responsive defense gene *PR1* and ion leakage. The inflorescence of *npr3/npr4* mutant also exhibited enhanced resistance to *Fg* compared to wild-type plants. Similarly, missense mutations in wheat *NPR3* (*WhNPR3*) and *NPR4* (*WhNPR4*) promoted resistance to FHB, which was accompanied by reduced mycotoxin accumulation. The ability to knockdown *NPR3* and *NPR4* function to promote resistance to FHB in wheat holds promise for developing non-GMO approaches utilizing natural variants with missense and nonsense mutations that knockdown the activity of *WhNPR3/WhNPR4*. These natural variants could provide novel genetic material for integration into FHB resistance breeding programs.

The *Fusarium graminearum* Effector Protease FgTPP1 Suppresses Immune Responses and Facilitates Fusarium Head Blight Disease

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Most plant pathogens secrete effector proteins to circumvent host immune responses, thereby promoting pathogen virulence. One such pathogen is the fungus *Fusarium graminearum*, which causes Fusarium Head Blight (FHB) disease on wheat and barley. Transcriptomic analyses revealed that *F. graminearum* expresses many candidate effector proteins during early phases of the infection process, some of which are annotated as proteases. However, the contributions of these proteases to virulence remains poorly defined. Here, we characterize a *F. graminearum* endopeptidase, FgTPP1 (FGSG_11164), that is highly upregulated during wheat spikelet infection and is secreted from fungal cells. To elucidate the potential role of FgTPP1 in *F. graminearum* virulence, we generated *FgTPP1* deletion mutants (*ΔFgtpp1*) and performed FHB infection assays. While the number of completely bleached spikes infected by *F. graminearum* wild-type reached 50% of total infected spikes, the number of fully bleached spikes infected by *ΔFgtpp1* mutants was 25%, suggesting FgTPP1 contributes to fungal virulence. Transient expression of green fluorescent protein (GFP)-tagged FgTPP1 revealed that FgTPP1 localizes, in part, to chloroplasts and attenuates chitin-mediated activation of mitogen-activated protein kinase (MAPK) signaling, reactive oxygen species production, and cell death induced by an autoactive disease resistance protein when expressed *in planta*. Notably, the FgTPP1 protein is conserved across the *Ascomycota* phylum, making it a core effector among ascomycete plant pathogens. These properties make FgTPP1 an ideal candidate for decoy substrate engineering, with the goal of engineering resistance to FHB, and likely other crop diseases caused by ascomycete fungi.

Marker-Free Transfer of *Fhb7* to Barley

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Scab is a devastating disease in wheat barley. Considerable progress has been made in understanding and improving host resistance in wheat with molecular cloning of the major QTL *Fhb1* and *Fhb7*; however, similar research with barley has lagged due to the lack of highly resistant genotypes, which makes it difficult to effectively control FHB and DON contamination. Supported by the USWBSI-TRSC program, we are developing marker-free transfer of *Fhb7*, encoding a glutathione S-transferase functioning in the detoxification of mycotoxins including DON, to barley via CRISPR-mediated targeted gene insertion. We used chemically modified *Fhb7* donor DNA and a CRISPR–Cas9 construct targeting the *mlo* locus to transform the immature embryos of the elite two-rowed malting barley cultivar Excelsior Gold (EG). From about 300 T_0 plants, we identified 13 *Fhb7* insertion lines. We also developed 20 transgenic plants in Gold Promise (GP) carrying two T-DNA cassettes, one for *Fhb7* gene and another for transformation selection marker *HygR* by *Agrobacterium* mediation. We have obtained marker-free *Fhb7* transgenic plants in EG and GP in the T_1 generation. Detached leaf assay showed Fhb7 function in resistance to *Fusarium graminearum*. Two EG transgenic lines together with non-transgenic EG as a control were tested for FHB resistance in the field and greenhouse conditions. The results showed that Fhb7 reduced FHB serenity by up to 64%. We are in the process of characterizing the insertion junctions and engineering *Fhb7* for new alleles.

Genetic Dissection of Mycotoxin Variation in Barley

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Fusarium head blight (FHB) is a devastating disease in barley because grain becomes unusable for food, feed, forage or malt due to the production of mycotoxins, for example - deoxynivalenol (DON). In barley, only partial resistance to FHB is currently available and much of that resistance is due to phenological or morphological avoidance. Increased levels of DON not only spoil grain but aid in pathogen infection. Our research aims to genetically dissect the variation in DON in a barley biparental mapping population. We evaluated a population of 117 RILs from a cross between Bearpaw and Pinnacle. Although the parents were not significantly different for FHB severity, they did vary for the presence of DON, with Bearpaw tending to have less. Through initial screening of the population at Eastern Agriculture Research Centre, Montana and DON testing at the University of Minnesota, we observed variation in DON among the RILs. The population was screened using the 50K SNP chip and a genetic linkage map of 4434 SNP markers built. Preliminary QTL analysis identified 4 QTL associated with variation in DON on chromosomes 4, 5, 6 and 7, none of which coincide with heading date QTL on chromosomes 1, 2 and 5. Three of the alleles from the Bearpaw parent were associated with decreased DON at QTL on chromosomes 4,6 and 7, but the Bearpaw allele was associated with increased DON on chromosome 5. On examining the QTL regions, we found known disease resistance genes underlying QTLs on chromosomes 5 and 6. We will re-evaluate the experiment in a replicated trail in 2025. By pyramiding alleles associated with decrease in DON, we hope to decrease the impact of FHB for barley growers and end-users.

Key words: Fusarium head blight, deoxynivalenol (DON), barley, *Fusarium graminearum*, QTL, SNP marker

Differential Transcriptomics Identifies the Key Wheat Genes and in Planta-Expressed Fungal Genes Associated with *Lpx3*-Conferred Resistance to Fusarium Head Blight

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In recent years, Fusarium head blight (FHB) has become a widespread disease that significantly impacts wheat yield and quality. Gaining insights into the molecular basis of wheat resistance to FHB is essential for developing effective disease management strategies. Our study has identified a susceptibility gene, *Lpx3*, associated with the 9-lipoxygenase (9-LOX) pathway, which plays a role in oxylipin biosynthesis and may influence fungal growth and cross-kingdom interactions. During *Fg* infection, wheat cells undergo dynamic shifts in gene expression, creating complex host-pathogen interactions. To explore the mechanisms of resistance conferred by *Lpx3* knockdown, we characterized the impact of lipoxygenase derived products on fungal growth and virulence along with discovering the transcriptional networks underlying the impact of *Lpx3* on fungal invasiveness. Differential expression analysis uncovered specific defense responses in FHB-resistant *Lpx3*-knockdown line, including upregulation of genes involved in polyketide biosynthetic processes. Additionally, *F. graminearum* showed varied expression of mycotoxin biosynthesis pathways during infection, allowing us to identify potential pathogenicity factors. Pharmacological experiments showed that the direct application of one of the oxylipin, 9-HOT restored the susceptibility in *Lpx3*-knockdown lines, as well as enhanced the expression of mycotoxin biosynthesis genes when added to liquid culture of fungus, but it did not have a direct impact on fungal growth on potato dextrose agar plates. Taken together, these results serve as a foundation for further study on the regulatory mechanism of wheat-*Fg* interactions as well as enhanced resistance resulting from *Lpx3* knockdown.

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Analysis of a Wild Barley Introgression Line Population for Resistance to Fusarium Head Blight and Deoxynivalenol Accumulation

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Disease resistance is an important component of an integrated strategy to manage Fusarium head blight (FHB) disease and to prevent the accumulation of mycotoxins in the grain. Extensive evaluations of barley germplasm for FHB resistance have been conducted over the past two decades but resulted in the identification of only a limited number of accessions with partial resistance. The wild progenitor of barley (*Hordeum vulgare* ssp. *spontaneum*) is a rich source of disease resistance for breeding. A set of wild barley introgression lines (S42IL) was developed by crossing the wild barley accession ISR42-8 from Israel with the German malting cultivar Scarlett. This population was subsequently genotyped with the 3K SNP chip to characterize the introgressions from ISR42-8 in the Scarlett background. Evaluations of this population (N=64) over three years at two sites (Crookston and St. Paul) in Minnesota with multiple replications (2-3) revealed a wide range of variation with respect to FHB severity (0.45% to 50.67% with an overall mean of 11.6%) and DON accumulation (0.47 to 42.3 ppm with an overall mean of 9.3 ppm). For Scarlett, the mean and range of FHB severity was 8.6% and 2.8%-14.9% and for DON accumulation 8.5 ppm and 1.3-15.8 ppm, respectively. No data were obtained for ISR42-8 because it did not produce robust plants in the field. A linkage map for the S42IL population was constructed with the 3K data using IciMapping software. The analysis revealed 12 QTLs for FHB severity in at least one environment: ten on chromosome 2H, one on 4H, and one on 5H. All but two of these FHB QTL co-located with heading date, suggesting a pleiotropic effect. Fifteen QTLs for DON accumulation were found in at least one environment: one each on chromosomes 2H and 5H, two on 4H, three on 6H and seven on 7H. One DON QTL on 7H was observed over multiple environments. None of the QTLs identified for DON co-located with QTLs for FHB or any agro-morphological trait. Scarlett contributed the favorable allele for lower FHB and DON in six of 12 and 12 of the 15 identified QTLs, respectively. ISR42-8 contributed four favorable alleles for low FHB on 2H and three favorable alleles for low DON on 7H. Introgression lines S42IL-124, S42IL-126, S42IL-176, and S42IL-102 exhibited the lowest DON levels across the population and may be useful for breeding programs for malting barley.

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A Forward Genetics Approach to Identifying Novel Sources of FHB Resistance and Susceptibility in Wheat and Barley

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Fusarium Head Blight of wheat and barley is a complex and ever evolving disease. Resistance to fungal progression is under the control of multiple plant genes of varying effectiveness. The major QTL FHB1 provides the best resistance to fungal progression, although it does not appear to facilitate resistance to initial infection nor is the consequence of FHB1 expression a complete elimination of FHB spread. Novel sources of FHB resistance are in constant need and the identification and characterization of sources of susceptibility may also aid in the development of more stable resistant wheat and barley lines. Over the past 3 years our lab has screened through roughly 400 lines of an EMS mutagenized family of wheat cultivar Kronos and another roughly 3000 lines of an EMS mutagenized family of barley cultivar Conlon. From that initial set of mutagenized Kronos lines, we have identified 30 lines as being more susceptible and another 25 lines as being more resistant than the parental line. These mutagenized Kronos lines have been retested to verify their phenotype and we have identified 12 lines which are reproducibly susceptible and another seven which are reproducibility resistant to FHB. To identify the map locations of the increased resistance, we are developing populations from the crosses of the resistant lines to the wheat cultivar Langdon. We have also initiated a search for Kronos lines with predicted deleterious mutations in metabolic pathway genes known to be up-regulated during *Fusarium graminearum* infection. Similarly, we identified 45 and 83 lines of the mutagenized Conlon family as being either more resistant or more susceptible than the controls, respectively. These resistant and susceptible lines will be retested in the summer of 2025 to verify their phenotype and to begin narrowing down potential target lines for further analysis.

Identification and Characterization of Fusarium Head Blight Susceptibility Genes in Durum Wheat

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Durum wheat is highly susceptible to Fusarium head blight (FHB), a destructive fungal disease mainly caused by *Fusarium graminearum*. It has narrow genetic variation for FHB resistance, and breeding programs have been challenged by the lack of reliable resistance sources within the elite gene pool. Moreover, studies have indicated the presence of susceptibility genes/factors in the durum wheat genome, which may further make the crop more susceptible to FHB. Generating mutant populations and analyzing the effect of gene mutations is a useful approach for identifying novel genes involved in plant disease susceptibility. A durum wheat mutant population was previously developed through EMS treatment of the cultivar 'Kronos' and characterized by exome capture and resequencing to detect the mutated genes. In this study, we evaluated 500 M4 mutants from this population across multiple greenhouse experiments to identify candidate genes involved in FHB susceptibility in durum wheat. Our results showed significant differences in FHB severity among the examined Kronos mutants, ranging from resistant to completely susceptible. We identified 11 mutant lines that consistently showed more resistance with average disease severity (DS) ≤ 33% to FHB compared to the wildtype Kronos (average DS = 70%). Analysis of all genes mutated in the selected resistant mutants using the information obtained from the wheat TILLING database (https://dubcovskylab.ucdavis.edu/wheat-tilling) identified 22 genes with non-synonymous or nonsense mutations shared across at least three resistant mutants. Our ongoing work focuses on knocking out the identified candidate genes in durum wheat through the wheat × maize hybridization coupled with genome editing method that has been established in our lab. This research will provide new insights into genes related to FHB susceptibility and facilitate breeding durum wheat cultivars with improved FHB resistance.

