USDA-ARS | U.S. Wheat and Barley Scab Initiative

FY22 Performance Progress Report

Due date: July 26, 2023

Cover Page

USDA-ARS Agreement ID:	N/A
USDA-ARS Agreement Title:	Field and spike diversity of Fusarium species
Principle Investigator (PI):	Briana Whitaker
Institution:	USDA-Agricultural Research Service
Institution UEI:	N/A
Fiscal Year:	2022
FY22 USDA-ARS Award Amount:	$57,570
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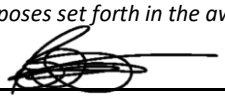
Period of Performance:	May 1, 2022 - April 30, 2023
Reporting Period End Date:	April 30, 2023

USWBSI Individual Project(s)

USWBSI Research Category*	Project Title	ARS Award Amount
MGMT	Fusarium Species Diversity within Spikes and Fields: Implications for FHB Management	$28,311
PBG	Fusarium Species Diversity within Spikes and Fields: Implications for FHB Management	$29,259
	FY22 Total ARS Award Amount	$57,570

I am submitting this report as an: ☒ Annual Report

I certify to the best of my knowledge and belief that this report is correct and complete for performance of activities for the purposes set forth in the award documents.



Principal Investigator Signature

07/20/23

Date Report Submitted

ǂ BAR-CP – Barley Coordinated Project
DUR-CP – Durum Coordinated Project
EC-HQ – Executive Committee-Headquarters
FST-R – Food Safety & Toxicology (Research)
FST-S – Food Safety & Toxicology (Service)
GDER – Gene Discovery & Engineering Resistance
HWW-CP – Hard Winter Wheat Coordinated Project
MGMT – FHB Management
MGMT-IM – FHB Management – Integrated Management Coordinated Project
PBG – Pathogen Biology & Genetics
TSCI – Transformational Science
VDHR – Variety Development & Uniform Nurseries
NWW –Northern Soft Winter Wheat Region
SPR – Spring Wheat Region
SWW – Southern Soft Red Winter Wheat Region

Project 1: 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 Management program goal is being addressed through the specific objective:
Objective 1 (MGMT Goal): 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.

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

a) What were the major activities?

Objective 1 – In the summer of 2022 we sampled 19 farms of winter wheat, primarily in southern Illinois where most wheat in Illinois is planted (Fig. 1). We collected ~60 symptomatic heads per field and collected data where available on local agronomic practices for that field.

Head samples were brought back to the USDA in Peoria and divided into one of three symptomology categories: 1) low disease with 1 visible point of infection, 2) high diseases with 1 visible point of infection, and 3) low disease with 2 distinct points of infection. Based on the symptomology category, we cultured 1-2 symptomatic spikelets per head on Fusarium-selective media (Nash-Snyder), then subcultured until pure isolates were obtained. 1,190 pure isolates were obtained, all of which were stored as spores in glycerol stocks after single sporing in a -80C freezer in the Whitaker Lab at the USDA-Peoria. The same single-spore derived culture used for archiving was extracted for DNA and genetically identified by TEF sequencing. Sequence editing and species identification is ongoing.

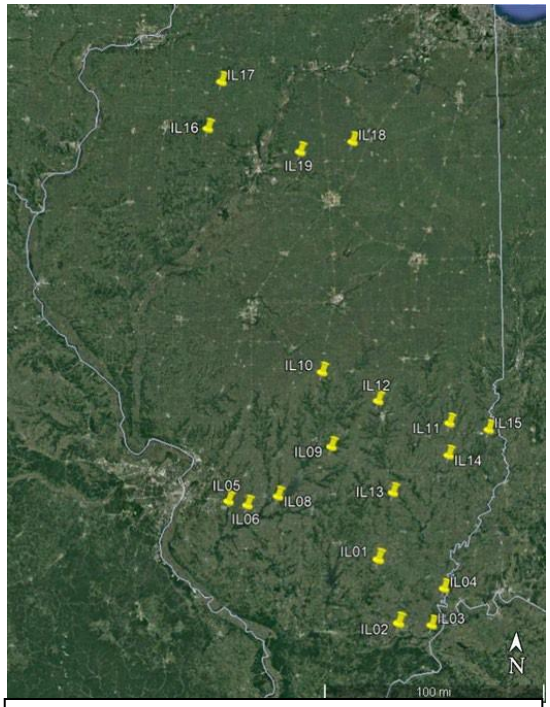

Fig. 1 – Map showing location of 19 Illinois winter wheat fields sampled for FHB and Fusarium spp. genetic diversity.

