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

Cover Page

Principle Investigator (PI):	Frances Trail
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Fiscal Year:	2018
USDA-ARS Agreement ID:	59-0206-6-004
USDA-ARS Agreement Title:	Resistant and Susceptible Interactions of Fusarium graminearum with Wheat and Barley.
FY18 USDA-ARS Award Amount:	$ 56,485
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:	04/24/18 - 04/23/19
Reporting Period End Date:	04/23/19

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
	FY18 Total ARS Award Amount	$ 56,485

July 12, 2019

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

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Project 1: *Control of Scab in Barley through Reduction of Infection and Sporulation.*

1. What are the major goals and objectives of the project? A recent report has documented the efficacy of spray application of dsRNA (double stranded RNA) to induce gene silencing (turn off 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 propose 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.

Due to delays in receipt of funding in 2018, this project was initiated in November 2018.

2. What was accomplished under these goals?
Objective 1
1) major activities
To develop a positive control and to work through the methodology of silencing, we have accomplished gene silencing *in vitro* using dsRNA specific to the GFP gene. We have successfully silenced GFP gene expression across spore (conidia) germination stages **(Figure 1)**. This was accomplished by synthesizing dsRNA that aligns to the entire GFP coding region. The GFP dsRNA is applied at a concentration of 40 ng μL^{-1} along with *F. graminearum* spores at a concentration of 10^6 spores mL^{-1} to cellophane covering germination medium. Spore germination is initiated by 3-4 hours and hyphal branching begins 10-12 hours. 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, the end of the experiment. Our previous studies have shown that the hyphal branching

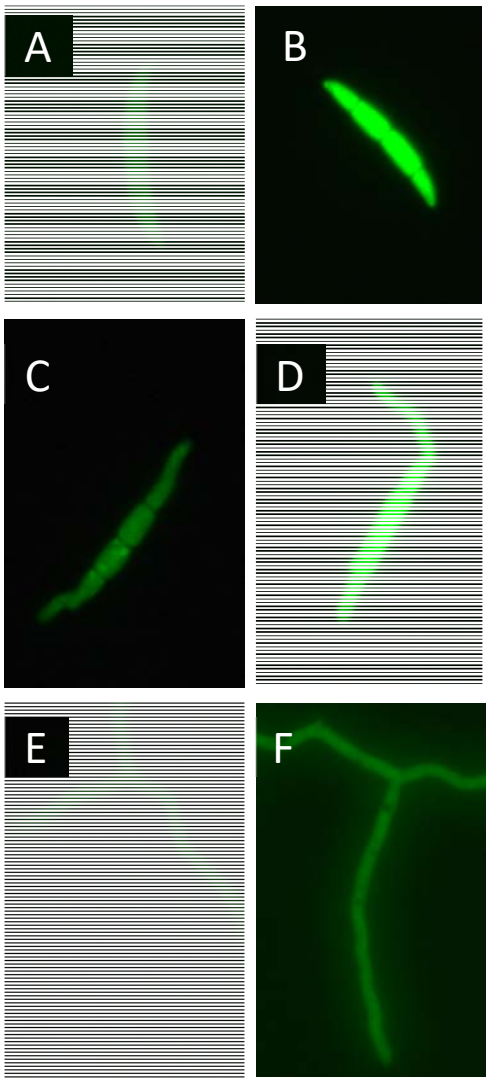

Figure 1. dsRNA induced gene silencing in F. graminearum in vitro at three stages of germination: **A,B**. isotropic swelling of spores. **C,D.** initiation of germination. **E,F.** First hyphal branches after germination**. A,C,E** are treated with GFP dsRNA to induce silencing as can be seen in the lighter fluorescence. **B,D,F** are untreated, as can be seen in the brighter fluorescence. Spores are inherently brighter in treated and untreated samples compared to hyphae due to the greater thickness of the spores (3um) versus hyphae (1um). Images were photographed using a Leica DM5000B equipped with GFP specific fluorescence capabilities. All images were viewed at 400X and photographs have not been adjusted for intensity. Photos are representative of several observations

stage in culture roughly corresponds to the timing of appressorium maturation on the plant, indicating that the length of time for silencing activity is sufficient if similar activities are seen *in planta*.

2) specific objectives. We are now assembling dsRNA synthesized for genes target genes essential for DON synthesis, hydrophobin genes (responsible for beer gushing), spore germination and pathogenicity. Testing of these *in vitro* will be straightforward, as the silencing of these genes produce a testable phenotype in culture.

3-4) significant results and key outcomes

To this end we have optimized the protocol for generating dsRNA specific for silencing a target gene. We have shown that GFP silencing occurs and and the decrease in fluorescence is being quantified through ImageJ software. Levels of RNA will also be determined.

Objective 2

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

1) major activities

We are just initiating our experiments on barley by applying what has been worked out in Objective 1.

2) specific objectives. This objective will focus on applying what we learn in Objective I to plant infection, mycotoxin production by the fungus, hydrophobin production 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.

3) significant results.

Nothing to report at this time.

4) key outcomes or other achievements.

Nothing to report at this time.

3. 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.

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

We have presented all of our results from the Scab funding to the regional growers on Wheat field day, June 12, 2019, at MSU.

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**

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 (04/24/18 - 04/23/19). 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 presentation with an abstract:

> 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)

Nothing to report.

Journal publications.

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

Other publications, conference papers and presentations.