Project FY22-MG-003: Fusarium Species Diversity within Spikes and Fields: Implications for FHB Management

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1. What are the major goals and objectives of the research project?

Goal: To survey the occurrence of minority *Fusarium* pathogens in FHB-symptomatic wheat and barley; understand environmental factors driving higher frequencies of minority species, including emerging mycotoxin producers; and determine how interactions between *F. graminearum* and weaker *Fusarium* pathogens impact FHB progression and mycotoxins.

The MGMT (Management) program goal is being addressed through Objective 1: Conduct a broad geographic survey of emerging/traditional *Fusarium* spp. and mycotoxin diversity and assess environmental factors (e.g., weather/climate, crop management) driving *Fusarium* diversity in FHB-symptomatic wheat and barley spikes.

The PBG (Plant Biology & Genetics) program goal is being addressed through Objective 2: Identify whether less aggressive *Fusarium* spp. reduce FHB caused by the aggressive pathogen *F. graminearum* if inoculated first or co-inoculated.

2. What was accomplished under these goals or objectives? *(For each major goal/objective, address these three items below.)*

What were the major activities?

1) Objective 1 – In 2023, we sampled 12 farms of spring barley and winter wheat from North Dakota (Fig. 1). We collected ~40-60 symptomatic heads per field and collected data where available on local agronomic practices for that field. 507 pure isolates were isolated from Nash-Snyder, single-spored, and stored as glycerol stocks in the Whitaker Lab.
    a. Sequence editing of the partial TEF-1α sequence and species identification is ongoing.
    b. The remainder of the heads were bulked by field and frozen. As with our 2022 sampling, the plan is to freeze-dry, hand-thresh, mill the developing seeds to a flour-like consistency, and then perform toxin analysis for Deoxynivalenol, Nivalenol, and Culmorin.
2) Objective 1 – In 2023, we also cultured fungal endophytes from paired FHB-symptomatic and asymptomatic heads from four North Dakota fields (n=30 reps/field). 320 fungi were isolated from Malt Extract Agar and identified by ITS-LSU sequencing.
3) Objective 1 – We completed sequence editing and genetic identification of the 2022 winter wheat *Fusarium* isolates collected from 19 farms in Illinois and improved the statistical analysis of the toxin concentration data.
4) Objective 2 – Completed hormone analyses and improved statistical analysis of the in-head competition assays between *F. graminearum* (15-ADON/3-ADON) and *F. poae* isolates in Alsen and Norm.

What were the significant results?

During the 2023 season, FHB risk was low in much of North Dakota during barley flowering. Despite this, we collected between 30-60 symptomatic heads per field, indicating an underlying presence of FHB in most fields. Total isolations per field ranged from 18-68 (Fig. 2). Among the spring barley fields, about an equal number of isolates came from heads with >1 or only 1 zone of infection (Fig. 2). Across the winter wheat fields, most isolates came from high disease heads with only a single visible point of infection (Fig. 2).

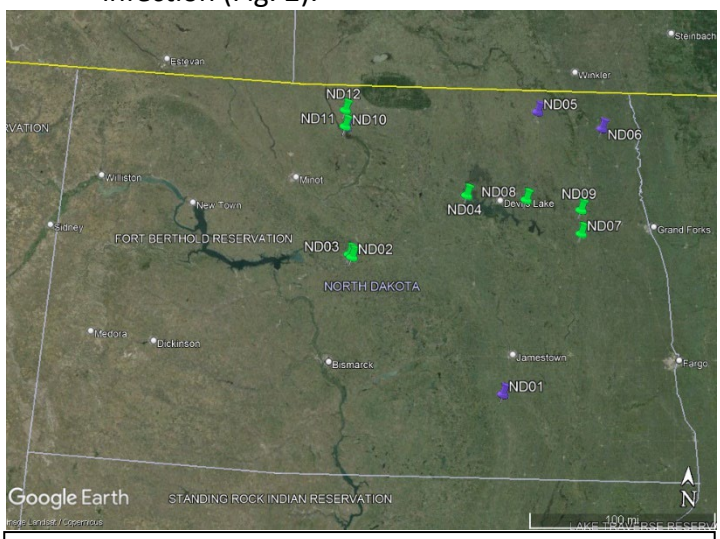

Fig. 1 – Map showing location of 12 North Dakota spring barley (green) and winter wheat (purple) fields sampled for FHB and Fusarium spp. genetic diversity.

