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# 2025 National Fusarium Head Blight Forum

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# Opening Session Presentations

## Design, Analyze, and Predict: Open-Source Tools to Advance Breeding and Screening for Fusarium Head Blight Resistance

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Public agricultural research programs excel at collecting data; however, there is a gap from collection to analysis that could be addressed by using tools and technologies that support interoperability for quicker decision-making. Incorporating digital solutions that connect experimental design, data analysis, and predictive modeling would allow researchers without programming skills to manage their experiment design and analysis, as well as develop predictive models for genomic or phenomic selection using high-throughput phenotyping (HTP) data. The tools include FieldHub, MrBean, PredictPro, and AgSkySight. FieldHub facilitates the design of field and greenhouse experimental trials. Its web-based interface makes it easy to create simple or complex designs of experiments (DOE). For example, the spatial design with repeated checks in FieldHub works well for mist-irrigated FHB nurseries. Analyzing this type of data is regularly done with MrBean, an application that combines descriptive analysis, measures of dispersion and central tendency, visualizations, and linear mixed models (LMMs) analysis for single- and multi-environment trials. Additionally, PredictPro helps develop, validate, and deploy predictive models for genomic or phenotypic selection, using statistical-, machine-, and deep-learning algorithms. Lastly, AgSkySight converts image data into vegetative indices for further analysis. When used together, FieldHub, MrBean, PredictPro, and AgSkySight create a fully connected analytics ecosystem that enhances transparency, interoperability, and scalability for genomic selection and HTP prediction. This approach aligns with the goals of the U.S. Wheat and Barley Scab Initiative (USWBSI), demonstrating that open collaboration and shared digital tools can accelerate genetic gains and improve resilience in modern agricultural research.

## **Better Together: Industry Relationships with Research are More Important Than Ever**

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In the current challenging political climate, where the value of science is not well understood, industry must join with researchers to speak out on the important work that underpins our safe, reliable food system.

# **FHB Management**



## Research Needs in Organic Grain Production

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Organic grain production is a growing sector in U.S. agriculture, particularly in the Midwest and Northeast. Although it still accounts for a small fraction of total grain acreage, the sector is expanding rapidly. In 2021, over 17,000 farms managed 4.9 million acres under organic production, with 811,676 acres dedicated to organic grain and oilseed with an estimated value of \$737 million. To support this growth and improve yield, quality, and sustainability, targeted research on disease management in organic grain systems is critical. Organic grain producers face several unique challenges in managing disease effectively. One major issue is variable disease pressure; in low-pressure years or regions, the benefits of disease control products can be difficult to demonstrate, which then require greater effort in generating data from multiple locations and years to determine effectiveness. Additionally, organic-approved (OMRI-listed) products often lack standardized application guidelines for timing and rate, complicating cross-study comparisons and on-farm implementation. Biological product performance can further be affected by formulation, shelf life, and environmental factors, introducing variability in efficacy. Cultural practices—central to organic systems—also influence disease dynamics, and trials that fail to incorporate these elements may yield misleading results. In addition, we are unable to inoculate in organic certified land which limits ability to determine pathogen specific efficacy of products in research. Moreover, cost and labor constraints limit the feasibility of large, replicated, multi-site trials on many organic farms, slowing the pace of evidence generation. Key research priorities include first identifying the major disease issues to then develop integrated disease management (IDM) strategies tailored to organic systems, identifying and breeding disease-resistant crop varieties suited for organic-certified seed production, and evaluating alternative cultural practices such as crop rotation, cover cropping, soil fertility management, soil health and suppressive soils. There is also significant opportunity to assess the efficacy and practical use of biocontrol agents and other alternative inputs within real-world organic farming contexts. Collaborative, interdisciplinary research will be essential to address these challenges and gaps. By aligning efforts across plant breeding, agronomy, and plant pathology—while engaging organic producers in participatory research—scientists can help build more resilient, productive organic grain systems. Advancing knowledge in these areas will empower organic farmers with effective tools and strategies for disease management, ultimately contributing to a more sustainable and economically viable organic grain sector.

## **Cropping with Complexity: How the Diversity of Soft Winter Wheat and Barley Production Systems in the U.S. Challenge Our Scab Management**

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How do the elements of our growing systems influence and complicate our ability to reduce Fusarium head blight and mycotoxin contamination? In many regions of the United States, small grains are important but must compete for priority with several other critical crops. Growing wheat and barley following corn is something producers in these regions often have no way to avoid. Economic considerations can force growers to give precedence to forage production and limit rotational options. Cultural and policy incentives toward environmental improvement make no-till production prevalent and crop residue abundant. These regions are also now experiencing more frequent weather extremes, resulting in unpredictable and intense temperature and moisture swings, making them difficult to account for in disease prediction models. Management for scab reduction in these systems presents numerous decision-making challenges for farmers and their advisors. We will explore how the convergence of factors in the diverse cropping systems of the southeast, mid-Atlantic and northeast U.S. influences our ability to sufficiently understand risk and efficacy of management strategies. Cases will be presented from small grain production regions in North Carolina, Pennsylvania and Kentucky to drive discussion about how we weigh management strategies and to understand where previous recommendations have broken down. How should we frame our research questions to address the intricacies of these systems and what are the elements missing from our focus that can drive the next phase of integrated management investigation?

## More Fungicides Than Ever for Fusarium Head Blight and Deoxynivalenol Management: How Good are They?

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As part of the Fusarium head blight (FHB) Management Coordinated project (MGMT\_CP), Uniform Fungicide (UFT) and Integrated Management (IMT) trials have been conducted in all major wheat and malting barley market classes across states commonly affected by FHB. Over the years, the UFT program has evolved from simply testing experimental fungicides and biological control agents to evaluating commercially available fungicides under highly FHB-favorable conditions that are promoted by mist irrigation and/or artificial inoculations of plots planted with susceptible varieties. Studies conducted during the early years of the UFT program led to the discovery that Demethylation Inhibitor (DMI) fungicides were more effective against FHB and DON than the other chemistries evaluated at the time, that Metconazole, Prothioconazole and Tebuconazole were the most effective of the available DMIs; that heading and early-anthesis were the optimum growth stages for treatment application to effectively manage FHB and DON in barley and wheat, respectively; and the establishment of Prosaro® and Caramba® as industry standards for FHB management. Thanks in no small part to the MGMT\_CP, several fungicides have received federal registration for FHB and DON management, and invaluable information has been generated regarding the efficacy of pre- and post-anthesis fungicide applications; the negative effects of Quinone Outside Inhibitor fungicides on DON contamination of grain, even when applied at early growth stages for foliar disease management; the efficacy and economic benefit of sequential fungicide applications; the efficacy and stability of integrating a well-timed fungicide application and genetic resistance for FHB/DON management; and more recently, the efficacy of newer DMI-DMI and DMI-SDHI (succinate dehydrogenase inhibitor) premix fungicides relative to the industry standards. Findings from these studies have led to the development of management programs with FHB and DON control efficacy consistently greater than 70%, and relatedly, changes to in-field FHB/DON management recommendations that have helped to reduce the negative impacts of FHB and DON in multiple production regions and grain classes.

## 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 2025 we observed the disease and yield impact of inoculation with *Fusarium graminearum*, and treatment with ChampION™ a copper-based organic fungicide at two timings on a susceptible (*var* Robust) and moderately susceptible (*var* ND Genesis) variety of barley. The ChampION fungicide was applied at heading and at heading plus 4 days after heading. The 2025 growing season has above average rainfall throughout the spring but dry conditions throughout the remainder of the growing season. Varieties differed in their DON concentrations with ND Genesis having significantly lower DON concentrations compared to Robust. All fungicide treatments and timings, including the control and the *Fusarium* inoculated plots, had DON concentrations below 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 0.660 ppm and was only significantly higher compared to the control. 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 variety in combination with application timings of a copper based organic fungicide 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 2025 we observed the disease and yield impact of inoculation with *Fusarium graminearum*, and treatment with ChampION a copper-based organic fungicide at two timings on a susceptible (*var* Robust) and moderately susceptible (*var* ND Genesis) variety of barley.

## 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 14, 2025. The plots were sown with Robust and ND Genesis barley at 350 seeds per m<sup>2</sup> with a Great Plains grain drill (Salinas, KS). The experiment was set up as a completely randomized block design in a split plot arrangement with variety as the main plot and fungicide treatments as the split plot. There were five replicates. 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, 2025 to ND Genesis and June 17, 2025, to Robust. 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. ChampION was also applied both at heading and 4 days after heading which occurred on June 17, 2025 for ND Genesis and June 23, 2025 for Robust. Fungicide and *F. graminearum* treatments were applied with a CO<sub>2</sub> backpack sprayer with paired TJ-60 8003vs nozzles mounted at an angle (30° from horizontal) forward and backward, 20-in. apart, pressurized at 30 psi, and calibrated to deliver 20 gal/A. Grain was harvested using an Almaco plot combine (Nevada, IA) on July 18, 2025. Grain moisture, plot yield, and test weight were recorded. Yield and test weight were adjusted to bushels ac<sup>-1</sup> 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 2025 growing season can be characterized as moderate temperatures with excessive spring rains and dry summer conditions. Temperatures were at or slightly above the 30-year average. Rainfall was well above the 30-year average with 5.12 inches of precipitation, more than normal in the month of May. However, dry and warm conditions persisted throughout the remainder of the growing season.

Selecting varieties can be a first step in reducing the risk of FHB infection of a grain crop. In this study the moderately susceptible variety had significantly less DON concentrations compared to the susceptible variety (Table 2). However, there was no variety by fungicide treatment interaction indicating that the varieties responded similarly to the fungicide treatments.

All fungicide treatments and timings, including the control and the Fusarium inoculated plots, had DON concentrations below 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 0.66 ppm and was significantly higher only to the control. 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
<i>Fusarium graminearum</i>			40,000 spores/ml
Champ ION <sup>++</sup>	NuFarm	Copper hydroxide	1.5 lbs ac <sup>-1</sup>

**Table 2.** Effect of barley variety on deoxynivalenol (DON) contamination and grain yield at Alburgh, VT, 2025.

Variety	DON	Test weight	Yield
	ppm	lb bu <sup>-1</sup>	bu ac <sup>-1</sup>
ND Genesis (moderately susceptible)	0.336	43.7	69.2
Robust (susceptible)	0.665	46.6	72.8
LSD (p=0.05) †	0.156	NS	NS

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

**Table 3.** Effect of organic fungicide + timing on deoxynivalenol (DON) contamination and grain yield at Alburgh, VT, 2025.

Fungicide + timing	DON	Test weight	Yield
	ppm	lb bu <sup>-1</sup>	bu ac <sup>-1</sup>
Non-sprayed, non-inoculated control	0.340	44.8	65.4
Inoculated FGS at heading	0.535	44.4	55.4
ChampION at heading	0.660	43.8	60.4
ChampION at heading plus 4 days after heading	0.470	45.7	59.7
LSD (p=0.05) †	0.220	NS	NS

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

## Effective Inhibition of Fungal Growth and Mycotoxin Biosynthesis in Wheat Pathogens *Aspergillus* and *Fusarium* spp. Through Pulsed Light Treatment

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The ongoing contamination of cereal grains by *Aspergillus* and *Fusarium* species poses a significant food safety risk, as these fungi are highly resistant to standard decontamination techniques and produce harmful mycotoxins. This study investigated pulsed light (PL) treatment as a means to inactivate fungi and reduce mycotoxins in wheat kernels inoculated with *Aspergillus flavus* (ATCC 15548) or *Fusarium graminearum* (ATCC 46779) (~7 log CFU/g). Two approaches were compared: grains stored at 5°C, 15°C, or 30°C for 5 days before PL treatment, and grains treated with PL (0.018–0.037 J/cm<sup>2</sup>) immediately after inoculation, then stored for 5 days at the same temperatures. PL at an effective fluence of 0.037 J/cm<sup>2</sup> resulted in over 5-log reductions in fungal counts for both fungi across both treatment methods. Pre-existing mycotoxins in wheat kernels were notably harder to eliminate than those in kernels treated with pulsed light prior to incubation. When pulsed light was applied to kernels already containing toxins, reductions reached up to 75% for deoxynivalenol (DON) and 90% for aflatoxin B1. Nonetheless, higher residual mycotoxin levels were observed at 30°C, underscoring the influence of storage temperature on toxin persistence and overall reduction efficiency.

## Fusarium Head Blight: Species Concepts in the Midwest and East Coast

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Fusarium Head Blight (FHB) is one of the most economically devastating diseases of wheat globally and caused estimated losses of \$171,000,000 in 2024 in the United States. FHB is caused by fungal phytopathogens from the genus *Fusarium*. While the predominant species in the United States is *F. graminearum*, several less prevalent species are known to coexist within the same grain tissue. To further complicate the identification of *Fusarium* species implicated in this disease, the genus *Fusarium* is comprised of several species complexes which require multilocus genotyping to accurately determine the species identity of isolates. This study assesses the species diversity of *Fusarium* isolates derived from various cereal grains in the United States Midwest and East Coast. We have successfully obtained 400+ isolates from symptomatic cereal grains from 15 states in the Midwest and East Coast from 2021-2025. In this collection, *Fusarium* has been isolated from wheat, barley, oats and rye. So far, we have confirmed the species identity of 205 isolates collected from 2021-2024 in the Midwest using the TEF1 $\alpha$  and RPB2 loci. To our understanding, we have identified a potential global first report of *F. nanum*. The isolate came from a two-row barley spike from Minnesota in 2024. Interestingly, it was isolated along with *F. graminearum* from a single kernel. Kochs postulate must be tested for the *F. nanum* to confirm its ability to be a causal agent of FHB. The collection of *Fusarium* isolates will be subjected to fungicide sensitivity testing across various modes of action to assess whether insensitive populations of *Fusarium* are present in the Midwest or East Coast small grain growing regions. The information obtained from this study will improve our understanding of FHB management in the Midwest and East Coast states.

## Dual Fungicide Applications for Fusarium Head Blight Management in Winter Wheat: Results From the IM-CP UFT in Kansas, 2024-25

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Fusarium head blight (FHB), caused by *Fusarium graminearum*, it is one of the most economically significant fungal diseases, causing substantial losses to producers in various regions of the world. Integrated management of wheat scab combines the use of resistant cultivars and the application of fungicides at the beginning of flowering (Feekes 10.5.1). We hypothesize is that, for FHB management, a second fungicide application performed four days after the initial treatment at Feekes 10.5.1 may result in greater disease control efficacy. The experiments were conducted in Manhattan and Ottawa during the 2024-25 season. The variety 'Green Hammer', classified as susceptible to FHB, was used in this study. Plots were inoculated with *F. graminearum*- infested corn spawn. The experimental fields were irrigated using a sprinkler system. Treatments followed the USWBSI MGMT-CP Uniform Fungicide Trial (UFT) protocol and consisted of 1) Non-treated control, 2) Prosaro® 6.5 fl oz/A at flowering, 3) Miravis® Era 10.3 fl oz/A at flowering, 4) Miravis Ace 13.7 fl oz/A at flowering, 5) Prosaro Pro 10.3 fl oz/A at flowering, 6) Sphaerex® 7.3 fl oz/A at flowering, 7) Miravis Ace 13.7 fl oz/A followed by Prosaro pro 10.3 fl oz/A, 8) Miravis Ace 13.7 fl oz/A followed by Sphaerex 7.3 fl oz/A, 9) Miravis Ace 13.7 fl oz/A followed by TebuStar® 4 fl oz/A, 10) TebuStar 4 fl oz/A followed by Miravis Ace 13.7 fl oz/A. FHB severity was evaluated as the average symptomatic area of heads infected out of 90 total heads per plot. Data collected were subjected to mixed-model analysis of variance, and means were compared using Tukey's Honest Significance Difference (HSD) at a 5% level of significance. Fungicide applications had no significant effect on yield or test weight in Manhattan, although the non-treated check generally yielded lower than the plots that received a fungicide treatment. Similarly, in Ottawa, no significant difference was observed among treatments for yield; however, a significant effect was observed for test weight ( $p = 0.0032$ ), with higher values observed for treatments 7 and 8. Significant differences were observed in disease-related variables at both locations. In Manhattan, treatments had a significant effect on disease index ( $p < 0.0001$ ), FDK ( $p < 0.0001$ ), and DON ( $p < 0.0001$ ). Similar results were observed in Ottawa. Overall, a consistent reduction in disease levels was observed with fungicide use, particularly with sequential applications, with treatments 7 and 8 showing the greatest level of disease control.

## Image Analysis-Based, High-Throughput Screening for Fungicide Sensitivity to Pydiflumetofen and Tebuconazole in U.S. *Fusarium* Head Blight Populations

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*Fusarium* head blight (FHB), primarily caused by *Fusarium graminearum*, remains a critical threat to global cereal production. This threat is exacerbated by increasing concerns over fungicide resistance, particularly to widely used demethylation inhibitor (DMI) fungicides like tebuconazole. This study aimed to develop a high-throughput method for assessing in vitro fungicidal efficacy, and to evaluate the sensitivity of 500+ FHB isolates collected across the United States to pydiflumetofen (SDHI) and tebuconazole (DMI). We developed and validated a novel image analysis pipeline utilizing the Segmentation Anything Model (SAM) for automated quantification of mycelial growth area. This automated method demonstrated high accuracy and a strong correlation with manual measurements ( $R^2 > 0.87$ ). This automated process was subsequently employed to calculate the EC<sub>50</sub> value for 60 representative *F. graminearum* isolates across five different concentrations of tebuconazole and pydiflumetofen. The EC<sub>50</sub> distribution for pydiflumetofen showed a highly similar range to previous studies (median variation from 0.050 to 0.056 mg L<sup>-1</sup>), whereas the tebuconazole EC<sub>50</sub> distribution exhibited an increase in the median value (from 0.23 to 0.42 mg L<sup>-1</sup>) compared to historical data. A linear regression approach further confirmed that EC<sub>50</sub> values could be estimated using only two specific fungicide concentrations, significantly accelerating the screening process. Utilizing this high-throughput system, we conducted a large-scale fungicidal sensitivity screening. The screening across the *Fusarium* spp. populations successfully confirmed the distribution and range of EC<sub>50</sub> values for both tebuconazole (0.13-7.72 mg L<sup>-1</sup>) and pydiflumetofen (0.002-190.5 mg L<sup>-1</sup>). Furthermore, correlation analysis revealed a tendency for isolates with higher EC<sub>50</sub> values to exhibit slower mycelial growth rates. In summary, this research established an efficient, objective, and reproducible method for FHB fungicide sensitivity testing. It also revealed shifting fungicide sensitivity profiles within U.S. FHB populations, necessitating proactive resistance management strategies.



