USDA-ARS/

U.S. Wheat and Barley Scab Initiative FY16 Final Performance Report

Due date: July 28, 2017

Cover Page

Cover rage				
Santiago Mideros				
University of Illinois				
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217-265-6340				
2016				
59-0206-6-011				
Pathogenesis of Fusarium graminearum.				
\$ 36,797				
The Board of Trustees of the University of Illinois				
Grants & Contracts Office				
1901 S. First Street, Suite A				
Champaign, IL 61820				
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5/7/16 - 5/6/17				
05/06/17				

USWBSI Individual Project(s)

	USWBSI Research Category*	Project Title	ARS Award Amount
	PBG	Identifying F. graminearum Pathogenesis Genes Under Field Conditions.	\$ 36,797
Ī		FY16 Total ARS Award Amount	\$ 36,797

Mideros Mora, Santiago X Digitally signed by Mideros Mora, Santiago X DN: cn=Mideros Mora, Santiago X, o=University of Illinois at Urbana-Champaign, ou=Dept. of Crop Sciences, email=smideros@illinois.edu, c=US Date: 2017.07.27 13:47:16-05'00'

Principal Investigator

Date

*	MCMT	FHD	Management	
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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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USDA-ARS Agreement #: 59-0206-6-011

Reporting Period: 5/7/16 - 5/6/17

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? Address items 1-4) below for each goal or objective.

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

- Samples for RNA extraction and for isolations where collected from five locations in Illinois. At each location, samples were collected from five wheat lines known levels of resistance.
- mRNA was extracted from 12 samples collected in the field.
- Illumina TruSeq stranded libraries were prepared and sequenced on a single lane of a HiSeq 4000 at the Roy J. Carver Biotechnology Center at the University of Illinois.
- Bioinformatics data analysis has been initiated.

2) specific objectives

- Identify essential effectors that are present in all pathogenic strains
- Identify effectors that are only present in strains that infect specific resistant or susceptible wheat lines.

3) significant results

- 197 isolates were collected from scabby heads from five locations in Illinois and from five winter wheat lines with different levels of resistance.
- High quality RNA was extracted from 12 samples of the wheat-fusarium interaction. One extra RNA extraction was conducted from an axenic control sample.
- Sequencing resulted on 13 paired end libraries that range between 20 and 35 Million reads for a total of 783 million reads. All libraries passed preliminary quality control.

4) key outcomes or other achievements

- Samples of the interaction were collected as planned.
- RNAseq data is available and bioinformatics analysis is underway.
- An agreement has been reached to use the services of the High Performance Biological Computing group at the Roy J. Carver Biotechnology Center for RNAseq data analysis (bioinformatics and statistics).

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Objective 2: Conduct functional aggressiveness assays with effector characterized strains.

1) major activities

- An aggressiveness field assay was conducted in the spring and summer of 2017 by inoculating isolates corresponding to the 12 samples used for RNA library on three wheat lines with different levels of resistance to FHB. We evaluated disease severity three times during the season, and percent of kernels infected after harvest.
- Green-house inoculation experiments have been initiated and the first batch of plants has been vernalized.
- A humidity chamber was constructed for greenhouse inoculations.

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

• Preliminary data analysis of the AUDPC from the disease severity data of the field experiment indicated a significant interaction (p = 0.03) between strain and cultivar. Within each cultivar, the strains produced significantly different levels of AUDPC (p < 0.001, Fig 1).

4) key outcomes or other achievements

- Strain characterization has started and preliminary aggressiveness assays suggests three types of strains: i) strains that cause significant damage on resistant and susceptible lines; ii) strains that cause damage only on susceptible lines, and iii) strains that cause little damage on both resistant and susceptible lines.
- Greenhouse aggressiveness assays for the fusarium strains has started.

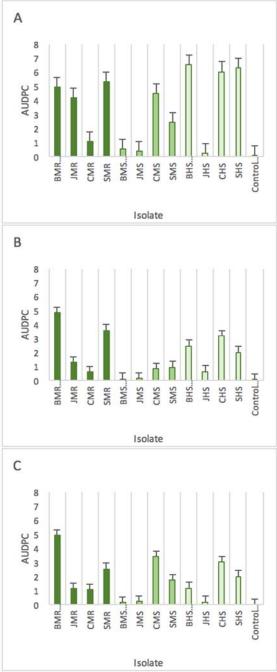


Figure 1. Area under the disease progress curve (AUDPC) from field aggressiveness assays of 12 isolates on A) highly susceptible cultivar, B) moderately susceptible cultivar, and C) moderately resistant cultivar. *MR strains (dark color), *MS strains (light color), and *HS strains (lightest color) were isolated from a moderately resistant, a moderately susceptible, and a highly susceptible wheat line respectively. Error bars represent standard error.

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

A graduate student (M.Sc.) is being supported under this project and has conducted all 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 and successfully, extracted RNA, and inoculated field experiments with *F. graminearum*.

The graduate student has also been involved in professional development opportunities by enrolling on a bioinformatics workshop for the analysis of RNAseq data.

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, and an abstract was submitted for the Annual meeting of the American Phytopathological Society in 2017.

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Training of Next Generation Scientists

Instructions: Please answer the following questions as it pertains to the FY16 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 FY16 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 FY16 award period?

No

If yes, how many?

3. Have any post docs who worked for you during the FY16 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 FY16 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 <u>full or partial</u> support through the USWBSI during the <u>FY16 award period</u>. 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

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Publications, Conference Papers, and Presentations

Instructions: Refer to the FY16-FPR_Instructions for detailed instructions for listing publications/presentations about your work that resulted from all of the projects included in the FY16 grant. Only include citations for publications submitted or presentations given during your award period (5/7/16 - 5/6/17). If you did not have any publications or presentations, state 'Nothing to Report' directly above the Journal publications section.

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, awaiting poster presentation

Acknowledgement of Federal Support: Yes (poster), No (abstract)