USDA-ARS
U.S. Wheat and Barley Scab Initiative
FY19 Final Performance Progress Report
Due date: November 30, 2021

Cover Page

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Fiscal Year:	2019
USDA-ARS Agreement ID:	58-5062-8-016
USDA-ARS Agreement Title:	Role of Mating-type Genes in Pathogenicity of Fusarium graminearum to Wheat
FY20 USDA-ARS Award Amount:	$ 43,143
Recipient Organization:	University of Kentucky Research Foundation University Station Lexington, KY 40506-0057
DUNS Number:	939017877
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Project/Grant Reporting Period:	9/1/19 – 9/30/21
Reporting Period End Date:	9/30/2021

USWBSI Individual Project(s)

USWBSI Research Category*	Project Title	ARS Award Amount
PBG	The Role of Mating-type Genes in Pathogenicity of Fusarium graminearum to Wheat	$ 43,143
	FY19 Total ARS Award Amount	**$ 43,143**

Lisa Vaillancourt

11/30/2021

Principal Investigator Date

* MGMT – FHB Management
FST – Food Safety & Toxicology
R – Research
S – Service (DON Testing Lab)
GDER – Gene Discovery & Engineering Resistance
PBG – Pathogen Biology & Genetics
EC-HQ – Executive Committee-Headquarters
BAR-CP – Barley Coordinated Project
DUR-CP – Durum Coordinated Project
HWW-CP – Hard Winter Wheat Coordinated Project
VDHR – Variety Development & Uniform Nurseries – Sub categories are below:
SPR – Spring Wheat Region
NWW – Northern Soft Winter Wheat Region
SWW – Southern Soft Red Winter Wheat Region

Project 1: *The Role of Mating-type Genes in Pathogenicity of Fusarium graminearum to Wheat*

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

The major goal of this project was to understand the basis for a previously observed reduction in aggressiveness to wheat of strains that were deleted for the MAT1-1-1 or the MAT1-2-1 mating type genes. The objectives were as follows:

1) Produce complementation strains for the gene knockouts (KOs) and confirm the function of the genes in aggressiveness to wheat heads.
2) Conduct a cytological analysis of KO transformants expressing fluorescent proteins in inoculated wheat heads, to characterize the reduced aggressiveness of the gene KOs in detail.
3) Carry out a comparative Illumina RNA-seq analysis of the wild type (WT) and KO transcriptomes in wheat heads, to reveal genes that are altered by activity of the heterodimeric mating specificity proteins versus by the non-dimerized forms.

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?

- Five independent MAT1-1-1 and MAT1-2-1 KO strains were inoculated onto hard red spring wheat varieties Wheaton and Alsen, to confirm the reduced pathogenicity that was observed previously with two strains of each on the winter wheat variety Pioneer 2555. The five strains were also tested on stalks and ears of Golden Jubilee maize and Fast Flowering Mini Maize (FFMM), respectively. Co-segregation analyses of the MAT1-1-1 and MAT1-2-1 KOs with reduced pathogenicity and other phenotypes was conducted.
- Infection and colonization of wheat (Wheaton) and maize (FFMM) by a fluorescent MAT1-1-1 KO strain was studied by epifluorescence microscopy.
- A comparative Illumina seq experiment was conducted to compare MAT1-1-1 and MAT1-2-1 KO strains with the MAT1 whole locus KO and WT strains in Wheaton wheat heads.

b) What were the significant results?

- Analysis of additional MAT1-1-1 and MAT1-2-1 KO strains in highly replicated experiments on Wheaton and Alsen showed that the gene deletions were not consistently associated with reduced aggressiveness: four of the five MAT1-2-1 KO strains were reduced in aggressiveness to both wheat varieties; although three of the five MAT1-1-1 KO strains were less aggressive on average than the wild type (WT) PH-1 strain, the differences were not statistically significant. None of the KO

strains differed significantly from the WT on maize stalks or ears. We chose to use co-segregation analyses rather than complementation, to avoid another round of transformation, in order to check whether the MAT1-2-1 KO is associated with reduced aggressiveness: the crosses have been made, random progeny have been isolated and marker segregation confirmed, and the pathogenicity experiments are ongoing. We assume that the MAT KOs will not co-segregate with reduced aggressiveness, since some of the KO strains are not different from the WT.

- Observation of a MAT1-1-1 KO strain expressing GFP in wheat heads and maize ears showed in each case that the fungus colonized glume tissues and moved readily, especially between cells, in the rachis. In wheat heads, we observed what appeared to be phloem cell death in the rachis of inoculated plants in areas that were not always colonized by fungus. This may be related to DON mycotoxicity. We did not see major differences between the deletion mutant and the WT fungus. Unfortunately our confocal microscope failed during these experiments, and we were limited to epifluorescence which limited the quality of our images. We have just obtained a new confocal microscope, so we hope to extend this work in the future.
- Sequencing of our transcriptome samples has been completed and we have received all the data. Our analysis of the data is ongoing. Several hundred genes are differentially expressed in the MAT1-1-1 vs MAT1-2-1 KOs, and between each of these knockouts and the WT or whole MAT1 locus KO strains. We are currently evaluating the identities and functions of these genes.

c) List key outcomes or other achievements.