Targeted Mutagenesis of Two *Fhb7* Alleles in Common Wheat through Wide Hybridization with Transgenic Maize Expressing Cas9 and Single guide RNA

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Fhb7 is an FHB resistance gene derived from chromosome 7E of tall wheatgrass species *Thinopyrum ponticum* (2n = 10x = 70) and *Th. elongatum* (2n = 2x = 14). *Fhb7*^{Thp} was the first allele of *Fhb7* cloned from *Th. ponticum*, which encodes a glutathione S-transferase (GST) and confers FHB resistance through detoxifying trichothecene. This allele has been integrated into wheat chromosome 7D by wide crosses and homoeologous recombination. More recently, a novel allele (*Fhb7*^{The2}) of *Fhb7* from *Th. elongatum* has been transferred to wheat chromosome 7B by translocation, which can be used for FHB resistance improvement in both common and durum wheat. To verify the function of GST in the *Fhb7* resistance, we conducted targeted mutagenesis of the GST-encoding alleles through wide hybridization between wheat lines carrying *Fhb7* and transgenic maize expressing Cas9 and single guide RNA (sgRNA). We generated transgenic maize plants by *Agrobacterium*-mediated transformation using a binary vector expressing Cas9 and sgRNA for targeting the *Fhb7* alleles. Three *Fhb7*-carrying wheat lines, named CS-*Fhb7* with *Fhb7*^{Thp} and PI 702949 with *Fhb7*^{The2} both in Chinese Spring background, and FW23-09-80 with *Fhb7*^{The2} in elite hard red spring wheat (HRSW), were used as female parents for pollination with the transgenic maize plants and a total of 170 haploid plants were generated through embryo rescue. PCR amplification and Sanger sequencing indicated that 22%, 24% and 13% of the haploid plants derived from CS-*Fhb7* (*Fhb7*^{Thp}), PI 702949 (*Fhb7*^{The2}), and FW23-09-80 (*Fhb7*^{The2}), respectively, had mutations at the target sites. Doubled haploid (DH) mutant lines were produced by colchicine treatment of the haploid plants and evaluated for FHB resistance in greenhouse. The results indicated that DH lines with *Fhb7* alleles mutated were significantly more susceptible to FHB compared to their wild types carrying the original *Fhb7* alleles. Our study not only confirmed the important role of GST in FHB resistance for both *Fhb7*^{Thp} and *Fhb7*^{The2} alleles, but also demonstrated the power of the wheat × maize hybridization combined with genome editing technology in functional characterization of genes in wheat.

Acknowledgement and Disclaimer

This material is based upon work supported by North Dakota Wheat Commission and the U.S. Department of Agriculture under Agreement No. 59-0206-2-156. This is a cooperative project with the U.S. Wheat & Barley Scab Initiative (USWBSI). 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.

Mapping the Spatial Expression Atlas of Wheat's Cellular Defense Against FHB

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Fusarium graminearum (*Fg*) interaction with wheat in an infected spikelet alters core plant immunity via modulating resistance and susceptibility genetic factors and allows opportunistic expression of *Fg*'s effectors and mycotoxin-producing genes, adding to the already ongoing complex and asymmetric gene expression in developing grain. This interaction can result in alternative host responses depending on pathogen virulence and host resistance status. *Fhb1* is the most effective and stable source of deployed Fusarium head blight (FHB) resistance in wheat and primarily follows FHB Type II resistance, which prevents the spread of infection from affected spikelets. However, the resistance conundrum includes varied *Fhb1* effectiveness in diverse genetic backgrounds and the confounding spatiotemporal activation and/or suppression of more than one functional gene family that contribute to *Fhb1* resistance phenotype. Thus, this genetic complexity piqued the interest of our collaborative group to create an unbiased, spatially resolved transcriptomics cell atlas during wheat-*Fg* interaction and answer a few of above-mentioned questions. Spatially resolved tissue specific transcriptomics and targeted spatial gene expression approaches are powerful gene network discovery approaches that can simultaneously capture inaccessible view of tissue biology to truly explore the conserved transcriptional reprogramming or genetic factors driving FHB resistance, which are often overlooked in bulk analysis techniques. Our group has taken initial steps and is currently utilizing spring wheat varieties, i.e., the *Fhb1*-carrying variety 'Brick' and a susceptible control 'Boost', inoculated with fluorescently tagged *Fg* strain PH-1 that derives GFP expression through *Tri5* promoter to optimize the 10x Genomics Visium spatial assay. A wild-type PH-1 strain was also initially used for assay optimization. We collected fresh-frozen, 10 µm-thick 'spikelet-rachis-spikelet' mega tissue sections at 5 days post-point inoculation and achieved fitting it in a 5mm fiducial frame on Visium gene expression slide. This fiducial frame contains ~5000 barcoded capture spots for sequencing. At this stage, we have also optimized the balance between the complexity of microscopy and RNA stabilization, a major bottleneck in capturing the plant-pathogen interaction simultaneously during a spatial expression assay. We are currently processing these tissue sections for RNA release optimization and sequencing library preparation. We aim to spatially resolve the intertwined and complex orchestra of gene expression interaction between diverse pathogens and plant tissues in infection court leveraging wheat's cellular defense against FHB as a prioritized model.

Proximity-dependent Biotinylation of Proteins – A Versatile Tool to Identify Effectors and Their Potential Targets in Plants

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Proximity-dependent biotinylation technologies are versatile tools to unravel interactions between macromolecules in vivo. The approach relies on the inducible activity of enzymes (biotin ligases or peroxidases) that promiscuously biotinylate macromolecules within a short range (10-20 nm). For this presentation, typically, the enzyme (biotin ligase) is fused to a protein of interest and expressed in living cells (fungi, plants) to biotinylate the amino acids of binding partners in proximity. The biotinylated molecules can then be easily affinity purified under denaturing conditions and analyzed by mass spectrometry. This approach has numerous advantages over the standard affinity purification strategies, such as immunoprecipitation and GST pull-downs, etc., including the identification of transient interactions as well as interactions among low-expressed molecules. This presentation will give examples of the use of BioID in both plants and fungi *(F. graminearum*) to identify effectors and their targets.

Transcriptomic Study to Reveal Genes Governing Fusarium Head Blight Resistance in Contrasting Durum Genotypes

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Fusarium head blight (FHB), mainly caused by *Fusarium graminearum (Fg),* is a devastating fungal disease in wheat that causes significant damage by compromising both the yield and the quality. In addition, mycotoxin contamination in the infected grains can cause severe health risks to both humans and animals. Although there are useful genetic sources of FHB resistance in common wheat, this is very limited for durum wheat. To explore and identify useful genetic variations that promote resistance, we analyzed disease responsive transcriptome data from durum wheat to determine the gene expression levels under fungal infection. A greenhouse experiment was carried out at Fargo, North Dakota over three years (2022, 2023 and 2024), with six durum lines with differences in resistance against FHB. Lines were subjected to transcriptomic study under the disease pressure to find the DEGs and explore the possible pathways involved in conferring resistance against this disease. The related durum germplasm, contain varying percentages of genetic sequence from resistant source Sumai3 background and correspondingly, display different levels of resistance against FHB. Spikes collected five days after inoculation with milli-Q water as mock and mixed race of *Fusarium* as treatment were used for RNA extraction and RNA-seq analyses. Sequencing information along with disease scores were used to perform differential expression study with the Hisat2-Stringtie-Ballgown (newer "tuxedo suite") pipeline. In this study, we evaluated the pairwise comparisons between inoculated compared to mock, lines with or without *Fhb1* gene and resistant reaction compared to susceptible reaction. A large number of DEGs were obtained for each condition (25,052 for inoculated vs mock, 12,605 for lines with vs without *Fhb1* gene and 11,175 for resistant vs susceptible). Gene ontology evaluation showed that several defense responsive genes were being activated and highly enriched under disease pressure. In addition, several stress signaling pathways were being highly expressed along with cell wall reinforcement, different catalytic processes and pathways in response to biotic stimulus. KEGG pathway analysis revealed that many of the genes identified are part of several plant-pathogen interaction pathways, the MAPK signaling pathway and several metabolites biosynthesis pathways. STRING protein-protein interaction revealed the complex interconnectivity network across different proteins. Our current study revealed the important genes and their corresponding chromosomal coordinates. In the future functional analysis and further haploblock study will give an insight into fractions of DEGs that are associated with Sumai3 or P1277012 background and broaden our understanding on functioning of major FHB resistance genes.

A *Fusarium graminearum* Effector FgTPP1 Interacts with Stromal Chaperone HSP70 and Suppresses Plant Immunity

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Fusarium head blight (FHB), caused mainly by *Fusarium graminearum*, is a common and destructive fungal disease that frequently occurs worldwide, leading to immense economic cost due to yield loss and grain contamination. However, sustainable genetic resources for FHB are still limited yet. Therefore, engineering resistance in wheat to FHB by creating decoy substrates for FHB effector proteases may be considered as one of promising coping strategies. Here we characterized one putative *F. graminearum* effector FgTPP1, which harbors two major isoforms. The mature FgTPP1 becomes proteolysis-active via cleaving its N-terminal autoinhibitory prodomain by itself and translocates from cytosol to chloroplast. Silencing *TaHSP70* by virus-induced gene silencing abolishes FgTPP1 chloroplast localization. Transient expression of FgTPP1 inhibits plant cell death and suppresses the hypersensitive response induced by an auto-active forms of AtRPS5 (RPS5^{D266E}) and bacteria, possibly by interfering with plant immunity. Overall, our results demonstrate the critical role of FgTPP1 in *F. graminearum* pathogenesis and potential application of decoy system to generate FHB resistance in wheat.

Map-Based Cloning of *Qfhb.Hwwg-2ds* for Fusarium Head Blight (FHB) Resistance in Hard Winter Wheat

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Fusarium head blight (FHB), mainly caused by *Fusarium graminearum*, is a devastating disease that poses a severe threat to global wheat production by reducing both grain yield and quality. Utilizing host resistance has been the most effective strategy for disease control. Among hundreds of resistance quantitative trait loci (QTL) identified for FHB resistance to date, only a few showed stable major effects on FHB resistance in wheat and have been repeatedly detected across multiple studies. One of the previously identified major QTL is *QFhb.hwwg-2DS* on chromosome arm 2DS and overlaps with a QTL for reduced plant height (Rht). This study conducted map-based cloning to pinpoint the candidate gene underlining *QFhb.hwwg-2DS* and investigated the genetic relationship between FHB resistance and the plant height. The fine mapping population were generated by continuous selfing of the plants heterozygous in the *QFhb.hwwg-2DS* region and the heterozygous plants were derived from one recombinant inbred line (RIL) of G97252W x G97380A. The fine mapping populations were genotyped by the flanking markers for *QFhb.hwwg-2DS,* and the selected critical recombinants were phenotyped for FHB resistance. The results delimited the *QFhb.hwwg-2DS* region to a 1.6 Mb interval between *KASP2D25112886* and *KASP2D26715133* with 12 high confidence genes in the region based on IWGSC Refseq v2.1. Gene expression analysis identified a candidate gene encoding a ribonuclease H-like domain protein (RNHL-D1) that underlines *rht8* for plant height in the candidate interval, displayed significantly higher FHB severity, but lower plant height. These CRISPR-Cas9 edited plants with the sequence mutation in current research demonstrated that enhanced plant height and reduced FHB severity by *QFhb.hwwg-2DS* are due to the pleiotropic effect of *rht8* encoding the RNHL-D1 protein. Consequently, use of *Rht8* as a major source for plant height reduction in FHB-prone regions may compromise FHB resistance therefore, alternative sources of *Rht*-genes should be considered in those regions.

