

**USDA-ARS/
U.S. Wheat and Barley Scab Initiative
FY16 Final Performance Report
Due date: July 28, 2017**

Cover Page

Principle Investigator (PI):	Frances Trail
Institution:	Michigan State University
E-mail:	trail@msu.edu
Phone:	517-432-2939
Fiscal Year:	2016
USDA-ARS Agreement ID:	59-0206-6-004
USDA-ARS Agreement Title:	Resistant and Susceptible Interactions of Fusarium graminearum with Wheat and Barley.
FY16 USDA-ARS Award Amount:	\$ 98,806
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
Project/Grant Reporting Period:	4/24/16 - 4/23/17
Reporting Period End Date:	04/23/17

USWBSI Individual Project(s)

USWBSI Research Category*	Project Title	ARS Award Amount
BAR-CP	Investigating the Basis of Resistance to Scab in Barley.	\$ 53,806
PBG	Initial Interactions of Fusarium graminearum with Wheat and Barley.	\$ 45,000
	FY16 Total ARS Award Amount	\$ 98,806

Frances Trail
Principal Investigator

7/27/17
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: *Investigating the Basis of Resistance to Scab in Barley.*

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

Our Objectives are:

1. Determine whether the resistance response we have documented in barley trichomes/silica cells is correlated with cessation of fungal penetration.
2. Characterize the resistance response in two- and six-row barley lines to determine if the response differs between these classes of barley. Use progeny of a two- and six-row barley cross to determine segregation pattern of resistance and barley type.
3. Determine if known barley powdery mildew pathogenesis-related genes *MLO* and *ROR2* alter the observed resistance response associated with barley trichomes/silica cells.

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

Objective 1. Determine whether the resistance response we have documented in barley trichomes/silica cells is correlated with cessation of fungal penetration. Note that mature trichomes in most grasses are filled with silica and these trichome/silica cells are sites of fungal penetration.

1) major activities- Tissue has been prepared for this Objective, but the histology and microscopy has not yet been completed.

Objective 2. Characterize the resistance response in two- and six-row barley lines to determine if the response differs between these classes of barley. Previously we noted that domed shaped trichomes are characteristic of two-row and prickles (pointed trichomes) are characteristic of six-row barley. The dome-shaped trichomes of two-row were associated with a resistance response.

- 1) major activities- We used barley lines from near-isogenic Bowman (2-row, domed) backcrosses for two-row and six-row loci at candidate alleles and examined focal accumulations (resistance responses) at the different trichome types.
- 2) specific objectives
- 3) significant results- Stander barley (6-row, prickle) is significantly reduced in the number of focal accumulations ($p < 0.05$) per palea compared to all Bowman backcrossed varieties, which confirms past results of fewer accumulations in Stander (six-row) than in two-row. Six-row locus *vrs1.c* barley has the most differential impact in foci formation, as compared to the wild-type Bowman, from all the Bowman backcross near-isogenic lines.
- 4) key outcomes- We have identified a locus, *vrs1.c*, associated with increased focal formation for resistance to fungal ingress. We will be examining the effect of this locus in more genetic backgrounds. We need to generate more data about this association before we will be confident that it should be used for screening.

Objective 3. Determine if known barley powdery mildew pathogenesis-related genes *MLO* and *ROR2* alter the observed resistance response to FHB associated with barley trichomes/silica cells.

- 1) major activities- We hypothesize that the defense compound accumulation seen associated with trichomes is decreased in the powdery mildew resistant lines, allowing *F. graminearum* to penetrate barley florets more readily. We have been investigating this hypothesis.
- 2) specific objectives
- 3) significant results- Preliminary data indicates that the barley line with complete powdery mildew resistance (*MLO*) has fewer focal accumulations of cellulose and lignin than the variety with partial resistance to powdery mildew (*mlo-5*) in response to *F. graminearum*. Ongoing work will determine the significance of this relationship. Samples will be cross-sectioned at the trichomes with and without focal accumulations to determine if fungal penetration has ceased in association with foci.
- 4) key outcomes. Preliminary evidence suggests that complete resistance to powdery mildew correlates with higher susceptibility to FHB, possibly because the defense compound accumulation seen in trichomes is decreased in the complete powdery mildew resistant lines, allowing *F. graminearum* to penetrate barley florets more readily.