## Quantifying the Additive Effects of a Sequential Fungicide Treatment Program FHB and DON Management in SRWW in Ohio

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Fusarium head blight (FHB), caused by the fungus *Fusarium graminearum*, reduces wheat yield and leads to grain contamination with mycotoxins, including deoxynivalenol (DON), which poses health risks for humans and animals. Under favorable weather conditions, a single fungicide application at anthesis may not be sufficient to reduce FHB and DON. In this study we continue and expand on ongoing research to evaluate the efficacy of double/sequential fungicide application programs by investigating the additivity of an anthesis application of Miravis® Ace followed by a post-anthesis application of Prosaro® for FHB and DON management. To accomplish this objective, separate plots of a susceptible wheat cultivar were treated either with Miravis Ace at early anthesis (MIR\_A), Prosaro at 4 days after anthesis (PRO\_4), Miravis Ace at early anthesis followed by Prosaro 4 days later (MIR\_PRO), or left untreated. Trials were conducted in 2018, 2019, 2020 and 2021, with treatments arranged in a randomized complete block design with four replications. FHB index (IND) was rated at Feekes 11.2, and grain samples were collected at harvest and assayed for DON. The tested fungicide treatments resulted in significantly lower mean IND and DON than the non-treated check. In all cases, means were significantly lower for MIR\_PRO than MIR\_A or PRO\_4, and significantly lower for MIR\_A than PRO\_4 for both IND and DON in three of the four years. The sequential application of Miravis Ace at anthesis followed by Prosaro 4 days after resulted in greater percent control of FHB index and DON than either treatment alone. Observed percent control (CO) values for MIR\_PRO were comparable to the expected percent control (CE) values under the assumption of additivity in three of the four years (2018, 2019 and 2021). For IND, CO and CE were 0.96 and 0.95 in 2018, 0.92 and 0.95 in 2019 and 0.94 and 0.96 in 2021, whereas for DON, CO and CE values were both 0.85 in 2018, 0.88 and 0.84 in 2019 and 0.89 and 0.93 in 2021. Based on the principle of treatment independence, the combination of an anthesis application of Miravis Ace followed by a post-anthesis application of Prosaro was additive in terms of percent control. Further analyses will be conducted to evaluate this additive effect under a range of baseline FHB and DON levels in different wheat market classes.

## Effect of Fungicides on Winter Barley Yield and Deoxynivalenol Contamination of Harvested Grain in Kentucky

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Fusarium head blight (FHB), caused by *Fusarium graminearum*, is a major disease affecting yield and quality of winter barley (*Hordeum vulgare*) grown in Kentucky. Field trials were conducted in Kentucky between 2020 and 2025 to evaluate the effects of fungicides on 2-row and 6-row winter barley cultivars for protection against deoxynivalenol (DON) contamination of harvested grain and for impact on yield. Integrated Management Trials (IMT) assessed fungicide products, application timing, and cultivars ('Calypso' and 'Violetta' for 2-row barley and 'Secretariat' and 'Thoroughbred' for 6-row barley), while Uniform Fungicide Trials (UFT) focused solely on fungicide products and application timing. Fungicide treatments included Prosaro®, Prosaro Pro, Miravis® Ace, Sphaerex®, Caramba®, Quadris®, and a tebuconazole fungicide, applied as single applications at Feekes growth stages 10, 10.3, or 10.5, single delayed applications 3–7 days after Feekes 10.5, or sequential applications after Feekes 10.5. Grain yield (kg/ha) at harvest and DON concentration (µg/g) in harvested grain were measured. Each trial was analyzed separately in SAS using *PROC GLIMMIX*. Fungicide treatment was a fixed effect, and year and replicate (nested within year) as random effects. In 2-row IMTs, fungicide and cultivar significantly affected DON and yield. Results from IMTs showed Miravis Ace produced the highest yield (5,423 kg/ha), while delayed Sphaerex 3–7 days after Feekes 10.5 resulted in the lowest DON (1.05 µg/g). The cultivar 'Calypso' achieved the highest yield (5,219 kg/ha), where 'Violetta' had the lowest DON (1.40 µg/g). In 2-row UFTs, fungicide significantly affected DON but not yield. Sequential applications of Miravis Ace followed by Sphaerex, or delayed applications of Prosaro Pro or Sphaerex were most effective at reducing DON (0.97–1.08 µg/g), with Miravis Ace followed by tebuconazole attaining the highest yield (4,731 kg/ha). In 6-row IMTs, fungicide significantly affected DON but not yield, while cultivar significantly affected both yield and DON. IMT trials showed 'Thoroughbred' had the highest yield (5,727 kg/ha) and lowest DON (1.71 µg/g), and a single application of Caramba at Feekes 10.5 resulted in the lowest DON (1.90 µg/g). In the 6-row UFTs, fungicide significantly affected DON but not yield. Sequential applications of Miravis Ace followed by Prosaro or Caramba, and delayed Miravis Ace 3–7 days after Feekes 10.5, were most effective at reducing DON (1.22–1.38 µg/g), while maintaining high yields (4,616–5,257 kg/ha). This study provides insights into FHB management in Kentucky and can inform future integrated disease management practices for winter barley growers.

## The Role of Seeding Rates When Integrated with Fungicide Applications and Genetic Resistance in Fusarium Head Blight Management in Kansas

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Fusarium head blight (FHB), caused primarily by *Fusarium graminearum*, remains one of the most economically important diseases of wheat worldwide and has become an increasing concern in Kansas in recent years. Effective management requires an integrated approach, combining genetic resistance, fungicide applications at early anthesis (Feekes 10.5.1), and cultural practices. Recent studies suggest that higher seeding rates may promote more uniform flowering periods, potentially improving fungicide coverage and disease suppression under favorable conditions for FHB. Additionally, denser canopies resulting from higher plant populations may physically reduce the exposure of wheat heads to *F. graminearum* spores, acting as a partial barrier to infection. We hypothesize that higher seeding rates enhance the performance of fungicide applications and genetic resistance under high FHB pressure. Field experiments were conducted in five site-years, Parsons, KS (2022–2023), Manhattan and Ottawa, KS (2023–24, 2024–25) using 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, 1,000,000, and 1,500,000 seeds/A), and two fungicide treatments (Miravis® Ace 13.7 fl oz/A applied at Feekes 10.5.1 versus untreated). Results showed that higher seeding rates reduced FHB index and deoxynivalenol (DON) accumulation across both varieties. The combination of the moderately resistant variety, highest seeding rate, and a single fungicide application resulted in the lowest FHB index, lowest DON concentration, and highest yield. These findings highlight the potential of optimizing seeding rate as a complementary strategy to improve fungicide efficacy and strengthen integrated FHB management recommendations for Kansas wheat producers.

## Integrated Management of Fusarium Head Blight in Winter Wheat in Nebraska

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Fusarium head blight (FHB), primarily caused by *Fusarium graminearum*, continues to be a challenge for wheat production in the US Great Plains. We evaluated the effects of combining cultivar resistance with fungicide application on FHB intensity and yield in winter wheat under field conditions at the University of Nebraska–Lincoln Havelock Research Farm (40°51'17.7"N 96°36'32.2"W). A split-plot randomized complete block design was implemented with four replications. The cultivars served as whole plots: Overland and Zenda, which are moderately resistant, and Wesley and Siege, which are susceptible. There were six subplot fungicide treatments: untreated inoculated check, Prosaro<sup>®</sup>, Miravis Ace<sup>®</sup>, Prosaro Pro<sup>®</sup>, Sphaerex<sup>®</sup>, and untreated non-inoculated check. Fungicides were applied with a CO<sub>2</sub>-powered backpack sprayer during anthesis and plots were spray-inoculated with *F. graminearum* at a concentration of  $1 \times 10^5$  conidia mL<sup>-1</sup> 24 hr after fungicide application. Corn kernel inoculum was applied weekly, starting three weeks before anthesis. FHB index was assessed 21 days after fungicide application. *Fusarium*-damaged kernels (FDK) and deoxynivalenol (DON) were measured. Mixed-model ANOVA revealed a significant main effect of fungicide treatment on FHB index ( $F_{5, 60} = 5.29$ ,  $p = 0.0004$ ) and on FDK ( $F_{5, 60} = 3.34$ ,  $p = 0.010$ ). There was a marginal cultivar  $\times$  treatment interaction for FHB index ( $p = 0.091$ ). Tukey separation, pooled across cultivars, indicated that the untreated inoculated check had the highest FHB index. All fungicide treatments resulted in significantly ( $P = 0.05$ ) lower FHB index compared to the untreated non-inoculated check. Prosaro Pro<sup>®</sup> had the lowest FDK. DON concentrations were consistently low or negligible across all treatments and cultivars at  $< 0.20$  ppm, and there were no effects of cultivar, fungicide treatment or their interaction. Linear regression showed no significant relationship between the FHB index and DON ( $R^2 = 0.003$ ,  $p = 0.622$ ). The low to negligible DON levels were due to low FHB intensity caused by unfavorable environmental conditions for FHB development. Yield and test weight showed no significant differences among fungicide treatments ( $p > 0.05$ ). 1000-kernel weight varied by cultivar ( $F_{3,12} = 22.16$ ,  $p < 0.001$ ) but not by fungicide treatment ( $p = 0.27$ ). Overall, the results support use of fungicide application at anthesis combined with cultivar resistance as an effective management strategy for FHB.

## Unexpected Effects of Trinexapac-ethyl on Fusarium Head Blight Severity and Yield in Wheat

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Growth regulators (GRs) are commonly applied to wheat in Illinois. While GRs may enhance yield in some environments, their impact on Fusarium head blight (FHB) has not been evaluated. Plant height is often negatively correlated with FHB symptom expression, with shorter varieties tending to manifest higher levels of disease. This may be because the wheat spikes of shorter plants are closer to the soil where they are more exposed to humidity and inoculum. We hypothesized that GRs could exacerbate FHB but improve yield by reducing plant height. To test this, we evaluated the effects of trinexapac-ethyl (active ingredient in Palisade® EC) and chlormequat chloride (active ingredient in Manipulator™) on FHB symptoms, yield, and height under intensive management. Two field trials were conducted during the 2025 season, one at Urbana and another at Belleville, IL. Each trial used a six-replicate split-plot design with chemical treatment as the main plot and variety as the subplot. Three commercial varieties with matching jointing and anthesis dates were tested under six treatment combinations: untreated control, Miravis® Ace applied at anthesis, and each GR with or without subsequent Miravis Ace application. Trials were not inoculated, though Belleville experienced high natural FHB infection. Data were collected for FHB severity (SEV), incidence (INC), Fusarium-damaged kernels (FDK), deoxynivalenol (DON), test weight, grain yield, and plant height. Lodging was not observed. Analysis of variance followed by Dunnett's post hoc tests were used to assess treatment effects relative to controls. The main effect of GR was significant for SEV, DON, test weight, grain yield, and plant height. Only trinexapac-ethyl significantly affected these traits compared to the control. Surprisingly, trinexapac-ethyl reduced SEV by 5 percentage points across sites and by 9 percentage points at Belleville. When examined by variety, the effect of trinexapac-ethyl on SEV was greatest for the variety 'AgriMAXX 513'. Trinexapac-ethyl also reduced yield but increased test weight relative to the control. This study is ongoing with a second year of data collection in progress. Current findings suggest that GRs do not hinder FHB control, but they also may not improve yield in the absence of lodging. Trinexapac-ethyl may reduce SEV in some varieties through mechanisms that are not yet understood.

## Optimizing Fungicide Application to Protect Yield and Mitigate Accumulation of Mycotoxins in Wheat Under High Disease Pressure

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*Fusarium graminearum*, a fungus that causes Fusarium head blight (FHB), affects both kernel quality and yield of wheat. In infected heads, the pathogen disrupts nutrient translocation to the kernels leading to poorly filled kernels which also accumulate mycotoxins. Deoxynivalenol (DON) is the most prevalent mycotoxin produced by *F. graminearum*. This study aimed to identify the optimal fungicide application strategy that maximizes wheat yield (bu/A) and kernel quality. The treatment structure included fungicides applied at (i) anthesis namely, Prosaro® (6.5 fl oz/ac), Miravis® Era (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), (ii) two fungicides applied in sequence, with the first application done at anthesis followed by a second application at the completion of flowering, and (iii) fungicides applied at the completion of flowering (post Anthesis) namely; Prosaro Pro (10.3 fl oz/ac), Sphaerex (7.3 fl oz/ac), and Miravis Ace (13.7 fl oz/ac). A randomized complete block design with four blocks was used to allocate fungicides to experimental units. Inoculation was conducted with corn spawn at boot stage and ascospores 24h after anthesis fungicide application. Data were analyzed using the generalized linear model (proc glimmix) with appropriate link functionalities. The sequential application strategy was the most effective for FHB management with the lowest DON content, Fusarium damaged kernels (FDK), and FHB severity, although there were no statistically significant ( $p \leq 0.05$ ) differences among treated plots. The sequential application strategy also produced 38% higher yield than the untreated and about 13% more than the anthesis and post anthesis applications. The sequential application of Miravis Ace (13.7 fl oz/ac) followed by Prosaro Pro (10.3 fl oz/ac) resulted in the lowest FHB related metrics (DON, FDK, and FHB severity). Positive Spearman associations ( $r=0.67$ ,  $p<.0001$ ) between FDK and DON, FHB severity and DON ( $r=0.53$ ,  $p<.0001$ ), were also observed. The results generated in this study suggest that applying fungicides at anthesis followed by a second application by the end of the flowering period is a superior strategy for managing FHB severity and maximizing yield, particularly under high disease pressure.

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## Summary of North Dakota Uniform Fungicide Efforts from 2022 to 2025

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Fungicide applications are a key component in the integrated strategies used to manage Fusarium head blight (FHB). Information pertaining to fungicide selection and timing are routinely requested by agricultural professionals when making decisions on over 7 million acres of small grains in North Dakota (ND). Uniform fungicide trials (UFT) were placed at several locations across ND to obtain local data and build into larger data summaries on fungicide performance in small grains. Throughout the UFT efforts from 2022 to 2025, a total of 23 field experiments were conducted with 9 being conducted on hard red spring wheat (HRSW), 8 on spring durum (DUR) and 6 on two-row spring barley (BAR). Research sites and the use of supplemental water varied among years and locations: Langdon (mist irrigated; 2022-2025), Fargo (dryland; 2022-2025), Davenport (dryland; 2022-2023), Minot (dryland; 2022-2025), and Nesson Valley (pivot irrigation; 2022-2025). All field experiments were conducted in a randomized complete block design with four to six replications. All plots were sown with a susceptible cultivar that has acreage relevance in ND. Disease intensity varied among environments (location by year) and data was summarized across fungicide treatments in each small grain market class for moderate to high disease environments. The FHB index, deoxynivalenol (DON), yield and test weight data were combined for common fungicide treatments that appeared in all environments and analyzed using PROC GLIMMIX. Fungicides evaluated included Prosaro® (prothioconazole + tebuconazole), Miravis® Ace (propiconazole + pydiflumetofen), Prosaro® Pro (prothioconazole + tebuconazole + fluopyram), Sphaerex® (metconazole + prothioconazole), and generic tebuconazole. Fungicide timings included Feekes 10.51/10.5 (early-anthesis HRSW or DUR / full-head BAR), 3 to 7 days after Feekes 10.51/10.5, or sequential applications of both timings. Fungicide treatments were able to reduce FHB index and DON in HRSW, DUR and BAR compared to a nontreated control. Treatments that provided the most amount of FHB and DON reduction were the sequential applications beginning with Miravis Ace and followed by either Sphaerex, Prosaro Pro, or tebuconazole. With one exception, single applications of Miravis Ace, Sphaerex, Prosaro Pro performed similar to each other in reducing both FHB and DON. All fungicides protected yield in HRSW and DUR, but no yield differences were observed in BAR. The summaries created from this research provide the critical information that will be communicated through Extension events to influence input decisions made by agricultural professionals in the Northern Great Plains.