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.

Application of Wheat Resistance Genes for FHB Control in Barley

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Fusarium head blight (FHB) caused by the fungal pathogen *Fusarium graminearum* is one of the most devastating diseases in barley (*Hordeum vulgare*). However, sources of effective resistance to FHB are very limited in barley germplasm. In the present study, we manipulated and employed host genes from wheat (*Triticum aestivum*), including those for *Fhb1* and *Fhb7*, to enhance barley resistance to FHB. *TaHRC* is a wheat gene that encodes a putative histidine-rich calcium-binding protein and has been identified to be involved in the *Fhb1*-mediated resistance. BLAST searches indicate that orthologues of *TaHRC* are widely conserved in cereal species. Using CRISPR-mediated mutagenesis, we have generated loss-of-function mutations in *HvHRC*, the barley ortholog of *TaHRC*. Additionally, we transferred *Fhb7* encoding a glutathione S-transferase (GST), driven by either the native promoter or a constitutive promoter, into barley cv. Bowman through *Agrobacterium*-mediated transformation. Homozygous loss-of-function *HvHRC* mutants and *Fhb7*-transformants were identified using Sanger sequencing and real-time PCR (q-PCR), respectively. Our preliminary field studies suggested that both the disruption of *HvHRC* and overexpression of *Fhb7* in barley improved resistance to FHB. Therefore, bioengineering cloned genes involved in FHB resistance in wheat may provide novel strategies to reduce impacts of this destructive disease in barley.

Which Gene is Responsible for the *Fhb1*-Mediated Resistance to Fusarium Head Blight in Wheat-*TaPFT* or *TaHRC*?

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Fusarium head blight (FHB) caused by *Fusarium graminearum* is a devastating disease, which causes significant grain yield and quality losses in wheat. Use of resistance is one of the most effective approaches for management of the disease. *Fhb1* originally derived from the Chinese wheat variety Sumai3 is a major QTL for FHB resistance, which has been widely used for fighting against FHB worldwide. Two genes (*TaPFT* and *TaHRC*) have been reported to be involved in the *Fhb1*-mediated resistance. *TaPFT* encodes a pore-forming toxin-like protein, while *TaHRC* encodes a histidine-rich calcium-binding protein. However, the role and function of these two genes in FHB resistance are still in controversy. In this study, we aimed to generate doubled haploid (DH) lines containing mutated alleles of *TaPFT* and *TaHRC* from various wheat genotypes via wide hybridization with transgenic maize expressing Cas9 and single guide RNA (sgRNA) for targeting the two genes, respectively. Five varieties (Bobwhite, Dayn, Fielder, ND VitPro and SD4539) without the *Fhb1* resistance and three varieties (Wangshuibai, Sumai3 and Alsen) with the *Fhb1* resistance were used in the targeted gene editing experiments. We generated 13, 9, 6, 4, and 6 DH lines with mutations in *TaHRC-S* (the susceptible allele of *TaHRC*) from Bobwhite, Dayn, Fielder, ND VitPro, and SD4539, respectively. We also obtained 3 and 19 DH lines with mutations in *TaHRC-R* (the resistant allele of *TaHRC*) from Sumai3 and Alsen, respectively. Evaluating some of the DH mutant lines along with their wildtype parents for FHB resistance in greenhouse didn't clarify the issue regarding if the *Fhb1*-mediated resistance is the result of a loss-of-function mutation of *TaHRC-S* or a gain of function of *TaHRC-R*. In addition, we identified one Wangshuibai-derived DH line (DH-P3$_{\mathrm{WSB}}$) with a 4-bp deletion in *TaPFT*, but observed no significant difference in FHB resistance between DH-P3$_{\mathrm{WSB}}$ and Wangshuibai. To solve the mystery of *Fhb1*-mediated resistance, we are evaluating more DH lines with mutations in *TaHRC-S* or *TaHRC-R* allele, and generating and evaluating DH lines with mutated *TaPFT* derived from Sumai3 and Alsen.

Acknowledgement and Disclaimer

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Pathogen Biology & Genetics

The Pan-secondary Metabolome of *Fusarium graminearum* Points to New Mycotoxins and Virulence Factors

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The dominant *fusarium* species and populations causing Fusarium head blight have been shifting on a global scale, a phenomenon that has increasingly been attributed to climate change. In other systems, similar changes have led to the emergence of new diseases and previously unknown secondary metabolites (SMs) that act as toxins and virulence factors. While recent work has shown that *Fusarium* populations on a global scale show patterns of long-distance dispersal and recent shifts, the secondary metabolites associated with these dynamics have remained unclear. We have mapped a new class of SM, isocyanides, across the fungal kingdom. Isocyanides have previously been associated with grass pathogens and ill-thrift disease in animals that consume them. We have demonstrated that these clusters are significantly enriched in Fusarium species, raising questions about their role in fungal pathogenesis. We use a dataset of ~450 genomes to explore population-specific variants of canonical SMs and of Isocyanides. We identify population-specific variants of many SMs, raising concerns about the coupling of emerging populations with emerging mycotoxins. To better understand the threat posed by understudied toxins, we perform molecular manipulation of a highly conserved isocyanide gene cluster and demonstrate that this cluster may be linked to disease progression in wheat heads. Overall, our results identify a significant reservoir of chemical diversity within and between *F. graminearum* populations that contributes to FHB progression and emphasizes a need to define emerging mycotoxins.

Recombination Analysis Reveals 3ADON Does Not Confer Higher Levels of Aggressiveness or Toxigenicity than 15ADON

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Fusarium Head Blight (FHB) in North America is primarily caused by three *Fusarium graminearum* populations known as NA1, NA2, and NA3. NA1 mainly produces the trichothecene toxins DON and 15ADON, while NA2 mostly produces 3ADON instead of 15ADON. The 3ADON chemotype reportedly confers higher levels of aggressiveness and toxigenicity than 15ADON, perhaps accounting for the steady expansion in range of the NA2 population from its apparent origin in the Canadian Maritimes. We are using a genetic approach to test this hypothesis. A self-sterile strain of *F. graminearum*, generated by deleting the *MAT1-1-1* locus in the NA1-15ADON isolate PH-1, was crossed with an NA2-3ADON strain that was more aggressive and toxigenic on wheat. A total of 230 single-ascospore progeny were collected from 20 perithecia. Ninety-three progeny were randomly screened with PCR primers targeting the *MAT1-1-1* and the TRI loci. The progeny exhibited expected Mendelian segregation for both markers. Eighty of these progeny, including 20 of each parental and recombinant type, were assayed for disease severity and mycotoxin production on ‘Wheaton’ and ‘Alsen’ wheat. No significant difference in either trait was observed between progeny with the 15-ADON versus 3-ADON chemotypes on either wheat variety. Twenty-one progeny strains consistently exhibited high or low aggressiveness (more than two standard deviations above or below the mean). After retesting on Wheaton, the strains comprised three groups: the “high” group was not statistically different from the more aggressive 3ADON parent; the “moderate” group was statistically equivalent to the 15ADON parent; and the “low” group was not statistically different from the water control. Each strain was individually genome-sequenced. Interestingly, this revealed that six of the "low" aggressiveness progeny were actually clonal with the 3ADON parent, and had come from a single selfed perithecium. Further investigation revealed that two of the 20 perithecia sampled were selfed. Thirteen progeny from these perithecia were removed from the original dataset, with no overall effect on the results. Our analysis so far suggests that factors other than chemotype are more important in conferring aggressiveness and toxigenicity. Comparative analysis of high-moderate progeny pools may help identify these factors.

Acknowledgement and Disclaimer

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

Fusarium Protein Toolkit: A Resource for Structural and Variant Analysis of *Fusarium* Species

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The genus *Fusarium* poses significant threats to global food security and safety due to its ability to cause destructive diseases and mycotoxin contamination in staple crops like maize and wheat. Climate change exacerbates these issues by altering environmental conditions—such as increased temperature and humidity—that favor the proliferation and virulence of *Fusarium* species. Addressing these threats necessitates innovative solutions, including advanced tools that facilitate the identification of targets for effective control strategies. To respond to this challenge, we developed the Fusarium Protein Toolkit (FPT), a web-based resource that enables users to explore the structural and variant landscapes of protein sequences within the *Fusarium* pan-genome. FPT features both AlphaFold and ESMFold-generated protein structure models from six *Fusarium* species, accessible through a user-friendly web portal. This toolkit facilitates comparative analysis and functional annotation inference within *Fusarium* species and lays the foundation for cross-species comparisons between the pathogen (*Fusarium*) and its cereal hosts (maize and wheat). By providing a common set of tools and models, FPT allows researchers to directly compare protein structures and functions across species, enhancing our understanding of host-pathogen interactions. Utilizing a protein language model, FPT predicts the impact of over 270 million coding variants in two agriculturally significant species, *F. graminearum* and *F. verticillioides*. Additionally, it offers variant effect scores for proteins in a *Fusarium* pan-genome based on 22 diverse Fusarium species, displayed as heatmaps using the PanEffectframework. This comprehensive dataset allows researchers to identify potential targets for breeding disease-resistant crop varieties. Building on this foundation, we used RFdiffusion to predict protein-protein interactions (PPIs) between *Fusarium* proteins and those of maize and wheat. We've identified over 15,700 potential PPIs in maize and 17,700 in bread wheat with this method thus far, and plan to refine our predictions using in silico analyses and experimental validation. By identifying and modeling these interactions, we can gain deeper insights into the mechanisms of pathogenicity and host defense. Future developments will include integrating visualization resources to display PPI networks, further enhancing the toolkit's utility in facilitating cross-species analyses and aiding in the development of innovative control strategies. The Fusarium Protein Toolkit is available at https://fusarium.maizegdb.org.

Fungal Endophyte *Sarocladium zeae* Delivers dsRNA to Suppress *Fusarium graminearum* Expressing GFP

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Fusarium head blight (FHB) caused by *Fusarium* species is a major threat to food safety and security by reducing crop yields and contaminating grains with harmful mycotoxins. RNA interference (RNAi) technology has been widely used to control plant diseases and pests. Two common RNAi application methods are host-induced gene silencing (HIGS) and spray-induced gene silencing (SIGS). Each method has its limitations. Therefore, our goal is to develop an alternative RNAi delivery platform using endophytes. Because endophytes colonize their hosts without causing damage, an endophyte-mediated RNAi system is expected to be cost-effective and sustainable. The fungal endophyte *Sarocladium zeae* has been shown to reduce fungal diseases in corn and wheat. To enhance *S. zeae*'s ability to reduce FHB and toxin contamination, we investigated whether *S. zeae* can deliver RNA silencing signals to *F. graminearum*. As a proof of concept, we expressed a hairpin RNAi construct in *S. zeae* 34560 that targeted a green fluorescent protein (GFP) gene (hereafter (SzGFPi). We found that SzGFPi strains suppressed GFP expression in a transgenic *F. graminearum* strain expressing GFP when co-cultured on plates and in liquid. RT-PCR and Northern blot analyses showed that SzGFPi strains produced GFP double stranded RNA (*GFP*-dsRNA) but did not process them to small interfering RNA (siRNA). Furthermore, we showed that *GFP*-dsRNA was present in conditioned media in which the Sz34560 GFP-RNAi strains had been grown, indicating that SzGFPi can secrete *GFP*-dsRNA to reduce GFP expression in *F. graminearum*. In addition, genome analysis revealed that *S. zeae* contains some RNA-induced silencing complex (RISC) members, including two Argonaute-like proteins (AGO1 and AGO2), one Dicer-like protein, and at least one RNA-dependent RNA Polymerase (RdRP). Further investigations are underway to determine if the dsRNA delivered by *S. zeae* can be processed into siRNA in plants and in *F. graminearum*.

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.

