USDA-ARS
U.S. Wheat and Barley Scab Initiative
FY17 Final Performance Report – NCE for FY18
Due date: July 12, 2019

Cover Page

Principle Investigator (PI):	Santiago Mideros
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Fiscal Year:	2017 (NCE for FY18)
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:	6/6/18 - 6/5/19
Reporting Period End Date:	06/05/19

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

Mideros Mora, Santiago X

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Principal Investigator Date

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[*] 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: *Identifying* Fusarium 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.

i) Major Activities. We previously reported the sequencing results of 13 RNA libraries from naturally infected wheat lines with different levels of resistance. We also reported on the preliminary results of the RNAseq analysis. During this reporting period, we conducted data mining on the results of differential gene expression analysis. We called variants of *F. graminearum* from the RNAseq data. Then, we generated a Neighbor-Net network with our samples and control strains with known population placement. We wrote a manuscript, submitted it to peer review, and addressed the suggestions from the reviewers and editor.

ii) 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

iii) Significant Results. Over 50% of the RNA reads aligned to the PH-1 genome for most of the samples. The percentage of reads mapped to the PH-1 genome was not dependent on the resistance level of the wheat-line. We found four *F.* graminearum genes differently expressed among wheat-line levels of resistance (Table 1). In addition, a total of 93 genes had differential gene expression at a more relaxed *P*-value (unadjusted *P*-value < 0.005; FDR <0.6). Among these 93 genes, there was significant enrichment for predicted secreted proteins. (For the complete list of genes mentioned here please see our publication). We also identified that the transcripts for six genes were highly expressed in wheat and had low levels of expression on axenic media (Table 2). Finally, variant calling produced 36,114 SNPs genotyped in all 12 samples. Eleven of our samples clustered with control isolates from the NA1 population. One sample did not cluster with any of the control strains (NA1, NA2, and NA3).

Table 1. *Fusarium graminearum* genes with significantly different levels of gene expression (FDR < 0.05) among samples collected from wheat lines with various levels of host resistance (analyzed with Model 1).

Gene ID[a]	Predicted Function[b]	FDR[c]	R vs S[d]	R vs I[d]	I vs S[d]
FGRAMPH1_01G13239	oxidation-reduction process	0.01	-3.04	-4.97	1.93
FGRAMPH1_01G01905	arginyl-trna synthetase	0.02	-3.39	-5.49	2.09
FGRAMPH1_01G28263	tpr domain	0.02	5.00	4.65	0.35
FGRAMPH1_01G09227	kinesin light chain	0.04	-3.33	-0.71	-2.62

[a] Gene ID from Ensembl (Kersey et al. 2018; King et al. 2017)

[b] Annotation from King et al. 2017

[c] False discovery rate adjusted *p* -value for treatment term

[d] Log2 fold change for pairwise comparisons between samples collected from wheat lines with various levels of host resistance (R = resistant, I = intermediate, and S = susceptible).

Table 2. *Fusarium graminearum* genes with significantly different levels of gene expression (FDR < 0.05) between samples collected from wheat lines with various levels of host resistance and a strain growing on sterile media (AX) (analyzed with Model 2).

Gene ID[a]	Predicted Function[b]	FDR[c]	R vs AX[d]	I vs AX[d]	S vs AX[d]
FGRAMPH1_01G14115	antigen 1; SP[e]	0.01	4.68	5.85	7.95
FGRAMPH1_01G25079	carbohydrate metabolic process; SP[e]	0.02	6.50	6.32	5.57
FGRAMPH1_01G16997	3-phytase precursor	0.02	3.22	4.04	5.86
FGRAMPH1_01G16533	cellobiose dehydrogenase; SP[e]	0.02	8.56	7.60	7.95
FGRAMPH1_01G12227	family inorganic phosphate transporter	0.04	-0.80	2.55	3.89
FGRAMPH1_01G12833	choline monooxygenase	0.04	8.01	7.25	7.23

[a] Gene ID from Ensembl (Kersey et al. 2018; King et al. 2017)

[b] Annotation from King et al. (2017).

[c] False discovery rate adjusted *p* -value for treatment term

[d] Log2 fold change for pairwise comparisons between samples collected from wheat lines with various levels of host resistance (R = resistant, I = intermediate, and S = susceptible) and the AX sample.