The Nash-Snyder medium was not as effective at restricting fungal growth in 2023 to only *Fusarium* isolates (likely due to poor distribution of the PCNB ingredient). Thus, we screened many isolates for non-*Fusarium* status using the ITS-LSU and are still in the process of sequence editing.

From the 2023 field season, four spring barley farms were additionally sampled for fungal endophytes from paired FHB-symptomatic and asymptomatic heads. Sequencing and sequence-editing is close to completion, with approximately ~85% of the 320 isolates identified. The most abundant culturable fungal endophyte from barley heads was an *Alternaria* sp. However, there were noted differences in the fungal endophyte abundance between FHB-symptomatic and asymptomatic heads, that could potentially be exploited for biocontrol potential.

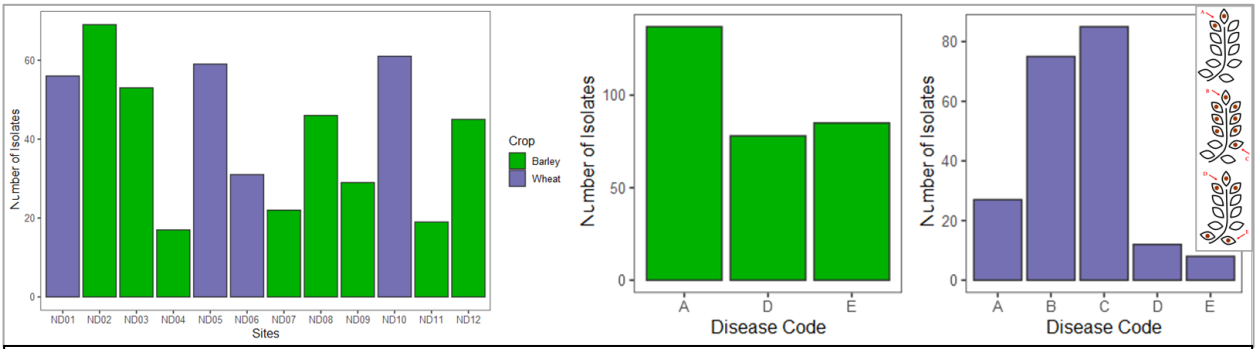

Fig. 2 – (left) Number of recovered Fusarium isolates per field. (center, right) Number of recovered isolates corresponding to disease severity of the sampled head and location of the diseased spikelet along head (center = barley, right = wheat). See inset for disease code to letter conversion.

In addition, we completed sequence editing and genetic identification from the 1,121 *Fusarium* strains isolated in 2022. More than 95% of the strains matched to *F.*