Chromosome-Scale Assembly of Wheat Cultivar Sumai 3, the Major Germplasm Source for Fusarium Head Blight Resistance

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Hard spring wheat (*Triticum aestivum* L.) production faces numerous challenges, including the threat of Fusarium head blight (FHB), also known as scab, caused by the fungal pathogen *Fusarium graminearum* Schwabe. FHB is a serious fungal disease of wheat spikes that severely reduces grain quality and leads to substantial yield losses. The Chinese wheat cultivar 'Sumai 3' has long been recognized for its strong resistance to FHB, primarily attributed to the *Fhb1* gene located on chromosome 3B. Here, we generated a chromosome-scale assembly of the Sumai 3 genome, generated using PacBio HiFi reads and chromosome conformation capture (HiC). The assembly yielded a genome size of 14.6 Gb with a contig N50 of 41.9 Mb and a BUSCO completeness score of 99.7%. We identified 104,620 high-confidence protein-coding genes. Our analyses included centromeric and subtelomeric regions, transposable element (TE) distribution, high and non-coding gene density, and GC content across the genome. A Circos plot visually represents these genomic features, providing a comprehensive view of Sumai 3's structural and functional landscape. Co-linearity analysis showed that Sumai 3 is mostly co-linear with the Chinese Spring genome, indicating high conservation in gene order. The region's gene content largely overlaps with annotations for Chinese Spring, although we discovered two genes at approximately 157.7 kb, including additional copy of a terpene synthase, suggesting a similarity between Sumai 3's 3B and Chinese Spring's 3D in FHB-related gene content. This structural insight raises the possibility that chromosome 3D could be a source of FHB resistance. A heatmap of differential gene expression (DGE) in the *Fhb1* region shows up-regulation of genes PFT, Sin, and PAP highlighting genes potentially involved in FHB resistance. This work advances our understanding of Sumai 3's genomic landscape, aiding in breeding strategies for FHB resistance in wheat.

Discovery of IssA, a Bacterial Protease that Cleaves flg22 to Allow Rhizosphere Colonization

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Compared to the surrounding soil, the root microbiome is limited in diversity, dominated by plant-recruited microbes. In this study we report that the commensal soil bacterium *Dyella japonica* secretes a protease—immunosuppressive subtilase A (IssA)—to dampen plant defenses and establish itself as part of the root microbiome by converting the immunity-eliciting peptide flg22 from a receptor agonist to an antagonist. A screen of 165 root-associated bacteria demonstrated that 67 strongly suppress flg22-induced activation of plant immunity in bioassays. These results, combined with a clue from the literature, identified *D. japonica* IssA as potentially involved in this suppression. To test this prediction, IssA was produced recombinantly and purified. Purified IssA suppressed flg22 activation in bioassays and cleaved flg22 *in vitro*. IssA cleaved flg22 at specific peptide bonds to create derivative peptides that are known to bind flg22 receptors without activating an immune response. While this research was performed on a model system, discovering how plants interact with the microbiome promises to generate new solutions for reducing wheat and barley scab.

Fusarium graminearum Effector *FgRGAE* is Critical for Fungal Initial Infection in Wheat and Barley

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Fusarium graminearum is the primary causal agent of Fusarium Head Blight (FHB), a devastating fungal disease on wheat, barley, and other grains. During infection, *F. graminearum* produces trichothecenes, predominately deoxynivalenol (DON), which contaminates grain and reduces grain yield and quality. Although DON functions as a virulence factor to promote *F. graminearum* spread in the wheat head, it is not essential for initial infection. When fungal pathogens, such as *F. graminearum,* infect a host plant, they secrete hundreds of small proteins called effectors that interfere with the plant immune system to promote infection and disease. A recent study identified approximately 150 putative effector-encoding genes that are conserved in *F. graminearum* species. Gene expression analysis of selected candidates from *F. graminearum* PH-1 identified 12 effector genes that were highly induced with various expression profiles in wheat heads over a seven-day infection period. To elucidate their role in FHB, deletion mutants were generated for ten effector genes and FHB virulence assays were conducted with dip inoculations. FHB virulence assays showed that deletion mutants of a rhamnogalacturonan acetylesterase homolog, FGSG_04848 (*FgRGAE),* significantly reduced initial infection and DON in wheat and barley spikes compared to wild-type controls. Replacing the $FgRGAE^{\Delta}$*::Hyg* construct with a $FgRGAE^{WT}$*::Gen* construct at the native locus restored FHB disease to WT levels in both wheat and barley heads. *In silico* analysis of *FgRGAE* suggests that this gene encodes a putative rhamnogalacturonan acetylesterase, a member of the SGNH-hydrolase family predicted to deacetylate pectin subunits. General esterase activity was experimentally shown using the colorimetric substrate 4-nitrophenyl acetate. *FgRGAE* may serve as an ideal target to reduce FHB and mycotoxin contamination.

Rapid Detection of *Fusarium graminearum* Chemotypes Using a Single-tube Multiplex High-Resolution Melting (HRM) Assay

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Fusarium graminearum is a primary causal agent of Fusarium head blight (FHB) on wheat and barley in North America. The fungus produces trichothecene mycotoxins that render grains unsuitable for food, feed, or malt. Strains of *F. graminearum* can differ in trichothecene production phenotypes (chemotypes), with single isolates producing predominantly one of the four toxins: 3-acetyldeoxynivalenol, 15-acetyldeoxynivalenol, nivalenol, or NX-2. Molecular tools to diagnose chemotypes remain inefficient. This study aimed to develop a single-tube, multiplex molecular assay to predict the four *F. graminearum* chemotypes. Conserved functional regions of three trichothecene biosynthetic genes (*TRI1, TRI8,* and *TRI13*) that impact chemotype were targeted to develop a high-resolution melting (HRM) assay. Multiplex HRM analysis produced unique melting profiles for each chemotype. The assay was validated on a panel of diverse 80 fungal isolates. We applied machine-learning-based linear discriminant analysis (LDA) to automate the classification of chemotypes from HRM data, achieving a prediction accuracy of 99.7%. The assay exhibited good analytical sensitivity, with a limit of detection below 0.02 ng of fungal DNA. Together, our results demonstrate that this simple, rapid, and accurate assay can be applied to *F. graminearum* molecular diagnostics and population surveillance programs.

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Genomic, Pathogenic, and Chemical Diversity of *Fusarium graminearum* Isolates Used in U.S. Barley Screening Nurseries

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Fusarium head blight (FHB) poses a significant threat to barley production by reducing both yield and grain quality. Genetic diversity of *Fusarium* species that cause the disease has potential to impact breeding efforts aimed at increasing FHB resistance of barley. To address this issue, we are assessing genomic variation, virulence, and toxin production of 97 isolates of *Fusarium* from barley screening nurseries in six U.S. states. Analysis of whole genome sequences revealed that all isolates were *Fusarium graminearum* but varied in trichothecene chemotypes. Population structure and SNP-based Splitstree analyses of the sequence data indicated that 57 isolates were from the NA1 population of *F. graminearum*; 30 were from the NA2 population; and 10 isolates were admixtures of the two populations. We examined 34 isolates, representing all six states, for their ability to cause FHB and produce deoxynivalenol (DON) on two barley varieties, AAC Synergy and Pinnacle. The results indicated that over half of the isolates tested were more virulent on Pinnacle than on AAC Synergy. Interestingly, disease severity did not always correlate with DON levels, as some isolates produced high DON levels in planta but showed variable FHB severity. Quantitative PCR revealed that fungal biomass varied in AAC Synergy and Pinnacle. Biomass levels were highly correlated with DON levels and moderately with Area Under Disease Progress Curve (AUDPC), suggesting that higher biomass is linked to increased DON and AUDPC. Metabolomic profiling using liquid chromatography-mass spectrometry combined with principal component analysis highlighted distinct differences between barley varieties and *Fusarium* isolates, independent of DON. These results highlight the complexity of *F. graminearum* virulence on barley and indicate that both genetic and environmental factors impact disease expression, providing insight into potential mechanisms to increase FHB resistance in barley.

Time Well-Spent: 28 Years in the Company of the Head Blight Fungus

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Fusarium graminearum causes a devastating disease of wheat and barley, resulting in grain moldy contaminated with toxins. For more than 25 years, my lab has focused on the life cycle of this fungus to better understand how it causes disease, how it survives through the winter, and how it disperses. We have learned that biofilms play a role in disease initiation on host plants, the first such finding among filamentous fungal plant pathogens. We have also studied the association of host trichomes and silica cells with disease development and progression. Fruiting bodies form in stomatal cavities and in cork/silica cell pairs on stems and flowers. We have investigated the mechanism of aerial dispersal of ascospores, which are forcibly discharged from microscopic water cannons, and form the primary inoculum of FHB. These investigations provide evidence of a finely tuned relationship between host and pathogen and have revealed new information for developing novel approaches to disease control.

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A New Inoculation Method Suggests that Resistance Observed in Barley Genotypes to FHB May Be Due to Physical Barrier

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Fusarium head blight (FHB), caused by *Fusarium* species, primarily *F. graminearum* is a destructive disease of barley (*Hordeum vulgare* L.) in many warm and humid growing regions of the world. Selection of barley varieties for FHB resistance and low DON accumulation is usually conducted in field nurseries or in controlled environments. However, the results obtained with the existing inoculation methods are highly variable and non-reproducible. This might be associated with the fact that the stage at which FHB infection takes place, primarily through anthers, happens while the anthers are still enclosed in the palea. Exposing the anthers by cutting the tip of the developing kernel at heading/early anthesis significantly increased disease infection rate most probably due to an increased chance of direct contact between spores and anthers during inoculation. Inoculation of barley lines previously reported as resistant following the standard spray inoculation procedure (kernels intact) resulted in a resistant reaction with low FHB severity. However, when the tip of the kernel/palea of these lines were excised and the macroconidia spray-inoculated on exposed anthers, FHB severity dramatically increased, and the lines became completely susceptible. The DON content on excised kernels was also significantly higher than on intact kernels. This suggests that the resistance observed on plants inoculated on intact palea may be due to physical barrier (of spores from coming in to contact with the anther) rather than genetic. The clip inoculation method may be a reliable method to confirm true genetic resistance of barley to FHB. Further investigation including gene expression analysis is required to fully understand this phenomenon.

Variety Development and Host Resistance

Evaluation of Wheat Scab Disease and Their Associations with Wheat Kernel Traits Through Grain Scan

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A significant and positive correlation up to 0.67 between visually observed FDK and DON values in Texas elite lines proved reliability of scab evaluation. Negative correlations of scab resistance ratings with thousand kernel weight and seed perimeter can be assessed as impacting seed size and quality and grain yield. The clustering analysis clearly differentiate three different clusters of traits (FDK and DON, INC and SEV, kernel traits) and refined PCA analysis in extreme lines showed that resistant lines with lower DON were positively associated with thousand kernel weight but negatively associated with FDK.

Field Evaluation of Fusarium Head Blight Resistance and Mycotoxin Accumulation in Winter Wheat in Southeast Idaho (2020-2024)

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Fusarium head blight (FHB) impacts wheat in the Pacific Northwest, increasing with climate change and corn production, elevating the prevalence of *Fusarium graminearum*. Disease occurs frequently in spring wheat, where IPM efforts have focused. Most winter wheat varieties are highly susceptible, resulting in high levels of deoxynivalenol (DON) when diseased. A five-year (2020-2024) screening study was conducted at Kimberly, Idaho to evaluate varieties and advanced lines for susceptibility to *F. graminearum*. Widely grown varieties and advanced lines of soft white winter (SWW) and hard winter wheat (HWW) from public and private breeding programs were tested in two-row, 5-foot plots with two replications in a randomized complete block design. Plots were planted with a Hege 1000 head row planter. Secondary mist irrigation systems fostered conditions for FHB infection, with corn spawn applied at 30 g/m² three weeks before anthesis. FHB incidence, severity, and index were assessed at the soft dough (Feekes 11.2). FDK and DON concentrations were measured from harvested samples. Data were analyzed using PROC GLM in SAS 9.4, with LSD ($\alpha = 0.05$) for mean comparisons. Disease pressure varied across years, with no varieties showing resistance to FHB. After multiple years of testing, four cultivars 'WB4510CLP' and 'WB4623CLP' (HWW) and 'Sockeye CL+' and 'Perrine' (SWW) showed moderate susceptibility. Overall, 82.7% of cultivars were rated as susceptible (S) or very susceptible (VS). In HWW, FHB index ranged from 5.1 in Brundage (check) to 54.7 in UI Bronze Jade, with DON content from 4.4 ppm in WB4510CLP to 58.7 ppm in MT1642. In SWW, FHB index values ranged from 11.2 in ORI2190025CL+ to 58.0 in WA8334, while DON levels across entries varied from 5.1 to 56.1 ppm. Eighteen entries in multi-year trials exhibited varied reactions (MS to VS), with mixed reactions observed in approximately 24.3% of cases. 70 entries were evaluated only in a single year, with 36 rated S or VS. In 2024, DON and FDK data for the other 34 single-year entries are pending. A weighted equation that includes DON, Incidence, Severity and FDK was developed (DISK value) as [(0.3 * DON) + (0.2 * Incidence) + (0.2 * Severity) + (0.3 * FDK)] for assessing FHB reaction. Significant positive correlations were observed between FHB index and DISK in both HWW ($r = 0.733$) and SWW ($r = 0.798$), with $p < 0.0001$. This study offers insights for selecting less susceptible varieties to reduce DON contamination in grain.