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

Graduate student Rebecca Shay has spearheaded this project and is doing a great job. She has become an expert in preparing and examining infected florets to determine responses to the fungus and has been working on all three objectives. She has presented a poster on her research at a national meeting (Mycological Society of America meetings in Athens Georgia the week of July 17th, 2017). She has also spearheaded multiple outreach activities bringing the concept of plant disease to the public.

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

Trail has participated in the Field Day for Wheat at MSU and interacted with growers and folks from industry. Rebecca Shay presented a poster on this project at the Mycological Society of America meetings in Athens Georgia the week of July 17th, 2017. We have sent in a revision of a manuscript on the trichome/silica cell responses to Molecular Plant Pathology, and anticipate that it will be accepted soon.

Project 2: *Initial Interactions of Fusarium graminearum with Wheat and Barley.*

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

We have identified two important stages of the life cycle of *F. graminearum* that are influenced by the presence of specific host cells that accumulate silica: the “silica cells” associated with stalk nodes, which support perithecium development; and the trichomes on lemma and palea that support fungal penetration. We have recently generated knockout strains for three genes encoding major intrinsic proteins (MIP) in *F. graminearum*. There is evidence that similar proteins are the transporters of Si into plants, so we hypothesize that they may be key sensors of Si for the fungus as well. Note that silica amendments precipitate when they are higher than 0.2 mM, so our amendment experiments did not go higher than this.

Our objectives are to

- (1) Test ability of *F. graminearum* wild-type and MIP mutants to grow on silica in culture and its effect on differentiation.
- (2) Test the influence of Si levels in barley florets on the pathogenicity and perithecium development of *F. graminearum*.
- (3) Perform transcriptomics under high and low Si conditions both in culture and on barley florets.
- (4) Knockout genes identified in (3) as having the largest change in expression associated with presence of Si to determine how the fungus senses Si and how Si affects pathogenicity.

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

Objective 1. Test ability of *F. graminearum* wild-type and MIP mutants to grow on silica in culture and the effect of silica on fungal differentiation.

1) major activities

Investigation of the effect of different levels of silica on growth of *F. graminearum* in culture, as this will provide a proxy for growth in the plant.

2) specific objectives

3) significant results

F. graminearum has a response in the presence of silica. In wild-type cultures amended with 0 or 0.2 mM silica, the fungus responds to the higher levels by producing significantly more aurofusarin: [0 mM Si] 308 +/- 30 mg compared to [0.2 mM Si] 703 +/- 90 mg/plate extracted. In addition, and probably more importantly in terms of interactions with the plant, in the presence of high silica, the young mycelia have a tendency to stick together in a hydrophobic way and the fungal growth is significantly more robust. The hyphae grown on the unamended plates stand apart from each other, and take on the “fluffy” or cottony phenotype characteristic of weak, apathogenic hyphae in cultures that are declining. Hydrophobicity would be an important characteristic of infection hyphae because it would allow the hyphae to interact with the hydrophobic plant surface for infection. This response may be promoted when hyphae are close to silica filled cells.

Absorbance spectra of extracts from wild-type cultures amended with 0, 0.1 or 0.2 mM silica, showed that there was an unidentified compound absorbing at 360 nm that decreased with

increasing silica from 0 to 0.2. The compound is aromatic, but we have not fully identified its structure.

Similar studies with the MIP mutants indicated that they produced an additional unknown compound in 0 silica that forms crystals. We are investigating the structure of this compound to determine if it is a mycotoxin.

4) key outcomes or other achievements.

F. graminearum responds differentially to varying levels of silica in culture media. The increased “stickiness” of the hyphae in the presence of high levels of silica may be important for pathogenicity and may stimulate the relationship of the fungus with trichomes and other silica cells. Our results are highly suggestive that in culture the manipulation of silica levels induces secondary metabolism in the fungus.