After using the 1-2 spikelets from each of the ~60 wheat heads for Fusarium culturing, the remainder of the wheat heads were bulked by field, freeze-dried, hand-threshed, and

the developing seeds milled to a powder. Toxin analysis for Deoxynivalenol, Nivalenol, and Culmorin was completed in triplicate per field from the milled developing seeds. Additional toxin analysis using an LCMS is in preparation. Genetic identification of the cultured isolates will be used to inform a *Fusarium* relative biomass assay using high-throughput qPCR.

In addition to the FHB sampling, we also sampled four fields for Fusarium crown rot (FCR) pathogens. FCR infections can sometimes result in translocation of mycotoxins into the grain head. Around 13 wheat plants were collected per field. From each plant, five crown sections and spikelets were plated on a Potato Dextrose Agar. The recovered isolates (~110) were single-spored and identified by TEF sequencing. GC-MS and LC-MS analyses were conducted to determine whether trichothecenes, or emerging toxins, were translocated from the crown to the head.

b) What were the significant results?

During the 2022 field season, FHB risk was low to moderate in much of southern Illinois during and just after wheat flowering; there was slightly higher risk for susceptible varieties or for farms where fungicidal disease management was limited. Despite this, collecting our target of ~60 symptomatic heads per field was relatively straightforward, indicating an underlying presence of FHB in most fields. Fusarium isolations per field ranged from 10-82 (Fig. 2). Most of the successfully isolated strains came from heads with low disease (<20% infected spikelets) and only a single visible point of infection (Fig. 2).

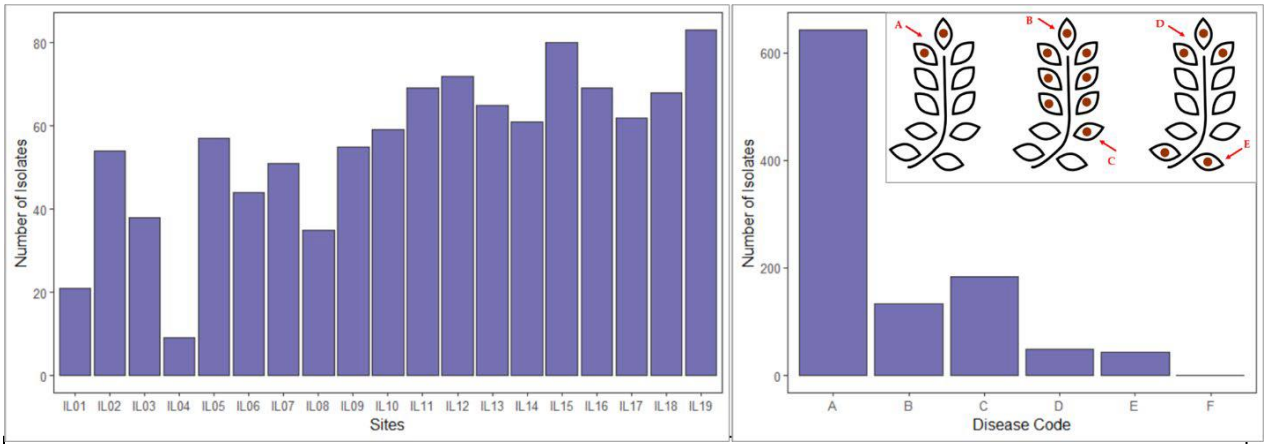

Fig. 2 – (left) Number of recovered Fusarium isolates per field. (right) Number of recovered isolates corresponding to disease severity of the sampled head and location of the diseased spikelet along head. See inset for disease code to letter conversion.

From a preliminary screening of the TEF sequences, most of the isolates mapped to the Fusarium sambucinum species complex (FSAMSC; ~96% of isolates), which includes *Fusarium graminearum*, *F. poae*, and *F. culmorum*, *F. crookwellense*, among others. The remaining 4% of isolates included members of the Fusarium tricinctum species complex, Fusarium incarnatum-equisetii species complex, and the Fusarium fujikuroi species complex. In addition, we also isolated and sequenced two strains of *Microdochium nivale* from symptomatic winter wheat.