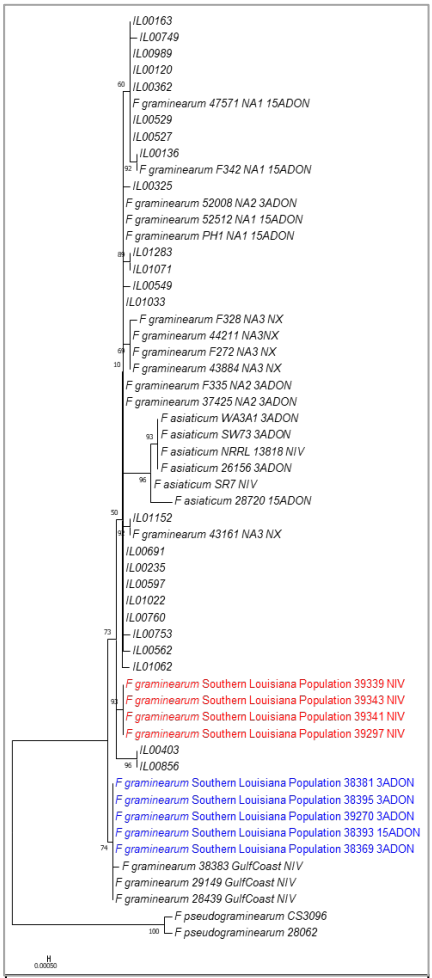

Fig. 3 – Maximum likelihood tree, representing zoomed in section with Illinois 2022 isolates (coded: ILXXXX) and reference strains from the F. graminearum Southern LA populations (red, blue).

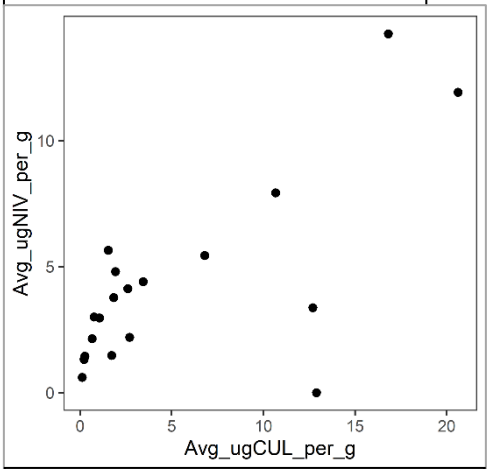

Fig. 4 – Correlation between Nivalenol (NIV) and Culmorin (CUL) across 19 Illinois wheat farms in 2022.

graminearum, with <5% belonging to 'minority' Fusarium species complex groups (i.e., FTSC, FSC, FIESC). Given the dominance of NIV and CUL contamination in the symptomatic wheat heads in that field season, it is therefore highly likely that the NIV and CUL contamination was produced by a population(s) of *F. graminearum* able to produce both toxins. Experiments are being planned to fully support this claim using chemotyping of a subset of strains with GCMS. Phylogenetic comparisons of the partial TEF1α sequences from the *F. graminearum* from Illinois with those from the southern Louisiana NIV-producing population (Gale et al 2011 doi: 10.1094/PHYTO-03-10-0067) indicate some degree of genetic divergence (Fig. 3).

From the toxin data collected in 2022, detection of DON was relatively low (0-2ppm), while abundance of NIV and CUL was very high (0-14ppm and 0.5-20ppm, respectively). To determine the strongest predictors of DON, NIV, and CUL concentrations in the symptomatic wheat heads, we performed a best subsets regression analysis. One of the dominant results from this analysis revealed that NIV and CUL concentrations were highly correlated (Fig. 4; $R^2 = 0.49$), with higher NIV concentrations in western longitudes and higher CUL concentrations at low average FHB severity within a field.

For the PBG portion of the grant, we made significant improvements to the analysis of the experiments where an *F. poae* strain (Fp) was either pre- or co-inoculated with either a 3-ADON *F. graminearum* strain (Fg3) or a 15-ADON *F. graminearum* strain (Fg15) in two host varieties (Alsen and Norm, spring hard red wheat). A paired set of experiments were performed. In the first, wheat heads were allowed to mature, and disease symptoms were measured; while in a second experiment, heads were collected every day for 4 days-post-infection and used for hormone and gene expression measurements. From the disease experiment, **the impact on DON mycotoxin accumulation of competing *F. poae* and *F. graminearum* strains was cultivar and strain dependent** (Fig. 5). Specifically, DON accumulation was greater in Alsen hosts when the Fp strain was either pre- or co-inoculated with the Fg15 strain, relative to infection by Fg15 alone (Fig. 5). There was also a non-significant trend toward increased DON accumulation when Fp was pre- or co-inoculated with the Fg3 strain. However, in Norm hosts the opposite pattern from the *F.*

