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

FY22 Performance Progress Report

Due date: July 26, 2023

Cover Page

USDA-ARS Agreement ID:	59-0206-2-162
USDA-ARS Agreement Title:	Mapping Genes Underlying Fusarium Head Blight Variation to Target
Principle Investigator (PI):	Christopher Toomajian
Institution:	Kansas State University
Institution UEI:	CFMMM5JM7HJ9
Fiscal Year:	2022
FY22 USDA-ARS Award Amount:	$57,143
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Period of Performance:	May 1, 2022 – April 30, 2024
Reporting Period End Date:	April 30, 2023

USWBSI Individual Project(s)

USWBSI Research Category*	Project Title	ARS Award Amount
PBG	Genetic Mapping of Genes Underlying Variation in Fusarium Head Blight Traits	$57,143
	FY22 Total ARS Award Amount	$57,143

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

July 24, 2023
Date Report Submitted

‡ 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: Genetic Mapping of Genes Underlying Variation in Fusarium Head Blight Traits

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1. **What are the major goals and objectives of the research project?**
   - **a)** In laboratory experiments, measure DON levels, fungicide sensitivity, ascospore discharge, and mycelial growth for 150 *Fg* isolates
   - **b)** Phenotype 150 *Fg* isolates for aggressiveness and DON production via greenhouse head inoculations on susceptible and resistant wheat
   - **c)** Perform genome-wide association to identify SNPs associated with variation in above traits

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?

   Before this period, all mycelial growth rate studies and perithecia formation/ascospore discharge experiments had been completed. Additionally, 1 season of greenhouse wheat head inoculations to measure aggressiveness and DON contamination had been performed. In this period, we completed experiments for *in vitro* measurements of DON levels as well as experiments to test sensitivity to two DMI fungicides, propiconazole and tebuconazole (**Obj 1**). We also phenotyped a 2nd replicate of nearly 150 isolates for aggressiveness and DON production via greenhouse head inoculations on a susceptible and a moderately resistant wheat variety (**Obj 2**). Some GWAS analyses has been performed for all of the traits measured (except DON levels, since we only have 1 replicate of *in planta* data so far and we are still waiting for the results of our USWBSI-supported DON tests for our *in vitro* DON experiments, **Obj 3**). Additionally during this period, we have analyzed four chromosome-level assemblies of *F. graminearum* isolates produced from a previous period of USWBSI funding. These analyses included genome comparisons to identify structural variants as well as transposable elements and other repetitive elements in each genome.

   b) What were the significant results?

   For our fungicide sensitivity experiments, we found broad distributions of EC_{50} values in our sample of NA1 isolates as well as a relatively strong correlation between the EC_{50} values for the 2 DMI fungicides for isolates in our sample (**Figure 1**). That said, EC_{50} values for propiconazole were on average 2x the values for tebuconazole (**Obj 1**). We had best luck in identifying significantly associated SNPs by using models that considered SNP effects as random to perform initial tests of association before subsequent SNP effect estimation and significance testing (e.g., **Figure 2**, **Obj 3**). The total number of significant SNPs varied by method used, but up to 30 significant SNPs were found for propiconazole sensitivity and up to 22 significant SNPs were found for tebuconazole sensitivity. Of the significant SNPs identified for each fungicide, the overlap was moderate, with many distinct loci found in GWAS for each fungicide. For comparison, up to 5 significant SNPs were found for aggressiveness on wheat (only 2 seasons of data), up to 3 significant SNPs were found for *in vitro* growth rate, and up to 6 significant SNPs were found for ascospore production. Another greenhouse season of

head inoculations is required to improve our results for GWAS of pathogen aggressiveness and *in planta* DON levels.

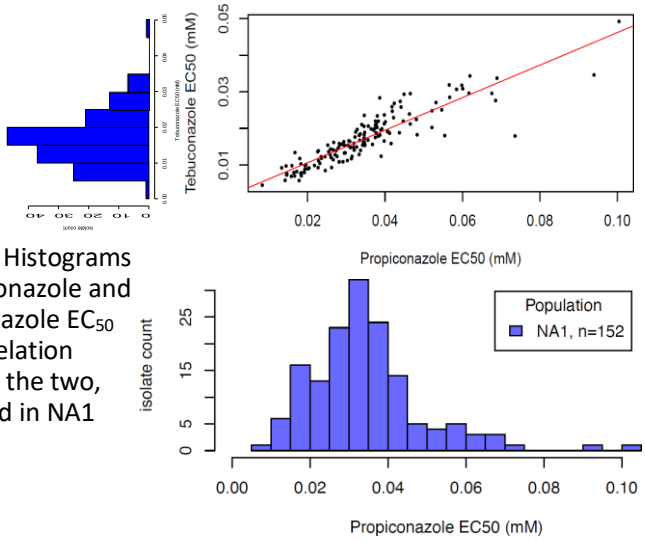
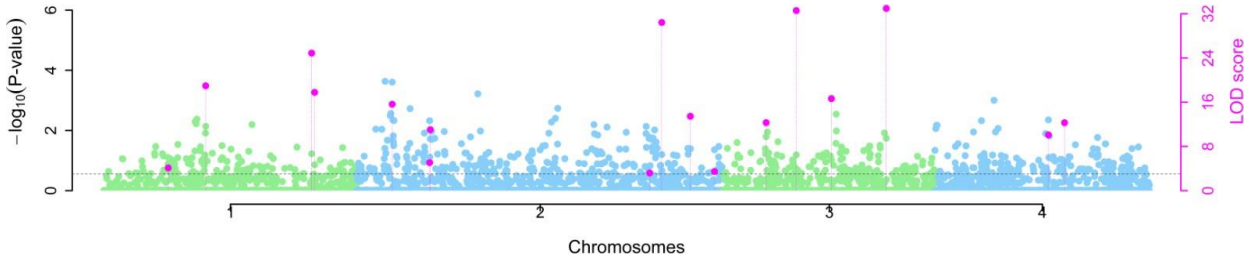


