FY23 USDA-ARS/USWBSI Project Summary of Progress & Requested Changes

Principal Investigator:	Briana Whitaker
PI Institution:	USDA-ARS
Project Title:	*Fusarium* Species Diversity within Spikes and Fields: Implications for FHB Management
Project ID:	FY22-PB-004/ FY22-MG-003

As the Principal Investigator on this request, I confirm that all provided information, including the budget documentation, is accurate. My required signature is included here as validation.

Principal Investigator Signature (required):	BRIANA WHITAKER Digitally signed by BRIANA WHITAKER Date: 2022.09.20 10:08:18 -05'00'
Date Signed:	

Summary of Progress

Include a brief summary of the overall progress achieved to date (since receipt of FY22 funding, May-September 2022) on this project by responding to the following items.

Report on progress relative to approved goals, objectives, and approach along with key accomplishments since receipt of FY22 funding.

The major objectives of the FY22 research program were to 1) conduct a broad geographic survey of *Fusarium* and mycotoxin diversity in FHB symptomatic wheat and barley and 2) identify whether less aggressive pathogens affect FHB and DON outcomes caused by the aggressive *F. graminearum* if inoculated first or co-inoculated.

To address the 1st objective of our project, in the spring and summer of 2022 we sampled 30 farms across the US in total, or specifically: 19 winter wheat fields in Illinois, 7 winter wheat fields in Virginia, as well as 3 winter wheat and 1 winter barley field in Maryland. To date >1200 *Fusarium* strains have been isolated from infected spikelets using Nash-Snyder selective medium and are in the process of being archived as glycerol stocks, as well as identified by sequencing the TEF-1α gene and chemotyped using the high-throughput Luminex system. In addition, we used an *in-silico* approach to identify a set of primers targeting a TEF-1α gene region that can be used to identify *Fusarium* spp. diversity using a next-generation sequencing-based metabarcoding approach. We intend to use these primers on wheat and barley heads collected in the field to validate our culturing effort, as well as reveal additional minority *Fusarium* spp.

Lastly, we were also able to assess Fusarium crown rot diversity from 4 farms in Illinois, and isolated 80 *Fusarium* species and 40 non-*Fusarium* isolates from symptomatic wheat crowns and heads. The *Fusarium* isolates were primarily *F. graminearum* (40%) and *F. acuminatum* (26%), with several other minority species from the *F. sambucinum*, *tricinctum*, and *fujikuroi* species complexes detected. Toxin analysis is ongoing to reveal whether toxins were translocated from the crown into the harvested grains.

To address the 2nd goal of our project, we have performed one field and three greenhouse inoculation experiments and are in progress on a fourth greenhouse experiment. In a paired greenhouse and field inoculation experiment, the winter wheat variety Shirley, which is high yielding but susceptible to FHB, was inoculated with *F. graminearum* to determine FHB outcomes and DON accumulation. Critically, we tested whether pre- or co-inoculation with the less aggressive strains *F. poae* or *F. acuminatum* would reduce *F. graminearum* disease severity or DON production. Environment was a major determinant of the disease outcome. Specifically, no pre-inoculation or co-inoculation treatment of *F. graminearum* with a less aggressive strain reduced AUDPC or DON accumulation in the greenhouse (Fig. 1,2). However, in the field, disease severity and DON were reduced 24.2% and 19.0%, respectively, when *F. acuminatum* was co-inoculated with *F. graminearum* relative to inoculation with *F. graminearum* alone. Disease severity also reduced 27.7% when *F.*

acuminatum was pre-inoculated. Lastly, results from the field trial showed that disease incidence was significantly reduced when *F. poae* and *F. acuminatum* were co-inoculated with *F. graminearum*, as well as when *F. acuminatum* was pre-inoculated (Fig. 1,2).