Breeding FHB Resistant Hard Red Spring Wheat for the Upper Midwest

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Fusarium head blight (FHB) continues as a threat to spring wheat production in the Upper Midwest in years in which climatic conditions are favorable. After more than 30 years of intensive breeding effort in the hard red spring wheat region, more than half of the available varieties have at least moderate levels of resistance, but none have complete resistance and even the most resistant materials available today can incur damage when environmental conditions are conducive for an epidemic. Phenotyping in two inoculated & irrigated nurseries remains the bedrock of our breeding efforts with this disease but have been complimented with genomic prediction and DNA marker selection. We routinely screen with DNA markers all of our pre-yield trial F_5 lines, between 2,000-3,000 lines per cohort, as well as BC_1 and TC_1plants segregating for *Fhb1* and the 5AS QTL. A training population of ~210 F_5 lines and their 40-50 parents is phenotyped for FHB traits each year and used to predict the remaining lines, helping us to reduce the number of preliminary yield trial candidates sent to and harvested from our winter nursery seed increase. Using historic data to train FHB genomic prediction models, beyond the previous years' cohort, has been of limed value. All lines entered in preliminary yield trials and retained for future evaluation undergo phenotypic selection for FHB resistance each year. After three or more years of FHB evaluation the most resistant lines, including those discarded from variety release consideration due to agronomic or end-use quality deficiencies, are evaluated in the spring wheat regional scab nursery.

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Development of Hard Red Winter Wheat ‘Scab Stryker’ for Adoption in Areas of Oklahoma Prone to Fusarium Head Blight

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Fusarium head blight (FHB), caused primarily by the fungus *Fusarium graminearum* Schwabe, is one of the most destructive diseases of wheat in humid and semi-humid regions globally. In the U.S. Great Plains, only two contemporary hard red winter wheat (HRWW) cultivars, ‘Everest’ and ‘Zenda’ are moderately resistant to FHB. However, none of the HRWW cultivars currently grown in the region carry *Fhb1*, the most effective gene with a major effect on FHB resistance. The main objective of this project was to release an *Fhb1*-carrying HRWW cultivar adapted to FHB-prone areas in Oklahoma. OK16103083 was an experimental line selected from a series of recurrent backcross progeny of Garrison*3/WesleyFhb1-106 by the Oklahoma State University (OSU) wheat breeding program. Progenies were developed from the marker-assisted backcrossing project by USDA Small Grains Genotyping Lab with support from the US Wheat and Barley Scab Initiative. OK16103083, named ‘Scab Stryker’, has similar agronomic traits to its parent, Garrison, but carries *Fhb1* for FHB resistance. Scab Stryker was evaluated in four yield trials in Oklahoma from 2018 to 2023 and for FHB resistance in the hard winter wheat FHB nursery, Manhattan KS from 2020 to 2023. The FHB nursery was arranged in a randomized complete block design with four replicates. The entries included in this nursery were experimental lines or recently released cultivars from breeding programs in the HWW-CAP project. Overley (FHB susceptible), Everest (FHB moderately resistant), and the OSU HRWW cultivar, ‘OK Corral’, with putative field tolerance to natural FHB infection in eastern Oklahoma were used as checks in the FHB nursery. Scab Stryker was ranked in the middle of the contemporary HRWW gene pool for grain yield and test weight in the yield trials but exhibited an exceptionally high level of FHB resistance in the FHB trials. Among 35 entries including check cultivars, Scab Stryker always produced lower area under disease progress steps (AUDPS) values across the four years from 2020 through 2023, which were significantly lower than those for Everest. The later heading date and taller stature of Scab Stryker possibly boosted the effectiveness of *Fhb1*. To our knowledge, FHB resistance of Scab Stryker is strong and unsurpassed by any currently available HRWW cultivar. Scab Stryker was released in 2024 and is currently the only commercial U.S. Great Plains HWW which carries the resistance gene *Fhb1.*

Acknowledgment and Disclaimer

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Pyramiding of Three Major Genes to Fusarium Head Blight Resistance in 15 U.S. Hard Winter Wheat

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Wheat Fusarium head blight (FHB) is one of the most destructive diseases of wheat worldwide. Using resistant cultivars is the most effective approach to control FHB. However, hard winter wheat (HWW) cultivars used in farmers' fields in the U.S. are mainly FHB susceptible. Large scale pyramiding of the major effect genes for FHB resistance in HWW wheat cultivars from FHB-prone states can enhance the resistance levels and durability of wheat cultivars in the region. To date, two major genes (*Fhb1* and *Fhb7*) have been cloned with diagnostic gene makers (*TaHRC-KASP* and *Fhb7-828bp*). *Fhb9* also showed a major effect with flanking markers (*KASP-10238* and *KASP-12056*) available. This study was designed to simultaneously transfer the three genes into the 15 U.S. HWW cultivars from six states (KS, CO, NE, OK, SD, ND and USDA-ARS) using marker-assisted selection (MAS). All recurrent parents were evaluated for FHB resistance in greenhouses. Among those, five were moderately resistant, three were moderately susceptible and others were highly susceptible. The four markers showed clear polymorphisms between donors and all recurrent parents. We first pyramided the three genes from different donors, and then crossed and backcrossed each recurrent parent to the heterozygous progeny carrying all three genes using MAS. This fall, we screened 8,320 plants with the four markers and selected 2,785 BC_2F_2 plants that carry the target alleles (either homozygote or heterozygote) at all three genes from 13 cultivars. Two other cultivars are in earlier stage of backcrossing due to later heading that did not match flowering time with the donor parents. The selected BC_2F_2 plants will be advanced and their BC_2F_3 lines will be genotyped with the four markers to select plants with at least two homozygous resistance genes. The selected BC_2F_3 lines will be genotyped with the genome-wide MRASeq markers for background selection. The BC_2F_4 seeds will be released to the seven breeding programs for further testing of agronomic traits to develop new cultivars or germplasms.

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

Advancing Wheat Resistance to Fusarium Head Blight Through Genomic Prediction

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Fusarium Head Blight (FHB) of *Triticum aestivum*, commonly known as head scab, is a disease caused by the fungal pathogen *Fusarium graminearum*. This infection causes reduced grain yield, test weight, and milling quality of the crop. Head scab can also lead to the production of harmful mycotoxins, such as deoxynivalenol (DON) that negatively affect animal and human health if consumed. Recent climate changes have intensified FHB occurrences, even in regions where it was not previously reported. This includes the Great Plains region of the US, where predominantly hard winter wheat is grown. These challenges highlight the need for research focused on rapidly and effectively developing wheat varieties resistant to FHB. Genetic resistance to FHB in wheat is primarily influenced by small-to-moderate effect Quantitative Trait Loci (QTL), and QTL mapping has been widely used to identify loci for Marker-Assisted Selection (MAS). Notably, the *Fhb1* locus is considered a stable and valuable QTL to be utilized in MAS. While MAS is effective for traits governed by a few major genes, wheat FHB resistance requires integrating MAS for moderate effect loci with genomic prediction. This project will incorporate genome-wide marker data and historical phenotypic information to generate genomic estimated breeding values (GEBVs) for relevant traits. Additionally, QTL haplotypes and crossing recommendations will be developed for collaborating programs in the Great Plains region. Genomic prediction cross-validation accuracies of currently available data are variable due to small training populations. Nevertheless, this analysis demonstrates the potential of the expanding dataset to provide predictions across collaborating institutions. Ultimately, this research aims to advance FHB resistance breeding techniques, contributing to the development of improved wheat cultivars.

Improving Prediction of FHB Resistance in Wheat Using Genotype-by-Environment Information

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Variability in FHB susceptibility is influenced by genotype, environment, and genotype-environment interaction (GxE). This study examines GxE in a multi-environment trial using data from the Uniform Regional Scab Nursery (URSN), a collaborative effort initiated in 1995 to assess resistant genotypes from spring wheat breeding programs across the Northern U.S. Of the 780 genotypes tested, 222 were genotyped using a 3K array, with a focus on the visual scabby kernel (VSK) trait.
Traditionally, GxE has been studied as a reaction norm over an environment index. Here, the environment index was predicted from a linear combination of a set of environmental covariables (ECs) specific to each environment. Three prediction scenarios were compared, all aimed at predicting untested genotypes with varying degrees of relatedness between training and target environments. GxE prediction was performed using joint-genomic regression analysis (JGRA) with two modeling approaches: (1) JGRA Reaction Norm (JGRA RN), which estimates genotype responses to the environment through intercept and slope regression against the environment index, and (2) JGRA Marker, where genomic selection (GS) was applied in each environment, and marker effects were regressed against the environment index. These methods were compared to a baseline genomic selection model without environmental covariates (GS), with predictive abilities calculated within and across environments. Results showed that within-environment predictions were more accurate for JGRA Marker than for GS or JGRA RN, though the differences were small (mean predictive abilities of 0.509 for JGRA Marker and 0.487 for GS). Predictive ability decreased when the target environment was less related to the training environments. For across-environment predictions, the advantage of the JGRA Marker was more pronounced, with a difference in predictive ability up to 0.203 compared to GS. Incorporating environmental covariables improved prediction accuracy and aided in selecting resistant genotypes across diverse environments.

Genotyping to Improve Scab Resistance

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The USDA-ARS Small Grains Genotyping Labs (SGGLs) provide researchers with access to advances in genotyping technology, playing an integral role in enhancing wheat resistance to Fusarium head blight (FHB). Early genotyping relied heavily on PCR-based methods requiring fragment separation of one or a few markers at a time. While time-consuming and expensive, this work led to identification of the Fhb1 locus and development of predictive markers that have been crucial to marker-assisted improvement of scab resistance. The high-throughput and cost-effective Kompetitive Allele Specific PCR (KASP) technology is now used for single-plex tracking of cloned resistance genes Fhb1 and Fhb7, markers associated with other FHB resistance QTL, and genes affecting a variety of other traits. Trait related markers and genome-wide variants are also genotyped using the new Illumina 3K multi-species and AgriSeq wheat targeted genotyping platforms designed by the SGGLs. Adoption of high-throughput genotyping techniques such as the Diversity Array Technologies (DArT), Illumina Infinity arrays and the genotyping-by-sequencing (GBS) technology, enabled the rapid analysis of thousands of SNP in large populations. These techniques facilitated association and QTL mapping studies in diverse sources of FHB resistance, including adapted cultivars (ie. NC-Neuse and Jamestown). Although these studies have generally not detected QTL of large effect, tools for integration of genomic data with phenotypic observations led to the deployment of the genomic selection (GS) breeding strategy. Collaborative FHB evaluation and data sharing across the Northern and Southern VDHR groups and the centralized genotyping done at the Raleigh SGGL fostered the development of regionally and locally useful training populations in the eastern soft wheat region. Genomic estimated breeding values obtained for FHB resistance traits now contribute to advancement decisions in breeding populations, as well as parent selection and prediction of cross performance. The continued reduction of next generation sequencing costs is further democratizing wheat researcher's access to genomic data. In addition to sequencing the exomes of more than 400 US wheat lines to build a database of variants in genes, the SGGLs are using long read sequencing to assemble whole genomes of FHB resistant lines, including locally relevant US cultivars. These efforts are contributing to the development of a haplotype database to facilitate identifying and validating haplotypes/genes associated with resistance. Continued investment in new resources and technologies for genotyping will enable more precise methods to develop superior wheat cultivars with improved FHB resistance.