## Fusarium Head Blight Management Coordinated Project: Uniform Fungicide Trials 2025

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Fusarium head blight (FHB) and associated grain contamination with mycotoxins such as deoxynivalenol (DON) continue to be of great economic importance in many wheat-producing regions of the United States. Prosaro has historically been one of the industry standards for FHB and DON management. However, newer fungicides such as Prosaro Pro<sup>®</sup>, Sphaerex<sup>®</sup>, and Miravis<sup>®</sup> Ace are now marketed for FHB and DON management, with efficacy, based on previous uniform fungicide trials (UFT), comparable to, or in some cases, greater than Prosaro. In 2025, UFTs were again conducted in 21 US states to evaluate and compare the efficacy of the aforementioned fungicides as well as an experimental fungicide, A23751C, for FHB and DON management in the following single- and double-application treatment programs: Prosaro (I), A23751C (II), Miravis Ace (III), Prosaro Pro (IV), or Sphaerex (V) applied at anthesis, or Miravis Ace at anthesis followed by an application of Prosaro Pro (VI), Sphaerex (VII), or Tebuconazole (VIII) at 4-6 days after anthesis, plus a non-treated check (CK). FHB index (IND) was assessed, and grain samples were collected and assayed for DON. Across environments, IND and DON in the checks ranged from 0 to 53% and 0.3 to 47.1 ppm, respectively. All fungicide programs resulted in significantly ( $P < 0.001$ ) lower mean IND and DON than CK. Among single-application treatments, pairwise differences in mean IND and DON contamination of grain between treatment I (Prosaro at anthesis) and the other four treatments were not statistically significant; treatment III (Miravis Ace at anthesis) resulted in significantly ( $P < 0.05$ ) lower mean IND than treatments I and IV; but mean DON was not significantly different among the five single-application treatments. Two of the three sequential



application fungicide programs, VI and VII, resulted in significantly ( $P < 0.001$ ) lower mean IND than all single-application programs, and significantly lower mean DON than all but one (treatment II) single application programs. The efficacy of each fungicide treatment was estimated in terms of percent control (C) for IND and DON relative to CK. Across environments, sequential application programs (VI, VII and VIII) resulted in higher C values for both IND and DON compared to single-application treatments. For instance, C values for IND and DON under sequential applications ranged from 66.7% to 69.3% and 40.1% to 52.4% respectively. Among single applications, treatment III provided the highest C values for both IND (61.2%) and DON (31.7%). Double-application treatments VI and VII were the most effective overall.

## Fusarium Head Blight Management Coordinated Project: Integrated Management Trials 2025

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The integration of two or more strategies such as a timely fungicide application in combination with genetic resistance is the most effective approach for reducing the impact of Fusarium head blight (FHB) and deoxynivalenol (DON) contamination on wheat grain yield and quality. As new fungicides such as Prosaro® Pro, Miravis® Ace, and Sphaerex® become available on the market for FHB/DON management and new FHB resistant varieties are released, studies such as this becomes necessary to (re)evaluate the efficacy, cost, and benefit of integrating the two strategies for managing this disease-toxin complex across a range of environments and grain market classes. During the 2025 growing season, separate replicated plots of susceptible (S), moderately susceptible (MS), or moderately resistant (MR) cultivars were established in 21 US states, 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* between 24 and 36 hours after treatment application. Mean FHB index (IND) and DON contamination of grain in the non-treated susceptible check (S\_CHK) ranged from 0 to 55% and 0.1 to 5.2 ppm, respectively. Relative to S\_CHK, all fungicide x cultivar treatment combinations resulted in significantly lower ( $P < 0.001$ ) mean IND and DON. Across all environments for which data were available at the time of this report, combining a moderately resistant cultivar with one of the tested fungicide treatments resulted in significantly lower mean IND and DON compared to when those same treatments were applied to a susceptible cultivar. The efficacy of each management combination was estimated as percent control (C) for IND and DON relative to S\_CHK. Across environments, the combination of an MR cultivar and a fungicide treatment

resulted in higher C values for IND and DON than treatments applied to a S cultivar. For instance, across the tested fungicide treatments, C for IND and DON ranged from 81.5 to 90.9% on MR cultivars and 48.5 to 91.8% on MS cultivars, compared to 54 to 73% on S cultivars. Relative to S\_CK, fungicide alone (S\_I-IV) reduced mean IND by 55.4 to 73.2% and mean DON 54.1 to 67.9%, whereas MR alone (MR\_CK) and MS alone (MS\_CK) reduced mean IND by 67.5% and 58.9%, respectively, and mean DON by 59.1% and 1.2%, respectively. The outcomes from this study, conducted under diverse environmental and agronomic conditions, provide valuable insights into the efficacy of the new fungicides relative to the industry standard Prosaro when used in combination with genetic resistance to manage FHB and DON.

## Get Spike Level FHB Screening on Real-Time Interface Before You Reach the Office

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FHB (Fusarium Head Blight) is a serious fungal disease that affects wheat and barley and lowers productivity because the affected grains are tiny, shrunken, of poor mass grain quality, and contaminated with mycotoxins that are harmful to animal and human nutrition. It requires precise and innovative methods for detecting and predicting disease symptoms at the earliest stages of pathogenicity. Traditional disease assessment of host plant genotypes is laborious, subjective (based on visual inspection), time-consuming, and costly, making it a limiting factor in plant breeding research. This study proposes a novel digital method for field-based FHB evaluation that makes use of a custom-built imaging device capable of taking close-up images of wheat heads from various angles. Ground-truth data were generated using polygon annotations of healthy and diseased spikelet in Roboflow. For model development, two deep learning pipelines were integrated: Attention U-Net for pixel-level segmentation and YOLOv11 for spike detection and disease classification. Together, they form a hybrid inference pipeline that quantifies disease severity per spike and row, generating masks, bounding boxes, and severity tables automatically. Technology automatically detects healthy and infected spikes by collecting high-resolution images from wheat plots and employing a complex predictive pipeline. Rather than relying on manual observation, the platform employs image-based learning to recognize disease symptoms and estimate infection severity. Results are visualized in an interactive web dashboard built using Flask, which allows users to upload field images, view colored severity maps, and download CSV or PDF reports within seconds. The process converts the raw images into quantitative summaries and color-coded maps indicating the percentage of FHB present in the wheat head. A web-based interface can help breeders, researchers, and producers use their tools more efficiently. Users can compare severity patterns across rows or plots, receive condensed reports, and evaluate analysis results immediately by uploading an image. The platform reduces the time between data collection and field decision making by providing feedback in almost real-time. This research demonstrates how cognitive computing and automated imagery can be used together to monitor crop health. It enables breeding operations to choose disease-resistant types more quickly by providing a dependable and scalable alternative to manual scoring. Future research can apply the method to other crop diseases, which would be a step in the direction of more intelligent, data-driven agricultural management. Keywords: FHB; Deep Learning; Attention U-Net; YOLOv11; 360-PICS; Disease Detection and Severity Estimation; Precision Agriculture

## Efficacy of Drone-Based Fungicide Applications for Control of Fusarium Head Blight in Illinois Wheat

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Fusarium head blight (FHB), caused by *Fusarium graminearum*, remains a major threat to wheat production in the U.S. Midwest due to yield losses and contamination of grain with deoxynivalenol (DON). This study evaluated the efficacy of aerial fungicide applications using a sprayer drone at different spray volumes. The field trial was conducted in a commercial plot near Hamel, Illinois in 2025 using a susceptible variety (GP 543) and a randomized complete block design with four replications. Plots were 30 ft long by 60 ft wide, and applications were delivered using a 10-ft swath width to ensure uniform spray distribution. Treatments included applications of Miravis<sup>®</sup> Ace (13.7 fl oz/ac) at spray volumes of 2, 3, and 5 gallons per acre (GPA), and an untreated control. Applications were made at Feekes 10.51 (50% anthesis) on May 5<sup>th</sup> using a DJI Agras T10 drone flying 10 ft above the canopy. Disease incidence and severity were evaluated four weeks after the sprays. Plots were harvested on June 23<sup>rd</sup>, and grain samples were analyzed for DON concentrations. Drone-based fungicide applications significantly reduced both FHB incidence and severity compared with the untreated control. The 5 GPA application achieved the greatest efficacy, lowering mean FHB severity from 19.3 % in the control to 9.3 % and reducing incidence from 28 % to 18.6 %. Intermediate reductions were observed with the 3 GPA and 2 GPA rates. Despite these significant disease reductions, grain yield did not differ statistically among treatments, although treated plots tended to produce higher yields. DON concentrations followed the disease trend, decreasing significantly from 2.15 ppm in untreated plots to 1.02 ppm at 5 GPA. These findings indicate that drone-based applications effectively suppressed FHB and mycotoxin accumulation in Illinois wheat, with higher spray volumes providing greater protection. Further research is warranted to refine UAV application parameters, optimize canopy coverage, and evaluate performance under diverse environmental conditions.

## Exploring Stakeholder Perceptions and Strategies for Fusarium Head Blight Management and Wheat Variety Selection

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Fusarium head blight (FHB) remains one of the most significant threats to wheat production globally. FHB management depends not only on scientific advances, but also on how those advances affect decisions made by key stakeholders. This study examines stakeholder perceptions, awareness, and management strategies for FHB across two contrasting production systems: Pennsylvania, USA, and Ethiopia, where disease risk is high but the challenges each production system faces differ. A total of twenty-eight semi-structured interviews were conducted with stakeholders, including nine farmers, two millers, and two extension agents in Pennsylvania, and fifteen agricultural professionals in Ethiopia, comprising plant breeders, pathologists, extension agents, agronomists, field assistants, and a biotechnologist. The study was conducted in accordance with a protocol approved by the Institutional Review Board at The Pennsylvania State University (STUDY00025627). Interviews were recorded, transcribed, and analyzed by themes in NVivo software, focusing on five broad areas: engagement with farmers, wheat production context, farmer decision-making, disease challenges, and management needs. Results revealed marked contrasts in awareness, seed systems, and management approaches. In Ethiopia, awareness of FHB has increased following severe outbreaks in 2019 and 2022, yet misidentification of the disease remains common. Farmers often attributed symptoms to early maturity rather than a pathogen. Awareness campaigns have since introduced training, photographs, and demonstration plots. Disease resistance is the foremost criterion for seed choice, but access to improved seed remains limited due to Ethiopia's informal seed markets and cost barriers. Fungicide options are scarce, and continuous wheat production policies undermine recommended rotation practices. In Pennsylvania, FHB is the primary disease concern due to strict grain quality standards and the risk of DON contamination. Farmers rely heavily on prophylactic fungicide applications and consider this "just a cost of doing business." Variety selection is dominated by yield and standability, with disease resistance viewed as a secondary trait. Although the FHB risk prediction tool is available, growers often ignore it, citing local microclimates and timing constraints. Instead, private agronomists and seed companies exert strong influence on decision-making. Comparatively, Ethiopian stakeholders prioritize resistance in the face of constrained access to inputs, while Pennsylvania growers emphasize profitability and logistical efficiency. Both systems demonstrate that awareness alone does not guarantee adoption of integrated disease management. Driving systems-level change in FHB management requires coordinated improvements in seed systems, clearer communication pathways between researchers and stakeholders, and a firm integration of social and economic realities.

## Efficacy of Fungicide Applications for Management of Fusarium Head Blight Across Wheat Varieties in Delaware

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Wheat (*Triticum aestivum* L.) is one of the most widely grown food crops globally, with over 215 million hectares under cultivation. However, its production is increasingly threatened by diseases such as Fusarium head blight (FHB), which not only deteriorates yield but also grain quality. FHB is primarily caused by the fungus *Fusarium graminearum*, which produces mycotoxins like deoxynivalenol (DON) in the infected grain. Management of FHB relies on partial resistance in wheat 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 the management of FHB under regional environmental conditions. In 2024 and 2025, susceptible variety Shirley and moderately resistant variety DG9172 were assessed in combination with four fungicide treatments, Prosaro®, Prosaro Pro, Miravis® Ace, and Sphaerex®, applied at anthesis for their efficacy in controlling FHB. The trial was set up as a split-plot design and included two controls: one inoculated and one non-inoculated, non-treated control, with five replications. All inoculated plots received 0.78 kg of colonized corn spawn per plot, and mist irrigation was set up in all plots. Plots were evaluated for FHB incidence, FHB severity, Fusarium damaged kernels (FDKs), DON, and yield. When comparing the non-treated inoculated controls of both varieties, DG9172 had significantly lower severity, FHB index, %FDK, and DON levels. In Shirley, plots treated with Prosaro Pro, Miravis Ace, and Sphaerex had higher yield and lower severity and DON levels, plots treated with Sphaerex had significantly lower incidence, and all fungicide treated plots had significantly lower %FDK than the non-treated inoculated plots. In DG9172, plots treated with Miravis Ace had significantly lower FHB index, and plots treated with Prosaro Pro, Miravis Ace, and Sphaerex had significantly lower incidence and DON levels and higher yield than the non-treated inoculated plots. While fungicide applications at anthesis can improve grain quality and yield across varieties, the use of host resistance allows for additive effects in FHB management, resulting in lower severity, index, %FDK, and DON accumulation. This study provides practical guidance for wheat growers in Delaware on effectively managing FHB by combining the use of moderately resistant varieties with well-timed fungicide applications.

## Approach to Addressing FHB And Other Disease Threats in Novel Perennial Grain Crops Such as Kernza®

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Perennial crops have the potential to accumulate disease over multiple years of continuous growth, though in practice this is not typically observed. As new perennial grain crops are introduced, disease pressure is inevitable. We are developing pipelines to identify, determine advisable primary approaches to reduce disease pressure, and develop tools that can be implemented in breeding programs and in grower fields. Fusarium head blight is one of the most important diseases to control in Kernza®, the first perennial grain crop developed by The Land Institute. Genetic resistance, timing of harvest, and avoiding harvesting lodged crops all reduce the level of Fusarium infection. Future efforts to translate disease forecasting models and evaluate fungicides for use in Kernza offer additional methods of control.



## Sensitivity of *Fusarium* Species Causing Fusarium Head Blight of Barley in Manitoba to Triazole Fungicides

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Fusarium head blight (FHB) is a major disease of barley that causes significant grain yield and quality losses in Western Canada. The disease is caused by a complex of *Fusarium* species, with *F. graminearum* as the primary pathogen, while *F. poae* and *F. sporotrichioides* have become increasingly prevalent. Triazole fungicides are widely used to manage FHB; however, information on the sensitivity of *Fusarium* species, particularly *F. poae* and *F. sporotrichioides*, against triazole fungicides remains limited. This study assessed the sensitivity of *Fusarium* isolates to metconazole, tebuconazole, and prothioconazole using a 96-well microplate assay. A total of 103 isolates were collected from infected barley heads across Manitoba during the 2023 and 2024 growing seasons, including 34 *F. graminearum*, 34 *F. poae*, and 35 *F. sporotrichioides* isolates. *F. graminearum* isolates exhibited significantly lower sensitivity to all three fungicides compared with the other two *Fusarium* species. Median EC<sub>50</sub> values for *F. graminearum* were 2.1, 4.1, and 0.2 mg L<sup>-1</sup> for prothioconazole, tebuconazole, and metconazole, respectively. In comparison, *F. poae* and *F. sporotrichioides* showed lower EC<sub>50</sub> values, indicating higher sensitivity against these fungicides. Four *F. graminearum* and two *F. poae* isolates displayed insensitivity to all triazoles, with minimal inhibition of mycelial growth at the concentrations 100x higher than medium EC50 values. In contrast, no such isolates were detected among *F. sporotrichioides*. Molecular characterization of resistant isolates is underway to identify potential target-site mutations.

# **Food Safety and Toxicology**

## Mycotoxins and Masked Mycotoxins as Potential Food Safety Risks

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Cereals such as wheat, barley and oats are frequently contaminated with *Fusarium* mycotoxins including type A trichothecenes (T-2 and HT-2 toxin) and type B trichothecenes (deoxynivalenol, DON; nivalenol, NIV), as well as their glucoside conjugates (masked mycotoxins). In Scottish cereals, contamination with T-2 and HT-2 and their masked forms pose a major challenge in oats, while wheat and barley are less frequently contaminated with DON and NIV and their masked forms. In oats, organic production and low-intensity cereal rotations were found to lower the risk of T-2 and HT-2 contamination (1). De-husking of oats removed the majority of mycotoxins with the outer husk, but significant levels remain and are carried over into final food products. Dietary mycotoxin exposure constitutes free mycotoxins and masked mycotoxins which are present in cereal foods. In vitro studies found high bioaccessibility of mycotoxins and masked mycotoxins from contaminated food, and masked mycotoxins were readily degraded and free mycotoxins released by human gut microbiota (2-4). In a urinary biomarker study, consumption of oat foods and cereal foods was associated with increased urinary excretion of HT-2 and DON in UK children. Dietary exposure frequently exceeded tolerable daily intakes (TDI), indicating exposure above safe levels (5-6). In summary, we demonstrate that contamination of cereal crops with free and masked mycotoxins results in carry-over into cereal foods and increases the risk of dietary mycotoxin exposure in cereal consumers.

