USDA-ARS
U.S. Wheat and Barley Scab Initiative
FY17 Preliminary Final Performance Report
Due date: July 31, 2018

Cover Page

Principle Investigator (PI):	Santiago Mideros
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Fiscal Year:	2017
USDA-ARS Agreement ID:	59-0206-6-011
USDA-ARS Agreement Title:	Pathogenesis of Fusarium graminearum.
FY17 USDA-ARS Award Amount:	$ 34,655
Recipient Organization:	The Board of Trustees of the University of Illinois Grants & Contracts Office 1901 S. First Street, Suite A Champaign, IL 61820
DUNS Number:	41544081
EIN:	37-6000511
Recipient Identifying Number or Account Number:	AC335
Project/Grant Reporting Period:	6/6/17 - 6/5/18
Reporting Period End Date:	6/5/2018

USWBSI Individual Project(s)

USWBSI Research Category*	Project Title	ARS Award Amount
PBG	Identifying F. graminearum Pathogenesis Genes Under Field Conditions.	$ 34,655
	FY17 Total ARS Award Amount	$ 34,655

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

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Project 1: *Identifying* F. graminearum *Pathogenesis Genes Under Field Conditions.*

1. What are the major goals and objectives of the project?
Goal: Identify diverse effector repertoires of *F. graminearum* from multiple field strains.
Objective 1: Describe the effector diversity of *F. graminearum* by characterizing the transcriptome of naturally infected wheat lines with various levels of resistance.
Objective 2: Conduct functional aggressiveness assays with effector characterized strains.

2. What was accomplished under these goals?
Objective 1. Describe the effector diversity of *F. graminearum* by characterizing the transcriptome of naturally infected wheat lines with various levels of resistance.
1) **Major activities.** We collected infected samples from wheat lines with three different levels of resistance (resistant, intermediate, and susceptible) from five locations in Illinois. We extracted mRNA from 12 samples collected in the field. Illumina TruSeq stranded libraries were prepared and sequenced on a single lane of a HiSeq 4000. We finalized the bioinformatics data analysis. We wrote the first draft of a publication.
2) **Specific objectives**
- Identify effectors that are only present in strains that infect specific resistant or susceptible wheat lines.
- Identify essential effectors that are present in all pathogenic strains

3) **Significant results.** Sequencing of 13 paired libraries yielded 20 and 35 Million reads for a total of 783 million reads. Despite the fact that the samples were collected from infected plant tissue for most of the libraries over 50% of the reads aligned to the PH-1 genome. There was a significant differential gene expression among isolate sources (resistant or susceptible wheat lines), although only four genes were significant at an FDR < 0.05. A total of 93 genes were significant with an FDR < 0.6 (not adjusted *p*-value < 0.005. Among the 93 genes there was a significant enrichment for predicted secreted and predicted effector proteins. A total of 306 genes had 32-fold more expression between all the isolate sources and the axenic control. These genes were also significantly enriched for predicted secreted and predicted effector proteins. RNAseq data was also used to call 120,086 SNPs with 36,114 of these being genotyped in all 12 RNAseq samples and nine strains with known placement among the *F. graminearum* populations. Eleven out of twelve samples clustered with the NA1 population. The other sample did not cluster with any of the *F. graminearum* populations suggesting that it was not infected by *F. graminearum*.
4) **Key outcomes or other achievements.** We have identified *F. graminearum* transcripts that have more expression in the susceptible line compared to the resistant line, and others that have more expression in the resistant line compared to the susceptible line. These genes are good candidates to be pathogenicity genes or targets of resistance mechanisms in wheat. We have identified *F. graminearum* transcripts that have low expression on an axenic control but high levels of expression in planta. These group of genes is enriched for predicted secreted and effector proteins making them good candidates to be essential genes required by *F. graminearum* to cause disease on wheat. We found that wheat lines with different levels of resistance in Illinois do not appear to be selecting for different populations of the pathogen.