- We have rejected the hypothesis that the deletion of MAT1-1-1 or MAT1-2-1 results in reduced aggressiveness to wheat. Reduced aggressiveness of some transformants may be related to off-site mutations that are introduced during transformation. We had not realized before how frequently mutations occur during transformation.
- Since the KOs do not cause decreased aggressiveness, we have been able to select two MAT1-1-1 KO strains with WT levels of aggressiveness and fitness to use as "test maters" in crosses with various WT strains of F. graminearum. These test maters mate produce fertile perithecia only in heterothallic crosses, and this facilitates differentiation of crossed perithecia from the majority of selfed perithecia. We intend to use these test maters to conduct genetic analyses of traits associated with disease including aggressiveness and toxigenicity. This will give us another important tool, in addition to forward genetics, to dissect pathogenicity and identify potential targets for disease management.

3. Was this research impacted by the COVID-19 pandemic (i.e. university shutdowns and/or restrictions, reduced or lack of support personnel, etc.)? If yes, please explain how this research was impacted or is continuing to be impacted.

Our research laboratories and greenhouses at the University of Kentucky were shut down in March of 2020, and remained closed, or operated at a reduced capacity, through January of 2021. Everyone was asked to work from home, including students, faculty, and support staff. This significantly impacted our research progress. We lost one set of wheat plants that were growing in the greenhouse. The student on the project was prevented from conducting bench research initially, and then made only slow progress once restrictions were partially lifted and he was allowed to come for a few hours each day. The laboratory was divided into shifts to comply with university requirements for social distancing. The transcriptome experiments, in particular, were significantly delayed, and that's why we are only now beginning to evaluate the data. The student spent his time working from home on learning "Rstats" and analyzing datasets and images that he had from before the shutdown. However, the inability to progress in his bench work during that time delayed him significantly.

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

Two graduate students have been trained as a result of this project. The first was a visiting scholar on a "Sandwich" study from Brazil for one year. I was a member of her advisory committee, and she defended her PhD in the summer of 2020. The second student is a Master's Degree candidate in our department. This student comes from an underprivileged background and belongs to an underrepresented minority (Afro-Latino). He has gained a wide array of experiences and skills from the project including fungal genetics and genomics, R programming, and wheat cultivation and inoculation. This student will defend his thesis next spring.

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

Results have been presented as posters or as oral reports at the USWBSI Annual Meetings (2019, 2020) and the Annual Meeting of the American Phytopathological Society (2019) as well as at the NC1183 Committee on Mycotoxins (2019, 2020, 2021). Some results were published in a refereed journal article (see 'Publications'.

Training of Next Generation Scientists

Instructions: Please answer the following questions as it pertains to the FY20 award period (9/1/19 - 9/30/21). The term "support" below includes any level of benefit to the student, ranging from full stipend plus tuition to the situation where the student's stipend was paid from other funds, but who learned how to rate scab in a misted nursery paid for by the USWBSI, and anything in between.

1. **Did any graduate students in your research program supported by funding from your USWBSI grant earn their MS degree during the FY19 award period?**
   ☐Yes ☒No ☐ Not Applicable
   If yes, how many? There is one student who will defend in Spring 2022

2. **Did any graduate students in your research program supported by funding from your USWBSI grant earn their Ph.D. degree during the FY19 award period?**
   ☒Yes ☐No ☐ Not Applicable
   If yes, how many? One

3. **Have any post docs who worked for you during the FY19 award period and were supported by funding from your USWBSI grant taken faculty positions with universities?**
   ☐Yes ☐No ☒ Not Applicable
   If yes, how many? Click to enter number here.

4. **Have any post docs who worked for you during the FY19 award period and were supported by funding from your USWBSI grant gone on to take positions with private ag-related companies or federal agencies?**
   ☐Yes ☐No ☒ Not Applicable
   If yes, how many? Click to enter number here.

Release of Germplasm/Cultivars

Instructions: In the table below, list all germplasm and/or cultivars released with full or partial support through the USWBSI during the **FY19 award period (9/1/19 - 9/30/21)**. All columns must be completed for each listed germplasm/cultivar. Use the key below the table for Grain Class abbreviations.

NOTE: Leave blank if you have nothing to report or if your grant did NOT include any VDHR-related projects.

Name of Germplasm/Cultivar	Grain Class	FHB Resistance	FHB Rating (0-9)	Year Released
N/A	Select Grain Class	Select what represents your most resistant check	Enter as text 0-9 rating	Select Year
Click here to enter text.	Select Grain Class	Select what represents your most resistant check	Enter as text 0-9 rating	Select Year
Click here to enter text.	Select Grain Class	Select what represents your most resistant check	Enter as text 0-9 rating	Select Year
Click here to enter text.	Select Grain Class	Select what represents your most resistant check	Enter as text 0-9 rating	Select Year
Click here to enter text.	Select Grain Class	Select what represents your most resistant check	Enter as text 0-9 rating	Select Year
Click here to enter text.	Select Grain Class	Select what represents your most resistant check	Enter as text 0-9 rating	Select Year
Click here to enter text.	Select Grain Class	Select what represents your most resistant check	Enter as text 0-9 rating	Select Year
Click here to enter text.	Select Grain Class	Select what represents your most resistant check	Enter as text 0-9 rating	Select Year
Click here to enter text.	Select Grain Class	Select what represents your most resistant check	Enter as text 0-9 rating	Select Year
Click here to enter text.	Select Grain Class	Select what represents your most resistant check	Enter as text 0-9 rating	Select Year
Click here to enter text.	Select Grain Class	Select what represents your most resistant check	Enter as text 0-9 rating	Select Year
Click here to enter text.	Select Grain Class	Select what represents your most resistant check	Enter as text 0-9 rating	Select Year