Figure 1. Histograms of tebuconazole and propiconazole EC_{50} and correlation between the two, measured in NA1 isolates



- **Figure 2**. GWAS Manhattan plot for tebuconazole EC50 using multi-locus models that first uses SNPs as random effects. Green and blue dots represent initial SNP scores (left axis p-values), while magenta dots represent multi-locus model LOD scores for SNPs passing an initial significance threshold.

Though not one of our planned objectives from this project, during this funding period we completed the error correction steps for the chromosome-level genome assemblies from four *F. graminearum* isolates. We then compared these assemblies to the PH-1 reference and 3 other publicly available chromosome-level assemblies in order to detect chromosomal rearrangements segregating within this species (**Figure 3**). Summing across all of the pairwise comparisons of seven query isolates against the PH-1 reference, we detected 87 inversions (some shared between isolates), hundreds of translocations and duplications, and thousands of insertions/deletions. Chromosomal rearrangements were overrepresented in regions of high recombination, and a significant proportion of rearrangements also overlap the repeat content of these genomes.

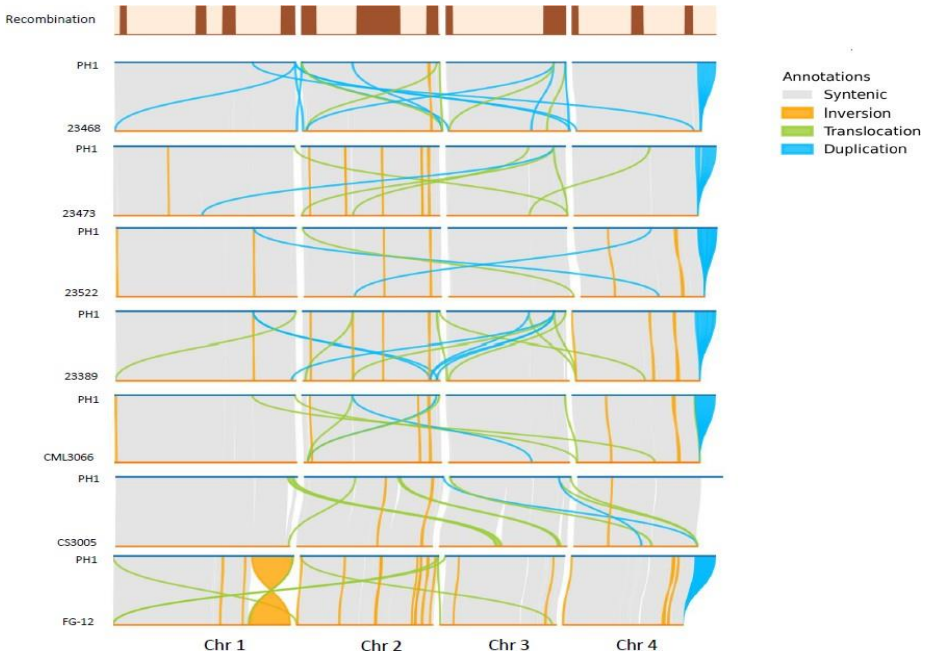
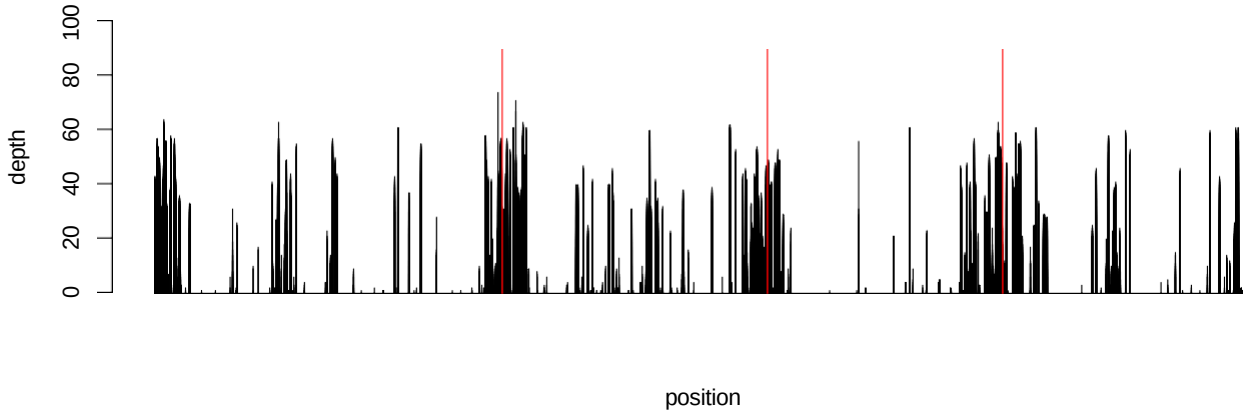