**Acknowledgements:** This project received funding from the Scottish Government (RESAS) and Interface Multiparty Fund. References: (1)Daud et al.(2023) doi:10.3390/toxins15040247; (2)Gratz et al.(2017) doi:10.1002/mnfr.201600680; (3)Daud et al.(2020) doi:10.3390/toxins12100654; (4)Daud et al.(2020) doi:10.1080/09637486.2019.1698015; (5)Charusalaipong et al.(2024) doi:10.3390/toxins16060251; (6)Gratz et al.(2020) doi:10.1021/acs.jafc.9b03964

## Decontamination of Fungal and *Fusarium* Mycotoxins in Wheat Grains Using Natural Photosensitizers

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In recent years, sprouted seeds have attracted increasing global attention owing to their superior nutritional value. For instance, sprouted wheat flour exhibits greater contents of fiber, as well as certain vitamins and minerals, compared to all-purpose flour. Meanwhile, sprouts are considered one of the most contamination-prone fresh produce items and have been recognized as a major source of foodborne pathogen outbreaks, thereby presenting a serious food safety issue. Photosensitization is a promising eco-friendly method to control microorganisms in cereal grains. Our previous study demonstrated that curcumin, as a natural photosensitizer, can inhibit *Fusarium* fungal growth and mycotoxin production *in vitro*. Therefore, this study aimed to evaluate the effectiveness of several natural photosensitizers (PSs) for decontaminating *Fusarium* head blight (FHB) infected wheat grains and sprouted wheat grains including curcumin, coumarin, and menadione sodium bisulfite (MSB). Initially, the specific fungal species contaminating FHB infected wheat were identified through sequencing and subsequently confirmed by comparing the sequences against the NCBI nucleotide database using Basic Local Alignment Search Tool (BLAST). The results showed that the fungi present on FHB infected wheat included *Arthrrium sp.*, *Aspergillus sp.*, *Fusarium graminearum*, *Alternaria alternata*, and *Cladosporium sp.* Afterwards, the three aforementioned natural PSs were evaluated for their ability to decontaminate wheat seed surfaces by counting surviving fungal colonies on 100 FHB infected wheat seeds. The antifungal results indicated that curcumin and coumarin, combined with light treatment, completely inhibited fungal colony growth at concentrations of 3 mM and 4 mM, respectively. In contrast, 4 mM of MSB significantly inhibited fungal growth as compared to control, but less effective than that of curcumin and coumarin. In terms of mycotoxin suppression in wheat seeds and sprouted wheat seeds, all three natural photosensitizers ( $p < 0.05$ ) significantly reduced overall mycotoxin production. Among them, MSB effectively reduced all tested mycotoxins (DON, 3-ADON, 15-ADON, Nivalenol, T-2, and Zearalenone), while curcumin and coumarin only significantly reduced DON, Nivalenol, T-2, and Zearalenone. Additionally, the effects of the three photosensitizers on seed germination rate were evaluated. The results indicated that coumarin and MSB did not affect germination rate, whereas curcumin significantly reduced the germination rate of wheat seeds as compared with control sample. In conclusion, MSB based photosensitization treatment shows great potential for controlling fungal contamination and mycotoxin production in highly contaminated wheat seeds and germinated wheat sprouts.

## High-Throughput LC-MS Quantification of Single-Kernel Mycotoxins to Identify Fusarium Damaged Kernels in Barley and Wheat

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In FHB infected cereals, Fusarium Damaged Kernels (FDKs) are the primary source of mycotoxin contamination, and even a small proportion of FDKs can elevate mycotoxin concentrations above regulatory limits. The percentage of FDKs is therefore widely used by plant pathologists and breeders as an indicator of FHB disease severity. In this project, we aim to develop a rapid and accurate LC-MS method to quantify major *Fusarium*-produced mycotoxins, including deoxynivalenol (DON), DON-3-glucoside (D3G), 3-Acetyl DON (3-AcDON) and 15-Acetyl DON (15-AcDON), at the individual kernel level. To establish the method, matrix-matched calibration curves were constructed for each toxin using barley and wheat kernel matrices; limits of detection (LOD), limits of quantification (LOQ), and analytical recoveries were rigorously validated. Finally, the method was applied to single kernels from a barley spike and a wheat spike to identify and spatially locate FDKs along the spike. This pipeline enables pathologists and breeders to accurately assess FHB incidence and spike-level disease progression in research applications.

## Rapid Quantification of Fusarium Damaged Kernels in Barley Using Hyperspectral Imaging and Machine Learning

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Pathologists and breeders assess Fusarium damaged kernels (FDKs) on spikes to evaluate FHB disease presence before harvest. However, the visible assessment alone cannot accurately quantify the disease severity. Additionally, the symptom on barley FDKs tend to fade as the grain matures. Rapid and accurate quantification of individual kernel DON levels, indicative of FDKs holds significant importance. Current detection methods, which rely on chromatography to analyze batch samples, are time-consuming and not realistic for a real-time measurement of individual kernels on spikes. Hyperspectral imaging (HSI) is capable of rapidly detecting toxins in kernels through infrared wave absorption at different wavelengths. The objective of this study is to establish a pilot-scale predictive model by combining HSI with machine learning data processing for identifying FDKs in malting barley. A total of 300 barley seeds is scanned using a HSI spectrum with a short-wave infrared camera (Specim, Oulu, Finland) at wavelengths of 1,000 - 2,500 nm. After preprocessing, each image is divided into 3×3 sub-pixel images using MATLAB 2023b (The MathWorks, Natick, Massachusetts). The scanned individual kernels are analyzed using LC-MS to quantify DON levels. Machine learning algorithms, such as Partial Least Squares Regression (PLSR), Multilayer Perceptron (MLP), Random Forest (RF) and Extreme Gradient Boosting (XGBoost), are applied to integrate LC-MS and hyperspectral data, establishing a comprehensive model for rapid and non-destructive analysis of kernel DON levels. A pilot-scale machine learning model is anticipated to predict the DON level in each kernel. Expected outcomes include a rapid, reliable, and non-destructive method for quantifying FDKs in a bulk barley sample. The HSI approach should provide accurate measurements for early detection of FDKs and mycotoxin contamination.

# **Gene Discovery & Engineering Resistance**

## Does Barley Exhibit Genetic Variation for Resistance to Fusarium Head Blight?

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Fusarium head blight (FHB), caused by *Fusarium graminearum*, significantly reduces barley and wheat yield and grain quality. The pathogen produces trichothecene mycotoxins such as deoxynivalenol (DON), which act as virulence factors during infection. All tested barley accessions display only type II resistance, suggesting limited natural genetic variation for this trait. Meta-analyses of barley genetic mapping studies indicate that most FHB resistance QTL are associated with morphological or physiological traits, implying that observed resistance often results from escape mechanisms rather than true genetic resistance. We identified a barley UDP-glucosyltransferase gene, *HvUGT13248*, that detoxifies DON by conjugating it to DON-3-O-glucoside (D3G). Barley lines overexpressing *HvUGT13248* exhibit reduced FHB severity and enhanced DON tolerance through rapid conversion of DON to D3G. Mutations in the enzyme's active site compromise this detoxification capacity, resulting in reduced type II resistance and increased DON sensitivity, demonstrating that *HvUGT13248* is required for type II resistance. Resequencing of diverse barley germplasm revealed that *HvUGT13248* is highly conserved, with few nonsynonymous substitutions that do not alter enzyme function. Functional assays using fungal strains producing a range of trichothecene toxins showed that *HvUGT13248* provides broad-spectrum detoxification and resistance. RNA-seq analysis of the *HvUGT13248* mutant compared to a wildtype sibling showed that overall gene expression was earlier and greater in the mutant; however, fungal trichothecene genes were more highly expressed in the wildtype genotype. Together, these findings demonstrate that *HvUGT13248* confers type II resistance in barley through the detoxification of multiple trichothecene mycotoxins.



## Genomics Tools and Germplasm Resources to Enhance FHB-Resistance Genes in Wheat

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Fusarium head blight (FHB) is a devastating disease that affects wheat and other small grain crops. The most sustainable way to improve wheat is through genetic resistance to FHB. Achieving genetic improvement requires ongoing discovery and the incorporation of valuable genes and alleles into breeding programs. It is essential to identify and deploy both typical and atypical resistance genes, along with mutants of susceptibility genes, to develop long-term, broad-spectrum resistance against FHB. We are utilizing sequence-indexed association panels, mutant populations, and established resistance genetic stocks to focus on discovering useful genes and rapidly deploying these genes and alleles through breeding assistance to increase wheat's resistance to FHB. By integrating gene discovery with genomics-assisted tools and an efficient development pipeline, we aim to develop and release resilient wheat cultivars.

## An Overview of the GDER: Recent Accomplishments and Future Directions

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The Gene Discovery and Engineering Resistance (GDER) research area is central to the USWBSI efforts to mitigate the threat of Fusarium Head Blight (FHB) by identifying novel genetic resistance mechanisms in wheat and barley. The GDER's primary focus is on understanding the molecular basis of host resistance and susceptibility and delivering new, validated gene variants for deployment in breeding programs. Past accomplishments have established potential new strategies and resources for FHB control. Key breakthroughs include the successful cloning of major resistance genes, such as *Fhb1*, providing crucial knowledge for marker-assisted selection, the introgression of novel sources of resistance, identification of susceptibility genes, and the development of gene editing tools to successfully validate the function of susceptibility genes. The GDER area has also invested in critical infrastructure, including specialized transformation facilities for both wheat and barley, to accelerate the testing and validating promising constructs. Future Directions should emphasize the transition from discovery to deployment and adoption. This will include increased coordination among GDER and other USWBSI research areas (such as VDHR and PBG) thereby promoting translational research pipelines from gene discovery to cultivar release.

## Barley Genetic Engineering Facility for FHB Research Community

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We have 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 client-based barley cultivars and for optimizing transformation efficiency to produce transgenic barley plants with client-provided constructs. After testing different explants, such as mature barley seeds, which save time needed to grow up plants that can provide immature embryos, or germinated seedlings, which provide meristematic tissues, we found that immature scutellum explants proved to be the optimal material for producing stable transgenic barley plants. Significantly, we observed that different barley cultivars require different hormone regimes at the initial stages of *in vitro* culture to produce regenerable embryogenic calli. We have developed tissue culture protocols for the *in vitro* regeneration of several barley cultivars, including the two-rowed spring barley “ND Genesis”, the winter cultivar “Thunder”, and the six-rowed cultivar “Morex”. Using these protocols, we generated barley embryogenic calli within 3 weeks that could then be transformed by either gene gun bombardment or *Agrobacterium*. After 8 weeks, transformed barley calli could be selected and used to regenerate transgenic plantlets. We have also demonstrated that co-bombardment with our transiently expressing barley *HvBBM* and *HvWUS* growth regulators, the barley regeneration rates were improved from single to multiple shoots per embryo. Our improved barley transformation and regeneration protocols have allowed us to transform Morex embryogenic calli with our custom integrative CRISPR-gene editing vector and to produce transgenic Morex plants that are phenotypically normal and able to produce seeds. We have also been able to produce transgenic ND Genesis plants that were transformed with our double-knock out (KO) CRISPR-gene editing vector to mutate the *HvHRC* gene. In addition, we have produced transgenic ND Genesis with client-provided construct over-expression Arabidopsis lipid transfer protein (LTP). 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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## Overexpression of Wheat Pore-Forming Toxin-like Gene in Transgenic Plants of Tomato and Strawberry Provides Broad Spectrum Resistance to Fungal Pathogens

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Wheat pore-forming toxin-like (PFT) gene was reported by Rawat et al. (2016) previously to provide *Fhb1* mediated resistance to *Fusarium graminearum* infection in resistant wheat cultivar Sumai 3. To investigate the effect of PFT in another plant system, we ectopically expressed it in dicot plant model plant *Arabidopsis thaliana*, which does not have any PFT ortholog or homolog. PFT gene in the dicot plant *Arabidopsis thaliana* provided resistance to not only *F. graminearum*, but to a broad-range of necrotrophic and hemi-biotrophic fungal pathogens including *Botrytis cinerea*, *Colletotrichum higginsianum*, and *Sclerotinia sclerotiorum*. In the present work, the wheat PFT gene was transferred to a diploid tomato cv. Moneymaker and an octaploid strawberry cv. Camerosa. Both of these varieties are susceptible to a number of pathogens. The transgenic tomato plants were challenged with *Fusarium oxysporum* f. sp. *lycopersici*, *Verticillium dahliae*, *Alternaria linariae*, and *Botrytis cinerea*. The transgenic strawberry plants were challenged with *Botrytis cinerea* and *Colletotrichum acutatum*. In both the experiments, transgenic plants of PFT tomato and strawberry showed significantly less disease severity index and fungal biomass with significant disease resistance against the fungal pathogens tested. This study demonstrates the broad spectrum-resistance of the wheat PFT gene to fungal pathogens in planta irrespective of the plant background. Keywords: *Fhb1*, Wheat PFT, Tomato, strawberry, broad-spectrum resistance, Fungal pathogens.

## High-Resolution Mapping of a Conserved Fusarium Head Blight Susceptibility Factor *Sf-Fhb-7AS* in Wheat

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Fusarium head blight (FHB), caused by *Fusarium graminearum*, severely impacts wheat yield and grain quality worldwide. Manipulation of plant susceptibility factors, genes that facilitate pathogen infection, offers a novel strategy for enhancing FHB resistance. In this study, we refined the genomic location and validated the role of a major susceptibility factor on the short arm of chromosome 7A (*Sf-Fhb-7AS*) in the wheat cultivar Chinese Spring. Nullisomic-tetrasomic analysis of group 7 chromosomes confirmed the dosage effect of the gene, with tetrasomic 7A lines showing enhanced susceptibility relative to wild-type Chinese Spring. The conserved nature of this susceptibility factor was further supported by its presence across diverse wheat backgrounds. Using recombination-independent approaches, including seed and pollen irradiation-derived deletion panels, we delimited *Sf-Fhb-7AS* to a shorter interval on chromosome 7AS. Screening of over 300 irradiated Chinese Spring and Jagger lines revealed that deletions spanning this interval imparted 50–60% Type II FHB resistance without any effect on plant development. Transcriptomic analysis of the 48 high-confidence genes in this region revealed differential expression of multiple candidate genes upon *F. graminearum* infection. Our findings establish the potential of *Sf-Fhb-7AS* as a promising target for novel FHB resistance breeding strategies in wheat. Keywords: *Fusarium graminearum*; Fusarium Head Blight; Susceptibility Factor; *Sf-Fhb-7AS*

## Disruption of *NPR3* and *NPR4* Genes in Wheat Confers Resistance to *Fusarium graminearum*

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Salicylic acid (SA) signaling mediated by *Nonexpresser of Pathogenesis-Related Genes 1* (*NPR1*) is a key component of plant immune responses against pathogens such as *Fusarium graminearum* (hereafter abbreviated as *Fg*), the causal agent of Fusarium head blight (FHB) in wheat and barley. Under controlled laboratory conditions, *Fg* can also infect the leaves and flowers of *Arabidopsis thaliana*. The *NPR1* homologs *NPR3* and *NPR4* have been identified as negative regulators of plant defense, as they promote *NPR1* degradation and interfere with the activity of defense-related transcription factors. Considering the pivotal role of the SA–*NPR1* pathway in both *Arabidopsis* and wheat resistance to *Fg*, we examined whether *NPR3* and *NPR4* contribute to plant susceptibility during interactions with *Fusarium graminearum*. Leaves of mutants lacking *NPR3* or *NPR4* in *Arabidopsis* showed defense-associated hypersensitive response (HR) phenotypes, including elevated reactive oxygen species accumulation, increased expression of the SA-responsive *Pathogenesis-Related 1* (*PR1*) gene, enhanced ion leakage, and decreased fungal biomass. In addition, the inflorescences of these mutants were more resistant to *Fg* infection than those of wild-type plants. Likewise, missense mutations in wheat *NPR3* and *NPR4* genes, identified through a TILLING population, led to reduced FHB symptoms and lower levels of mycotoxin contamination. Altogether, these results indicate that *NPR3* and *NPR4* act as susceptibility factors in plant–*Fg* interactions and that downregulation of these genes offers an effective strategy to improve FHB resistance in wheat. The naturally occurring alleles of wheat *NPR3* and *NPR4*, identified as part of this project, provide valuable non-GMO resources for developing FHB-resistant wheat varieties.

## Enhanced Fusarium Head Blight Resistance in *Fhb7* Transgenic Barley and Wheat

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Fusarium head blight (FHB), commonly known as scab, is a devastating disease affecting wheat and barley worldwide, leading to significant yield losses and contamination of mycotoxins. While progress has been made in wheat in understanding the host resistance to FHB by identification of the major QTLs *Fhb1* and *Fhb7*, breeding FHB resistance in barley is hindered by the lack of resources with sufficient resistance levels. We are developing marker-free *Fhb7* transgenic barley and successfully obtaining 14 transgenic lines in elite two-rowed barley Excelsior Gold (EG). Tests of two transgenic lines and EG in field and greenhouse conditions showed that insertion of *Fhb7* significantly reduced the FHB infection. We also cloned the *Fhb7* gene from the J1 genome of sea wheatgrass (*Thinopyrum junceaforme*) and transformed it into Fielder wheat for overexpression. Greenhouse inoculation experiments demonstrated that one transgenic line lowered disease incidence by ~90%. These results showed that *Fhb7* functions in both wheat and barley and the transgenic plants are valuable resources for breeding FHB resistance in these crops.

## Ancestral Germplasm as a Source of Fusarium Head Blight Resistance in Bread Wheat

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*Fusarium graminearum* is an economically important fungus causing Fusarium head blight (FHB), which has become an increasing threat to the security of global cereal production. The use of genetically resistant cultivars is a critical tool for managing FHB; however, modern elite wheat germplasm lacks genetic diversity, and only a limited number of known resistance genes are currently available. The germplasm of wild species is a diverse and underutilized source of novel alleles associated with many important traits, including disease resistance. Approximately a third of known FHB resistance genes originated from a wild species. *Aegilops tauschii* is the donor of the D-genome in wheat and only a limited amount of *Ae. tauschii* germplasm is represented in modern wheat germplasm. Four accessions of *Aegilops tauschii*, that showed FHB resistance in a prior screening have been used to develop 607 BC1F5 derived introgression lines (ILs) in the background of adapted cultivars. In this study, we aimed to identify resistant ILs and map underlying resistance genes. ILs were grown in Kansas State University's Fusarium head blight screening nursery for two seasons and phenotyped pre- and post-harvest for disease traits. 30 ILs either never headed or headed extremely late missing the disease outbreak, resulting in only 577 ILs being analyzed. 203 ILs were found to exhibit lower disease scores in all phenotyped traits compared to the recurrent winter wheat accession. For FHB incidence ratings, 146 of these lines were moderately resistance, while 57 lines were resistant. 93 ILs had mycotoxin content levels of 6.12 ppm or lower, which were approximately at least half of the recurrent accession's mycotoxin content levels. The primary mycotoxin present was deoxynivalenol. Plant height was found to have negative moderate correlation with all disease traits but deoxynivalenol content. Based on phenotypic data, genetic resistance present in *Ae. tauschii* accessions was introgressed into wheat. ILs were genotyped using low-pass whole genome sequencing, while parental lines were whole-genome sequenced. Variant calling is currently in progress, which will be followed by SNP imputation to identify and correct missing regions in the sequenced introgression lines. Due to equipment failure and a late disease outbreak, phenotypic data from the second field trial replication cannot be used. Field trials will be replicated for a third time in the coming spring. A genome-wide association study using the R package GAPIT v3 will be conducted to map resistance associated chromosomal regions. KASP markers for resistant regions will be developed.



