

**USDA-ARS/
U.S. Wheat and Barley Scab Initiative
FY18 Performance Report
Due date: July 12, 2019**

Cover Page

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| Phone: | 309-681-6520 |
| Fiscal Year: | 2018 |
| USDA-ARS Agreement ID: | N/A |
| USDA-ARS Agreement Title: | Control of FHB by Targeting Pathogen Effector and Host Protein Interactions. |
| FY18 USDA-ARS Award Amount: | \$ 52,819 |

USWBSI Individual Project(s)

| USWBSI Research Category* | Project Title | ARS Award Amount |
|----------------------------------|--|-------------------------|
| PBG | Control of FHB by Targeting Pathogen Effector and Host Protein Interactions. | \$ 52,819 |
| | FY18 Total ARS Award Amount | \$ 52,819 |

Guixia Hao

Principal Investigator

7/1/19

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: *Control of FHB by Targeting Pathogen Effector and Host Protein Interactions.*

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

The major goal of this project is to aid in the development of FHB resistance and DON reduction by characterizing *Fusarium graminearum* effectors that interact with plant molecules to initiate infection and suppress plant defense responses. Effectors play an important role in the pathogenesis of many bacterial and fungal pathogens. As a hemibiotrophic pathogen, *F. graminearum* likely secretes effectors to suppress plant defenses during initial infection and induce cell death in later stages. We have selected thirty-seven putative effectors via bioinformatic analyses of genome sequences from sixty strains representing three different *F. graminearum* populations found in the U.S. (NA1, NA2 and NA3).

The objectives of this proposal are 1. Determine the expression profile of selected candidate effectors during pathogenesis. RT-qPCR will be performed for gene expression studies. 2. Characterize the effect of candidate genes on FHB pathogenesis via mutagenesis experiments. Mutants will be created for selected effectors by split marker or *Agrobacterium*-mediated mutagenesis. The impact of mutations on pathogenesis will be evaluated on wheat heads by dip inoculation (for type I resistance) and/or point inoculation (for type II resistance). DON production of mutants will be determined using GC/MS and compared with controls. 3. Characterize effector-interaction partners. Once critical effectors associated with FHB pathogenesis are identified, effector localization in planta will be verified by GFP fusion and microscopic examination. Host protein targets of critical effectors will be pursued by co-immunoprecipitation assays.

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

1) major activities:

Objective 1: We selected 28 genes encoding effectors and designed primers for each gene. We inoculated wheat heads by dip inoculation and collected samples at various time points after inoculation. We isolated RNA, synthesized cDNA and performed RT-qPCR. We determined the expression profile of 28 candidate effectors during early infection.

Objective 2: We generate mutants for highly induced genes and perform FHB virulence assays on wheat head by dip and point inoculations. These included mutants of FGSG_01831, FGSG_03599, FGSG-04563 and FGSG_12160. We also determined DON production of these mutants in liquid medium and during wheat infection.

Objective 3: We created FGSG_04563 and GFP fusion protein and examined the fusion protein localization via transient gene expression.

2) specific objectives: Identify effector genes induced during pathogenesis; Generate mutants for highly induced effectors and perform virulence assays

3) significant results:

Objective 1: We found that four effector-coding genes were upregulated immediately upon infection, and four effector-coding genes were highly induced during infection. The remaining 20 candidates, which were primarily expressed under axenic culture conditions, were not induced or were only slightly induced during infection under our test conditions.

Objective 2:

- a) None of the deletion mutants had altered DON production in liquid medium.
- b) Although highly induced during infection, the deletion mutants of FGSG_03599 and FGSG-12160 did not significantly affect FHB development on wheat head either by dip inoculation or point inoculation.
- c) The deletion mutants of FGSG_04563 reduced FHB development in wheat head by point inoculation, but not by dip inoculation. These results indicate FGSG_04563 is associated with disease spread in wheat.

Objective 3: We observed FGSG_04563-GFP fusion protein primarily localized in the plant nucleus. We will pursue its target in plant by co-immunoprecipitation assays (Object 3).

4) key outcomes or other achievements.

None

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

One technician (Jamie Blumberg), hired through ORISE, has been trained in molecular biology and pathogenesis assays including DNA isolation, cloning, mutagenesis, RCR and RT-PCR, preparation fungal culture for inoculation, inoculation and scoring of virulence assays.

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

Poster presentation at the 2018 National Fusarium Head Blight Forum, St. Louis. Dec. 2-4, 2018

Invited to present “*Fusarium graminearum* effector targeting plant nucleus is associated with wheat head blight” at the 30th Fungal Genetics Conference, Pacific Grove, CA. Mar.12-17, 2019.

Invited to present “*Fusarium graminearum* effector targeting plant nucleus suppresses plant immunity” at the Fusarium Workshop, Pacific Grove, CA. Mar. 11th, 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 |
|----------------------------|-------------|--|------------------------|------------------|
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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. 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 presented at the FHB Forum:

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.

Hao G., Vaughan MM, and McCormick S. 2018. “*Fusarium graminearum* effectors suppress plant immunity.” In: S. Canty, A. Hoffstetter, B. Wiermer and R. Dill-Macky (Eds.) *Proceeding of the 2018 National Fusarium Head Blight Forum* (p. 85). East Lansing, MI/Lexington, KY: U. S. Wheat & Barley Scab Initiative.

Status: Abstract Published and Poster Presented

Acknowledgement of Federal Support: YES (Poster), NO (Abstract)

Hao G., Kim H.S., Proctor R., and McCormick S. 2019. *Fusarium graminearum* effector targeting plant nucleus is associated with wheat head blight. 30th Fungal Genetics Conference, Pacific Grove, CA. Mar.12-17, 2019.

Status: Talk given and Poster Presented

Acknowledgement of Federal Support: YES (Talk and Poster)