Fhb7^{The2} Confers Significant FHB Resistance in Spring, Winter, and Durum Wheat

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Wheat breeding for Fusarium head blight (FHB) resistance has been limited by the shortage of resistance genes and the complexity of the current resistance sources in terms of inheritance and interaction with environments. The discovery, characterization, and deployment of novel resistance genes in wheat, especially in durum wheat, has been a major research priority in the FHB community. We transferred a novel *Thinopyrum elongatum* (2n=2x=14, EE)-derived FHB resistance gene *Fhb7*^{The2} to wheat through a wheat-*Th. elongatum* 7B-7E translocation (7BS·7BL-7EL) by genomics-enabled chromosome engineering and developed an FHB-resistant germplasm containing *Fhb7*^{The2}, which was released as PI 702949 to the public in 2023. Obvious deleterious effects have not been observed with the *Th. elongatum* chromosome segment of the 7B-7E translocation in the germplasm. PI 702949 has been distributed to most, if not all, of the USWBSI-funded wheat FHB breeding programs, and to USDA-ARS, CIMMYT, and many private breeding companies in the US and other countries. Also, we developed diagnostic PCR allelic competitive extension (PACE) markers specific for *Fhb7*^{The2}, which have largely increased the efficacy and throughput of *Fhb7*^{The2} introgression and deployment in US wheats. More recently, we have incorporated *Fhb7*^{The2} into elite HRSW, HRWW, and spring and winter durum genotypes for FHB-resistant germplasm/variety development by marker-assisted backcrossing breeding. Three HRSW introgression lines containing *Fhb7*^{The2} were developed and evaluated for FHB resistance in the FHB nurseries at Lincoln, NE and Fargo, ND and greenhouses in 2023 and 2024. They consistently exhibited FHB resistance significantly better than their recipient parents under all field and greenhouse environments and had similar agronomic performance as their respective parents. In addition, we have developed a number of HRWW and spring and winter durum introgression lines containing *Fhb7*^{The2}, which have been evaluated for FHB resistance in the field and greenhouse. All of the *Fhb7*^{The2} introgressions significantly outperformed their recipient parents on FHB resistance under all environments. In summary, the wild grass-derived FHB resistance gene *Fhb7*^{The2}confers significant resistance in both common and durum wheat backgrounds and the alien translocation involving wheat chromosome 7B does not result in obvious deleterious effects on the overall agronomic performance of the introgressions. The monogenic nature of *Fhb7*^{The2} in wheat backgrounds dramatically improves its utility and efficacy in marker-assisted breeding pipelines. The deployment of *Fhb7*^{The2} in different classes of US wheats will strengthen and diversify their resistance to FHB and reduce the economic losses caused by the disease in wheat production.

Ensembles of Genomic and Hyperspectral Imaging-Based Predictions Enable Selection for Reduced Deoxynivalenol Content in Wheat Grains

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Breeding for low deoxynivalenol (DON) mycotoxin content in wheat is challenging due to the complexity of the trait and phenotyping limitations. Recent advances in prediction-based breeding using phenomic and genomic data have improved breeding strategies for such traits. Since phenomic prediction relies on non-additive effects and genomic prediction on additive effects, their comparison and complementation are necessary. Five Bayesian generalized linear regression models and two machine learning models were trained for phenomic and genomic predictions using advanced breeding lines evaluated in 2021 and 2022 independently, and the calculated BLUEs across years to account for year variation. Across all training sets and models, phenomic predictions using wavebands in the visible light spectrum (400-700 nm) had higher predictive ability than genomic predictions or phenomic predictions using the full waveband range (400-1000 nm). Forward prediction and model averaging were conducted on two sets of $F_{4:5}$ selection candidates evaluated independently in 2022 and 2023. The phenotypic and genetic correlations, as well as indirect selection accuracies, of the model averages of phenomic predictions and combined phenomic and genomic predictions, were higher than those of genomic predictions alone, but depended on the combination of training set and selection candidates. Unsupervised K-Means clustering-based ensembling of predicted values indicated distinct groupings of candidates with varying DON content, depending on the models, predictors, and training sets used. This study demonstrates the potential of hyperspectral imaging-based phenomic prediction to complement genomic prediction, highlighting considerations for prediction-based selection of low DON in soft winter wheat.

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

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Fusarium head blight (FHB) is a major disease of wheat that can cause yield losses exceeding 50%. Breeding for resistance remains the most effective control method, however, traditional scoring methods are labor-intensive and subjective. This study presents a deep learning pipeline for wheat spike detection and FHB quantification, trained using images collected by the Google Moonshot Mineral X phenotyping rover. The pipeline detects and segments spikes and diseased tissue to quantify disease severity at a spike and plot scale. To validate this high throughput FHB phenotyping pipeline, disease inferences from field-based images were compared to disease scoring in the field and manual image analysis of diseased wheat spikes by raters. Disease assessments from the imaging pipeline correlated strongly with rater disease scores at the spike and plot level, and outperformed traditional methods in precision and throughput. Furthermore, the pipeline was found to be generalizable across years, environments, and disease progressions, which allows for reliable assessments under diverse conditions. To improve access to this phenotyping tool, the image analysis pipeline has been adapted to analyze mobile images at single row plot resolution. This new tool is an objective and accessible phenotyping method for the wheat breeding and research community interested in low cost and scalable FHB field phenotyping.

Pyramiding FHB Resistance QTL Affects DON, Flavor, and Aroma in Soft Red Winter Wheat

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Wheat (*Triticum aestivum L.*) is one of the three most important crops produced worldwide and accounts for 50 percent of the world's diet. Wheat crops are devastated annually by fungal diseases, with a major one being Fusarium head blight (FHB), caused by *Fusarium graminearum*. While deployment of resistance QTL has reduced crop losses from FHB, the effect that these QTL have on grain quality and flavor is unknown. The goal of this research is to test if linkage drag associated with pyramided FHB resistance QTL affects agronomic and post-harvest quality of soft red winter wheat (SRWW). In this study, an F_2 population was developed from parents with different QTL combinations, and the QTL status of the 120 F_2 individuals was characterized by genomic predictions. We hypothesized that an increased number of QTL will lower FHB severity but would also negatively affect yield and other traits. To analyze QTL status effects on agronomic traits, twenty $F_{2:4}$ lines were planted in a randomized complete block at two locations in Kentucky. Post-harvest grain quality measurements including predicted protein concentrations were measured using near-infrared spectrometry. Flavor profiles were analyzed using roti flatbread from each lines flour and a set of 10 tasters assessed various flavor traits. We found significant differences between deoxynivalenol (DON) content, flavor intensity and likeness, and aroma intensity based on the number of pyramided QTL in the line. Pyramiding QTL led to a significant decrease in DON content. Pyramiding QTL did not consistently affect flavor and aroma in the wheat lines. This research will provide valuable information for breeding decisions regarding disease resistance and maintaining grain quality. A better understanding of FHB resistance in wheat will further development of resistant cultivars while maintaining high quality grain.

Developing Fusarium Head Blight Resistant Durum Wheat for Montana

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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 we have acquired durum lines from our collaborator, Dr. Xiwen Cai, with *Fhb*1, *Fhb5,* and non-*Fhb* sources of resistance integrated from hexaploid wheat [1]. Populations were developed from crosses with these lines and tested for FHB resistance. Additionally, Dr. Cai worked with us to move *FHB7*^{The2} resistance sources into Montana adapted line MTD18348. Additional crosses will be made to combine all available forms of resistance in new breeding populations. The introgression of *FHB7*^{The2} into bread wheat and durum wheat from *Thinopyrom ponticum* has been shown to greatly reduce disease severity and DON accumulation [2,3]. We conducted field-based screening for FHB resistance in 2024 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. EARC is located on the border of North Dakota which has the highest durum production of any state, making this area the most relevant for testing FHB Resistant Durum. The development and release of FHB resistant lines will substantially decrease yield losses due to FHB and reduce DON levels in MT grown durum.

Germplasm Development for FHB Resistance in USDA-ARS

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Germplasm enhancement for Fusarium head blight resistance is built into research programs of USDA-ARS geneticists across the United States. The regional genotyping laboratories provide support for genetic marker development and application of both allele-specific markers and genome-wide genotyping for genomic prediction. In addition, ARS germplasm enhancement efforts collectively expand availability of well adapted parents with valuable resistance alleles for commercial breeding programs. At Manhattan, KS, near-isolines of the soft red winter (SRW) wheat ‘Clark’ with *Fhb1* were released (PI 668559 – PI 668563). These were followed by distribution of introgression of *Fhb1* into the Nebraska hard red winter wheat (HRW), ‘Wesley.’ Subsequently, Wesley-*Fhb1* was used to develop *Fhb1* introgressions into eleven regional HRWs from five breeding programs. One such line, an introgression into ‘Garrison’ named ‘Scab Stryker,’ was released in 2024 by Oklahoma State University. Building upon this work, pyramids combining *Fhb1*, *Fhb7* and *Fhb9* are being built into 15 backgrounds from 7 programs. At Fargo, ND, germplasm enhancement efforts in hard red spring wheat are using the ‘Sumai 3’ resistance and the Chr 5A resistance from the spelt, PI 277012. This resistance from PI 277012 also is being used for germplasm enhancement at Raleigh, NC, where efforts are underway to bring this resistance into coupling with the winter allele at *vrnA1* in SRW. The Raleigh program also brought *Fhb1* into coupling with the adult plant stem rust gene, *Sr2*, and distributed seed for evaluation in regional nurseries. The physiological black chaff associated with *Sr2* in these lines has limited adoption. At Manhattan, *Fhb1* and *Sr2* were similarly brought into coupling in HRW and distributed for evaluation/breeding. This material was recycled in breeding to address late maturity, soil-borne mosaic virus susceptibility, and weak gluten. The Lincoln, NE program released PI 702949, an introgression of $Fhb7^{The2}$ on 7BL into ‘Chinese Spring.’ This translocation is not associated with yellow flour pigment. The Raleigh, Manhattan, and Lincoln programs have initiated introgressions of $Fhb7^{The2}$ into soft and hard winter wheat. Scientists at Lincoln also have initiated introgressions of $Fhb7^{The2}$ into durum and are constructing pyramids of $Fhb7^{The2}$ with *Fhb1* and PI 277012 resistance in adapted germplasm. The Manhattan program also developed a hard winter wheat *Ms3*-facilitated recurrent selection population incorporating both exotic and regionally adapted resistance sources; release is anticipated in early 2025. Collectively, these germplasm enhancement efforts are dedicated to increasing the frequency of valuable resistance alleles in the broader pool of U.S. breeding germplasm.

Pyramiding Fusarium Head Blight Resistance in Wheat using Double Haploid Development

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Wheat doubled haploid (DH) could decrease the time to develop pure wheat lines by 3-4 years. We have been using the wheat DH to develop lines from F_1 or BC_1F_1 plants derived from various combinations of FHB resistance genes, such as *Fhb1*, *Fhb5*, Fhb6, *Fhb7* and some major QTL in both hard and soft wheat. About 200 DH lines are planted in the field in the scab nursery in College Station for testing and they are also planted in the field for yield testing. Harvested seeds will be distributed to collaborators for field testing in 2025. Available DHLs were sent to the genotyping center for *Fhb1* marker testing.