Our mycotoxin analysis of the bulked and threshed wheat heads is still ongoing, but preliminary results indicate a greater than expected amount of Nivalenol (NIV) and Culmorin (CUL) present in scabby wheat heads from southern Illinois. In particular, we found up to 14 ug of NIV per gram of plant material and up to 20 ug of CUL per gram of plant material in some fields (Fig. 3). By contrast, we only found up to 2 ug of DON per gram of plant material across all 19 fields. The presence of CUL was greatest in the southern latitudes (Fig 3) and there was a strong correlation between the presence of NIV and CUL (R^2 = 0.49; Fig. 3), with smaller correlations between DON and CUL (R^2 = 0.30; Fig. 3) or between DON and NIV (R^2 = 0.08).

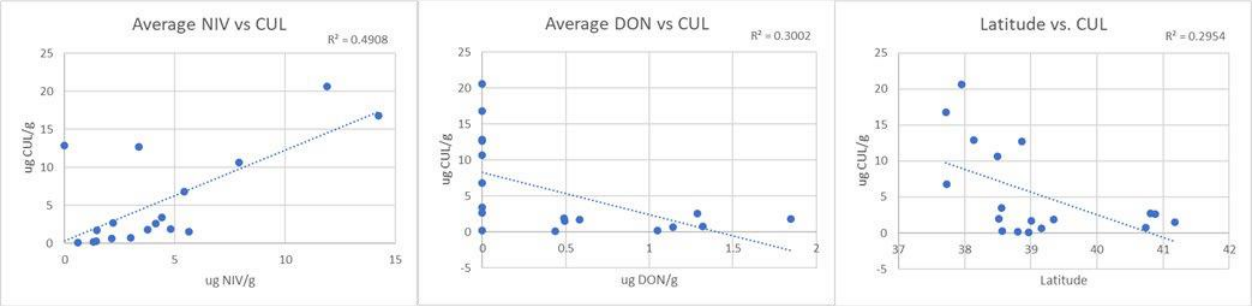

Fig. 3 – Mycotoxin results from bulked scabby heads in Illinois farms. Shown are correlations between (left) average NIV and CUL, (center) average DON and CUL, (right) average CUL and latitude of the farm.

For the FCR survey, we identified a prevalence of FCR in southern Illinois. Of the FCR symptomatic plants we isolated around 70 *Fusarium* and 40 non-*Fusarium* isolates. The most predominant *Fusarium* spp. were *F. graminearum* (44%) and *F. armeniacum* (22%) (Fig. 4). Several other species from the F. sambucinum, tricinctum, incarnatum-equiseti, and fujikuroi species complexes were also detected in low frequencies (Fig. 4). However, toxin analyses did not detect any translocated mycotoxins in the wheat heads.

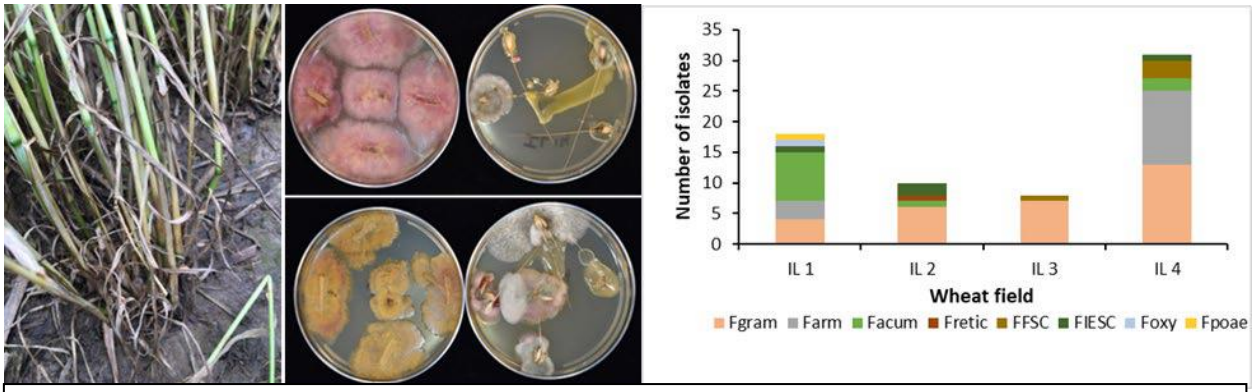

Fig.4: (left) Stand of FCR symptomatic wheat and symptomatic crown or spike tissues plated on potato dextrose medium. (right) Number of recovered Fusarium isolates per each of four fields.

c) List key outcomes or other achievements.