NOTE: List the associated release notice or publication under the appropriate sub-section in the 'Publications' section of the FPR.

Publications, Conference Papers, and Presentations

Instructions: Refer to the PR_Instructions for detailed more instructions for listing publications/presentations about your work that resulted from all of the projects included in the FY20 grant award. Only citations for publications published (submitted or accepted) or presentations presented during the **award period (9/1/19 - 9/30/21**) should be included. If you did not publish/submit or present anything, state 'Nothing to Report' directly above the Journal publications section.

NOTE: Directly below each citation, you **must** indicate the Status (i.e. published, submitted, etc.) and whether acknowledgement of Federal support was indicated in the publication/ presentation. See example below for a poster presentation with an abstract:

Winn, Z.J., Acharya, R., Lyerly, J., Brown-Guedira, G., Cowger, C., Griffey, C., Fitzgerald, J., Mason R.E., and Murphy, J.P. (2020, Dec 7-11). Mapping of Fusarium Head Blight Resistance in NC13-20076 Soft Red Winter Wheat. p. 12. In: Canty, S., Hoffstetter, A. and Dill-Macky, R. (Eds.), *Proceedings of the 2020 National Fusarium Head Blight Forum*. https://scabusa.org/pdfs/NFHBF20_Proceedings.pdf.
Status: Abstract Published and Poster Presented
Acknowledgement of Federal Support: YES (Abstract and Poster)

Journal publications.

Bec, S., G.E. Yulfo-Soto, and L.J. Vaillancourt (2021) "Relative efficiency of split-marker versus double-crossover replacement protocols for production of deletion mutants in strain PH-1 of *Fusarium graminearum*," Fungal Genetics Reports: Vol. 65, Article 1. https://doi.org/10.4148/1941-4765.2175
Status: Published
Acknowledgement of Federal Support: Yes

Books or other non-periodical, one-time publications.

Nothing to report.

Other publications, conference papers and presentations.

de Barros, A. V., Bec, S., Machado, F., Trail, F., Van Sanford, D. A., Alves, E., & Vaillancourt, L. J. (2019). The role of mating-type genes in pathogenicity of Fusarium graminearum to wheat. *Plant Health 2019*. https://apsjournals.apsnet.org/doi/10.1094/PHYTO-109-10-S2.1
Status: Abstract Published and Poster Presented
Acknowledgement of Federal Support: Yes

Sladana Bec, Franklin J. Machado, Mark Farman, Aline Vieira de Barros, Scott Schwartz, Richard Metz, Charles Johnson, David Van Sanford, Emerson Del Ponte and Lisa Vaillancourt. 2019. "Highly Aggressive and Toxigenic Transgressive Progeny from a Cross of Model *Fusarium graminearum* Strains PH-1 and GZ3639 are Associated with a Recombination Hotspot on Chromosome2" In: Canty, S., A. Hoffstetter, B. Wiermer and R. Dill-Macky (Eds.), *Proceedings of the 2019 National Fusarium Head Blight Forum*. East Lansing, MI/Lexington, KY: U.S. Wheat& Barley Scab Initiative.
Status: Invited Talk Presented, Abstract Published.
Acknowledgement of Federal Support: Yes

Yulfo Soto, G.E., Viera de Barros, A., Bec, S., Machado, F.J. Trail, F., Van Sanford, D., Vaillancourt, LJ. 2019 Exploring the role of mating-type genes in *Fusarium graminearum*. In: Canty, S., A. Hoffstetter, B. Wiermer and R. Dill-Macky (Eds.), *Proceedings of the 2019 National Fusarium Head Blight Forum*. East Lansing, MI/Lexington, KY: U.S. Wheat & Barley Scab Initiative.
Status: Poster presented, Abstract Published.
Acknowledgement of Federal Support: Yes

Yulfo Soto, G.E., Bec, S., Viera de Barros, A., Machado, F.J., Van Sanford, D., Trail, F., Del Ponte, E., Vaillancourt, LJ. (2020, Dec. 7-11). Heterothallic mutants of *Fusarium graminearum* and their potential use for genetic analysis of fungal pathogenicity and toxigenicity. In: Canty, S., A. Hoffstetter and R. Dill-Macky (Eds.), *Proceedings of the 2020 National Fusarium Head Blight Forum.* https://scabusa.org/pdfs/NFHBF20_Proceedings.pdf.
Status: Poster presented, Abstract Published.
Acknowledgement of Federal Support: Yes