Genome-wide Association Analysis of Fusarium Head Blight Resistance in an Association Mapping Population Derived from Backcrosses of Synthetic Wheat with Spring Wheat Germplasm

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Fusarium head blight (FHB), caused by *Fusarium graminearum*, is currently one of the most destructive diseases affecting wheat (*Triticum aestivum*) and other cereal crops in North America and many regions of the world. Outbreaks of this disease can seriously impact the grain yield and end-use quality due to mycotoxin contamination. Improving FHB resistance has been a major focus in wheat genetics and breeding programs globally. However, only a few major loci have been effectively deployed, limiting progress in breeding for FHB resistance. The objectives of this study were to identify and map novel FHB resistance loci in synthetic wheat and hard red spring wheat (HRSW) germplasm using a genome-wide association study (GWAS) approach. We evaluated a panel of 274 spring wheat lines derived from backcrosses of four synthetic wheat lines from *T. dicoccum* (AABB) × *Aegilops tauschii* (DD) with eight HRSW varieties and breeding lines along with their parents for FHB response under two greenhouse seasons and four field environments over two years (2023–2024). Genotyping the population with single nucleotide polymorphism (SNP) markers generated by the 90K SNP Infinium array revealed 33,000 polymorphic markers, which were used for association mapping. A total of 16 significant marker-trait associations for FHB disease severity were identified on chromosomes 2A, 4A, 2B, 3B, 6B, 1D, 2D, and 5D, explaining 1-25% of phenotypic variation. Notably, the QTL on chromosome 4A not only has a major effect on FHB resistance but is also stable across different environments. Further validation of these QTL could facilitate successful introgression of new FHB resistance loci into breeding programs, potentially accelerating the development of FHB-resistant varieties.

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Quantification of FHB Using Aerial Phenotyping in Montana's Winter Wheat Breeding Lines

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Winter wheat (*Triticum aestivum* L.) is an important crop in Montana. Since 2015, the winter wheat breeding program has focused on improving Fusarium head blight (FHB) resistance in winter wheat. As part of the ongoing efforts, each year advanced lines in various stages of the breeding pipeline get evaluated in a misted nursery established at Southern Agricultural Research Center in Huntley, Montana. While manual disease scoring is still an important aspect of identifying disease severity in breeding lines, use of aerial phenotyping approaches is also being explored to evaluate the disease development and severity in the FHB nursery to increase precision. The objective of this study is to evaluate the potential of multispectral based aerial phenotyping to estimate FHB severity in winter wheat breeding lines. For this purpose, a M200 drone fitted with multispectral camera was utilized to collect data during the crop season-2024. The images were collected at different time points and heights viz. 10 ft, 33 ft. and 66 ft. While data is currently processing, the initial processed images indicated variations among in the breeding lines for disease severity, ranging from moderately resistant to highly susceptible, at visual level. We are using a hybrid approach utilizing both images and vegetative indices to train machine learning models for achieving high precision in the quantification of FHB severity.

The 2024 Uniform Southern Soft Red Winter Wheat Scab Nursery

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The Uniform Southern Soft Red Winter Wheat Scab Nursery gives public and private sector breeders the opportunity to obtain valuable data from multi-environment evaluations of FHB resistance for advanced generation breeding materials. The 2024 nursery was comprised of 54 advanced generation lines, three resistant checks 'Ernie', 'Bess', 'Jamestown', and two susceptible checks 'Coker 9835' and 'SS8641'. Seven U.S. public programs (Georgia, Louisiana, North Carolina, South Carolina, Texas, Virginia, and Maryland), and one private company (KWS) submitted entries. Data were collected for the FHB traits Rating, Severity, Incidence, FDK, and DON for breeding lines and compared with data from the long-term resistant check varieties. Data were also collected on important fungal and viral diseases, Hessian fly resistance, and agronomic characteristics. Field data were returned from up to nine locations in the US. Two USDA-ARS laboratories conducted evaluations for Hessian fly resistance and marker genotyping. Genotyping analyses for major QTL identified alleles present at 19 important loci. Genomic Estimated Breeding Values (GEBV) for nursery entries were provided as part of the continuing evaluation of the FHB training population for the Coordinated Project. The training population was comprised of nursery entries from 2011 to 2023. A combined mixed model analysis of the phenotypic data was performed using R (v 4.3.2) and best linear unbiased estimates (BLUEs) for each genotype were calculated. The genomic selection (GS) model utilized phenotypic BLUEs and 37,041 SNP markers to predict GEBVs for individuals in the 2024 nursery with the R-package RR-BLUP (v 4.6.3). GS model accuracy was evaluated by Pearson correlation between GEBVs and BLUEs for the 2024 entries. Correlation varied between 0.38 for DON and 0.69 for FHB 0-9 Rating. Machine learning models were used to provide estimates of presence/absence for 34 QTL of interest. Additionally, crosses were simulated among the 2023 and 2024 nursery entries using the R package PopVar (v 1.3.1) and estimates of progeny performance were reported. Copies of the full report will be available at the 2024 National Fusarium Head Blight Forum and subsequently online at the USWBSI web site at https://scabusa.org.

Acknowledgement and Disclaimer
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Effects of Fusarium Head Blight Resistance Genes on Flavor and Yield in Soft Red Winter Wheat

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Fusarium Head Blight (FHB) is an economically devastating disease of wheat causing low yields and poor quality. FHB, caused by *Fusarium graminearum*, disrupts the grain filling phase and results in bleached spikes and undeveloped seeds. Deoxynivalenol (DON) is a mycotoxin produced by the fungal pathogen and is harmful when consumed by humans and causes feed rejection in livestock. Using FHB resistant varieties and timely applications of fungicides is the optimum strategy for management of this disease. However, there has been little research focusing on the impact that FHB resistance genes have on wheat yield, flavor, and baking quality. The objective of this study is to evaluate the impacts of different combinations of resistance genes on yield and flavor. Knowing these impacts will improve breeding efforts for FHB resistance breeding of wheat while maintaining acceptable yield and flavor.

This study involves two populations: yield and quality populations. Both populations were created from a three-way cross between parents containing either FHB resistance genes, desirable characteristics (strong gluten, high yield, etc.), or both. From each of these two populations, 150 lines were derived and are being evaluated for presence of FHB resistance genes. There will soon be two years of agronomic data, one year of multi-location agronomic data, a flavor profile study, and genomic predictions. This study will also explore the reliability of machine learning and bioinformatic models to predict the resistance genes present in each line to identify lines that have the optimal balance between FHB resistance, yield, and flavor. This study aims to evaluate effects caused by breeding for FHB resistance to better focus breeding efforts on ways to achieve the highest FHB resistance and minimize negative effects on important agronomic and end-use characteristics of wheat.

FHB Detection Using 360-degree Deep Scanning Method

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Fusarium Head Blight (FHB) results in massive yield and quality losses in wheat and barley annually. Evaluating and estimating the infection level on FHB-resistant lines is time-consuming, labor-intensive, and needs expertise. This paper describes an innovative method to detect and assess the stage of FHB disease in wheat and barley by implementing the advancements in artificial intelligence (AI) and image processing and utilizing an innovative system. The 360° phenotyping robot, developed by the Precision Ag team at SDSU, captures close-up images of wheat heads to detect FHB symptoms even at early stages. In this study, 10,000 images were captured from an FHB-inoculated wheat field at SDSU's research farm in Volga, SD, forming a dataset for deep learning models. Images were annotated using the Roboflow platform's smart polygon tool, which precisely outlined irregular shapes and improved detection accuracy. The dataset was categorized into "Healthy" and "Unhealthy" classes and split into training (70%), validation (15%), and testing (15%) sets. Faster R-CNN was utilized initially for object detection, effectively predicting bounding boxes and segmenting infected areas. The model training included 80 epochs, with early stopping triggered at epoch 38 to avoid overfitting. Key hyperparameters included a learning rate of 0.005, momentum of 0.9, and stochastic gradient descent (SGD) optimizer. Metrics such as precision, recall, mean average precision (mAP), and Intersection over Union (IoU) evaluated model performance, with IoU improving from 0.1660 to 0.5942, precision from 0.0602 to 0.2961, and recall from 0.1328 to 0.4201. Results indicated effective learning, as train loss decreased from 1.7762 to 1.1850 and validation loss from 1.7436 to 1.2841, signaling successful model generalization. However, future approaches could integrate multiple models, such as attention U-Net, U-Net, PSPNet, MANet, SSD, and DeepLabV3+, to enhance segmentation and detection accuracy. These advanced architectures, especially those incorporating attention mechanisms, could focus on disease-affected areas and improve detection sensitivity.

Characterization of the Effects of Three Major Genes on Fusarium Head Blight Resistance in Different U.S. Hard Winter Wheat Backgrounds

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Fusarium Head Blight (FHB) is a devastating disease in wheat worldwide, causing significant yield losses and mycotoxin contamination in infected grain. Although hundreds of resistance genes or quantitative trait loci (QTL) have been reported to date, wheat cultivars used in farmers' fields in the Great Plains are mainly FHB susceptible. Recently, two major effect genes (*Fhb1* and *Fhb7*) have been cloned, and their gene makers (*TaHRC-KASP* and *Fhb7-828bp*) are available. *Fhb9* is a novel QTL from a Chinese cultivar with a major effect and has flanking markers (*KASP-10238* and *KASP-12056*) available. However, none of them have been deployed in any U.S. hard winter wheat (HWW). Therefore, it remains unknown how they perform in diverse U.S. HWW genetic backgrounds. This study used marker-assisted backcrossing to transfer the three genes into six HWW varieties (Guardian, KS16DH0010-17, NE18445, OK19225, Providence, and U7494) with various levels of FHB resistance. We stacked the three genes from different donors, and then crossed and backcrossed each recurrent parent to the donor carrying all three genes using marker-assisted selection (MAS). We selected 1-19 homozygous plants containing all three genes, none of the three genes, each of the three genes, or two genes in different combinations for each cross after screening 3,592 BC_2F_2 plants with four markers. About 400 BC_2F_2 plants were selected and advanced, and their BC_2F_3 and BC_2F_4 lines will be phenotyped for FHB resistance in both greenhouse and field experiments. The selected plants will be screened using multiplex restriction amplicon sequencing (MRASeq) to evaluate the donor-genomes' contribution in each selected line. The results from this study will lead to understanding of FHB resistance performance of *Fhb1*, *Fhb7*, and *Fhb9* across diverse HWW genetic backgrounds and release of improved germplasm with these major genes in adapted HWW genetic backgrounds.

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

Thirty Years of the Hard Red Spring Wheat Uniform Regional Scab Nursery

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The USDA-coordinated hard red spring wheat uniform regional scab nursery (URSN) was initiated in 1995 in response to the emerging threat posed by Fusarium head blight in US spring wheat growing regions. 2024 marked the 30th consecutive year of testing breeding material in these artificially inoculated nurseries. Here I will describe how the number and location of testing sites, data collected, number of entries screened, as well as number and type of participants has evolved over the URSN's history. All URSN data is deposited in the T3 database and is publicly available on the GrainGenes website. These data represent a valuable resource and have been accessed by USDA and academic collaborators to develop a genomic prediction model to aid in the advancement of experimental lines in breeding programs. We invite industry, academic, and government cooperators to submit seed for testing and look forward to continuing to provide valuable scab related data to our stakeholders.

Training Population Optimization for within-Family Predictions of Fusarium Head Blight Traits

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Genomic prediction is most useful in early generations, but the family structure in these generations can reduce selection efficacy. To test genomic prediction strategies at this stage, a population representing an early generation breeding program stage (450 genotypes) was planted in two locations in November of 2023 and harvested at the beginning of June in Baton Rouge and Winnsboro, Louisiana. During the field season, heading data was collected in both locations, and plant height was collected in Winnsboro. Visual FHB ratings were taken after the appearance of the disease. After harvest, Fusarium damaged kernel (FDK) rates were taken on harvested grain, and ground samples were assayed for Deoxinivalenol (DON) content. Multi-trait models utilizing correlations between traits were used to generate predictions between and within families.