Objective 2. Conduct functional aggressiveness assays with effector characterized strains.
1) **Major activities.** Field assays to evaluate the aggressiveness of strains isolated from the same samples used for RNAseq were conducted in 2017 and 2018. We evaluated disease severity three times during the season. One green-house assay to evaluate the aggressiveness of the same strains was conducted in 2017. Data analysis of field and greenhouse experiments is underway. We started the development of an HPLC protocol to accurately quantify the mycotoxin production levels of our twelve selected strains.
2) **Specific objectives**

- Confirm that predicted aggressiveness based on effector repertoire reflects aggressiveness observed in field and greenhouse assays.
- Characterize (phenotypically) a collection of Illinois isolates.

3) **Significant results.** In the field, higher levels of disease development were observed in 2018 than in 2017. There were significant differences for the aggressiveness of strain groups in 2017, 2018, and the greenhouse experiment. In addition, independent strains varied on their levels of aggressiveness. The strains collected from the susceptible wheat line, were consistently the most aggressive. Strain group was also a significant determinant of the number of spores produced in vitro. The strains also differed on the level of spore production.
4) **Key outcomes or other achievements.** Field, greenhouse, and laboratory experiments have been conducted and our preliminary analysis supports the hypothesis that strain group (wheat line source) determines the level of aggressiveness in *F. graminearum*.

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

A graduate student (M.S.) activities are being supported under this project and has conducted most of the experiments. The graduate student and an undergrad (supported under different funds) have received training and learned techniques to isolate, store, and produce inoculum of *Fusarium graminearum*. In addition, the graduate student has received training to extract RNA, and has been involved in professional development opportunities by enrolling on a bioinformatics workshop for the analysis of RNAseq data.
A Research specialist was hired in 2018 to help with the project and we have worked one-on-one on the bioinformatics analysis, she took a RNAseq data analysis workshop, and we are now working on the preparation of a manuscript for submission.

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

- Partial results were presented at the Agronomy Day of the University of Illinois at Urbana-Champaign in 2017 to attendants. The talk was entitled: major wheat diseases.
- A poster was presented at the National Fusarium Head Blight Forum in 2016.
- A poster was presented at the Annual meeting of the American Phytopathological Society in 2017.
- An abstract was submitted to the International Congress of Plant Pathology and a poster will be presented there between July 29th and August 3rd.

Training of Next Generation Scientists

Instructions: Please answer the following questions as it pertains to the FY17 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 FY17 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 FY17 award period?**
   No
   If yes, how many?

3. **Have any post docs who worked for you during the FY17 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 FY17 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?

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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 FY17 award period. All columns must be completed for each listed germplasm/cultivar. Use the key below the table for Grain Class abbreviations. *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 FY17-FPR_Instructions for detailed instructions for listing publications/presentations about your work that resulted from all of the projects included in the FY17 grant. Only include citations for publications submitted or presentations given during your award period (6/6/17 - 6/5/18). 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.

Journal publications.

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

Other publications, conference papers and presentations.

Salazar, M., Kolb, F., and Mideros, S. 2016. Identification and characterization of *Fusarium graminearum* pathogenesis genes. Proceedings of the 2016 National Fusarium Head Blight Forum. St. Louis, Missouri. Poster # 30.
Status: Abstract Published and Poster Presented
Acknowledgement of Federal Support: Yes (poster), Yes (abstract)

Salazar, M., Kolb, F., and Mideros, S. 2017. Aggressiveness levels of *Fusarium graminearum* isolates from wheat lines with diverse levels of resistance. APS Annual Meeting Program Book. San Antonio, Texas. 342-P.
Status: Abstract Published, and Poster Presented
Acknowledgement of Federal Support: Yes (poster), No (abstract)

Salazar, M., Fall, L-A., Butts-Wilmsmeyer, C., Kolb, F., Mideros, S. 2018. Determinants of aggressiveness in *Fusarium graminearum*. 11th International Congress of Plant Pathology. Boston, Massachusetts. 1049-P
Status: Abstract Published (Online), and Poster will be presented on August 2nd 2018.
Acknowledgement of Federal Support: Yes (poster), No (abstract)