## From In Silico to Function: A Bioinformatic Workflow for Discovering Novel Multi-Domain Lipid Transfer Proteins (nsLTPs)

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Non-specific lipid transfer proteins (nsLTPs) are a diverse superfamily of small, cysteine-rich proteins that play key roles in plant defense, including direct inhibition of fungal pathogens such as *Fusarium graminearum*. Beyond their roles in cuticle formation and stress signaling, many nsLTPs act as antimicrobial peptides (AMPs), disrupting fungal membranes and limiting infection. Despite their importance, tens of thousands of nsLTP sequences in public databases remain uncharacterized, and the full extent of their structural diversity, particularly multi-domain variants, has not been systematically explored. We developed a robust bioinformatic workflow to identify, classify, and structurally model nsLTPs from large-scale sequence datasets. Starting with 12,880 proteins from the InterPro database (IPR000528), a custom Python script parsed the non-redundant dataset to detect all proteins containing one or more eight-cysteine motifs (8CMs). The analysis revealed 11,861 single-domain nsLTPs, 35 with two domains, three with three domains, and one protein with four domains. Among these, two multi-domain nsLTPs from African wild rice (*Oryza barthii*), A0A0D3HHF7 (two 8CMs) and A0A0D3HQM8 (four 8CMs), represent previously undescribed architectures within the family. Three-dimensional structures predicted using AlphaFold3 revealed compact yet highly variable multi-domain configurations, expanding the known structural landscape of nsLTPs. Because structure underpins antimicrobial function, these newly identified architectures may confer novel or enhanced activity against *Fusarium* and other fungal pathogens. This computational pipeline thus provides a foundation for discovering and prioritizing nsLTPs with superior antifungal potential for functional validation and, ultimately, deployment in breeding programs to reduce *Fusarium* head blight in wheat and barley.

## Integrated Host-Pathogen Transcriptomics Elucidates the Role of Wheat *Lpx3* in Fusarium Head Blight Resistance

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Fusarium head blight (FHB), caused by *Fusarium graminearum* (*Fg*), is a major disease that threatens global wheat production by reducing grain yield and contaminating grain with trichothecene mycotoxins. While fungicides are widely used for disease management, their effectiveness is limited, and their use raises environmental concerns. Therefore, identifying host genes that influence FHB susceptibility or resistance is critical for developing sustainable control strategies. Oxylipins, lipid-derived signaling molecules produced by both plants and fungi, play multifaceted roles in defense and inter-kingdom communication. Our research identified the a oxylin synthesizing 9-lipoxygenase, *Lpx3*, as a susceptibility factor in wheat interaction with *Fg*. *Lpx3* knockdown enhanced resistance to *Fg* and significantly reduced mycotoxin accumulation compared to the wild type. To elucidate the molecular mechanisms underlying this enhanced resistance, dual RNA sequencing of wheat and the pathogen was conducted using spikelets collected from mock- and *Fg*-inoculated wild type and *Lpx3*RNAi lines at 24 and 72 hours post-inoculation. Comparative transcriptomic analyses revealed that *Lpx3* knockdown led to differential regulation of host genes associated with fatty acid metabolism, plant hormone signaling, and defense-related secondary metabolism. Among the most highly induced pathways was flavonoid biosynthesis, including strong upregulation of chalcone synthase (*CHS*) genes. Given the known antimicrobial and signaling roles of flavonoids, these results suggested a link between oxylin metabolism and secondary metabolite-mediated defense. To explore this relationship, we examined *Arabidopsis thaliana* mutants deficient in *CHS* (*tt4*). These mutants displayed enhanced susceptibility to *Fg*, providing functional evidence that flavonoid biosynthesis contributes to floral defense. On the fungal side, several virulence and pathogenicity associated genes, including those encoding effector proteins and detoxification enzymes, exhibited altered expressions in the *Lpx3*RNAi line. Together, these findings reveal that suppression of a single host lipoxygenase gene can reprogram both lipid and flavonoid derived defense networks and modulate fungal pathogenic responses, likely through cross-kingdom lipid and secondary metabolite signaling. Overall, our results highlight *Lpx3* and *CHS* as key molecular players influencing FHB outcome and demonstrate the potential of targeting oxylin flavonoid crosstalk to develop wheat cultivars with durable resistance to *Fg*.

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## The Role of the UDP-glucosyltransferase UGT13248 in Resistance to Fusarium Head Blight: What it Can and Cannot Do

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Fusarium head blight (FHB) is a complex disease affecting wheat and barley. Finding stable resistance to infection and disease spread has proven a difficult endeavor. Our lab has focused on the role of a barley UDP-glucosyltransferase, HvUGT13248, in the glycosylation and subsequent detoxification of the major FHB mycotoxin deoxynivalenol (DON). Here, we summarize our results across multiple experiments that aimed to understand the role of HvUGT13248 in the resistance of wheat and barley to FHB. We utilized a nonfunctional mutant allele of HvUGT13248 to show that type I resistance to FHB is unaffected by the loss of this enzyme. The infection rate and maximum number of infection points per plant were unchanged in barley plants lacking a functional HvUGT13248. Disease spread was greatly increased in the mutant line, confirming the role of HvUGT13248 in type II resistance. To expand our understanding of the substrate specificity of HvUGT13248 we observed the increased spread of *Fusarium graminearum* chemotypes producing 15-acetyl-DON, 3-acetyl-DON, Nivalenol and NX2 and reduced glycosylation ratios against those toxins in the HvUGT13248 mutant compared to wild-type plants. Similarly, overexpression of HvUGT13248 in the wheat cultivar 'Bobwhite' resulted in increased and faster glycosylation of DON, 3-acetyl-DON, 15-acetyl-DON and NX2 toxins in the transgenic compared to wild-type plants. When HvUGT13248 was constitutively expressed in the wheat cultivar 'Rollag' in the presence or absence of the FHB1 resistance QTL, HvUGT13248 provided significant resistance to FHB spread. This effect was as strong and less variable than the resistance provided by the native FHB1 resistance QTL. RNA-seq analysis of the HvUGT13248 mutant and wildtype plants indicated a more rapid and substantial change in gene expression in the mutant compared to wildtype. Furthermore, a weighted gene co-expression network analysis identified a large module of genes with similar expression patterns, which was enriched for genes associated with defense, metabolite processing, protein and macromolecule modification, and response to stimuli. This module was similarly more rapidly and highly expressed in the HvUGT13248 mutant compared to wildtype during infection with *F. graminearum*. Conversely, differential gene expression analysis of *F. graminearum* genes revealed that the genes involved in trichothecene biosynthesis were more highly expressed when inoculated in wildtype barley plants with a functional HvUGT13248.

## Discovering Marker-Trait Associations for Fusarium Head Blight and Kernel Traits in Texas Wheat Breeding Lines

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Fusarium head blight (FHB) primarily caused by the fungal pathogen *Fusarium graminearum* is a very devastating wheat disease causing significant losses in grain yield and quality. It infects wheat spikes and gradually causes discoloration and deterioration of grain, and contamination with mycotoxins, mainly deoxynivalenol (DON). FHB resistance is very complex due to its quantitative nature of inheritance involving several genes. This study was designed to explore allelic diversity within the Texas A&M wheat breeding lines for FHB disease components (severity, incidence, Fhb-index, FDK, and DON) and kernel traits (area, perimeter, length, width, TKW). A set of 97 and 195 lines from Texas wheat breeding nurseries in 2024 and 2025, respectively were evaluated for FHB using a randomized complete block design in College Station, TX. Kernel traits were collected using the hp Scanjet G4010 photo scanner for image capturing and GrainScan v3. software for image analysis. Genomic libraries were prepared and run on Illumina NovaSeq 6000 to generate 150 bp paired end reads. Sequences were aligned to the IWGSC RefSeq genome assembly v2.1 using the Burrows Wheeler Aligner for SNP calling. Genomic sequences were filtered at 1% minor allele frequency and 5% heterozygosity which finally retained 109,323 and 55,051 polymorphic SNP markers for 2024 and 2025 mapping panels, respectively. To identify significant genomic regions associated with phenotyping traits, Blink, FarmCPU and MLM genome-wide association study models were implemented using the R package GAPIT version 3.5. Principal component analysis revealed that the first two components explained 62 to 67% phenotypic variations for Scab2024 and Scab2025 panels, respectively. Seed traits were highly correlated with  $r$  values ranging from 0.60-0.89 in Scab2024 and 0.37-0.94 in Scab2025. For disease components, INC and SEV had significant correlation ( $r=0.61$ ), FDK correlated with seed length, SEV ( $r=0.32$ ), and INC ( $r=0.4$ ) in 2024. FDK correlated with seed length and SEV ( $r=0.26$ ) in 2025. FDK had significant correlation with DON in both years ( $r=0.52$  and  $0.33$ ). Breeding lines in both panels had high relatedness due to narrow genetic basis and common pedigrees in the breeding program. Preliminary results revealed several significant marker-trait associations for DON and other traits. The identified significant SNPs could reduce DON by 1.7 to 8.8 ppm. *Fhb1* gene on chromosome 3BS is the most common source of resistance but we did not detect through genome wide markers. Texas wheat breeding line might potentially carry some unique resistances for FHB which needs further confirmation.

## Transcriptomic Study to Reveal Genes Governing Fusarium Head Blight Resistance in Durum Genotypes with Introgressed Major Resistance Genes

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*Fhb1* (*Qfhs.ndsu-3BS*) is the most important quantitative trait locus (QTL) conferring resistance against *Fusarium* head blight disease (FHB). Although this QTL has been extensively deployed in breeding programs worldwide, the presence of *Fhb1* does not always guarantee strong resistance across different genetic backgrounds. Understanding the genetics of resistance has been impeded by uncertainty regarding the causal gene within the *Fhb1* locus, and in durum, the underlying molecular mechanisms remain largely unexplored. To improve our understanding of FHB resistance in durum, we performed a transcriptomic analysis coupled with a haplotype study of six related durum lines in response to *Fusarium graminearum* using a novel reference genome, Sumai 3. These lines were derived from crosses between Sumai 3 and PI277012 cultivars, and represent lines with or without *Fhb1*, 5AS (*Qfhb.rwg-5A.1*) and 5AL (*Qfhb.rwg-5A.2*), showing varying levels of FHB resistance. Results revealed no significant change in the expression of the *PFT* gene within the *Fhb1* locus, while *TaHRC* expression was observed only in lines with the *Fhb1* QTL. However, the expression pattern of *TaHRC* did not support its role as the causal gene conferring resistance. Instead, results showed that the interaction of the *Fhb1* locus with genes from 5A plays a key role in defining FHB resistance. *Fusarium* head blight susceptibility of lines carrying *Fhb1* was further studied using haplotype analysis to understand how genes within each haploblock, inherited from respective parents, contribute to variations in FHB susceptibility or resistance. To further investigate gene expression dynamics, we will employ Quantitative Real-Time PCR and Enzymatic Methyl-seq assays.

## Enhancement of Fusarium Head Blight Resistance and DON Reduction by Expressing Optimized *FgTRI101* in Moderately Resistant Wheat

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The fungal pathogen *Fusarium graminearum* causes Fusarium head blight (FHB) and produces various mycotoxins that contaminate grains and cause profound health problems in humans and animals. Deoxynivalenol (DON) is the most prevalent trichothecene mycotoxin found in contaminated grains. Our previous study showed that transgenic wheat cultivar, Bobwhite, expressing *F. graminearum* trichothecene 3-*O*-acetyltransferase (*FgTRI101*) can increase FHB resistance and DON reduction. To further enhance FHB resistance and reduce mycotoxin contamination, *FgTRI101* was modified based on wheat translational codons to increase its expression; Then, the modified *mTRI101* was synthesized and introduced into moderately FHB resistant wheat cv Fielder and Forefront, driven by the maize ubiquitin promoter (Ubi-1). Three independent Fielder transgenic lines and one Forefront line containing *mTRI101* were identified. Gene expression studies revealed high expression levels of *mTRI101* in both wheat leaf and spike tissues in the transgenic lines. The phytotoxic effects of purified DON on the root growth of transgenic wheat expressing *mTRI101* were compared to non-transgenic and parent controls. The *mTRI101* transgenic seedlings had significantly longer root lengths on media containing DON than the controls. The conversion of DON to 3-ADON in the *mTRI101* transgenic wheat florets was detected. FHB evaluation assays showed that transgenic wheat plants expressing *mTRI101* enhanced FHB resistance. Significantly less DON accumulation was observed in *mTRI101* expressing lines, but no 3-ADON was detected in infected wheat spikes, suggesting that 3-ADON is unstable in wheat. Our study suggests that the introduction of a codon-optimized *mTRI101* gene in a moderately resistant wheat background is a promising strategy to control FHB and mycotoxin contamination. 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.

# **Pathogen Biology & Genetics**

## Revisiting the Evolutionary History of *Fusarium graminearum*

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*Fusarium graminearum* has been reported to possess a “two-speed” genome, with fast-evolving compartments exhibiting elevated nucleotide diversity and enrichment in genes associated with pathogenicity and preferentially expressed *in planta*. However, the evolutionary processes that gave rise to this two-speed genome—and the forces maintaining conserved positions of compartments across strains—remain poorly understood. Although admixture has been suggested to play only a minor role in *F. graminearum* population structure, published phylogenetic trees and split-network analyses instead suggest that the reshuffling of standing variation acquired through admixture is the dominant evolutionary force. This prediction was confirmed using genome-wide scans, which revealed that the fast-evolving compartments comprise sequences introgressed from other species within the *F. graminearum* species complex, including *F. asiaticum*, *F. austroamericanum*, *F. boothii*, *F. cortaderiae*, *F. gerlachii*, *F. louisianense*, and *F. meridionale*. Intriguingly, while the fast compartments imply extensive gene flow among the NA1, NA2, and NA3 lineages, the slow compartments suggest that such exchange is largely absent. This compartment-specific pattern of gene flow presents an evolutionary conundrum that challenges traditional models of speciation.



## An Update for Pathogen Biology and Genetics (PBG): Accomplishments and Challenges

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For the past USWBSI funding cycle, PBG has had two major goals: characterize plant-fungal interactions to identify important genes, proteins, or small molecules that may be used to develop FHB resistance or reduce DON contamination in barley and wheat; and discover epiphytic and endophytic microbes and microbial communities useful for development of FHB control. The following significant accomplishments have been made: 1) discovered that aggressiveness does not segregate with the 3ADON/15ADON chemotypes in progeny from a genetic cross; 2) identified single nucleotide polymorphisms (SNPs) associated with fungal growth, fungicide resistance, and toxin production using genome-wide association studies; 3) identified multiple *Fusarium* species and chemotypes in surveys of symptomatic wheat and barley, including nivalenol (NIV) strains present in the collection from Illinois; 4) developed and tested a new method for identifying and quantifying FHB-causing species in grain samples; 5) determined that barley resistance varied by the strains used for inoculations; 6) characterized G protein-coupled receptors (GPCRs) critical for *F. graminearum* pathogenesis, and characterized the regulatory roles of *TRI6* and *TRI10* in DON production. To utilize the critical fungal pathogenesis genes to reduce FHB and mycotoxin contamination, two RNAi delivery systems, spherical nucleic acid nanomaterials and fungal endophyte-mediated RNAi silence, were developed. Although both RNAi delivery systems appeared to work, they did not lead to a practical application. Since we have gained abundant knowledge in *Fusarium* genomics, transcriptomics, secondary metabolites, and pathogenesis, the future needs and challenges are to translate this knowledge to address FHB and mycotoxin contamination in wheat and barley.

## Transcriptional Regulation of DON Biosynthesis in *Fusarium graminearum*

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Deoxynivalenol (DON), a type B trichothecene, is the most frequently detected mycotoxin in cereal grains. To better understand transcriptional regulation of DON biosynthesis in *Fusarium graminearum*, we conducted stranded RNA-seq analysis with the *tri6* and *tri10* mutants cultured under DON-inducing conditions and identified antisense transcripts of key *TRI* genes and a lncRNA of *TRI5* transcribed from the promoter region of *TRI5* (named RNA5P). The *TRI10* and *TRI6* transcription factor genes regulate each other's expression. They both are involved in regulating the expression of *TRI5* sense and antisense transcripts as well as RNA5P, which has *cis*-acting inhibitory effects on *TRI5* expression and DON biosynthesis. We also characterized the functional relationship between Tri6 and Tri10 and the effects of chromatin modifications as global regulators on *TRI* gene expression. Results from these studies indicate that complex regulatory mechanisms are involved in controlling DON biosynthesis in *F. graminearum*.

