USDA-ARS
U.S. Wheat and Barley Scab Initiative
FY17 Final Performance Report
Due date: July 31, 2018

Cover Page

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Fiscal Year:	2017
USDA-ARS Agreement ID:	59-0206-6-004
USDA-ARS Agreement Title:	Resistant and Susceptible Interactions of Fusarium graminearum with Wheat and Barley.
FY17 USDA-ARS Award Amount:	$ 98,517
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:	4/24/17 - 4/23/18
Reporting Period End Date:	04/23/18

USWBSI Individual Project(s)

USWBSI Research Category*	Project Title	ARS Award Amount
BAR-CP	Investigating the Basis of Resistance to Scab in Barley.	$ 53,608
PBG	Initial Interactions of Fusarium graminearum with Wheat and Barley.	$ 44,909
	FY17 Total ARS Award Amount	$ 98,517

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: *Investigating the Basis of Resistance to Scab in Barley.*

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

Our overall project goal is to better define the mechanism of a resistance response (called "focal accumulation" of lignin and cellulose) that we have commonly observed specifically in trichomes and silica cells in two row barley, but only very infrequently in six-row barley, and appears to halt ingress of *F. graminearum* into the palea.

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

3.

1. major activities: Major activities: We used NILs to identify the locus that were involved in the focal accumulation. We demonstrated that this resistance response is indeed an inhibitor of fungal ingress in one of the major penetration pathways of F. graminearum into barley florets.

2. specific objectives: 1. Determine whether the resistance response we have documented in barley trichomes is correlated with cessation of fungal penetration. See below 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. See below. 3. Determine if known barley powdery mildew pathogenesis-related genes *MLO* and *ROR2* alter the observed resistance response associated with barley trichomes. These were shown to be similar in response to *F. graminearum,* and no trichome resistance was documented.

3) significant results:

- Stander barley (six-row) is significantly reduced in the number of focal accumulations ($p < 0.05$) per palea compared to all Bowman backcrossed varieties, which confirms our past results of fewer accumulations in Stander than in two-row varieties (Figure 1). Results from detached floret assays were confirmed with on-plant assays that showed the same relative focal accumulations to the detached assays.

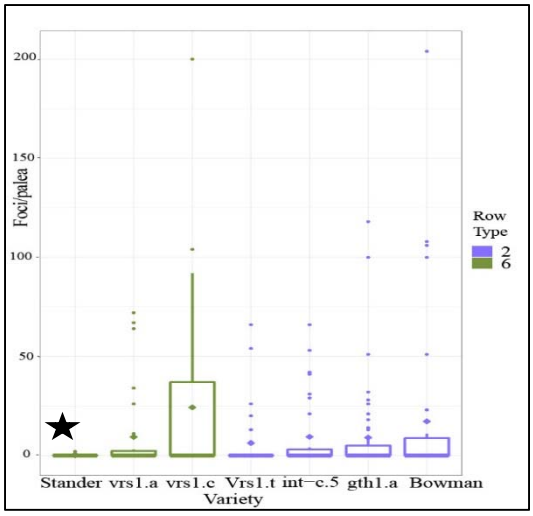

Figure 1. Presence of foci in barley varieties. Diamonds indicate the mean value for each sample. Stars indicate significantly different ($p < 0.05$, calculated by a Student's T Test) responses compared to the wildtype Bowman. Focal accumulation ranges were large, with up to 200 foci per palea.

- Locus *vrs1.c* barley has the most differential impact as compared to the wild-type Bowman from all the Bowman backcross near-isogenic lines (Figure 1).
- Cross sectioning shows that at foci no fungal penetration has occurred. Fungal penetration can occur at areas without foci (Figure 2).

4) key outcomes or other achievements:

Our overall project goal is to better define the mechanism of a resistance response (called "focal accumulation" of lignin and cellulose) that we have commonly observed specifically in trichomes and silica cells in two row barley, but only very infrequently in six-row barley, and appears to halt ingress of *F. graminearum* into the palea. This is an important additional resistance response that could be incorporated into barley lines. The next step is to determine the genes controlled by the locus, and if the locus harbors known defense response genes. Testing efficacy of resistance induction with pathogenicity mutants will indicate the phases of fungal ingress important to stimulation of this resistance response. This is worth pursuing.

