USDA-ARS
U.S. Wheat and Barley Scab Initiative
FY18 Performance Report
Due date: September 23, 2019

Cover Page

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Fiscal Year:	2018
USDA-ARS Agreement ID:	58-5062-8-016
USDA-ARS Agreement Title:	The Role of Mating-type Genes in Pathogenicity of Fusarium graminearum to Wheat.
FY18 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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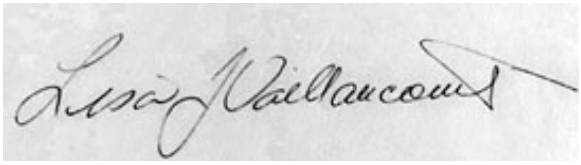
Recipient Identifying Number or Account Number:	3200002191
Agency PI:	H. Corby Kistler
Project/Grant Reporting Period:	9/1/18 - 8/31/19
Reporting Period End Date:	08/31/19

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
	FY18 Total ARS Award Amount	$ 43,143



Lisa J. Vaillancourt 9-26-2019
Principal Investigator Date

* MGMT – FHB Management
FST – Food Safety & Toxicology
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 project?

The goal of the proposal is to test the hypothesis that MAT1-1-1 and MAT1-2-1 proteins, when present in isolation, regulate genes that negatively impact aggressiveness and toxigenicity of the heterothallic strains.

We have three objectives:

1. 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. Cytological analysis of KO transformants expressing fluorescent proteins in inoculated wheat heads, to characterize the reduced aggressiveness of the MAT1-1-1 and MAT1-2-1 specificity gene KOs in detail.
3. Produce complementation strains for each of the specificity gene KOs and confirm function in aggressiveness to wheat heads.

2. What was accomplished under these goals? *Address items 1-4) below for each goal or objective.* Activities related to our research goal.

1) major activities

Our original experiment that indicated a negative impact of the MAT Kos on aggressiveness was done in a single experiment on winter wheat, with two Mat1-1-1 and Mat1-2-1 KO strains compared with the WT. We thought that it was important to confirm our findings in a larger study, so we repeated the inoculations in the spring wheat cultivar Wheaton, including three additional Mat1-1-1 KO strains and three additional MAT1-2-1 KO strains (Results shown in Figure 1). The inoculations were repeated twice with a large number of replications (20 per experiment) so we can be quite confident of the results. Mature heads were collected from plants in both experiments for mycotoxin analysis, which is on-going. We also wanted to optimize inoculation conditions in the much more experimentally tractable spring wheat for use in objectives 1 – 3.

2) specific objectives

Objectives 1 and 2, We have been working with fluorescently labeled WT strains to develop a detailed understanding of the time course of infection in Wheaton, and a "baseline" for comparison with the MT strains. Results of our inoculations have enabled us to choose two KO strains for each locus that are representative for use in the RNAseq experiments.

Objective 3. We have been working on cloning the MAT1-1-1 and MAT1-2-1 loci into a vector containing the selectable marker nourseothricin, and optimizing the protocol for transformation.

3) significant results

Results of our inoculation experiment indicated that most (but not all) of the KO mutants were significantly reduced in pathogenicity in comparison to the WT (Figure 1). The fact that some of the strains were similar in aggressiveness to the WT might indicate that the

reduced aggressiveness of the other strains are NOT due to the KO of the MAT loci. We will need to test that hypothesis with the complemented strains.