## The Pan-Genomic Effectome of the *Fusarium sambucinum* Species Complex Reveals Novel Virulence Factors

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The *Fusarium sambucinum* species complex (FSAMSC) comprises prominent phytopathogenic fungi that collectively place a severe burden on global agriculture. Understanding the interaction between host plants and fungi is crucial to delineate mechanisms of plant defence and mitigate agricultural losses. Many phytopathogens deploy suites of secreted proteins, called effectors, to modulate host immunity and promote colonization; however, fungal phytopathogenic effectors are poorly understood in comparison to those of bacterial phytopathogens. To promote characterization of FSAMSC effectors, we developed a pan-genomic effectome for this species complex using a combination of bioinformatics tools. Our pipeline led to the prediction of 125,777 candidate-secreted effector proteins (CSEPs) from 195 isolates, including 235 conserved (“core”) effector families. A subset of core families demonstrated diversifying selection, and we hypothesized that these epitopes may be critical to host recognition and are thus likely to interact with host defence factors. We developed structural comparisons to known fungal effectors using AlphaFold and Foldseek, and used multiple virulence assays to demonstrate effector-like, and immune-eliciting function for a subset of conserved effectors. Host interaction partners of fungal effectors will be identified via proximity-based labelling with TurboID, to ultimately resolve a plant-pathogen interaction network.

## A Pangenome Graph Approach to Characterize Genomic Variation in *Fusarium graminearum*

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*Fusarium graminearum*, the causal agent of Fusarium Head Blight (FHB) in wheat and barley, consists of genetically distinct populations. Variation within and between populations can be composed of both small-scale changes, such as single nucleotide polymorphisms (SNPs), and larger structural variants (SVs) of the genome. However, analysis based on short-read sequences and a single reference genome is not designed to detect large SVs, leading to an underestimation of total genetic variation. To reduce reference bias, we constructed a pangenome graph using long-read genome assemblies representing different populations of *F. graminearum* and performed graph-based read mapping and variant calling with Mini-graph Cactus and Vg Giraffe, respectively. The pangenome approach identified a greater number of SVs compared to a linear-reference workflow. Graph-based variant calling also enabled the identification of high-confidence, polymorphic SNPs, with the NA1 population showing more SNPs than NA2. The Minigraph-Cactus pangenome approach detected significantly more large (>1 kb) insertions (479) and deletions (410) across isolates than the linear-reference workflow using SyRI (98 and 83, respectively). Minigraph-Cactus also identified more inversions (88 vs. 58) and duplications (4,981 vs. 234) compared to SyRI, while SyRI detected more translocations (139 vs. 45). These results demonstrate that pangenome graphs can more efficiently identify genomic variation, also highlighting the need for continued refinement of graph-based methods for detecting inter-chromosomal SVs. Our ongoing work aims to improve the detection and interpretation of these SVs to better understand pathogenicity and adaptation in *F. graminearum*.

## Differential Response of *Fusarium graminearum* Isolates to DMI Fungicides

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*Fusarium* Head Blight (FHB) caused by a fungal pathogen *Fusarium graminearum* is one of the significant constraints to wheat production in the United States due to its reduced yield and mycotoxin contamination. The intensive application of fungicides on cereal crops, such as wheat, has led to the development of resistance in many fungal pathogens that infect them. Historically, populations of *Fusarium graminearum* have been mainly treated as homogeneous at the level of management and resistance breeding. However, this clashes with documented variation in populations in terms of pathogenic traits, such as fungicide sensitivity and toxin production. This work aimed to determine the variation in sensitivity of the *F. graminearum* NA2 population to tebuconazole and propiconazole, and to identify the genetic basis of phenotypic variation, providing targets for disease management. Sensitivity of 150 *F. graminearum* isolates towards the two demethylation-inhibitor (DMI) fungicides was tested using a flat-bottom 96-well plate assay. Absorbance readings were taken before incubation, and at 5 and 7 days at 405 nm. Effective concentration 50 (EC50) values were computed using R packages. We used standard statistical methods to detect associations between genetic variants derived from whole-genome sequencing and pathogen traits. Using genome-wide association studies (GWAS), we aim to identify the genetic basis of variability in fungicide sensitivity. We anticipate our results will aid in developing strategies to manage FHB outbreaks and will suggest targets for pathogen control.

## Assessment of Pathogenicity and Aggressiveness of *Fusarium poae* on Wheat and Barley

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*Fusarium* head blight (FHB), primarily caused by *Fusarium graminearum*, poses a major threat to wheat and barley production. Although *Fusarium poae* is increasingly reported in cereal-growing regions worldwide, its pathogenic potential in U.S. wheat and barley remains underexplored. We compared the pathogenicity and aggressiveness of four *F. poae* isolates (GA18W 2.1.6, GA18W 5.2.4, GA18W 6.1.4, GA19W 13.2.1II) and one reference *F. graminearum* isolate (GA18W 3.1.4) across seven cultivars (three soft red winter wheat, two durum wheat, two barley) classified as moderately resistant or susceptible. Greenhouse evaluations were conducted in 2021, 2022, and 2024 using single floret inoculation (SFI) and direct spray (DS). Disease severity (SEV), *Fusarium* damaged kernels (FDK), thousand kernel weight (TKW), and mycotoxins (DON, HT-2/T-2) were assessed. Main effects of isolate, cultivar, and inoculation method were significant for SEV, FDK, and TKW ( $P < 0.0001$ ). After finding no significant differences among the four *F. poae* isolates for SEV, FDK, or TKW, we pooled the *F. poae* data to report species-level summaries. Across cultivars, methods, and years, *F. graminearum* GA18W 3.1.4 produced higher SEV (47–63%) and FDK (54–76%) and reduced TKW (to 18 g) relative to pooled *F. poae* (SEV 14–36%; FDK 32–56%; TKW up to 24 g), with the greatest impacts in durum wheat. Preliminary toxin data showed DON frequently  $>40 \mu\text{g g}^{-1}$  with *F. graminearum*. HT-2/T-2 was sporadically detected only in durum wheat inoculated with *F. poae* (maximum  $2,300 \mu\text{g kg}^{-1}$  in ‘Divide’ with GA18W 6.1.4). These results show that *F. poae* can contribute to FHB in U.S. small-grain cereals and support including this species in resistance screening.

## Competitiveness of Segregating 3ADON versus 15ADON *Fusarium graminearum* Progeny on Susceptible Wheat, With or Without Fungicides

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Variation in trichothecene chemotype among *Fusarium graminearum* populations has been linked to shifts in the incidence and severity of Fusarium Head Blight (FHB) epidemics across North America. The NA2 population, which is usually associated with the 3ADON chemotype and higher toxin levels than the predominant 15ADON-producing NA1 population, has been expanding its range. However, it is unclear whether the 3ADON chemotype has contributed directly to this expansion. To test the hypothesis that the 3ADON chemotype increases pathogen competitiveness during infection of wheat heads, we conducted co-inoculation experiments with segregating progeny from a cross between a 15ADON MAT-deletion heterothallic tester strain and a 3ADON wild-type homothallic isolate. Seven progeny pools were constructed, each comprising five 3ADON and five 15ADON strains selected randomly. Pools were inoculated onto spikes of the susceptible spring wheat line Wheaton with or without fungicide. Disease severity was assessed at 7-, 10-, and 14-days post-inoculation and summarized as area under the disease progress curve (AUDPC). Mycotoxin quantities and profiles were measured in wheat heads, and the trichothecene genotypes of recovered progeny were determined by using TRI gene-based multiplex PCR assays. In all three trials, the progeny pools produced less disease and accumulated less mycotoxin than the parental strains in the absence of fungicide, possibly due to antagonistic interactions. Fungicide treatment reduced overall disease and mycotoxin levels, although the 3ADON parent was less sensitive to the fungicide than the 15ADON parent. Analysis of the mycotoxin profiles showed that there was no significant bias in favor of 3ADON versus 15ADON in the wheat heads inoculated with any of the pools, in either the presence or the absence of fungicide. Analysis of 24 single-spored strains per pool that were recovered from the wheat heads also mostly showed no significant deviation from a 1:1 ratio of 3ADON to 15ADON trichothecene genotypes across all pools, regardless of fungicide treatment. Individual pools did exhibit dominance by certain clones, but this was not associated with the trichothecene genotype. These results indicate that the 3ADON chemotype alone does not confer a competitive advantage in mixed infections, and that the recent expansion of 3ADON populations in North America is likely driven by other factors. The recovery of dominant fungal clones from mixed infections supports the hypothesis that some of these competitiveness factors are genetic. These genetic factors will be explored in future studies to understand their function and identify associated markers to track them in the population.

## Effect of Resistance on *Fusarium graminearum* Populations

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Quantitative host resistance is the best method to control *Fusarium* head blight (FHB). However, the impact of wheat resistance on the pathogen population remains poorly understood. This study aimed to determine if the presence or absence of wheat resistance influenced the fitness components and genomic regions of *F. graminearum*. We collected isolates across five locations in Illinois from two naturally infected wheat varieties: IL11-28222 (moderately resistant) and 25R47 (highly susceptible). We obtained 31 isolates from the highly susceptible and 25 isolates from the moderately resistant variety. We evaluated the isolates aggressiveness in the greenhouse on 'Norm' spring wheat. The experiment was established in a randomized complete block with three replications. Wheat plants were inoculated at anthesis with. FHB severity was evaluated at one, two, and three weeks post inoculation. Isolate aggressiveness was quantified using the area under the disease progress curve (AUDPC), *Fusarium* damaged kernels (FDK), and deoxynivalenol (DON) contamination. We also measured in vitro growth rate and spore production for each isolate. DNA was extracted from all the isolates for whole-genome resequencing using Illumina short-read technology. Variant calling was completed using GATK and a dataset of 333,297 SNPs was used to complete two whole-genome scans for selection: one across the entire sample, and another comparing isolates from moderately resistant and highly susceptible wheat. Isolates from highly susceptible wheat were significantly more aggressive than the isolates from moderately resistant wheat. Conversely, isolates from moderately resistant wheat exhibited significantly higher in vitro growth rates. These results suggest that isolates with greater saprophytic ability may be less aggressive in planta. AUDPC, FDK, and DON were all significantly correlated. We identified several regions of the *F. graminearum* genome with signatures of selection. A total of 936 nonsynonymous SNPs were found in these regions, which included the trichothecene gene cluster, the TRI101 locus, and other loci previously associated with DON production or pathogen aggressiveness. Three genomic windows showed evidence of divergent selection between isolates from the two wheat varieties. Our findings suggest that the moderately resistant wheat varieties do not select for more aggressive isolates, supporting the durability of genetic resistance as a strategy for FHB control.



## Genome-Wide Perspectives on *Fusarium graminearum* Adaptation in Barley, Soybean, and Potato

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*Fusarium graminearum* causes Fusarium head blight, damaging small-grain crops including barley, and produces the mycotoxin deoxynivalenol (DON), which poses health risks to humans and animals. Crop rotation is a sustainable management strategy often used in North Dakota to control various pests and pathogens. However, crop residues can harbor pathogens such as *F. graminearum*, serving as inoculum sources for subsequent crops. Although *F. graminearum* is mainly a fungal pathogen of cereals, it has also been reported to cause dry rot in potatoes and root rot in soybeans. The pathogen adapts to different crops, which makes crop rotation alone insufficient for managing the disease. We hypothesize that, in addition to its preference for cereals, *F. graminearum* might also favor non-cereal crops in a rotation system. To examine this, we cross-infected a total of seven *F. graminearum* isolates collected from barley (2), potato (1), soybean (3), and winter rye (1) onto barley, potato, and soybean. Results of our cross-infectivity experiments show that *F. graminearum* isolates vary in aggressiveness levels. For instance, isolates from potato and barley caused similar levels of disease when inoculated into potato tubers, whereas soybean and winter rye isolates caused no to low disease in potato. We sequenced isolates and examined how genomic features affect host preferences. Each genome had 4–5 contigs totaling 36.5–37.5 Mb, consistent with *F. graminearum* size. The largest contigs reached 11.8 Mb, with an N50 over 9 Mb, indicating high contiguity. GC content was stable at 47.8%, with no ambiguous bases. Genomic analysis showed that *F. graminearum* isolates from barley and potato had higher variant counts and rates than those from soybean and winter rye. In addition, barley and potato had the most SNPs, insertions, and deletions, while soybean and winter rye had fewer variants. Variant rates were also higher in barley and potato (1 per 233–236 bp) than in other isolates (1 per 337–390 bp). All seven isolates share a large core genome of 11,505 orthogroups, indicating conserved functions, but comparing accessory genes reveals two groups: barley and potato, and soybean and winter rye. Our findings indicate that while *F. graminearum* has conserved regions (core), a small part of its genome (accessory) is variable and may influence the fungal pathogen's ability to infect specific crops. Our study is the first to demonstrate the connection between cross-infectivity patterns and genomic variation in *Fusarium graminearum*.

## Chemotypes, Population Structure and Genome-wide Association Study of *Fusarium graminearum* Isolates Collected From Eastern North Dakota and Western Minnesota

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*Fusarium* head blight (FHB), caused by *Fusarium graminearum*, is a major disease of wheat worldwide. Regular monitoring of the local pathogen population is critical for understanding pathogen dynamics and developing effective management strategies. In this study, we conducted surveys and collected FHB-symptomatic spikes from wheat fields across eastern North Dakota and western Minnesota during the growth seasons of 2021 and 2022. A total of 300 *F. graminearum* pure cultures were isolated and used to assess chemotype diversity, aggressiveness and DON production. Chemotyping indicated that 69.40% and 30.58% of the isolates were 15ADON type and 3ADON type, respectively. NX-2 chemotype was only found for one single isolate collected from a wheat field located in Norman County, Minnesota. Aggressiveness and DON production were evaluated on two wheat genotypes, Alsen (moderately resistant to FHB) and Wheaton (highly susceptible to FHB). No significant difference was observed in aggressiveness between 15ADON and 3ADON isolates, although the latter produced significantly higher DON. Genome sequencing and single nucleotide polymorphism (SNP) analysis classified the isolates into three genetic clusters, with two corresponding to the two different chemotypes (15ADON and 3ADON), respectively, and one admixture of the populations. Furthermore, the genome wide association study identified multiple SNPs associated with aggressiveness and DON production on chromosomes 1, 2 and 4, some of which are potential novel candidate genes associated with aggressiveness. Overall, our work provides a new information on current *F. graminearum* populations in this region, which is important for the development and deployment of resistant cultivars.

# Variety Development and Host Resistance

## Corteva Hybrid Wheat and FHB Resistance Efforts

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Corteva will launch hybrid wheat in the Hard Red Winter market class in Fall 2027. Thanks to its unique, non-transgenic sterility system, hybrid seed can now be produced reliably and at scale and independent of genetic background. This breakthrough eliminates production challenges and leverages hybrid vigor to set a new standard for wheat yield in the U.S. Internal trials show that the yield advantage of hybrids is +10% greater than varietal checks, and up to 20% higher under drought stress. Fusarium head blight (FHB) resistance is also an important focus in Corteva's breeding programs. For example, in the Soft Red Winter market class, FHB resistance is one of the competitive advantages of Corteva (Pioneer) commercial varieties; progress for FHB resistance is being achieved through a combination of whole genome predictions strategies and selection for major FHB genes. FHB screenings are conducted in field nurseries, controlled conditions in greenhouse, and in collaboration with universities.

## The Effect of Weather Conditions on Flowering and the Selection for Loci That Contribute to Durable Resistance

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Fusarium head blight (FHB) infection occurs at anthesis in wheat (*Triticum aestivum* L.) and is influenced by weather conditions in and around flowering. Timing of flowering relative to weather conditions causes inherent genotype-by-environment interactions, making it difficult to select for resistant varieties. Understanding which loci contribute toward stable resistance across environmental conditions versus those that are sensitive to weather is critical for improving quantitative resistance to FHB. This study evaluated 879 genotyped lines in misted FHB-inoculated nurseries in Warsaw, Virginia, over four cropping seasons (2019-2022). Here, we present a phenological model of host response to disease infection and progression under variable weather conditions relative to flowering. Weather covariates, including temperature, precipitation, and humidity, were adjusted by the flowering date of each plot, such that weather variables were indicative of conditions at flowering and at one-day intervals for 7 days before and after flowering. Main genetic effects of markers, phenologically adjusted weather variables, and their interactions were included in a Bayesian mixed model to separate the effects into three categories: 1) stable genetic marker effects across environmental conditions, 2) effects of weather on infection and disease progression per se, and 3) unstable genetic effects sensitive to weather conditions, respectively. The deviance information criterion statistic found the phenological model that included all three terms the best for three FHB traits, indicating marker-by-weather interactions were significant. High temperatures at post-anthesis have the greatest effect, reducing the rate and severity of FHB infection and deoxynivalenol (DON) accumulation. Total precipitation had little effect on FHB infection in the misted nursery, but it is likely to have an effect under standard growing conditions. We recommend breeders focus on stable genetic effects on host resistance and explicitly ignore or select against those with unstable effects.

## Variety Development to Improve Wheat's Resistance to Fusarium Head Blight: Tremendous Progress with Some Unfinished Business

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In the past 4-year funding cycle, over 50 cultivars were released across four major wheat classes: spring, durum, hard red winter, and soft red winter. The Variety Development and Host Resistance (VDHR) Coordinated Projects that cover each of these market classes have made breeding for FHB resistance a top priority, and the dedication has paid off for the wheat industry and the many thousands of producers that support it. Resistance genes from both native and exotic sources are now commonly found in elite lines tested by the 25 wheat breeding programs that receive USWBSI funding. Many cultivars released from these programs possess more than one known FHB resistance QTL, and this gene stacking has escalated due to year-over-year FHB phenotyping in inoculated nurseries and with routine marker screening. The exotic resistance gene *Fhb1*, originating from Sumai 3, is now pervasive throughout gene pools of most major wheat classes, with more than 20 varieties released in the past four years harboring this effective gene. Many of these releases with *Fhb1* or multiple resistance QTL from native sources are doubled haploids (DHs), which has expedited the breeding cycle. Many programs now set their sights on incorporating *Fhb7* from *Thinopyrum elongatum* to further boost FHB resistance and reduce the reliance on *Fhb1* and popular native resistance alleles. In complement, breeders are utilizing genomic selection to stack favorable alleles and build more robust resistance to remain ahead of *Fusarium graminearum*'s ability to overcome isolated resistance mechanisms. Approaches like genomic prediction and the onset of automated or semi-automated field phenotyping are enabling FHB resistance screening earlier in the breeding pipeline at higher population sizes, which will lead to more accelerated gains.