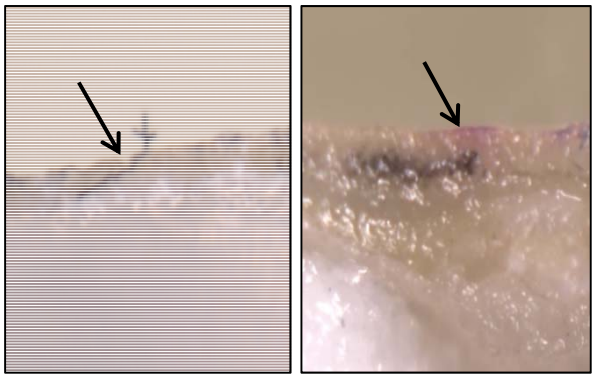

Figure 2. Cross section of inoculated barley paleae demonstrate defense responses cause cessation of fungal penetration. Left, Fungal penetration occurs after *F. graminearum* inoculation in Stander (arrow, hypha entering palea surface). Right, The defense response arrested fungal penetration (arrow) with accumulations of cellulose and lignin (purple stain on surface of palea) in Bowman. Dark stripe below epidermis in Right figure is not related to infection.

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

This research was performed primarily by Rebecca Shay, a PhD student, who was funded by the project.

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

Posters:Shay R., Trail F. 2017. Investigating barley defense response to Fusarium Head Blight. Poster presented at the Mycological Society Meeting. August, Athens, GA.

Shay R., Trail F. 2017. Investigating barley defense responses and interactions with *Fusarium graminearum.* Poster presented at the Fusarium Forum, Milwaukee, WI December.

Shay, R., Imboden, I., Afton, D. and Trail, F. 2018. Investigating the barley defense response to Fusarium Head Blight. In: Plant Science Graduate Student Research Symposium; Michigan State University.

Shay, R., Imboden, I., Afton, D. and Trail, F. 2018. Genetics of differential defense responses to Fusarium graminearum. In: Proceedings of the XI International Mycological Congress; San Juan, PR.

Published paper: Imboden L, Afton D, Trail F. 2017. Surface interactions of *Fusarium graminearum* on barley. Molecular Plant Pathology, DOI: 10.1111/mpp.12616.

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

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

Previously, we have shown that *Fusarium graminearum* infects and sporulates in associate with silica containing cells (trichomes for the former, stomates and "silica cells" for the latter). Our goal with this work is to determine the effect of silica on *Fusarium graminearum* to try to tease apart these reactions, including the mechanism of sensing silica. Since silica amendment of fields is encouraged, this project was focused on whether this would enhance or attenuate head blight.

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

1) major activities: Analysis of effects of different levels of silica in culture and in barley florets.
2) specific objectives: (a) Test ability of *F. graminearum* to grow on Si *in vitro* and the effect of Si on differentiation.
3) significant results: Figure 3 shows that lowering the levels of silica in culture lowers the levels of aurofusarin, a red pigmented mycotoxin associated with head blight infection.
4) key outcomes or other achievements: This result is interesting because aurofusarin is associated with *F. graminearum* in crop residues and in senesced plant tissue. In these tissue, silica is concentrated due to the skeletalization of the plant tissue. Aurofusarin is an antimicrobial protectant for the fungus. If we amend our crops with silica, will the fungus survive better?

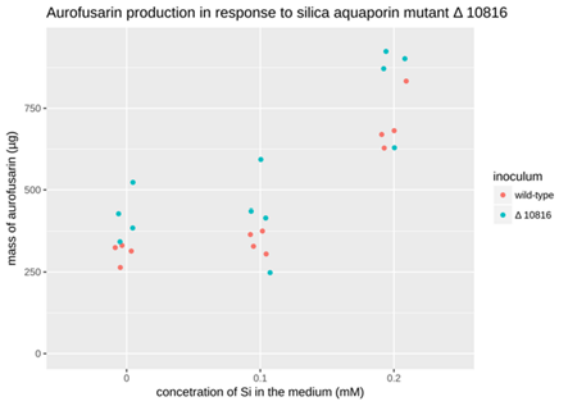

Figure 3. Generation of aurofusarin from wild-type and aquaporin mutant 10816 (see below). For both genotypes, the production of aurofusarin was significantly greater at 0.2 mM than at lower levels.

1) **major activities**: Analysis of the function of aquaporins in the fungal response to silica.

2) **specific objectives**: (b) Test the influence of Si levels in barley florets on the pathogenicity and perithecium development of *F. graminearum.* Analysis of low and high silica plants necessitates growing low silica plants, as we found, not an easy task. (c) Knockout genes associated with the presence of silica to determine how the fungus senses silica and how it is affecting pathogenicity. We focused on the analysis of aquaporin genes as sensors of silica due to the recent literature pointing to these as silica sensors in animals and plants.

