

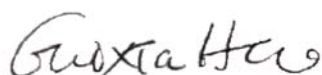
USDA-ARS
U.S. Wheat and Barley Scab Initiative
FY19 Performance Report
Due date: July 24, 2020

Cover Page

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| Phone: | 309-682-6520 |
| Fiscal Year: | 2019 |
| USDA-ARS Agreement ID: | N/A |
| USDA-ARS Agreement Title: | Control of FHB by Targeting Pathogen Effector and Host Protein Interactions |
| FY19 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 |
| | FY19 Total ARS Award Amount | \$ 52,819 |



7/9/2020

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

1. What are the major goals and objectives of the research 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.

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 or objectives? (For each major goal/objective, address items a-b) below.)

a) What were the major activities?

Objective 1: In addition to determining the expression profiles of 28 effectors last year, we selected additional 10 effector genes and examined their expression during wheat head infection.

Objective 2: To determine the role of FGSG_01831, FGSG_03599, FGSG-04563 and FGSG_12160 during FHB pathogenesis, we performed and replicated FHB virulence assays on wheat heads to evaluate FHB initial infection by dip inoculations and FHB spread by point inoculations.

We also performed virulence assays on barley heads to assess initial infection with these mutants.

We generated mutants for two highly induced effector genes encoding xyloglucanase (FGSG_05851 and 11037).

We examined DON production of FGSG_05851 and 11037 mutants in liquid medium and during wheat infection.

Objective 3: We performed co-immunoprecipitation assays using tobacco leaves transiently expressing FGSG_04563 (designate FgNls1) and GFP fusion protein.

We created an RNAi construct targeting FgNls1 and generated transgenic wheat expressing FgNls1 RNAi construct.

b) What were the significant results?

Objective 1: We identified two effector-coding genes, FGSG_05851 and 11037, which were highly induced during infection. The remaining 8 candidates were not induced or were only slightly induced during infection under our test conditions.

Objective 2:

- 1) We discovered that the deletion mutants of FGSG_05851 significantly reduced 15-ADON production in liquid medium. However, the mutants did not reduce FHB spread and DON content in infected wheat head.
- 2) Although highly induced during infection, the deletion mutants of FGSG_03599 and FGSG-12160 did not significantly affect FHB pathogenesis on wheat and barley.
- 3) FGSG_01831 mutants did not affect FHB spread, but significantly reduced FHB initial infection.
- 4) The deletion mutants of FgNls1 reduced FHB development in wheat and barley. These results indicate FgNls1 is required for FHB pathogenesis.

Objective 3:

- 1) We confirmed that the FgNls1-GFP fusion protein is primarily localized in the plant nucleus by microscopic examination and western blot. However, co-immunoprecipitation assays did not identify its interaction partner in tobacco.
- 2) Transgenic wheat expressing RNAi targeting FgNls1 reduced FHB in our preliminary study.

c) List key outcomes or other achievements.

Identified one effector involved in DON production in liquid medium and several effectors associated with FHB pathogenesis. More importantly, transgenic wheat expressing an RNAi construct targeting FgNls1 appeared to reduce FHB development.

3. Was this research impacted by the COVID-19 pandemic (i.e. university shutdowns, reduced or lack of support personnel, etc.)? If yes, please explain how this research was impacted or is continuing to be impacted.

Yes. Due to COVID-19 pandemic, the experiments for mutant complementation and virulence assays have been postponed.

4. 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.

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

Poster presentation at the 2019 National Fusarium Head Blight Forum, Milwaukee, WI. Dec. 8-10, 2019

Poster presentation at the 2019 Plant Health APS annual meeting, Denver, CO. Aug. 11-15, 2019 (Virtual)

Training of Next Generation Scientists

Instructions: Please answer the following questions as it pertains to the FY19 award period (N/A). 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 FY19 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 FY19 award period?**

No

If yes, how many?

- 3. Have any post docs who worked for you during the FY19 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 FY19 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 FY19 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 FY19-FPR_Instructions for detailed more instructions for listing publications/presentations about your work that resulted from all of the projects included in the FY19 grant award. Only citations for publications published (submitted or accepted) or presentations presented during the **award period (N/A)** should be included. If you did not publish/submit or present anything, state ‘Nothing to Report’ directly above the Journal publications section.

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

De Wolf, E., D. Shah, P. Paul, L. Madden, S. Crawford, D. Hane, S. Canty, R. Dill-Macky, D. Van Sanford, K. Imhoff and D. Miller. 2019. “Impact of Prediction Tools for Fusarium Head Blight in the US, 2009-2019.” In: S. Canty, A. Hoffstetter, H. Campbell and R. Dill-Macky (Eds.), *Proceedings of the 2019 National Fusarium Head Blight Forum* (p. 12), Milwaukee, WI; December 8-10. University of Kentucky, Lexington, KY.

Status: Abstract Published and Poster Presented

Acknowledgement of Federal Support: YES (Abstract and Poster)

Journal publications.

Hao G., McCormick S., Usgaard T., Tiley H., and Vaughan MM. Characterization of *Fusarium graminearum* effectors and their roles during Fusarium head blight. Front. in Plant Science

Status: Submitted

Acknowledgement of Federal Support: Yes

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

Other publications, conference papers and presentations.

Hao G., McCormick S., Naumann T., Kim HS., Proctor R. 2019. “*Fusarium graminearum* effector FgNls1 reduces Fusarium head blight.” In: S. Canty, A. Hoffstetter, H. Campbell and R. Dill-Macky (Eds.) *Proceeding of the 2019 National Fusarium Head Blight Forum* (p. 72). Milwaukee, WI; December 8-10. University of Kentucky, Lexington, KY.

Status: Abstract Published and Poster Presented

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