## Barley Coordinated Project: Advancing Fusarium Head Blight Resistance through Breeding, Genomics, and National Collaboration

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The Barley Coordinated Project (BAR-CP) continues to protect U.S. barley from Fusarium Head Blight (FHB) and its mycotoxin, deoxynivalenol (DON), through integrated breeding, genomics, and collaborative research. From 2022–2025, BAR-CP supported more than 20 projects across ten states, accelerating the development of resistant spring and winter barley varieties. Key achievements include establishing new winter barley FHB nurseries, enabling doubled haploid production to speed breeding cycles, and lowering genotyping costs through a multi-crop 3K barley SNP chip. Fundamental advances have also been made toward understanding the genetic basis of Type II resistance, deploying gene editing of the *Fhb1* ortholog, and transferring *Fhb7* into barley. Looking forward, the project emphasizes data management under FAIR principles and the need for stronger engagement with private breeding programs to ensure rapid adoption of resistant germplasm. These coordinated efforts strengthen the resilience of U.S. barley production and safeguard grain quality against FHB.

## Improvement of Wheat Disease Resistance Through the Global Wheat Health Alliance (GWAH) Project

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The Global Wheat Health Alliance (GWAH) project addresses the growing threat of Fusarium Head Blight (FHB), wheat blast, and rusts to global wheat production, food security, and safety. By linking Advanced Research Institutes (ARIs), National Agricultural Research Systems (NARES), and the CGIAR Global Wheat Program, GWAH aims to accelerate the discovery, validation, and deployment of disease resistance genes. The project's primary objective—especially for FHB—is to develop wheat varieties with enhanced, stable resistance for improved yields and grain quality. GWAH will harness advanced genetic technologies, including gene stacking and genome editing, to leverage both existing and newly discovered resistance sources, integrating well-characterized genes like *Fhb1*, *Fhb5*, and *Fhb7*, quantitative trait loci (QTL) such as *Qfhb.cim-2D*, and novel loci identified through genomics. Strategic efforts also focus on removing resistance inhibitors and susceptibility factors for maximum gene effectiveness. Accelerated pre-breeding and breeding pipelines, particularly benefitting vulnerable regions such as East Africa and South Asia, will enable rapid release of improved, locally adapted varieties. The project also strengthens local and regional research capabilities for long-term sustainability and resilience. Within three years, GWAH expects to expand current global collaborations to significantly reduce wheat disease risks, safeguard food production for vulnerable populations, and future-proof breeding against evolving pathogens.



## Advancing Wheat Resistance to Fusarium Head Blight through Genomic Prediction

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Fusarium Head Blight (FHB), or head scab, is a disease of *Triticum aestivum* caused by the fungal pathogen *Fusarium graminearum*. It reduces grain yield, test weight, and milling quality, and leads to the production of mycotoxins, such as deoxynivalenol (DON), which threaten human and animal health. Recent climate shifts have intensified FHB outbreaks, including in the Great Plains region of the U.S., where hard winter wheat is predominantly grown, underscoring the need for rapid development of resistant cultivars. Resistance to FHB in wheat is primarily governed by small-to-moderate effect Quantitative Trait Loci (QTL), with QTL mapping widely used for Marker-Assisted Selection (MAS). Notably, the *Fhb1* locus is considered a stable and valuable QTL for MAS, but due to the polygenic nature of resistance, combining MAS with genomic prediction is essential to capture broader genetic effects. This project integrates genome-wide marker data and historical phenotypic records to generate genomic estimated breeding values (GEBVs) for key traits supporting selection decisions. Annual line contributions from collaborating universities were genotyped using a 25K SNP array. In 2025, approximately 450 CSU breeding lines were screened at the University of Illinois Urbana-Champaign (UIUC), with visual ratings collected in-person and Fusarium-damaged kernels (FDK) data gathered post-harvest. Preliminary analyses combining historical phenotypic records and 25K SNP genotypes yielded a mean Pearson's correlation of 0.43 for FHB visual ratings. While current prediction accuracies are limited by small training populations, results highlight the potential of expanding datasets to improve genomic prediction and accelerate FHB resistance breeding.

## 30 Years of Breeding for Fusarium Head Blight Resistance in Wheat: A Success Story

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There has been considerable work on increasing the genetic resistance to Fusarium head blight (FHB) in hard red spring wheat. Harnessing the phenotypic data from uniform regional nurseries, the genetic gain over the last 30 years was assessed for three FHB-related traits, namely disease index, visual scabby kernels, and deoxynivalenol concentration. Specifically, two nurseries were compared: the uniform regional nursery (URN), used to test variety release candidates with good agronomic qualities, and the uniform regional scab nursery (URSN), used to facilitate the phenotyping of FHB resistance and to exchange germplasm among the cooperating breeding programs. The objective of the study was three-fold: (i) studying the frequency of the major FHB-related quantitative trait locus (QTL) *Fhb1*, (ii) assessing the genetic gain for three FHB traits, and (iii) estimating the effect of this QTL on FHB and agronomic traits. Within these objectives, a comparison was made between the two nurseries. We showed that FHB resistance has increased significantly over the years for FHB-related traits, with a higher rate for the initially more susceptible URN materials. More recently, there has been little difference in the susceptibility level between the two nurseries. The frequency of the *Fhb1* resistance allele has dramatically increased in frequency over the years, from 0% in 1995 to 40% in 2024 for the URN nursery. We observed a higher frequency of the resistance allele in the URSN compared to the URN nursery, but the differences tended to diminish over time. The *Fhb1* resistance allele was significantly associated with increased FHB resistance for the three traits, with up to a 39% decrease for the disease index trait. We tested the effect of *Fhb1* on agronomic traits using phenotypic data from the URN dataset and found that there was no detrimental association between the presence of resistance alleles and important agronomic traits. This work underscores progress achieved through breeding, likely due to a successful transfer of resistance alleles from the URSN to the URN material.

## Strengthening Durum Wheat Resistance Against Fusarium: *Fhb7<sup>The2</sup>* Delivers Consistent Resistance Across Diverse Cultivars

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Fusarium head blight (FHB) remains a persistent threat to durum wheat production, particularly due to the limited availability of effective resistance genes and the susceptibility of elite cultivars. In this study, we incorporated the *Thinopyrum elongatum*-derived resistance gene *Fhb7<sup>The2</sup>* into four U.S. durum wheat cultivars, ND Riveland, Divide, MTD18348, and Miwok using a marker-assisted backcrossing breeding pipeline. Each cultivar was crossed with WGC002 (*Fhb7<sup>The2</sup>* donor) and backcrossed four times to develop near-isogenic lines carrying *Fhb7<sup>The2</sup>*. FHB severity was assessed under controlled greenhouse conditions at 14- and 21-days post inoculation (DPI), and in the field at 21 days post flowering using corn spawn inoculation. Fusarium-damaged kernel (FDK) percentages were visually scored from greenhouse-harvested seed. Across all environments, the introgression lines demonstrated a consistent and significant reduction in FHB severity and FDK compared to their recurrent parents. For instance, Miwok-*Fhb7<sup>The2</sup>* showed a dramatic decrease in FHB severity (5.77% vs. 49.55% at 14DPI\_GH) and FDK (17.52% vs. 82.72%) compared to Miwok. Similar results were observed in Divide-*Fhb7<sup>The2</sup>* (5.82% vs. 35.59%; 7.19% vs. 68.33%), MTD18348-*Fhb7<sup>The2</sup>* (6.51% vs. 38.40%; 12.07% vs. 70.76%), and ND Riveland-*Fhb7<sup>The2</sup>* (5.09% vs. 26.39%; 6.08% vs. 41.52%). These results were statistically significant ( $p < 0.05$ ) and consistent across greenhouse and field trials. The four recipient cultivars represent a diverse genetic and agronomic background: ND Riveland and Divide are high-yielding, regionally adapted cultivars in North Dakota; MTD18348 is a Montana-bred rainfed variety with strong pasta quality; and Miwok is a low-cadmium desert durum tailored for export markets. Despite their differences, all responded positively to *Fhb7<sup>The2</sup>* introgression, with no observable agronomic penalties. This study confirms the broad utility of *Fhb7<sup>The2</sup>* in durum wheat breeding and highlights its potential to enhance FHB resistance across diverse genetic backgrounds. The monogenic nature of the resistance and the absence of deleterious effects from the alien translocation make *Fhb7<sup>The2</sup>* an effective gene for accelerating resistance breeding in durum wheat. Its deployment could substantially reduce disease-related losses and improve food safety in durum production systems.

## Genomics and Functional Genomic Studies Reveal Novel Host Resistance Mechanisms and Microbiome Roles in Cereal Defense Against Pathogens

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Our research is focused on understanding cereal-*Fusarium*-microbiome interactions in order to enhance control of Fusarium Head Blight (FHB) disease. We work on FHB disease of wheat and oats. At a fundamental level, we work to better understand how the mycotoxin deoxynivalenol (DON) affects wheat and through such studies we have revealed that DON plays a role in the very early stages of the plant-pathogen interactions and its effects are dose-dependent (beyond what is known regards its role in disease spread). We characterized novel gene pathways associated with FHB resistance in wheat. For example, our studies of a novel wheat gene led to the identification of several genes that can improve FHB resistance. Our studies of the wheat genome identified genomic hotspots associated with FHB resistance, i.e. regions enriched in genes responsive to FHB disease and hence potential disease resistance genes. We developed a pipeline to identify 44 FHB-associated disease responsive gene clusters, including one with antimicrobial activity and another co-located with a FHB resistance genetic locus. Ongoing work is assessing the relationship between wheat, *F. graminearum* and the wheat head microbiome. Based on our completed oat x FHB x microbiome studies, we know that specific microbial assemblages are associated with low levels of T2/HT-2 toxin production by *Fusarium langsethiae*. Thus, we question whether the same thing occurs in the wheat x FHB x microbiome interactions. Using diverse wheat genotypes from the Watkins collection, the goal is to determine if (a) there are microbial cohorts associated with FHB resistance, and (b) if there are wheat genetic loci that support the establishment of these microbes in wheat heads. Additionally, we are assessing the relationship between FHB, wheat genotype and the environment using multi-locational field trials. Acknowledgments: This research was supported by the Research Ireland project 'WheatHeath' (project No. 22/FFP-A/10284), Science Foundation Ireland project No. 14/1A/2508, the Department of Agriculture, Food and the Marine (DAFM) and The Department of Agriculture, Environment and Rural Affairs (DAERA) project 'Mycotox-I' (project No. 2021R460), the EU Wales-Ireland Programme 2014-2020 project "HealthyOats" (part funded by the European Regional Development Fund through the Ireland Wales Cooperation Programme 2014-2020, project number 82128), the Disruptive Technologies Innovation Fund project 'E-Crop' (project number DT20200139) and the European Union Interreg North Periphery and Arctic Programme Project 'OatFrontiers' (financed through European Regional Development Fund (ERDF) and ERDF equivalent funding from the Non-EU partner countries, project number NPA0500123).

## Improving Montana Durum Wheat Production through Utilization of *Fhb1*, *Fhb5*, and *Fhb7<sup>The2</sup>*

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Montana ranks second in U.S. durum (*Triticum turgidum* subsp. *durum*) wheat production, with about 250,000 hectares grown annually. Rain during the growing season increases Fusarium Head Blight (FHB) incidence and elevates deoxynivalenol (DON) levels. Since complete resistance to FHB is unavailable, pyramiding known resistance genes offers the most effective management strategy. To combat FHB in durum, we have developed populations by crossing durum with hexaploid wheat lines carrying *Fhb1* and *Fhb5*. Additionally, to decrease FHB infection and DON even further, we are also collaborating to introgress *Fhb7<sup>The2</sup>*, a *Thinopyrum elongatum*-derived resistance gene, into durum. All resistance genes were tracked using KASP markers in greenhouse trials, followed by multi-year field evaluations at Montana State University's Eastern Agricultural Research Center (EARC) in Sidney, MT—Montana's primary durum-growing region. In 2024, field tests of eight populations totaling 176 genotypes with at least one fixed *Fhb1* or *Fhb5* resistance gene identified individuals with varying DON levels. From these genotypes, a third of the individuals with lowest DON levels were selected and advanced for field testing in 2025, with results to be presented. Further, a durum genotype with *Fhb7<sup>The2</sup>* was tested in the field for FHB resistance in 2025 with results forthcoming. Developing and releasing FHB-resistant lines with improved yield and reduced DON levels is essential to sustain high-yielding durum production in the state.

## Effects of FHB Resistance QTL and Nitrogen Rate on Agronomic and Quality Traits of SRW Wheat

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Wheat (*Triticum aestivum* L.) is one of the three most important crops produced worldwide but is devastated annually by fungal diseases. Fusarium head blight (FHB), caused by *Fusarium graminearum*, affects wheat both worldwide and in Kentucky. While deployment of resistance QTL has reduced crop losses from FHB, their effect on grain quality is still being explored. In addition to FHB resistance, nitrogen management is an important factor in wheat production. This research aims to assess if different nitrogen rates and pyramiding FHB resistance genes impact agronomic and post-harvest qualities of soft red winter wheat in Kentucky. In this study, an F<sub>2</sub> population was developed from parents with different QTL combinations, and the QTL status of the 120 F<sub>2</sub> – derived lines were characterized by Kompetitive Allele Specific PCR (KASP). We hypothesized that an increased number of QTL will lower FHB severity but would also decrease grain protein. To analyze effect of nitrogen applications, thirty F<sub>2:5</sub> lines were planted in a replicated split-block field design with four rates ranging from 0 to 140 lbs/ac at two locations in Kentucky. Based on index selection, the top performing lines contained the QTL combinations of *Fhb1*, *Fhb1* plus *1A Neuse*, and *1A Neuse* plus *4A Neuse*. *Fhb1* and *Fhb1* plus *1A Neuse* had the lowest DON concentrations of the three QTL combinations. QTL combinations had varying responses to increased nitrogen rates, despite no significant difference across all combinations. Grain protein did not differ significantly among the three QTL combinations. A significant gain in grain protein across all QTL combinations was seen as nitrogen rates increased. The effect of nitrogen on FHB and post-harvest quality provides valuable insights to the breeder and producer. This research will lead to a better understanding of FHB resistance QTL and aid in providing producers with management guidelines.

## Developing Durum Wheat Germplasm with Fusarium Head Blight Resistance Through Interspecific Crosses

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Fusarium head blight (FHB) is one of the most devastating fungal diseases affecting durum wheat in North Dakota. Breeding for FHB resistance in durum wheat (*Triticum turgidum* L. subsp. durum) remains particularly challenging due to the limited availability of resistant germplasm. ND-WGE-22S2393 is a BC<sub>1</sub>F<sub>3</sub>-derived line developed from an interspecific cross between the hard red spring (HRS) wheat resistant line ND-WGE-20S558 and the local durum wheat cultivar 'ND Riveland'. ND-WGE-20S558 is a male-sterile half-sib line selected from an HRS recurrent selection population for FHB resistance. Selection of progenies from the interspecific cross was based on phenotypic performance under both greenhouse and field conditions. ND-WGE-22S2393 was evaluated for FHB resistance over four years at two locations and exhibited significantly lower disease severity and deoxynivalenol (DON) concentration compared with the check cultivar ND Riveland. Genotyping using the KASP marker Fhb1-FM227 confirmed that ND-WGE-22S2393 carries the resistant allele of the Fhb1 gene. ND-WGE-22S2393 showed similar plant height to the cultivar 'ND Grano' but was shorter than ND Riveland. Flowering occurred approximately two days earlier than in ND Riveland. Overall, ND-WGE-22S2393 represents a valuable FHB-resistant pre-breeding germplasm well adapted to the Northern Great Plains.

## The 2025 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 2025 nursery was comprised of 54 entries, including 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. Field data were collected for the FHB traits Rating, Severity, Incidence, Index, FDK, and DON for breeding lines and compared with data from the long-term resistant check varieties. Data were also collected on important agronomic characteristics, including heading date and plant height. Field data were collected from up to 11 locations in the US. The USDA-ARS Eastern Regional Genotyping Laboratory conducted marker genotyping, identifying alleles for major FHB QTL and other QTL of agronomic importance. 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 2024. A combined linear mixed model analysis of the phenotypic data was performed and best linear unbiased estimates (BLUEs) for each genotype were calculated. The genomic prediction model utilized phenotypic BLUEs and 45,695 SNP markers to predict GEBVs for individuals in the 2025 nursery with the R-package RR-BLUP (v 4.6.3). Predictive ability was calculated using Pearson correlation between GEBVs and BLUEs for the 2025 entries. Predictive abilities from 0.57-0.58 were observed for FHB 0-9 Rating, FDK, and DON. Machine learning models were used to provide estimates of presence/absence for 34 QTL of interest. Additionally, a new set of crosses were simulated among the 2024 and 2025 nursery entries using the R package PopVar (v 1.3.1) and estimates of progeny performance were reported. The full 2025 report will be available online at the USWBSI web site at <https://scabusa.org>.