3) **significant results**: We developed a system to grow low silica plants in hydroponic medium. The wild-type *F. graminearum* produced significantly ($p<0.05$) more perithecia on low silica plants than on high silica plants (grown in 0.1 mM vs. 1.0 mM; a mean of 10 perithecia per floret vs a mean of 0.5 perithecia per floret, respectively). Aquaporin mutants (mutants of FGSG10816 and FGSG811) produced more perithecia than wild-type on low silica plants. The data supports a reduction in perithecia with increased silica in plants. In addition, low silica increased disease. Finally, aquaporin knockouts were less aggressive pathogens. Several types of evidence suggest that 10816 is necessary for silica sensitivity and may be a silica transporter. Chemical analysis is in progress to determine if the knockout 10816 accumulates less silica than the wild-type. In addition, aquaporin 811 exhibits an inability to outcross although this is not explained by silica in the environment.

4) **key outcomes or other achievements:** These findings indicate that there is not an association with increased disease increased silica. However, the head blight pathogen senses and responds to silica. Pursuing the basis of that interaction may result in findings that would be helpful in reducing the disease. We are taking a different approach to that in our other study (see previous summary).

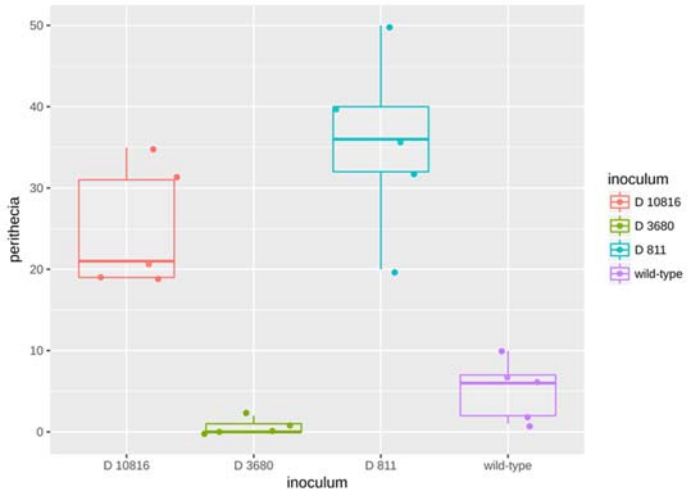

Figure 4. Perithecium production in the low silica hydroponic system, measured 14 dpi. (D next to gene number means "delta" or knockout).

Moving forward: There are 2 other genes that are members of the broad aquaporin family in *F. graminearum.* We are in the process of knocking these 2 out to determine potential involvement in silica transport. The chemical analysis of silica in wild-type and mutants will resolve the question of a tie of aquaporin 10816 to silica transport.

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

Benjamin Smith worked on this project as a technician. He is moving on to graduate school in biostatistics at MSU and gained a lot of experience in developing the hydroponic system and designing experiments. Molly Cavanaugh is a local high school student who has assisted on this project for the last 2 years. Molly has learned how to perform gene knockouts and

phenotyping and has been characterizing the aquaporin mutants. She is headed off to University of Michigan to the engineering program this fall.

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

We are working on a paper on these results which will be submitted this fall.

We have presented a poster:

Smith B., Trail F. 2017. The response of *Fusarium graminearum* to silica. Poster presented at the Fusarium Forum, Milwaukee, WI December.

Training of Next Generation Scientists

Instructions: Please answer the following questions as it pertains to the FY17 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 FY17 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 FY17 award period?** No

   If yes, how many?

3. **Have any post docs who worked for you during the FY17 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 FY17 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 FY17 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

Publications, Conference Papers, and Presentations

Instructions: Refer to the FY17-FPR_Instructions for detailed instructions for listing publications/presentations about your work that resulted from all of the projects included in the FY17 grant. Only include citations for publications submitted or presentations given during your award period (4/24/17 - 4/23/18). 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.

Journal publications.

Imboden L, Afton D, Trail F. 2017. Surface interactions of *Fusarium graminearum* on barley. Molecular Plant Pathology, DOI: 10.1111/mpp.12616.
Status: Published
Acknowledgement of Federal Support: YES

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

Other publications, conference papers and presentations.

Shay R., Trail F. 2017. Investigating barley defense response to Fusarium Head Blight. Poster presented at the Mycological Society Meeting. August, Athens, GA.

Shay R., Trail F. 2017. Investigating barley defense responses and interactions with *Fusarium graminearum*. Poster presented at the Fusarium Forum, Milwaukee, WI. December.

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Shay, R., Imboden, I., Afton, D. and Trail, F. 2018. Investigating the barley defense response to Fusarium Head Blight. In: Plant Science Graduate Student Research Symposium; Michigan State University.

For all posters:
Status: Abstract Published and Poster Presented
Acknowledgement of Federal Support: YES