graminearum-strains was found. In other words, DON accumulation was greater in Norm hosts when the Fp strain was either pre- or co-inoculated with the Fg3 strain, but no significant effect was detected for the Fg15 strain (Fig. 5). *F. graminearum* biomass was determined from a high-throughput qPCR assay and largely correlated with the DON results. Our analyses of DON accumulation and *F. graminearum* biomass showed consistent trends within cultivars but did not correlate well with disease severity (data not shown) – indicating mycotoxin accumulation may not correspond synonymously with visible symptoms. Lastly, we were able to fix the GC/MS used for phytohormone analysis and complete the sample processing for the second paired experiment. Analyses are ongoing in comparison to the disease experiment.

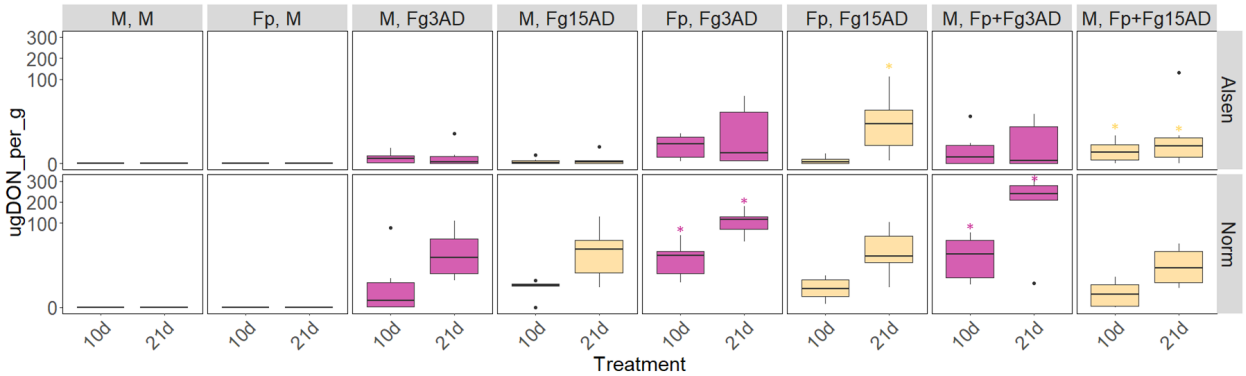

Fig. 5 – DON toxin accumulation across two wheat varieties and eight Fusarium competition treatments. M = mock, Fp = F. poae, Fg3AD = F. graminearum 3ADON, Fg15AD = F. graminearum 15ADON. Alsen (top panels) and Norm (bottom panels) varieties. Treatments where Fp was inoculated two days prior to an Fg strain are separated by a comma; where they were simultaneously inoculated a plus symbol is used.

Lastly, an additional $18,081.30 was used for a one-time equipment purchase of a cryogenic grinding mill to aid in sample preparation for sensitive materials, including RNA gene expression profiling.

List key outcomes or other achievements.

1) Nivalenol risk may be of rising importance in Illinois wheat, or the midwestern US more broadly. Results from 2024 (a higher incidence scab year in Illinois) are expected to confirm or refute the 2022 findings.
2) *F. graminearum* was the primary causal agent behind Nivalenol and Culmorin contamination in Illinois wheat (2022).
3) Fusarium Tricinctum Species Complex (FTSC) species, including *F. avenaceum* and *F. acuminatum*, are known producers of emerging mycotoxins. However, FTSC contamination was minimal in IL Wheat in 2022
4) Simultaneous dual infections of *Fusarium* strains are common in field grown wheat and barley.
5) The impact on DON mycotoxin accumulation of competing *F. poae* and *F. graminearum* strains is cultivar and strain dependent.
6) Where *Fusarium* species compete *in planta*, mycotoxin accumulation may not correspond synonymously with visible symptoms of disease.