1) 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

2) Simultaneous dual infections of competing Fusarium strains are common in field grown wheat.
3) Nivalenol risk may be of rising importance in Illinois wheat, or the midwestern US more broadly.
4) Translocation of mycotoxins from FCR symptomatic wheat crowns into wheat grain was not identified on the Illinois wheat farms sampled.

Key outcome: 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.

3. What opportunities for training and professional development has the project provided?
This project has already provided training and professional development to 4 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 2022 conference in lightning talk and poster formats. This project also funded a temporary 6-month technician position for Karly Cazzato, who received training in field sampling, fungal isolation, and molecular detection. Karly used the skillsets learned to pursue a career in statistical analysis. 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 in part at the USWBSI forum as a poster and lightning talk by Pete Oppenheimer in 2022. These results will be presented at the USWBSI forum as an invited talk by Briana Whitaker for the MGMT section in 2023. The project goals and preliminary research were presented to the Illinois Wheat Association Annual Southern Plot Tours in May 2022 by Briana Whitaker, with assistance from Imane Laraba.

Project 2: 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 Plant Biology & Genetics program goal is being addressed through the specific objective:
   Objective 2 (PBG Goal): 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.)*

   a) **What were the major activities?**
   Objective 2 – We conducted a set of paired experiments under greenhouse conditions to assess whether pre- or co-inoculation with the less aggressive pathogen *F. poae* would affect the disease caused by the more aggressive pathogen *F. graminearum*. We used one strain of *F. poae*, but tested two strains of *F. gramineraum*, one each from the North American populations NA1 and NA2 known to produce 15ADON and 3ADON, respectively. Both greenhouse experiments were conducted in two spring wheat cultivars, Alsen and Norm, which are moderately resistant and susceptible to FHB, respectively.

   In the first experiment, we tested how *F. poae* affected *F. graminearum* disease progression, deoxynivalenol accumulation, and fungal biomass. In the second experiment, we tested whether host immune responses are altered by *F. poae* and *F. graminearum* interactions.

Table 1: Experimental treatments	
1st Inoculation	**2nd Inoculation**
Mock	Mock
Mock	NRRL 38746 NA2 *F. graminearum*
Mock	NRRL 37525 NA1 *F. graminearum*
F. poae	Mock
F. poae	NRRL 38746 NA2 *F. graminearum*
F. poae	NRRL 37525 NA1 *F. graminearum*
Mock	*F. poae*+NRRL 38746 NA2 *F. graminearum*
Mock	*F. poae*+NRRL 37525 NA1 *F. graminearum*

Experiment 1 – At anthesis, 24 heads per treatment were point inoculated by injecting 10 µL mock solution (0.04% Tween 20) or 10^5 conidia mL^{-1} into a single floret (i.e., the "1st Inoculation", see Table 1). After 48 h, the spikes were again point-inoculated using

either a mock or 10^5 mL^{-1} conidial suspension (i.e., the "2nd Inoculation", Table 1). Symptomatic spikelets were counted every three days post inoculation (dpi) until 21 dpi. At 10 and 21 dpi, 12 heads were harvested and bulked in duplicate, resulting in six experimental replicates per time point. Samples were ground to a powder and subsampled for DNA and toxin extractions. We quantified several mycotoxins (DON, Culmorin, and Nivalenol) using a GC-MS. DNA extractions were prepared for high-throughput qPCR to determine *F. poae* and *F. graminearum* biomass using the Biomark HD Fluidigm system.