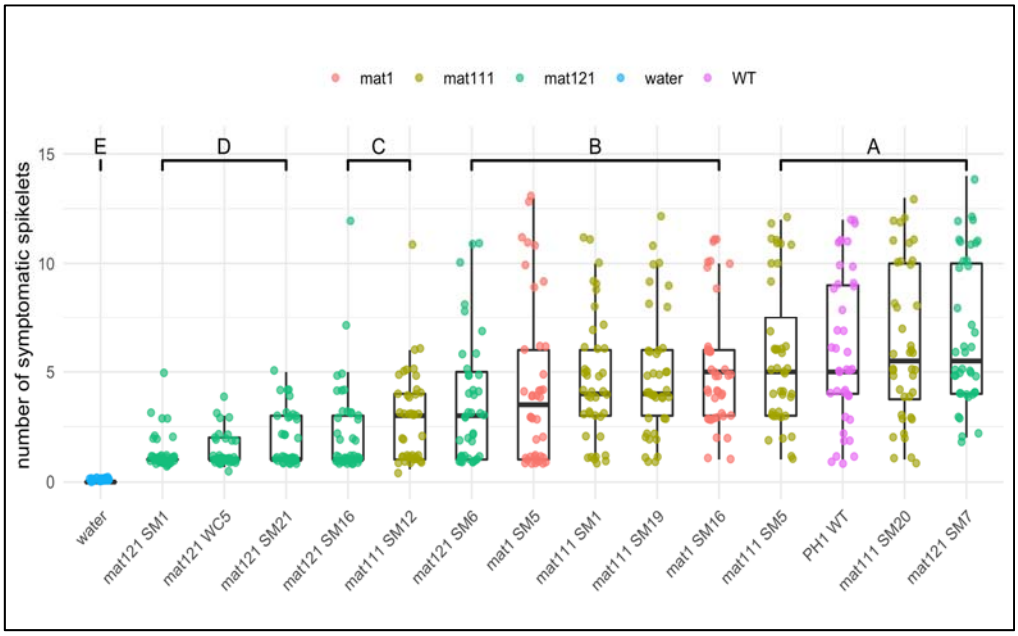


Figure 1. Effect of MAT gene deletions on aggressiveness to Wheaton hard red spring wheat. Each bar is an independent, single spored KO transformant that was confirmed by Southern hybridization and/or by a newly developed PCR assay. Colors are used to indicate the different genes that were knocked out. KOs were produced by either whole-cassette replacement (WC) or by the split-marker method (SM). Note that one MAT1-2-1 KO and two MAT1-1-1 KOs were not significantly different from the WT PH-1 (in purple). This graph shows the combined results of two experiments, each with 20 reps per treatment. Strains behaved similarly in both trials and letters indicate groups that are statistically different.

4) key outcomes or other achievements

- We confirmed the phenotypes of five representative MAT1-1-1 and MAT1-2-1 KO strains in Wheaton spring wheat.
- We have developed a time course of infection in Wheaton spring wheat for optimized collection of tissue for the RNAseq experiment (Objective 1). We have learned that the fungus has colonized individual spikelets by 48 hpi, and entered the rachis by 72-96 hpi.
- We have developed methods for tissue staining and fixation, and we have started to use these to compare the MT and WT strains during their colonization of wheat heads. We decided to use this method to avoid the need for re-transformation of the MT strains, which would potentially change their behaviors.
- We have cloned the MAT1-1-1 gene into the nourseothricin vector in preparation for complementing the MAT1-1-1 KO strains.

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

The project has included two graduate students (one PhD and one MS, neither of whom is yet finished) and a visiting scholar. The PhD student is a dual degree student who is simultaneously earning doctoral degrees from UK and from the Universidade Federal de Viçosa in Brazil. The MS student is an URM from Puerto Rico. The visiting scholar is from Brazil, where she is pursuing her PhD degree in microscopy techniques. These students have had opportunities to learn new techniques including cloning, RNA extraction and analysis, and fungal transformation. They have become familiar with the execution of pathogenicity and developmental assays and microscopy with *F. graminearum* in wheat.

All three students have had the chance to attend professional meetings including the USWBSI meeting in St. Louis in 2018, the American Phytopathological Society meeting in Cleveland in 2019, and meetings of the NC1183 Mycotoxins, Biosecurity, Food Safety, and Biofuels Byproducts Multistate Research Committee in 2018 and 2019. At these meetings they have made important professional contacts and benefitted from various activities focused on their career and skills development.

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

Results were presented as a poster at the meeting of the American Phytopathological Society in Cleveland OH, in August 2019, and also as a talk at the annual meeting of the NC1183 Mycotoxins, Biosecurity, Food Safety, and Biofuels Byproducts Multistate Research Committee in Blacksburg Virginia, September 2019.

Training of Next Generation Scientists

Instructions: Please answer the following questions as it pertains to the FY18 award period. 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 FY18 award period?**
   No.
   If yes, how many?

2. **Did any graduate students in your research program supported by funding from your USWBSI grant earn their Ph.D. degree during the FY18 award period?**
   No.
   If yes, how many?

3. **Have any post docs who worked for you during the FY18 award period and were supported by funding from your USWBSI grant taken faculty positions with universities?**
   No.
   If yes, how many?

4. **Have any post docs who worked for you during the FY18 award period and were supported by funding from your USWBSI grant gone on to take positions with private ag-related companies or federal agencies?**
   No.
   If yes, how many?

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 FY18 award period. 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** (S, MS, MR, R, where R represents your most resistant check)	**FHB Rating** (0-9)	**Year Released**

Add rows if needed.

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

Abbreviations for Grain Classes

Barley - BAR
Durum - DUR
Hard Red Winter - HRW
Hard White Winter - HWW
Hard Red Spring - HRS
Soft Red Winter - SRW
Soft White Winter - SWW

Publications, Conference Papers, and Presentations

Instructions: Refer to the FY18-FPR_Instructions for detailed instructions for listing publications/presentations about your work that resulted from all of the projects included in the FY18 grant. Only include citations for publications submitted or presentations given during your award period (9/1/18 - 8/31/19). If you did not have any publications or presentations, state 'Nothing to Report' directly above the Journal publications section.

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

> Conley, E.J., and J.A. Anderson. 2018. Accuracy of Genome-Wide Prediction for Fusarium Head Blight Associated Traits in a Spring Wheat Breeding Program. In: Proceedings of the XXIV International Plant & Animal Genome Conference, San Diego, CA.
> Status: Abstract Published and Poster Presented
> Acknowledgement of Federal Support: YES (poster), NO (abstract)

Journal publications.

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

Other publications, conference papers and presentations.

Vieira de Barros, A., S. Bec, F.J. Machado, F. Trail, D. Van Sanford, E. Alves, and L.J. Vaillancourt, L.J. 2019. The role of mating type genes in pathogenicity of *Fusarium graminearum* to wheat. Presented at the Annual Meeting of the American Phytopathology Society, Plant Health 2019, in Cleveland OH. August 2-7.
Status: Poster presented and abstract submitted, in press.
Acknowledgement of Federal Support: YES (poster); NO (abstract).