Key outcomes: 1) We identified greater than expected concentrations of Nivalenol in FHB-symptomatic wheat, which has not been previously detected in Illinois wheat. Our results indicate that Nivalenol contamination of wheat may be of rising importance in the central US and highlights the risk for Nivalenol contamination during epidemic years or long-term grain storage. 2) In addition, our *Fusarium* competition study indicates that predicting mycotoxin risk from competing *Fusarium* strain infections *in planta* is complicated by strain and cultivar-dependent effects.

3. **What opportunities for training and professional development has the project provided?**
This project has already provided training and professional development to 5 individuals at various career stages. Specifically, Imane Laraba served as a co-investigator while an ORISE funded postdoctoral scientist. She received training in mentorship of a graduate student and intern, as well as developed skillsets in plant hormone analysis. Dr. Laraba also participated in an outreach activity, by providing expertise in wheat pathology to local farmers. Pete Oppenheimer is a PhD student in the Cowger lab at NCSU, who received training in field sampling, fungal isolation, inoculation projects, and is developing methods in high-throughput *Fusarium* detection. Pete presented his research at the Scab 2023 conference in lightning talk and poster formats. This project also partially funded two post-degree interns Karly Cazzato and Nate Tyler, who received training in field sampling, fungal isolation, and molecular detection. Karly used the skillsets learned to pursue a career in statistical analysis, while Nate aims to pursue a PhD in agro-ecology. Lastly, Odalis Curzio was an undergraduate student intern from NEIU (a primarily minority serving institution), who assisted and received training in the laboratory isolations for this project.

4. **How have the results been disseminated to communities of interest?**
These results were presented at the USWBSI 2023 forum (Cincinnati, OH) as an invited talk for MGMT by Briana Whitaker, as a poster by ORISE intern Nate Tyler, and as a poster and lightning talk by Pete Oppenheimer.
Briana Whitaker presented the project goals and previous results from the 2022 Illinois Wheat sampling to the Illinois Wheat Association Annual Southern Plot Tours in Belleville, IL in May 2024 and to the Crop Management Conference in Collinsville, IL in Feb 2024.

5. **What do you plan to do during the next reporting period to accomplish the goals and objectives?**
To continue Objective 1, we are doing a repeated sampling of wheat farms in southern Illinois in the year 2024 (technically completed outside the reporting period for Year 2). Most of the wheat in Illinois is grown in the southern portion of the state, but there is still wide variation maturation date. With the spring showers in early to mid-May this year, the co-incidence of rain and flowering was highly variable across the state. We expected this sampling to provide a fuller picture of the risk of Nivalenol contamination during higher scab risk years. In addition, we are planning to pair our previous sampling scheme (sampling

40-60 diseased heads from across the field) with a test of grain samples from elevators with the help of the Illinois Department of Agriculture (which the MPM unit has a previous collaboration with). The combination of these data should provide a strong description of toxin risk across geographic regions, and the genetic sources of infection.

To continue Objective 2, we will finalize the analyses of the hormonal data from the paired set of experiments evaluating competing *F. poae* and *F. graminearum* strain infection in wheat. We are currently in the process of designing qPCR primers that can successfully distinguish strains within the F. sambucinum species complex (including *F. poae* and *F. graminearum*). We believe additional information on *F. poae* biomass will greatly inform the differential toxin and disease severity patterns between the Alsen and Norm wheat varieties, as well as between the 15-ADON and 3-ADON producing strains of *F. graminearum*. If the hormonal data also reveals informative patterns of Jasmonic and Salicylic acid accumulation, we will also repeat the exact experiments for a second replication and move to publish the data as is. Alternatively, we are also considering examining the competition between Nivalenol-producing *F. graminearum* strains *in planta* derived from the field work in Objective 1.