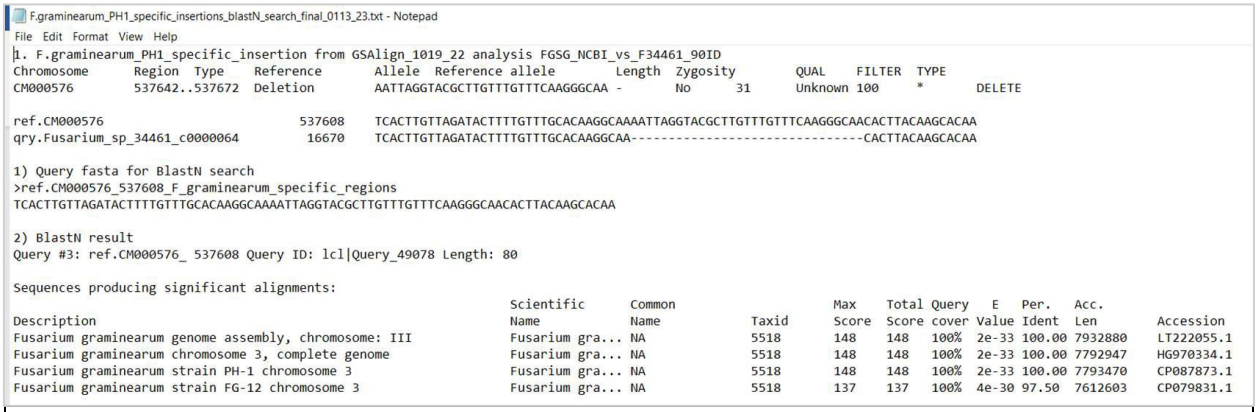
Experiment 2 – For this experiment, we used dip inoculations instead of point inoculations to avoid inducing a wound-induced plant immune response. At anthesis, 24 heads per treatment were dip inoculated in a mock 0.04% Tween 20 solution or 10^4 conidia mL-1 suspension of either *F. graminearum* or *F. poae* isolates (i.e., the "1st Inoculation", see Table 1). After 48 h, the spikes were dip-inoculated using either a mock or 10^4 mL-1 conidial suspension (i.e., the "2nd Inoculation", Table 1). Six heads per treatment were collected at 1, 2, 3, and 4 dpi. For each treatment, heads were bulked in duplicate, resulting in three biological replicates per treatment at each time point. Samples were ground and divided into two aliquots for transcriptomic and metabolomic analyses. Specifically, we are working to quantify plant defense phytochromes, salicylic acid (SA) and jasmonic acid (JA), at the transcriptomic and metabolomic levels. The transcription pattern of genes involved in SA and JA signaling pathways (LOX1, LOX2, PAL, ICS, and NPR1) and CCOMT, a regulator of lignin synthesis, were assessed using the high-throughput Biomark HD Fluidigm system. SA and JA were extracted from the ground plant tissues, and their quantification is ongoing using a GC-MS.

b) What were the significant results?

Both greenhouse projects were completed during the FY22 reporting period. For the first experiment, we collected data on visible disease progression, and collected harvested grain for quantifying toxin and fungal biomass. Samples collected from the first experiment have been processed for toxin analysis, as well as for fungal biomass determination of *F. graminearum* and *F. poae* – which required the development and testing of novel qPCR primers (see below for more details). For the second experiment, we collected harvested grain for quantifying plant phytohormone transcripts and metabolites. Samples were processed for RNA transcript analysis, including stabilization by making complementary DNA.

Unfortunately, we experienced a few setbacks that have prevented a full analysis of the data collected so far. First, some data cleaning will be required to process the disease progression data from the first experiment, due to small error in excel organization. Second, the GC-MS that we would use for phytohormone metabolite quantification was broken for several months. Therefore, analyses from the wheat FHB progression and phytohormone experiments are ongoing but expected to be completed within the next couple of months.

For the design of the species-specific primer-probe regions, we used a comparative genomic approach. Genomes of the species of interest were aligned with genomes of closely related species. Regions of high overlap within, but not between, species were identified using the program GSAlign. For example, we identified a 31 bp insertion in the genome of *F. graminearum* PH1 that was not present in its close relative (i.e., *Fusarium* sp. NRRL 34461; Fig. 5). Using this approach, we were able to design 22 unique primer pairs, including for *F. graminearum, F. poae, F. avenaceum, F. acuminatum, F. culmorum,* and *F. cerealis*.