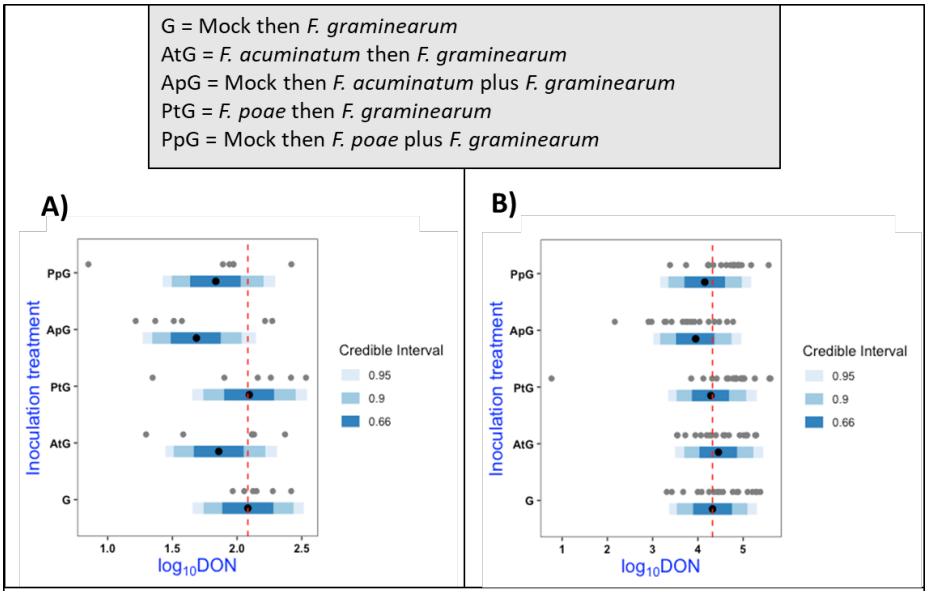
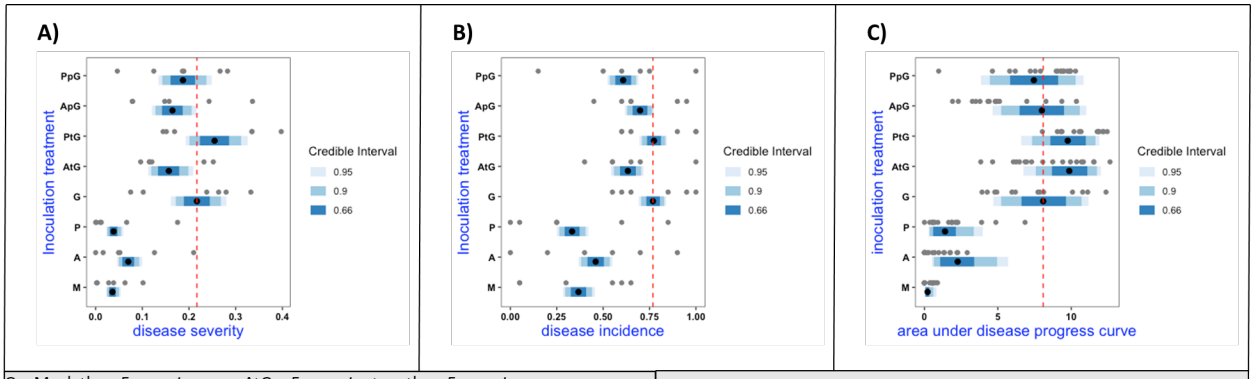


Fig. 1. Predicted and observed deoxynivalenol (DON) concentrations in wheat spikes inoculated with *Fusarium graminearum* conidia together with a pre- or co-inoculation of the weaker *Fusarium* species *F. poae* or *F. acuminatum* in **A)** a field trial and **B)** a greenhouse trial. Gray dots = observed $\log_{10}$ DON, black dots = predicted mean $\log_{10}$ DON, blue-shaded bars = predicted credible interval for $\log_{10}$ DON, dashed red line = predicted mean $\log_{10}$DON for the G treatment. DON data were $\log_{10}$-transformed to achieve a normal distribution.



G = Mock then *F. graminearum* AtG = *F. acuminatum* then *F. graminearum*
A = Mock then *F. acuminatum* ApG = Mock then *F. acuminatum* plus *F. graminearum*
P = Mock then *F. poae* PtG = *F. poae* then *F. graminearum*
M = Mock then Mock PpG = Mock then *F. poae* plus *F. graminearum*

Fig. 2. Predicted and observed FHB symptoms **A,B)** field and **C)** greenhouse trials. **A)** disease severity and **B)** disease incidence (DI) 23dpi. For **C)** disease severity as the area under disease progress curve (AUDPC) Gray dots = observed per-plot means (A and B) or per-spike means (C). Black dots = predicted mean values; blue-shaded bars = predicted credible intervals; and dashed red line shows disease metric for the G treatment.

