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

FY22 Performance Progress Report

Due date: July 26, 2023

Cover Page

USDA-ARS Agreement ID:	59-0206-2-160
USDA-ARS Agreement Title:	Genetics of Aggressiveness in Fusarium graminearum
Principle Investigator (PI):	Lisa J. Vaillancourt
Institution:	University of Kentucky
Institution UEI:	H1HYA8Z1NTM5
Fiscal Year:	2022
FY22 USDA-ARS Award Amount:	$40,937
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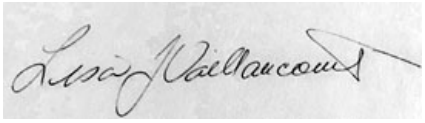
Period of Performance:	May 1, 2022 – April 30, 2026
Reporting Period End Date:	April 30, 2023

USWBSI Individual Project(s)

USWBSI Research Category*	Project Title	ARS Award Amount
PBG	Role of chemotype in aggressiveness and toxigenicity of Fusarium graminearum to wheat	$40,937
	FY22 Total ARS Award Amount	$40,937

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.



July 25, 2023

Principal Investigator Signature | 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: Role of chemotype in aggressiveness and toxigenicity of Fusarium graminearum to wheat

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1. **What are the major goals and objectives of the research project?**
   The goal of our project is to test the hypothesis that the *F. graminearum* 3ADON chemotype confers higher levels of aggressiveness, toxigenicity, and competitiveness than the 15ADON chemotype, regardless of genetic background. The objectives are: **1)** Determine whether trichothecene toxin chemotype, mating type locus, and other genetic makers exhibit Mendelian segregation among progeny from crosses of strains from different *F. graminearum* populations; **2)** Determine whether individuals and mixtures of progeny from outcrosses differ in aggressiveness, toxigenicity, and competitiveness in susceptible and moderately resistant wheat in the presence and absence of fungicides; and **3)** Identify DNA markers associated with aggressiveness and high toxin production by analyzing whole genome sequence data from pools of progeny that differ in these traits.
2. **What was accomplished under these goals or objectives?** *(For each major goal/objective, address these three items below.)*
   For **Objective 1,** our goal is to test the hypothesis that crosses with Δ*mat1-1-1* tester strains will produce expected Mendelian segregation patterns of markers across all four chromosomes. The mapping populations we will generate as part of this objective will be stored as a permanent collection, and the strains and relevant data (sequence reads, genome assemblies, SNP comparison lists, and marker segregation data) will be published and made available to the community on request.
   - **a) What were the major activities?**
     1. We received a collection of 37 North American *F. graminearum* strains from wheat, representing four different chemotypes (15ADON, 3ADON, NIV, NX2)**.** These strains have been stored as a permanent collection on silica gel and in glycerol in our freezers.
     2. The chemotype of each of the strains was confirmed by PCR, and each strain was phenotyped for growth and sporulation *in vitro*, sexual fertility, aggressiveness to the susceptible spring wheat variety Wheaton, and toxigenicity (still in progress) to aid selection of the most suitable parents for our crosses.
   - **b) What were the significant results?**
     1. The strains varied markedly in the traits analyzed. So far, **we have selected the crossing parents for this objective based on their growth in vitro and their aggressiveness to Wheaton**. Strains that grew , produced few conidia, exhibited low levels of fertility in crosses with the tester, or had very low levels of aggressiveness were removed from consideration as parents in the crosses.
   - **c) List key outcomes or other achievements.**
     1. Four strains (3ADON strains NRRL46434 and KY410; NIV strain NC016; and NX2 strain NRRL66040) were chosen as the most suitable parents for crosses.
     2. The four strains have been crossed with the 15ADON mating type gene deletion tester strain. Progeny are currently being collected and genotyped by PCR**.**

3. The 3ADON crosses are being prioritized because the progeny will be used to inoculate wheat in the greenhouse this fall and winter**. We are on track with this objective.**

We haven't begun work on **Objective 2**, but the student has mastered the necessary technique for wheat inoculations.

For **Objective 3**, our goal is to conduct genome-wide SNP (single nucleotide polymorphism) analysis of bulked progeny pools from crosses among different chemotypes. The aim is to identify new genetic markers associated with aggressiveness, competitiveness, and toxigenicity. Genome sequence assemblies for all but one of the selected parental strains have been generated in the Proctor laboratory at the USDA-ARS in Peoria. We are currently generating the sequence data for the last strain (i.e., the strain with the NIV chemotype). For genome sequencing, strains were first grown on a growth medium amended with antibiotics to remove any potential bacterial contamination. Genomic DNA was isolated from liquid cultures using the Qiagen DNeasy Plant Mini Kit. Resulting DNA was then subjected to a 16S PCR screen to confirm the absence of bacterial DNA, and then used to prepare libraries for sequencing with an Illumina MiSeq instrument. Resulting sequence reads were processed with the computer program CLC Genomics Workbench. Adapter and low-quality sequences were removed from sequence reads, and then reads were screened against 73 bacterial genome sequences to remove low levels of bacterial sequence reads resulting from contaminated reagents and/or equipment. The unmapped sequences were then used to generate an assembled genome sequence using CLC Genomics Workbench. The student has received training in Linux and R programming in preparation for analysis of the data.

3. **What opportunities for training and professional development has the project provided?**
The previous M.S. student who I had in mind for this project decided in the end to accept a fellowship at the USDA mycotoxin lab in Peoria. He successfully defended his M.S. in July of 2022, and he has since been hired in a permanent position at the USDA lab in Beltsville. To replace him, we hired a new M.S. student on the project, who joined us in January 2023. She has a strong background in agronomy and classical plant breeding (wheat and maize), so this project gives her a chance to add plant pathology and fungal genetics to her repertoire, as well as to learn and apply molecular genetic and genomic protocols. She has quickly mastered all the relevant assays, and she is also learning R and Linux programming so she can work with her genome data in the future (Objective 3). When she completes her M.S. she will have a valuable combination of knowledge and skills relevant to crop improvement from both the host and the pathogen side.
4. **How have the results been disseminated to communities of interest?**
Since our project is only in its first year, we haven't published results yet. The student has shared results during our regular weekly lab meetings and also with our collaborators during informal research discussions. We also presented the goals of the project and some of the preliminary data at the NC1183 mycotoxins committee annual meeting in May 2023. We plan for her to present a poster at the upcoming USWBSI forum in Cincinnati.

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?

☒ 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.

We presented the project goals and some of the preliminary data at the NC1183 multistate meeting in Ames Iowa in May 2023 (virtual presentation).