Objective 2. Test the influence of Si levels in barley florets on the pathogenicity and perithecium development of *F. graminearum*.

1) major activities

We grew barley in different levels of Si and compared the susceptibility to *F. graminearum*. Note that in order to control silica levels, we must use distilled water tested for silica levels and grow the plants using hydroponics. It has taken some time to get this going, but we are now confident of the procedure (despite having to justify that we are using hydroponics for legal purposes).

2) specific objectives

3) significant results

In plants grown on 0 mM silica, the trichomes on the florets are smaller, and the young flowers, still in the boot, are very fragile. They appear normal once they emerge.

Of the 3 MIP gene mutants, one of them is expressed at very low levels during perithecium development, the two others are expressed at very high levels during perithecium development. The MIP gene mutant with low expression does not produce perithecia on leaves grown at low silica, but does produce them when inoculated onto leaves with high silica. Wild-type produces fewer perithecia on the low silica leaves than on the high silica leaves. We are in the process of determining the effects of the 2 other MIP mutants.

4) key outcomes or other achievements

Perithecia are associated with silica cells, and this supports that finding. It also supports the finding that the MIP gene may be a silica sensor.

Objective 3 Perform transcriptomics under high and low Si conditions both in culture and on barley florets.

This has been initiated, but nothing to report yet.

For Objective 4 results are pending.

FY16 Final Performance Report
PI: Trail, Frances
USDA-ARS Agreement #: 59-0206-6-004
Reporting Period: 4/24/16 - 4/23/17

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

The experiments were primarily carried out by Ben Smith, who graduated with a BS in Biochemistry and is preparing for graduate school. Ben has been able to increase his arsenal of techniques and has shown a great capacity to solve technical problems and think through experimental designs. Ben has participated in several outreach programs including taking exploration of secondary metabolites to an AP chemistry class and making mushroom kits with 4th graders.

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

Trail has participated in the Field Day for Wheat at MSU and interacted with growers and folks from industry.

FY16 Final Performance Report
PI: Trail, Frances
USDA-ARS Agreement #: 59-0206-6-004
Reporting Period: 4/24/16 - 4/23/17

Training of Next Generation Scientists

Instructions: Please answer the following questions as it pertains to the FY16 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 FY16 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 FY16 award period? No**

If yes, how many?

3. **Have any post docs who worked for you during the FY16 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 FY16 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?

FY16 Final Performance Report
 PI: Trail, Frances
 USDA-ARS Agreement #: 59-0206-6-004
 Reporting Period: 4/24/16 - 4/23/17

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 FY16 award period. All columns must be completed for each listed germplasm/cultivar. Use the key below the table for Grain Class abbreviations. *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

FY16 Final Performance Report
PI: Trail, Frances
USDA-ARS Agreement #: 59-0206-6-004
Reporting Period: 4/24/16 - 4/23/17

Publications, Conference Papers, and Presentations

Instructions: Refer to the FY16-FPR_Instructions for detailed instructions for listing publications/presentations about your work that resulted from all of the projects included in the FY16 grant. Only include citations for publications submitted or presentations given during your award period (4/24/16 - 4/23/17). If you did not have any publications or presentations, state 'Nothing to Report' directly above the Journal publications section.

Journal publications.

Imboden, I., Afton, D. and Trail, F. 201-. Surface interactions of *Fusarium graminearum* on barley.
Molecular Plant Pathology.

Status: *Revisions submitted.*

Acknowledgement of Federal Support: YES

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

Other publications, conference papers and presentations.

Shay, R., Imboden, I., Afton, D. and Trail, F. 2017. Investigating the barley defense response to Fusarium Head Blight. In: Proceedings of the LXXV Mycological Society of America Conference, Athens, GA.

Status: Abstract Published and Poster Presented

Acknowledgement of Federal Support: YES (poster), NO (abstract)