F.graminearum_PH1_specific_insertions_blastN_search_final_0113_23.txt - Notepad

File Edit Format View Help

1. F.graminearum_PH1_specific_insertion from GSAlign_1019_22 analysis FGSG_NCBI_vs_F34461_90ID

Chromosome	Region	Type	Reference	Allele	Reference allele	Length	Zygosity	QUAL	FILTER	TYPE
CM000576	537642..537672	Deletion	AATTAGGTACGCTTGTTTGTTTCAAGGGCAA	-	No	31	Unknown	100	*	DELETE

```
ref.CM000576                        537608    TCACTTGTTAGATACTTTTGTTTGCACAAGGCAAAATTAGGTACGCTTGTTTGTTTCAAGGGCAACACTTACAAGCACAA
qry.Fusarium_sp_34461_c0000064       16670    TCACTTGTTAGATACTTTTGTTTGCACAAGGCAA------------------------------CACTTACAAGCACAA
```

1) Query fasta for BlastN search

```
>ref.CM000576_537608_F_graminearum_specific_regions
TCACTTGTTAGATACTTTTGTTTGCACAAGGCAAAATTAGGTACGCTTGTTTGTTTCAAGGGCAACACTTACAAGCACAA
```

2) BlastN result

```
Query #3: ref.CM000576_ 537608 Query ID: lcl|Query_49078 Length: 80
```

Sequences producing significant alignments:

Description	Scientific Name	Common Name	Taxid	Max Score	Total Score	Query cover	E Value	Per. Ident	Acc. Len	Accession
Fusarium graminearum genome assembly, chromosome: III	Fusarium gra...	NA	5518	148	148	100%	2e-33	100.00	7932880	LT222055.1
Fusarium graminearum chromosome 3, complete genome	Fusarium gra...	NA	5518	148	148	100%	2e-33	100.00	7792947	HG970334.1
Fusarium graminearum strain PH-1 chromosome 3	Fusarium gra...	NA	5518	148	148	100%	2e-33	100.00	7793470	CP087873.1
Fusarium graminearum strain FG-12 chromosome 3	Fusarium gra...	NA	5518	137	137	100%	4e-30	97.50	7612603	CP079831.1

Fig.5: An example illustrating the process of identifying species-specific regions to be targeted for primer design in F. graminearum using the tool GSAlign.

c) List key outcomes or other achievements.

1) Using whole genome sequence data and cutting-edge bioinformatic tools we designed species-specific primers and probes for simultaneous detection and quantification of multiple *Fusarium* spp., including *F. poae*, *F. acuminatum*, and *F. graminearum*.
2) These primer-probe combinations will be able to quantify the fungal biomass of several known FHB-causing pathogens in parallel from up to 192 field or greenhouse samples simultaneously using high-throughput qPCR via the Fluidigm system.

Key Outcome: The expected outcome of this project will be to show how infection by multiple, competing *Fusarium* spp. impacts disease progression, mycotoxin accumulation, and plant immune response.

3. What opportunities for training and professional development has the project provided?

This project has already provided training and professional development to 4 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 2022 conference in lightning talk and poster formats. This project also funded a temporary 6-

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Publications, Conference Papers, and Presentations

Please include a listing of all your publications/presentations about your FHB work that were a result of funding from your FY22 grant award. Only citations for publications published (submitted or accepted) or presentations presented during the **award period** should be included.

Did you publish/submit or present anything during this award period May 1, 2022 – April 30, 2023?

X Yes, I've included the citation reference in listing(s) below.

☐ No, I have nothing to report.

Journal publications as a result of FY22 award

List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Include any peer-reviewed publication in the periodically published proceedings of a scientific society, a conference, or the like.

Identify for each publication: Author(s); title; journal; volume: year; page numbers; status of publication (published [include DOI#]; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).

Books or other non-periodical, one-time publications as a result of FY22 award

Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like.

Identify for each one-time publication: Author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (book, thesis, or dissertation, other); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).

Other publications, conference papers and presentations as a result of FY22 award

Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication.

Peter Oppenheimer, Quentin Read, Briana Whitaker, Imane Laraba, Susan McCormick, Mark Busman, and Christina Cowger. (2022). Biocontrol of FHB: Beyond the greenhouse. Proceedings of the 2022 National Fusarium Head Blight Forum; Tampa, FL. December 4-6, 2022. Retrieved from: https://scabusa.org/forum/2022/2022NFHBForumProceedings.pdf

"Impact of genetic and chemotypic diversity of Fusarium spp. on winter wheat in Illinois". Briana Whitaker and Imane Laraba. Illinois Wheat Association's Annual Plot Tours. 2022.