[e] This gene codes for a secreted protein (King et al. 2017)

iv) Key outcomes. We identified *F. graminearum* genes that have significantly different gene expression on wheat lines with different levels of host resistance (Table 1). These genes are candidates for resistance level specificity on wheat. In addition, we identified genes that appear to be important for wheat infection (Table 2). Some of these genes are secreted and likely effectors. Finally, we determined that 11 of the 12 infections on field wheat in Illinois in 2016 were caused by strains of *F. graminearum* NA1 population.

Objective 2. Conduct functional aggressiveness assays with effector characterized strains.
i) Major Activities. We previously reported the competition of field experiments to characterize the aggressiveness of a collection of strains obtained from wheat lines with diverse levels of resistance. During this reporting period, we finalized the statistical analysis of the aggressiveness assays. We quantified the levels of DON on the samples. A graduate student (Melissa Salazar), wrote a Master's Thesis.
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. Strains of *F. graminearum* collected from a susceptible wheat line were all aggressive. Conversely, the aggressiveness of *F. graminearum* strains collected from resistant wheat lines varied. While strains varied in their levels of spore production, sporulation did not appear to be correlated with aggressiveness.
4) Key outcomes. We found that highly aggressive strains of *F. graminearum* were consistently recovered from susceptible wheat lines. The ability to produce DON was also consistently high from strains collected from susceptible wheat lines as opposed to more variability on the strains collected from resistant wheat lines. These experiments were conducted by a graduate student who successfully defended her thesis.

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

- Melissa Salazar (MS student partially supported by the project) graduated in May of 2019. In this reporting period, she was coached by the PI on writing a thesis, and by other faculty members in the Department of Crop Sciences on statistical analyses.
- Leigh-Ann Fall (Research Specialist, partially supported by the project) was coached by the PI on bioinformatics, RNAseq data mining, and fungal pathogenesis.
- Mara Swapp (undergraduate student, supported by other funds) was trained on DON quantification.

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

- A poster was presented by Melissa Salazar at the International Congress of Plant Pathology between July 29th and August 3rd, 2018 about the results of the second objective.
- A Flash and Dash and a poster were presented by Leigh-Ann Fall at the Fusarium Head Blight Forum in 2018 about the results of the first objective.
- The PI gave an interview to personnel from the office of communications in the college and she wrote a press release about our publication.

Training of Next Generation Scientists

Instructions: Please answer the following questions as it pertains to the FY17-NCE 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-NCE period?**
   Yes
   If yes, how many?
   One (1)

2. **Did any graduate students in your research program supported by funding from your USWBSI grant earn their Ph.D. degree during the FY17-NCE period?**
   No
   If yes, how many?

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

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Publications, Conference Papers, and Presentations

Journal publications.

Fall, L. A., Salazar, M. M., Drnevich, J., Holmes, J. R., Tseng, M. C., Kolb, F. L., and Mideros, S. X. 2019. Field pathogenomics of *Fusarium* head blight reveals pathogen transcriptome differences due to host resistance. Mycologia. DOI: 10.1080/00275514.2019.1607135
Status: Published online
Acknowledgement of Federal Support: Yes

Salazar, M. M., and Mideros, S.X. 2019. First report of *Fusarium armeniacum* causing *Fusarium* head blight on soft red winter wheat in Illinois. Plant Disease
Status: Under review
Acknowledgement of Federal Support: Yes

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

Salazar, M. M. 2019. Identification and characterization of *Fusarium graminearum* pathogenesis genes and determination of aggressiveness. M.S. Thesis. University of Illinois at Urbana-Champaign.
Status: Approved and submitted to the Graduate College
Acknowledgement of Federal Support: No

Other publications, conference papers and presentations.

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. Poster presentation. 1049-P
Status: Abstract Published Online, and Poster presented on August 2nd 2018.
Acknowledgement of Federal Support: Yes (poster), No (abstract)

Fall, L.-A., Salazar, M., Drnevich, J., Holmes, J., Tseng, M.-C., Kolb, F. L., and Mideros, S. X. 2018. Field pathogenomics of *Fusarium* head blight reveals pathogen transcriptome differences due to host resistance. Proceedings of the 2018 National Fusarium Head Blight Forum: p. 82. East Lansing, MI/Lexington, KY: U.S. Wheat & Barley Scab Initiative
Status: Abstract Published; Flash and Dash, and Poster presented
Acknowledgement of Federal Support: Yes (poster), Yes (abstract)

Quinn, L. 2019. Resistance to Fusarium head blight holding in Illinois, study says [Press release]. Retrieved from https://emails.illinois.edu/newsletter/224039.html
Status: Published
Acknowledgement of Federal Support: Yes