Next, in a second set of greenhouse experiments, we focused on two spring wheat varieties, Alsen and Norm, which are moderately resistant and moderately susceptible to FHB, respectively. In these experiments, we tested whether pre- or co-inoculation with the less aggressive *F. poae* would reduce *F. graminearum* head blight spread or DON production in the two host varieties varying in disease resistance. In this experiment, we also tested the effect of *F. poae* on two separate North American populations/chemotypes of *F. graminearum* (i.e., NA1/15-ADON and NA2/3-ADON). Analyses are ongoing, but preliminary results from the first round of this experiment indicate that *F. poae* did not significantly reduce disease and accumulated DON levels from the two *F. graminearum* strains in the moderately susceptible Norm.

Lastly, we designed novel *F. poae*-specific primers and are working to design *F. acuminatum*-specific primers for quantitative PCR (qPCR), which will allow us to identify whether disease reduction *in planta* is the result of the less aggressive isolates outcompeting *F. graminearum* for space or a host immune system priming effect.

Describe any difficulties/problems encountered and actions to overcome in achieving planned objectives.
We were not able to sample as many barley fields in year 1 of our Objective 1, due to a general lack of barley production in the state of Illinois and surrounding areas as well as a reduced number of connections with farmers and extension agents. However, one barley field was sampled in Maryland and connections with a malting barley extension agents in Wisconsin and the Upper Peninsula of Michigan were established. It is expected that we will be able to target a greater number of barley fields in year 2 of funding with the new stakeholder and extension connections made in year 1.

For the inoculation experiments of Objective 2, traditional frequentist statistics were inadequate to analyze the results of the paired greenhouse and field experiment using the winter wheat variety Shirley. A Bayesian statistical approach is being used instead and analyses are ongoing. Additionally, we hypothesize that results from the co- and pre-inoculation trials were limited in the greenhouse because the *F. graminearum* inoculum concentration was too high (10^6 spores/ml). Overall, conditions were likely more FHB-conducive in the greenhouse than in the field. We plan to test lower concentrations of inoculum to understand whether the treatments respond to different magnitudes of disease pressure.

Lastly, we are still in the process of troubleshooting the *F. acuminatum* qPCR primers. We initially targeted housekeeping or enniatins/aurofusarin toxin genes, however the primers designed to be specific for *F. acuminatum* also amplified other members of the *F. tricinctum* species complex. Further *in silico* analyses have revealed a genomic insertion unique to *F. acuminatum*, which we are now targeting for the design of qPCR primers.

Requested Changes/Project Plan for FY23

Include a brief request and rationale for any changes needed in FY23 to the project plan. If no changes necessary, simply indicate that is the case. Any requested changes noted here should also be reflected in your Project Description file.

Provide a rationale for any requested changes in approach and strategies for the FY23 project plan compared to what was outlined in the FY22 approved and funded award.

No changes to the Project Plan are necessary.

Include a projected FY23 timeline for project implementation.

Tentative Timeline	**Objective 1**	**Objective 2**
Fall 2022	Generate single-spore isolates & extract DNA	qPCR & Toxin on Growth Chamber and Field Tests (Wheat Rep 1); Growth Chamber Tests (Wheat Rep 2)
Winter 2022-23	Identify isolates; qPCR, Toxin, & Metabarcoding on spikes, NCSU grad student travels to Peoria for training	qPCR & Toxin on Growth Chamber Test Wheat (Rep 2); Start Field Test (Wheat Rep 2)
Spring 2023	Sample >40 fields; Metabarcoding data analyzed	Field Test (Wheat Rep 2); Growth Chamber Tests (Barley Rep 1 or Wheat Rep 2 repeated as necessary)
Summer/ Fall 2023	Generate 2,400 single-spore isolates & extract DNA	Extract DNA, qPCR, & Toxin on Field Samples; Growth Chamber Tests (Barley Rep 1)