USDA-ARS/
U.S. Wheat and Barley Scab Initiative
FY15 Final Performance Report
Due date: July 15, 2016

Cover Page

Principle Investigator (PI):	Frances Trail
Institution:	Michigan State University
E-mail:	trail@msu.edu
Phone:	517-432-2939
Fiscal Year:	2015
USDA-ARS Agreement ID:	59-0206-1-120
USDA-ARS Agreement Title:	Interactions of Fusarium graminearum, the Head Scab Pathogen, with Wheat and Barley.
FY15 USDA-ARS Award Amount:	$ 99,707
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:	RC100624
Project/Grant Reporting Period:	04/24/15-04/23/16
Reporting Period End Date:	04/23/16

USWBSI Individual Project(s)

USWBSI Research Category*	Project Title	ARS Award Amount
BAR-CP	Development of Transgenic Barley for Control of Scab.	$ 53,206
PBG	Use of Genes Important to Penetration for Control of FHB in Wheat and Barley.	$ 46,501
	FY15 Total ARS Award Amount	**$ 99,707**

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: *Development of Transgenic Barley for Control of Scab.*

1. **What are the major goals and objectives of the project?**
   The overall goals of the project are to identify genes that are essential to very early penetration and colonization of barley by *F. graminearum.*
   Objective 1. Identify genes important to early infection and spread through the plasmodesmata.
   Objective 2. Genes identified in Objective 1 as important for fungal movement through the plasmodesmata will be functionally tested by initially generating knockout mutants in the fungus.
   Objective 3. Design silencing vectors for barley for targeting genes early in development. These will be forwarded to Lynn Dahleen for transformation into barley.

2. **What was accomplished under these goals?**
   Objectives 1 and 2:
   1 and 2) major activities and specific objectives:
   To accomplish this, we used Laser Capture Microdissection (LCM) to cells where penetrating hyphae were moving through plasmodesmata. We extracted RNA, sequenced, and are identifying genes upregulated at this stage of fungal ingress.

   3) significant results:
   In preparing the tissue for sectioning (laser capture), we learned more about the initial infection of *F. graminearum* into trichomes and the movement through plasmodesmata, illustrated in Figure 1.

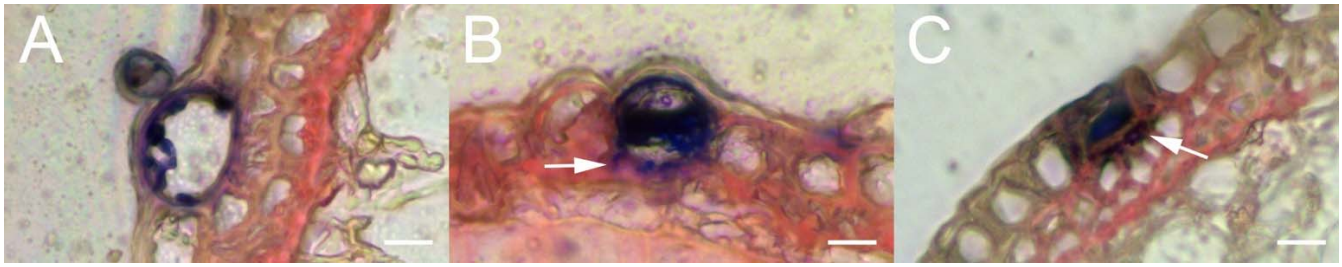

Figure 1. Initial infection sequence of barley paleae by *Fusarium graminearum*. (A) Early infection of barley trichome with bulbous hyphae inside the lumen of the trichome and colonization of the surrounding plant cell wall. (B) Heavily infected trichome with distinct hyphal invasion of neighboring plant cell and cell wall. White arrow points to ingress of infecting hyphae into the plasmodesmata as they move to the next cell. (C) Heavily infected non-trichome epithelium cell also showing distinct hyphae moving through plant cell walls to invade neighboring cells (white arrow). In all samples were stained with trypan blue and counterstained with Safranin O, resulting in fungus being purple and plant cell walls being light red. Scale bars are 10 μm.

   4) key outcomes or other achievements:
   This has been a challenging process. We are just completing a third set of RNA extractions. We will hopefully complete this soon, but will keep working on it until it is completed. We

will also complete the knockouts as soon as the sequencing is done. We have a team of undergrads trained in knockouts who will perform this task.

Objective 3.
1 and 2) major activities and specific objectives:
We worked with Lynn to generate RNAi expressing plants for the *VeA* gene from *F. graminearum.*
3) significant results:
We received putative barley transformants, some of which showed very strong resistance in a detached floret assay.

Figure 2. Florets from putative transgenic barley plants inoculated with *F. graminearu*m and are shown six days post-inoculation. Florets from putative transgenic plants with no macroscopic symptoms (left), or florets showing susceptibility (center), indistinguishable from inoculated wild type barley (right).

4) key outcomes or other achievements: We saw a very strong resistance in some of the transgenic plants. However, we were sent very little material, which took a long time to be sent and that was not well labeled, with controls missing. We are collaborating with Dr. Guo-qing Song, at MSU, who has a transgenic barley system. He has our set of vectors for the VeA gene and is doing the transformation so we can better test the outcome.

3. What opportunities for training and professional development has the project provided?
The project has trained a Postdoctoral associate in LCM usage and a second postdoc on analysis of transgenic plants.