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## Head-by-Head AI for High-Throughput FHB Scoring in Wheat & Barley

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Fusarium head blight (FHB) severely constrains wheat and barley breeding due to the time-, labor-, and expertise-intensive nature of field scoring. We developed and field-tested an innovative 360° Deep Scanning Robot that leverages advanced imaging, sensor fusion, and AI-driven analytics to detect FHB and quantify disease severity in real time. Preliminary deployments by the Precision Agriculture team at SDSU produced highly encouraging results, generating cardiograph-like graphical outputs that deliver objective, line-by-line ratings across breeding plots. We have proposed a next-level, automated Head-by-Head FHB scoring system featuring an adaptive, height-adjustable scanning mechanism for plant-head-level assessment for variable canopy heights. The system integrates trained computer-vision models with high-throughput field phenotyping to provide precise, rapid (<10 min) and reproducible resistance ratings. System integration, real-time algorithm optimization, and validation across multi-location field trials by benchmarking against expert manual assessments and stress-testing performance across genotypes, crop heights, and environments. Deliverables include an operational prototype, validated accuracy and throughput metrics, and user-ready training resources and best-practice guides. This project directly aligns with USWSI research priorities and, once fully optimized, promises scalable, and efficient FHB screening to accelerate genetic gain in wheat and barley breeding programs.

## Assessment of DON Accumulation in Durum Wheat Across North Dakota Variety Trial Sites

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North Dakota produces just over half of the U.S. durum wheat (*Triticum durum* Desf.). However, production is frequently threatened by Fusarium Head Blight (FHB) and associated contamination with the mycotoxin deoxynivalenol (DON), due to limited host resistance to *Fusarium graminearum*. The episodic nature of infections and variable environmental conditions further complicate disease development and DON accumulation. This study aimed to assess DON levels in 21 durum wheat varieties from variety trials conducted at nine locations across North Dakota — Carrington, Garrison, Hettinger, Langdon, Minot, Mohall, Nesson, Rugby, and Williston — during the 2023 and 2024 growing seasons. Grain samples were milled, and DON concentrations were quantified using Neogen's Reveal Q<sup>+</sup> DON test strips, with results read by the AccuScan Gold® Lateral Flow Strip Reader. Data were analyzed using JMP® Student Edition 18.2.2. Results showed a significant Variety × Location × Year interaction ( $P < 0.0001$ ). While varietal differences in DON levels were not statistically significant ( $P = 0.1084$ ), location had a strong influence ( $P = 0.0040$ ), with DON levels notably higher in Rugby and Mohall in 2024, while Langdon remained stable across both years. These findings underscore the challenges posed by inconsistent disease pressure and environmental variability, emphasizing the need for continued statewide monitoring of DON. Moreover, locations like Rugby and Mohall may benefit from more aggressive FHB management strategies, including the use of the FHB Risk Tool and timely fungicide applications, to reduce DON contamination. These insights are valuable for informing durum variety selection and guiding site-specific disease management across North Dakota.

## Response to Early Generation Selection for Resistance to Fusarium Head Blight and Stripe Rust in Winter Wheat Breeding Populations

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Early generation selection for disease resistance in segregating populations can increase the frequency of resistance alleles among derived inbred lines. A set of 12 populations segregating for resistance to wheat stripe rust caused by *Puccinia striiformis* Westend. f. sp. *tritici* Erikss. and 26 populations segregating for resistance to Fusarium head blight caused by *Fusarium graminearum* Schwabe. were split into replicated experimental groups undergoing selection for disease resistance and replicated control groups without selection. Selection for disease resistance was applied under greenhouse conditions at the F2 and F3 generations by culling susceptible plants and retaining resistant plants each generation while control populations were advanced without selection. Recombinant inbred lines (RILs) were randomly derived from replicates of selected and control populations at the F4 generation. A higher frequency of disease-resistant RILs were derived from populations undergoing selection in contrast to control populations where no disease selection pressure was applied. Selection signatures were detected using a Fisher's Exact Test of allele counts between RILs derived from selected and control populations. This study demonstrates phenotypic selection for disease resistance at the F2 and F3 generations increases the frequency of disease-resistant lines derived at the F4 generation and shifts the frequency of alleles associated with disease resistance.

## Improving Fusarium Head Blight Resistance in Durum Wheat Using Recurrent Phenotypic Selection and Genomic Selection

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Breeding for Fusarium head blight (FHB) resistance in durum wheat (*Triticum turgidum* L. subsp. *durum*) is particularly challenging due to the limited availability of resistant germplasm. Several durum wheat lines with moderate resistance, carrying different resistance genes, have been developed using resistant sources identified in tetraploid wheat relatives and hexaploid bread wheat. Given the complex genetic architecture of FHB resistance, recurrent selection provides an effective approach to improve resistance by integrating multiple resistance genes and increasing the frequency of favorable alleles. This strategy also enhances the likelihood of developing FHB-resistant inbred lines from the improved populations. A base population was developed by crossing ten moderately FHB-resistant lines with five elite durum wheat cultivars. Five cycles of phenotypic selection for FHB severity were conducted from 2019 to 2024, one cycle per year. The mean FHB severity decreased from 82.5% in Cycle 0 to 59.3% in Cycle 5. A genomic prediction model was developed using 318 breeding lines from the NDSU durum program and 438 S<sub>0:1</sub> lines from recurrent selection Cycle 3–5 populations. Prediction accuracies were 0.51 for FHB severity, 0.53 for plant height, and 0.79 for days to flowering. Two cycles of genomic selection (GS), initiated with the top 15 S<sub>0:1</sub> lines from the Cycle 5 population, were conducted from September 2024 to April 2025. Field evaluations in 2025 showed progressive improvement in FHB severity across GS cycles at both locations. The top 24 lines selected from GS-Cycle 0–2 were genotyped using the KASP marker for *Fhb1*, revealing that over 65% of lines carried the resistant allele. Several of these top lines also exhibited significantly lower FHB severity and deoxynivalenol (DON) content compared with the check cultivar ND Riveland at both Fargo and Prosper, ND. These results demonstrate the effectiveness of recurrent phenotypic selection and genomic selection in enhancing FHB resistance in durum wheat, providing a strong foundation for developing resistant cultivars adapted to the Northern Great Plains.

## Pyramiding the Fusarium Head Blight Resistance Genes Through Doubled Haploids to Accelerate Wheat Breeding

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Fusarium head blight (FHB) is a devastating wheat fungal disease that has caused significant grain yield and quality losses worldwide. Developing FHB resistant varieties is an effective, economical, and environmentally friendly approach to mitigate these losses. The FHB resistance is quantitatively inherited involving several genes which are further highly influenced by the environment, genetics backgrounds, susceptibility genes, and epistasis. Several FHB resistance genes, *Fhb1*, *Fhb6* and *Fhb7* with variable effects have been identified and utilized in breeding programs. To improve FHB resistance in hard winter wheat (HWW) region, a coordinated project for pyramiding these genes into adapted germplasm through doubled haploid and marker assisted selection was funded by US Wheat and Barley Scab Initiative. Since 2022, this project accomplished significant goals on standardizing protocols and improving success ratios on haploid embryos, chromosomal doubling with fertile wheat spikes. We rescued 2291 embryos from 25 crosses, with 707 haploid plants and 360 doubled haploid lines (DHLs) in 2023. In 2024, out of 2176 embryos 634 haploid plants and 351 DHLs were generated. In 2025, we received F<sub>1</sub> seed from four collaborators and planted for downstream DH development processing in October. Based on marker screening, we have identified 13 lines (TX22DH280, TX23DH209, TX23DH212, TX23DH217, TX23DH221, TX23DH227, TX23DH235, TX23DH236, TX23DH237, TX23DH249, TX23DH252, TX23DH92, TX23DH93) positive for *Fhb1* gene. We are increasing seeds, simultaneously field phenotyping for FHB, and continue assaying for FHB available markers to identify more lines with pyramided genes. The DHLs are available to all collaborators for further research and integration in the targeted recombination to accelerate FBH resistant wheat breeding process.

## A Genetic and FHB Phenotypic Analysis of Near-Isogenic Lines Carrying *Fhb1* in Different Genetic Backgrounds of Spring Bread Wheat and Durum Wheat

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Fusarium head blight (FHB) is a devastating disease affecting both bread and durum wheat, and use of host resistance is one of the major components in mitigating the impact of the disease. *Fhb1* is one of the most widely used resistance genes in wheat breeding programs, but its effectiveness in different genetic backgrounds of hosts is still not very well understood. In this study, we introduced *Fhb1* into six adapted spring wheat cultivars ('Linkert', WA8283, 'Glenn', 'ND VitPro', 'Surpass', and 'Dayn') and one durum cultivar (ND Riveland) by crossing with Alsen as the donor parent followed by backcrossing the progeny to the recipient parents. After seven cycles of backcrosses in combination with a greenhouse speed breeding protocol, which involved embryo rescue and marker-assisted selection, we developed six Near-Isogenic Lines (NILs) in spring common wheat backgrounds and one in durum wheat background at BC<sub>7</sub>F<sub>2</sub>. We genotyped these seven NILs plus four NILs (N-1, N-38, W-7, and W-9) carrying *Fhb1* from Sumai 3 in the genetic backgrounds of wheat cultivars Norm and Wheaton, respectively, along with their respective recurrent parents using the wheat 90K Infinium SNP array. The results indicated that the NILs with *Fhb1* shared 91% to 99% genetic background of their recurrent parents and contained a donor segment harboring the *Fhb1* locus on chromosome 3B. We evaluated the NILs along with the recurrent parents for FHB resistance under greenhouse and field conditions during the 2023-2024 seasons. The disease phenotyping data showed that the NILs carrying *Fhb1* generally had lower average FHB severity compared to their recurrent parents. In the greenhouse, three NILs carrying *Fhb1* (Linkert, W-7, and N-1) exhibited significantly lower disease severity than their parents, with reductions in disease severity values of about 11%, 20%, and 41%, respectively, compared to their recurrent parent. However, no significant differences in FHB severities between NILs and their respective parents were observed under field conditions. Collectively, these results suggest that *Fhb1* introgressed into elite wheat cultivars is not always effective, depending on the genetic background and environmental factors.

## Evaluating Within-Family Genomic Prediction of FHB Traits

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Fusarium Head Blight (FHB) remains a major barrier to wheat productivity in the Southern U.S., reducing yield and grain quality through contamination with deoxynivalenol (DON), a harmful mycotoxin. In the face of rising disease pressure and limited breeding resources, we explored how early-generation genomic prediction can be reimagined to improve efficiency, scalability, and resilience. A soft red winter wheat population of 450 genotypes (90 families × 5 siblings) was evaluated under artificial FHB inoculation at two Louisiana locations in 2023–2024. Traits assessed included FHB severity, Fusarium-damaged kernels (FDK), DON concentration, plant height, stripe rust, and heading date. Using GBLUP and a leave-one-family-out cross-validation strategy, we compared the predictive performance of individual genotyping, family means, and mid-parent marker imputation. Results revealed minimal accuracy loss when replacing individual genotyping with low-cost, family-based methods. GWAS identified key resistance loci including *Fhb1*, 1B (Jamestown), and 4A, confirming genetic targets for selection. Our results challenge the assumption that genotyping every plant is necessary at early stages, offering an adaptive, scalable approach for genomic selection in resource-limited programs. This work contributes to the design of faster, smarter, and more cost-effective breeding pipelines, enhancing FHB resistance while reducing fungicide dependence. In a climate of increasing disease risk and tightening budgets, this innovation supports more resilient agriculture for growers and breeding programs alike.

## Incorporation of *Thinopyrum elongatum*-derived FHB Resistance Gene *Fhb7<sup>The2</sup>* into 'Sumai 3', 'Frontana', and PI 277012

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Fusarium head blight (FHB) poses a significant threat to wheat production, severely affecting grain yield, grade, and end-use quality. Developing and utilizing host resistance remains the most effective and sustainable approach to manage the disease. However, the limited availability of resistance genes that can be effectively integrated into high-yielding wheat cultivars is one of the major hurdles. The primary objective of this study was to incorporate *Thinopyrum elongatum*-derived resistance gene *Fhb7<sup>The2</sup>* into major FHB resistance sources, including Sumai 3, Frontana, and PI 277012 for the development of germplasm with enhanced resistance. To achieve this, Sumai 3, Frontana, and PI 277012 were hybridized with the *Fhb7<sup>The2</sup>* source line WGC002 (PI 702949). The F<sub>1</sub> hybrids of these three crosses were backcrossed four times to their respective recipient parents (i.e. Sumai 3, Frontana, and PI 277012) through a marker-assisted backcrossing breeding pipeline. *Fhb7<sup>The2</sup>*-specific PACE markers were used to select the progeny containing *Fhb7<sup>The2</sup>* in each backcrossing generation. After four successive backcrossing generations, the near-isogenic introgressions harboring *Fhb7<sup>The2</sup>* were developed in each of the Sumai 3, Frontana, and PI 277012 backgrounds. They are designated Sumai 3-*Fhb7<sup>The2</sup>*, Frontana-*Fhb7<sup>The2</sup>*, and PI 277012-*Fhb7<sup>The2</sup>*, respectively. Their FHB severity was evaluated under controlled greenhouse conditions at 14- and 21-days post-inoculation (DPI), as well as in field trials at 21 days post-flowering (DPF) using corn spawn inoculation. Furthermore, we assessed Fusarium-damaged kernel (FDK) of the *Fhb7<sup>The2</sup>* introgressions and their recipient parents. Those analyses revealed that the *Fhb7<sup>The2</sup>* introgressions had enhanced resistance against FHB, exhibiting markedly reduced disease severity and lower FDK percentages than their respective recurrent parents. Frontana-*Fhb7<sup>The2</sup>* demonstrated a substantial reduction in FHB severity (16.89% vs. 47.84% at 21 DPI) and FDK (5.24% vs. 20.02%) relative to Frontana. Comparable trends were observed with Sumai 3-*Fhb7<sup>The2</sup>* in FHB severity (18.2% vs. 24.97% at 21 DPI) and FDK (2.36% vs. 11.17%), and with PI 277012-*Fhb7<sup>The2</sup>* in FHB severity (14.3% vs. 18.62% at 21 DPI) and FDK (4.77% vs. 7.23%). These improvements were statistically significant ( $p < 0.05$ ) and consistent across different greenhouse and field conditions. Apparently, *Fhb7<sup>The2</sup>* shows an additive effect in these three major FHB resistance source lines. In addition, *Fhb7<sup>The2</sup>* shows a monogenic inheritance pattern and has no obvious detrimental agronomic effects from the alien translocation. All these results highlight *Fhb7<sup>The2</sup>* as a robust and effective FHB resistance source in wheat breeding.



## Smart Phenotyping for Smarter Resistance Breeding: AI-Based FHB Assessment in Wheat

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Fusarium head blight (FHB), caused primarily by *Fusarium graminearum*, is a major fungal disease of wheat that reduces grain yield and quality and contaminates grain with mycotoxins. Accurate and scalable phenotyping of FHB severity and Fusarium-damaged kernels (FDK%) is critical for resistance breeding but remains constrained by subjective, labor-intensive visual scoring. In this study, two state-of-the-art deep learning models, YOLOv11 (You Only Look Once) and YOLOv12, were implemented using an oriented bounding box (OBB) annotated dataset to automate the detection and classification of healthy and infected spikelets and kernels from high-resolution RGB images. A large-scale benchmark dataset, the SD-FHBSD and SD-FDKD, was developed containing precisely annotated spikelet- and kernel-level images collected from a 2024 FHB screening nursery. YOLOv11 achieved superior detection and classification performance, with mAP@0.5 (mean average precision) values of 0.937 for spikelets and 0.932 for kernels, and the fastest inference time (9.5 – 14.2 ms per image), supporting real-time phenotyping applications. For FHB severity estimation, YOLOv11 achieved an  $R^2$  of 0.96 with the lowest RMSE (0.06) (Root Mean Square Error), while for FDK%, it achieved an  $R^2$  of 0.92 and an RMSE of 0.05, outperforming YOLOv12. The incorporation of brightness-based augmentation further improved robustness across various lighting conditions. The integration of OBBs enabled more precise localization of tilted and overlapping spikelets, enhancing detection accuracy compared with conventional axis-aligned boxes. This study introduces the first OBB-based FHB dataset and demonstrates an efficient, low-cost, and scalable framework for automated FHB disease severity and kernel damage estimation, paving the way for AI-driven, real-time phenotyping and precision disease monitoring in wheat breeding programs.

## Existing Haplotypes Associated with Fusarium Head Blight Resistance (FHB) in a Diverse Panel of Winter Wheat

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Fusarium head blight (FHB) is responsible for significant yield losses among the plant pathogenic diseases in wheat. In this study, we genotyped and evaluated a diversity panel of wheat cultivars representing most U.S. public breeding programs for field FHB disease severity metrics. The results show significant variations in the phenotypic FHB disease severity, including lines outperforming the moderately resistant check Everest (PI: 659807) and some underperforming compared to the susceptible check Overlay (PI: 634934). The correlation between traits and years was significant ( $r = 0.21\text{--}0.70$ ,  $p < 6.0 \times 10^{-04}$ ). Trait heritability ranged between 0.48–0.63. Using genomic variants called in the practical haplotype graph for genome-wide association studies, we identified SNPs within genomic intervals of haplotypes and alleles previously associated with FHB resistance. SNPs were mapped on all chromosomes except 4D and 7D. Previous associations with Jamestown, Bess, and NC-Neuse alleles were mapped, with the Jamestown haplotype exhibiting the strongest effect, lowering AUDPC and FDK by ~25% and mycotoxin DON level by 18%. Most associations with rare alleles (MAF < 0.05) were susceptible alleles, and the favorable alleles/haplotypes were common alleles.



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

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