Deoxynivalenol Accumulation in Lines Tested in the Winter North American Barley Scab Evaluation Nursery

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A winter barley scab evaluation nursery was planted at multiple locations across North America during the 2021–22 and 2022–23 field seasons. This trial is referred to as the winter North American Barley Scab Evaluation Nursery (NABSEN). Six North American public breeders submitted lines for inclusion the 2021–22 season, and seven the 2022–23 season. The nursery was planted at seven locations 2021–22 and eight locations 2022–23. Disease pressure was significantly higher at the Blacksburg Virginia and Wooster Ohio locations both seasons over that of the other trial locations. Comparison of DON levels between the Blacksburg and Wooster locations for the individual lines indicated a significant correlation for the two trial years. Ranking the lines for DON accumulation using only the Ohio and Virginia data was carried out, which showed stratification of the lines. A question raised was whether lines were escaping infection as a consequence of heading date, for example by avoiding high disease pressure when environmental conditions are most favorable for disease progression. To address this question, a correlation test and an analysis of variance (ANOVA) was carried out. Neither of these analytical methods supported there being a strong correlation between DON levels and heading date. However, the ANOVA suggested the possibility of a correlation between DON levels of the line and the program from which the line came from; a preponderance of low DON accumulating lines emanated from the University of Minnesota and Virginia Tech breeding programs. Thank you to Drs. Pierce Paul and Wanderson Moraes for providing the corn spawn and *F. graminearum* spore inoculum and for your guidance and help with inoculations, to all NABSEN participants planting the nursery and carrying out all steps to obtain the data, and to Dr. David Francis for providing help with some of the statistical analyses. This work is supported by the United States Department of Agriculture and the U.S. Wheat and Barley Scab Initiative. This material is based upon work supported by the U.S. Department of Agriculture, under Agreement No. 59-0206-0-174, and USWBSI Project ID FY22-BA-006. This is a cooperative project with the U.S. Wheat & Barley Scab Initiative. Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the author(s) and do not necessarily reflect the view of the U.S. Department of Agriculture.

Breeding Winter Wheat for Fusarium Head Blight (FHB) Resistance in Canada

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Fusarium graminearum (Schwabe) (FG) is the principal cause of Fusarium head blight (FHB) disease of wheat. Deoxynivalenol (DON) is the most important mycotoxin produced by FG. Winter wheat is mainly grown in Eastern Canada. Our goal is development of wheat resistant to FHB, without yield and quality penalties. Conventional breeding, doubled haploid method, and marker-assisted selection are applied in the program, with combination of exotic and adapted sources of FHB resistance. In population with 'Sumai 3' as a source of FHB resistance, the breeding lines grouped in the 3B QTL class had the lowest FHB index, DON content and FDK level and did not have a significantly lower yield compared to the lines grouped in QTL classes 3A and 5A, or their combinations. Using adapted FHB sources, we detected QTL for FHB/DON on chromosomes 2D, 4B, 4D and 7A, in a population 'Vienna' x '25R47'. The plant height dwarfing alleles of *Rht-B1* and *Rht-D1* were associated with increased FHB index/DON level. The same QTL, except 7A, were identified in 'Maxine x 'FTHP Redeemer' population. Recent genotyping in soft white population 'Superior' x 'D8006W' was performed using 90 K Infinium iSelect SNP array. FHB resistance QTL were detected on chromosomes 2D, 4B, 5A, and 7A across multiple environments, with resistance from both parents. Trait specific QTL were detected on chromosomes 1A (symptoms), 5D (FDK), 6B (FDK and DON), and 7D (DON). The plant height and days to anthesis QTL on chromosome 2D coincided with *Ppd-D1* and were linked with FHB traits. The Eastern winter wheat genomic selection project (EWGS) is an effort aimed to harmonize breeding efforts across four public breeding programs via the development of common genomic tools for complex traits, such as FHB resistance. An analysis of allele frequency of three major FHB resistance QTLs (*Fhb1, Fhb2*, and *Fhb5*) in a 352 winter wheat breeding lines was performed. *Fhb5* was the most common resistance allele (present in 61% of accessions), while *Fhb1* and *Fhb2* were rare (9% and 0.6%, respectively). Commercially grown wheat in Ontario is entered in the Performance Trial and tested for FHB resistance in the nurseries spray inoculated with FG. 'Marker', 'UGRC Ring', 'UGRC C2-5' and 'UGRC Comet' are soft red winter wheats developed by our breeding program (www.gocereals.ca). The cultivars had competitive yield in Eastern Canada, but their FHB resistance is moderately resistant or moderately susceptible.

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Determination of Trichothecene Mycotoxins in Wheat Grains from Cultivar Trials in Paraná State, Southern Brazil

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Fusarium head blight (FHB) epidemics on wheat have been recurrent in subtropical southern Brazil. The contamination of grains by mycotoxins associated with FHB has gained increasing importance in the Brazilian flour market and wheat–derived products. Efforts have been directed towards developing genetic resistance in wheat against FHB and mycotoxin contamination, particularly concerning deoxynivalenol (DON) and zearalenone (ZEA). However, the diversity of *Fusarium* species associated with FHB in southern Brazil must be considered due to their potential to produce different mycotoxins. A published study involving 317 strains from Paraná State highlighted the predominance of the *F. graminearum* species complex (FGSC) as FHB pathogens. *Fusarium graminearum s. str*. of the 15-acetyl(A)DON genotype accounted for 69%, followed by *F. meridionale* of the nivalenol (NIV) genotype at 19%, *F. cortaderiae* of the NIV and 3-ADON genotypes at 8%, and *F. austroamericanum* of the 3-ADON genotype at 3%, all of which are type B trichothecene producers. Other species included *F. poae* (type A trichothecene), *F. tricinctum, F. fujikuroi*, and *F. incarnatum-equiseti* complexes, each comprising less than 1%. This diverse array of species and trichothecene genotypes has influenced the inoculum composition used in genetic resistance trials. This study aimed to determine the range of mycotoxin contamination, including type A and B trichothecenes and ZEA, in wheat grains (whole grain flour) from experimental plots testing cultivar resistance to FHB from 2019 to 2023 in the southern region of Paraná. These plots were artificially inoculated with a macroconidial suspension of 10 isolates, representing the diversity of FGSC species and trichothecene genotypes described above, with concentrations ranging from 0,35–6,7x10^4 spores per mL, applied to wheat at the mid-anthesis stage. Mycotoxin content was measured using standard protocols at the Central Laboratory of Agraria Cooperative with an Acquity UPLC I-Class Plus System. DON was the predominant mycotoxin, with levels varying across genotypes and years [below detection level (BDL)–33341 µg/kg], alongside 15-ADON (BDL–320 µg/kg) and NIV (BDL–2887 µg/kg). Notably, 15-ADON and 3-ADON were detected only in the last three years, while NIV and DON-3-glucoside (D3G) (BDL – 3561 µg/kg) levels showed more stability than DON over the years. Type A trichothecenes such as diacetoxyscirpenol (DAS), T-2, and HT-2 mycotoxins were absent. In conclusion, DON is a primary concern, although NIV, D3G, and ZEA warrant attention, and the relevance of 15-ADON and 3-ADON requires further study. This information will be useful for mycotoxin management in Brazil.

Enhancing Predictive Accuracy for Fusarium Head Blight-Related Traits in Winter Wheat through Integrating Genomics, Phenomics, and Deep Learning

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Fusarium head blight (FHB) remains one of the most destructive diseases of wheat (*Triticum aestivum* L.), causing considerable losses in yield and end-use quality. Phenotyping of FHB resistance traits, *Fusarium*-damaged kernels (FDK), and deoxynivalenol (DON), is either prone to human biases or resource expensive, hindering the progress in breeding for FHB-resistant cultivars. Though genomic selection (GS) can be an effective way to select these traits, inaccurate phenotyping remains a hurdle in exploiting this approach. Here, we used an artificial intelligence (AI)-based precise FDK estimation that exhibits high heritability and correlation with DON. Further, GS using AI-based FDK (FDK_QVIS/FDK_QNIR) showed a two-fold increase in predictive ability (PA) compared to GS for traditionally estimated FDK (FDK_V). Next, the AI-based FDK was evaluated along with other traits in multi-trait (MT) GS models to predict DON. The inclusion of FDK_QNIR and FDK_QVIS with days to heading as covariates improved the PA for DON by 58% over the baseline single-trait GS model. We next used hyperspectral imaging of FHB-infected wheat kernels as a novel avenue to improve the MT GS for DON. The PA for DON using selected wavebands derived from hyperspectral imaging in MT GS models surpassed the single-trait GS model by around 40%. Finally, we evaluated phenomic prediction for DON by integrating hyperspectral imaging with deep learning to directly predict DON in FHB-infected wheat kernels and observed an accuracy (R2 = 0.45) comparable to best-performing MT GS models. This study demonstrates the potential application of AI and vision-based platforms to improve PA for FHB-related traits using genomic and phenomic selection.

Identification of Fusarium Head Blight (FHB) Resistance Loci in Winter Wheat Using Exome and Promoter Capture Single Nucleotide Polymorphisms (SNPs)

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Advancing Fusarium head blight (FHB) resistance relies on discovering new sources of genetic resistance through novel genomic loci linked to causal genes. Current adapted wheat cultivars exhibit only moderate genetic resistance, which has proven insufficient due to the quantitative nature of disease resistance. We developed a diverse panel of 269 winter wheat lines representing different U.S. wheat-growing regions to uncover novel loci and enhance breeding efforts. We employed exome and promoter capture sequencing to generate single nucleotide polymorphisms (SNPs) for the panel, focusing on gene coding and regulatory regions, which are typically informative based on other traits and disease interactions. Three years (2022, 2023, 2024) of field evaluations were conducted to assess each line's response to FHB, focusing on disease severity metrics, including Area Under the Disease Progress Curve (AUDPC), Fusarium Damaged Kernel (FDK), and Deoxynivalenol (DON) content. Using these phenotypic data, we performed genome-wide association studies (GWAS) to identify genomic loci associated with FHB resistance. The results show significant variability in disease severity metrics (p-value <0.05) across breeding lines, including some more resistant than the moderately resistant checks. Exome and promoter capture generated ~645,000 overlapping SNPs. Using the best linear unbiased estimates (BLUEs) values of the FHB disease severity metrics, we mapped novel QTLs conferring FHB resistance or susceptibility alleles using mixed linear models (MLM) in GWAS. Some significant novel QTLs were identified to be associated with FHB disease severity metrics with heritability of traits being 0.58 for AUDPC, 0.57 for FDK, and 0.32 for DON. These newly identified QTLs will undergo validation through Kompetitive Allele Specific PCR (KASP) assays on independent populations. Incorporating these rare loci into adapted wheat varieties has the potential to strengthen genetic resistance to FHB in winter wheat, providing farmers with more robust options to mitigate the disease.

Breeding Scab Resistant Wheat, Just South of the Mason-Dixon Line

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Kentucky is often described as belonging to the Upper South or the Southern Corn Belt; in other words, it does not fit naturally into standard geographies. Situated just below the Mason-Dixon Line, Kentucky is the epicenter of doublecropping, in which growers produce 3 crops in 2 years: corn is planted no-till in the spring and harvested in September; wheat is sown without tillage directly into corn stover in October and harvested in June, with soybean drills following closely behind wheat combines to plant “doublecrop beans”. This profitable rotation puts the SRW wheat crop at risk for FHB every year. Our resistance breeding approach has relied heavily on phenotyping breeding lines and cultivars in a mist-irrigated nursery, inoculated with grain spawn. In 2016, however, the UK Wheat Breeding Program began to use genomic selection (GS) for FHB, agronomic and quality traits. Initially, we simply added GS to the protocol we had previously followed, testing preliminary lines in an augmented design at one or two locations. After determining the augmented design was no more predictive of future performance than GS, we began to use GS alone at the preliminary line stage in 2021. $F_{3:4}$ headrows are selected phenotypically and remnant seed is genotyped with genome wide markers. Genomic predictions for $F_{4:5}$ progeny are generated using the genomic data coupled with phenotypic data from field-tested lines (the training population) related to the F_4 lines. Selections are ultimately based on genomic predictions with some attention to phenotype – height, maturity, vigor, clean leaves. Genomic predictions have been very useful for FHB resistance breeding: from 2019 - 2022, weather during the infection period made it impossible to create an epidemic in the irrigated scab nursery. Genomic predictions for FHB index and DON were used in guiding selections for resistance. In 2023 and 2024 we compared predictions developed from two very diverse sets of training populations: one set associated with Sungrains, a consortium of southern states, the second set with Norgrains, a consortium of northern states. Differences between the two sets of predictions were abundant, but certain parents were present in the pedigrees of top performing lines from both sets of predictions.

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