4. How have the results been disseminated to communities of interest?
We are working on a manuscript on the LCM findings, which will be published when the project is completed. A second manuscript will be written on the transgenic findings.

Project 2: *Use of Genes Important to Penetration for Control of FHB in Wheat and Barley.*

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

Our overall goal is to identify targets for control that would be useful to address the very early stages of infection. Experimental evidence suggests that the aquaporin (channels that transport water, small carbohydrates, and possibly ions) mutations arrest fungal ingress at a very early stage, perhaps at the entrance of the infection peg into the plant. In light of these current findings, we proposed the following objectives:

(1) Perform detailed histological analysis on barley plants infected with each of the aquaporin mutants to identify the stage at which the ingress is halted. This will allow us to identify the most effective way to develop transgenic plants that silence aquaporins.

(2) Fully examine and characterize the wheat interactions with the aquaporin mutants to determine if their pathogenicity on wheat is similarly compromised.

(3) Develop RNAi vectors targeting aquaporins for expression in barley and wheat.

2. What was accomplished under these goals?

1-2) major activities for specific objectives:

Single aquaporin gene knockouts slow the growth of mycelia in a petri dish. In addition, on a detached barley floret assay, which tends to make the plant more susceptible, the disease production was attenuated, with PH-1 producing significant disease by 4 dpi and the 3 individual knockout strains having slight necrosis at the site of inoculation by 4 days. The aquaporin knockouts produce fewer perithecia in culture and development is delayed. We have generated single and double mutants of the three aquaporin genes found in *F. graminearum.* These mutants showed enhanced phenotypes of the single mutants.

3) significant results:

The single mutants of all three aquaporing show nearly identical phenotypes of attenuated disease production, indicating that all three function together, perhaps in forming a single pore. Localization will be done to resolve this question. No triple mutant could be generated, indicating that the absence of these genes may be lethal. We have preliminary evidence that the aquaporin mutants do not penetrate well or move from cell to cell effectively. This may have to with our recent work on the association of *F. graminearum* with silica containing cells, such as trichomes. In plant, aquaporins import silica, particularly specialized NIP proteins. If fungal aquaporins are similarly involved in silica transport, the aquaporin mutants may not be properly recognizing the silica cells such as trichomes on the surface of barley, resulting in their attenuated ingress into the host. We are working on sorting this out with the funding we have just received from USWBSI.

4) key outcomes or other achievements:

These aquaporin genes appear to affect the surface interactions of the fungus with the plant—both wheat and barley. As such it is a good target for HIGS. Since Lynn Dahleen is no longer in the scab arena, we are working with a new collaborator, Dr. Guo-qing Song at MSU, on generating some transgenic barley.

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

The project trained on postdoctoral associate in working with fungal-plant interactions. It also trained one undergraduate in gene knockout technology.

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

We are working on 2 scientific papers regarding this project. One should be submitting within the month to New Phytologist.

Training of Next Generation Scientists

Instructions: Please answer the following questions as it pertains to the FY15 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 FY15 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 FY15 award period?** No.

   If yes, how many?

3. **Have any post docs who worked for you during the FY15 award period and were supported by funding from your USWBSI grant taken faculty positions with universities?** One postdoc is teaching at a community college.

   If yes, how many?

4. **Have any post docs who worked for you during the FY15 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 FY15 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

Refer to the FY15-FPR_Instructions for listing publications/presentations about your work that resulted from all of the projects included in the FY15 grant. If you did not have any publications or presentations, state 'Nothing to Report' directly above the Journal publications section.

Journal publications.

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

Other publications, conference papers and presentations.

Imboden, L, Trail, F. 2015. Exploring the function of genes involved in disease initiation by *Fusarium graminearum.* Poster presented at the Fusarium Forum, St. Louis, Mo, December.
Status: Abstract Published and poster presented
Acknowledgement of Federal Support: YES

Roze, LV, Springer, J, Trail, F. 2015. Comparison of the resistance mechanisms to *Fusarium graminearum* infection in two resistant wheat cultivars. Poster presented at Fungal Genetics Meeting in Asilomar California, March.
Status: Abstract Published and poster presented
Acknowledgement of Federal Support: YES

Related publications:

Quesada-Ocampo L, Al-Haddad J, Scruggs AC, Buell CR, Trail F. 2016. Susceptibility of maize to stalk rot caused by *Fusarium graminearum* deoxynivalenol and zearalenone mutants. Phytopathology: Published ahead of print. doi: http://dx.doi.org/10.1094/PHYTO-09-15-0199-R.

F. Trail and A. D. Jones. 2015. Compounds for Inhibition of Fungal Toxin Production U.S. Patent Application Serial No.: PCT/US2015/034543.

Related funding:

- Development of agents to control mycotoxin contamination of agricultural commodities. TSGTD-Midwestern Ag. Company. $51,440. 3/1/16-2/28/17.
- Harnessing the transcriptome of conidial germination for pathogen control. USDA-AFRI. $300,000 to Trail lab. Trail (PI) with co-PI Jeff Townsend. 1/1/15- 12/31/2017. Use of USWBSI derived preliminary data facilitated the funding.
- Collaborative Research: Evolution of systems biology underlying fruiting body development in fungi. NSF. $497,000 to Trail. 5/1/15-4/31/18.