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

Cover Page

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Fiscal Year:	2019
USDA-ARS Agreement ID:	59-0206-6-004
USDA-ARS Agreement Title:	Resistant and Susceptible Interactions of *Fusarium graminearum* with Wheat and Barley
FY19 USDA-ARS Award Amount:	$ 49,095
Recipient Organization:	Michigan State University Contract & Grant Administration Hannah Administration Building, Room 2 East Lansing, MI 48824-1046
DUNS Number:	193247145
EIN:	38-6005984
Recipient Identifying Number or Account Number:	RC106173 & RC106213
Project/Grant Reporting Period:	4/24/19 - 4/23/20
Reporting Period End Date:	4/23/2020

USWBSI Individual Project(s)

USWBSI Research Category*	Project Title	ARS Award Amount
BAR-CP	Control of Scab in Barley through Reduction of Infection and Sporulation	$ 49,095
	FY19 Total ARS Award Amount	**$ 49,095**

Frances Trail

July 23, 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 Scab in Barley through Reduction of Infection and Sporulation*

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

A recent report has documented the efficacy of spray application of dsRNA (double stranded RNA) to induce gene silencing (knockdown of expression) in *Fusarium graminearum* in the host plant. Genes showing a loss of pathogen growth or infection or a loss of DON production when mutated or knocked out in *F. graminearum* are useful targets. We proposed to take advantage of this technique to target genes that will improve the quality of barley products.

Our specific objectives for this proposal are to:

Objective 1: Generate dsRNA against stages important to beer production, disease initiation, and spread.

Objective 2: Test application on barley to determine what approaches or combinations thereof are most effective.

2. What was accomplished under these goals or objectives? *(For each major goal/objective, address items a-b) below.)*

Objective 1

a) What were the major activities?

Working through the precise methodology necessary to induce gene silencing has been a major activity. The GFP dsRNA is applied at a concentration of 40 ng μL^{-1} to *F. graminearum* spores at a concentration of 10^5 spores mL^{-1} in liquid germination medium. Spore germination is initiated by 2-3 hours and hyphal branching begins at 10-12 hours. Microscopic observations are performed at every hour post-inoculation and by 3 hours, GFP fluorescence has visibly diminished in the conidia that are exposed to the dsRNA compared to the control. Diminished fluorescence is maintained through 24 hours of observation, which is the end of the experiment. Our previous studies have shown that the hyphal branching stage in culture roughly corresponds to the timing of appressorium maturation on the plant, indicating that silencing will activity will match the timing *in planta*. Thus, silencing genes important to appressorium development could be an effective tool.

b) What were the significant results?

Because of the successful knockdown of the GFP gene *in* vitro, we are now assembling dsRNA synthesized for target genes essential for spore germination DON biosynthesis, hydrophobin genes (responsible for gushing of beer), spore germination, and pathogenicity. Testing of these *in vitro* is straightforward, as the silencing of these genes produce a testable phenotype in culture. Additionally, validation of microscopic observations showing decreasing fluorescence is being performed using RT-PCR for genes being targeted with dsRNA.

c) List key outcomes or other achievements.

We have accomplished gene knockdown *in vitro* using dsRNA that is complementary to the entire GFP gene coding region. The knockdown of the GFP gene expression has been observed and recorded across spore (conidia) germination stages. Results of silencing the GFP gene can be seen in the figure below.

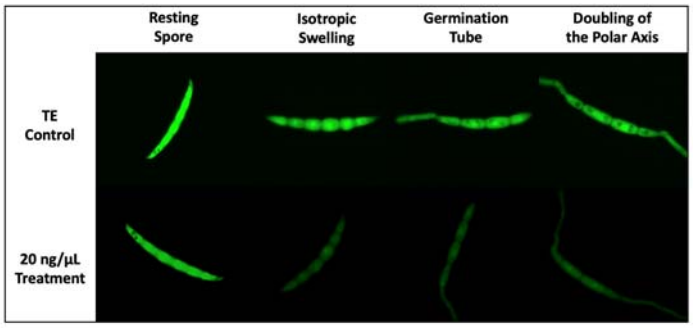

Figure 1. Gene silencing of the constitutively-expressed GFP gene after application of dsRNA treatment at 20 ng/uL per 10^5 conidia/mL in liquid Bird medium.

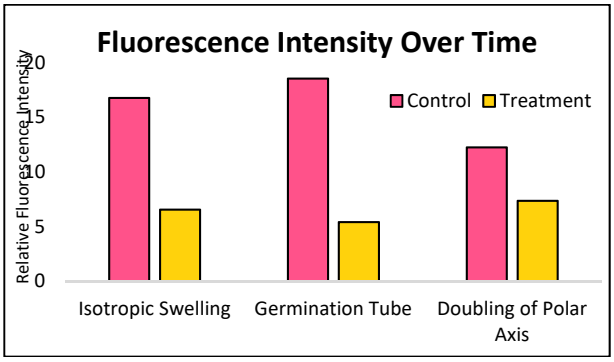

Figure 2. Relative fluorescence after dsRNA application at 20 ng/uL across conidial germination stages.

Objective 2

a) What were the major activities?

We are working through our experiments on detached barley sheaths and leaves by applying methodology that has been worked out in Objective 1.

b) What were the significant results?

This objective will focus on applying what we learn in Objective I to plant infection, mycotoxin and hydrophobin production by the fungus, and others as listed above. Recently, as part of a different project, we have identified 13 previously uncharacterized genes which, when knocked out, elicit reduced pathogen ingress (deMigual-Rojas and Trail, in prep). We are incorporating these as targets for RNA silencing along with those listed above.

c) List key outcomes or other achievements.

We have worked through all of the processes we need to complete the study, which we are starting up again as we get back into the lab.

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.

Though data analyzing and experiment planning continued in-home, laboratory research was halted for nearly four months due to university shutdown and state of Michigan government mandates.

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

The funding is used to support one PhD student, Tara Watkins, who is interested in novel applications to control diseases and testing new approaches in the field.

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

We have presented results from this funding to the regional barley growers, maltsters, brewers, and other industry people at the Great Lakes Hop and Barley Conference in Ann Arbor, MI, on March 5-7, 2020. In addition, a poster was presented at the FHB Forum Dec 2019 to scientists, growers, millers and others.

Training of Next Generation Scientists

Instructions: Please answer the following questions as it pertains to the FY19 award period (4/24/19 - 4/23/20). 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**

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 (4/24/19 - 4/23/20**) 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.

None at this time.

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

None at this time.

Other publications, conference papers and presentations.

Watkins T., deMiguel Rojas C., Trail F. 2019. "Spray-induced gene silencing to manage fusarium head blight in barley." In: S. Canty, A. Hoffstetter, H. Campbell and R. Dill-Macky (Eds.), *Proceedings of the 2019 National Fusarium Head Blight Forum* (p. 78), Milwaukee, WI; December 8-10. University of Kentucky, Lexington, KY.
Status: Abstract published and poster presented.
Acknowledgement of Federal Support: YES (Poster and Abstract).

Watkins T. Short talk at the Great Lakes Hop and Barley Conference in Ann Arbor, MI, on March 5-7, 2020.
Status: Talk presented.
Acknowledgement of Federal Support: YES.