Figure 3. Chromosomal rearrangements in *Fusarium graminearum* genomes, plotted in comparisons with PH-1

c) List key outcomes or other achievements.

We have tested different GWAS analysis methods, and have found the ones that appear to work best in our samples. Our earliest tests with samples composed of isolates from different populations indicated that the strong population structure in mixed samples could lead to poor results, even when using methods that have been adapted to account for population structure. In our experiments with the greatest level of replication and environmental control, such as our tests of fungicide sensitivity, we are obtaining dozens of significant SNPs to pursue for their involvement in natural trait variation. We have also found this assay to be very efficient, so that we plan to continue to use it with at least one more fungicide, even if this work is not completed until after the second year of funding. We have also completed chromosome-level genome assemblies of four *F. graminearum* isolates and found an important association between chromosomal rearrangements and both repetitive elements and high recombination regions. This result lays important groundwork for understanding the drivers of genome variation and evolution in an important plant pathogen. The high-quality genome assemblies also can serve as important reference genomes for *F. graminearum* isolates from different populations, especially the NA2 and NA3 populations (the canonical PH-1 reference genome is from the NA1 population). When discovering variants or genotyping samples by mapping sequence reads to a reference genome, the choice of a poorly-matching reference genome can mean a substantial portion of reads not mapping, and therefore missing important variation. As a demonstration, we mapped orphan contigs from a seminal *F. graminearum* population genomics study (Kelly & Ward 2018) that represented sequence not matching to the PH-1 reference, and we find the places in our reference genomes (usually in highly variable sub-telomeric regions) where most of these contigs correspond (**Figure 4**).



- **Figure 4**. Depth and position of orphan contigs from Kelly and Ward (2018) mapped against new reference *Fusarium graminearum* genome from isolate 23389. Red lines mark chromosomal boundaries.

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

The project has provided training for my PhD student Upasana Dhakal (including presenting Scab Forum poster and Flash and Dash). She has been trained on Fusarium lab culturing and experiments, greenhouse inoculations, data analysis and a range of bioinformatics and genomics analyses . Graduate student training continues with training for a new PhD student, Sandhya Gopisetty, who started on the project in Summer 2023. The project has provided research training for undergraduate hourly worker Emily Gipson on greenhouse inoculations and grain sample preparation for DON testing. It also provided training in fungicide sensitivity assays for 1st-year undergraduate student Jennie Tucker. Tucker participated in a course where she was matched with PI Toomajian as a research mentor, to perform at least 12 hours of laboratory research and present a poster at an end of semester undergraduate research symposium. PI Toomajian used data from this project in teaching his graduate Population Genetics class to 11 students in the Spring of 2023. The project also helped to provide professional development and training to 20 participants in the 2022 Fusarium Laboratory Workshop.

4. How have the results been disseminated to communities of interest?

The results of this project have so far been disseminated through presentations at the annual Scab Forum (December 2022) and will be submitted for publication in peer review journals as the final analysis and write-up is completed. PI Toomajian also presented some results in June 2022 through his lecture on population genetics for the Fusarium Laboratory Workshop at Kansas State University. PI Toomajian also provided an update and overview of his project during the USWBSI GDER and PBG Joint Mid-year Meeting (Virtual) on April 27, 2023. PI Toomajian participated in Kansas State University's annual open house for the purpose of outreach to enhance public understanding of plant pathology and increase interest in careers in this field.

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.

Dhakal, U., Leslie, J. F., Toomajian, C., (2022). Genetic basis of variation in DMI fungicide sensitivity in U.S. Fusarium graminearum isolates. Proceedings of the 2022 National Fusarium Head Blight Forum; Tampa, FL. December 4-6, 2022. Retrieved from: https://scabusa.org/forum/2022/2022NFHBForumProceedings.pdf
Status: abstract published and poster presented; Acknowledgement of federal support: yes