USDA-ARS/ U.S. Wheat and Barley Scab Initiative FY14 Final Performance Report July 15, 2015

Cover Page

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Fiscal Year:	FY14	
USDA-ARS Agreement ID:	59-0206-1-120	
USDA-ARS Agreement	Interactions of Fusarium graminearum, the Head Scab Pathogen,	
Title:	with Wheat and Barley.	
FY14 USDA-ARS Award	\$ 99,803	
Amount:	φ <i>77</i> ,00 <i>5</i>	

USWBSI Individual Project(s)

USWBSI Research Category*	Project Title	ARS Award Amount
BAR-CP	Development of Transgenic Barley for Control of Scab.	\$ 53,258
PBG	Use of Genes Important to Penetration for Control of FHB in Wheat and Barley.	\$ 46,545
	FY14 Total ARS Award Amount	\$ 99,804

Principal Investigator

Date

WES-CP – Western Coordinated Project

^{*} MGMT – FHB Management

FSTU - Food Safety, Toxicology, & Utilization of Mycotoxin-contaminated Grain

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

FY14 (approx. May 14 – May 15) PI: Trail, Frances USDA-ARS Agreement #: 59-0206-1-120

Project 1: Development of Transgenic Barley for Control of Scab.

1. What major problem or issue is being resolved relevant to Fusarium head blight (scab) and how are you resolving it?

Approaches that address early fungal ingress would be most effective in eliminating head blight disease, so we are focusing on early ingress of the fungus into the plant. Early infection genes are being identified from initial infection of the barley trichomes and associated plant tissue. This tissue has been collected by cutting out individual infected plant cells. RNA extraction and sequencing is being used to identify genes important to this process. We want to silence these genes using HIGS in barley. Because the identification of these early genes is taking longer than we expected, we have developed a pipeline for HIGS in parallel. We generated an RNAi silencing construct from *F. graminearum* gene *VeA*, which regulates toxin production at the chromatin structure level, and Lynn Dahleen transformed it into Barley plants. Our reasoning was that this gene would prevent DON production, and thus limit disease, but also, it would not be lethal to the fungus, which would allow beneficial fungi to continue to grow and live in the plant.

2. List the most important accomplishments and their impact (i.e. how are they being used) to minimize the threat of Fusarium Head Blight or to reduce mycotoxins. Complete both sections; repeat sections for each major accomplishment:

Accomplishment: Transgenic barley harboring the *VeA* RNAi construct appear to be highly effective in inhibiting disease. We are increasing seed so we can perform Southerns and expression tests on these lines.



Florets from putative transgenic barley plants generated in a single transformation event and wildtype barley were inoculated with *F. graminearu*m and are shown six days post-inoculation. Some putative transgenic plants have florets with little to no macroscopic symptoms (left) while other other plants have florets (center) that display symptoms indistinguishable from wildtype barley (right).

Impact:

We are very encouraged by these initial trials, which were repeatable with the limited material we had. Due to the departure of Lynn from the program, the material we received was very limited. We need to use the seed increases to generate more material for further

testing. In addition, we will redo the transformation, to determine the repeatability of the results.

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

1. What major problem or issue is being resolved relevant to Fusarium head blight (scab) and how are you resolving it?

This proposal addresses the 2014-2015 research priority for PBG: Develop new strategies for reducing impact of FHB disease and mycotoxin contamination in barley and wheat. Focus on pathogen genes and responses, including specific host target genes.

Our research goal is to develop new approaches to developing plant resistance to F. *graminearum* that enhance the resistance being developed by breeders. One such approach is the use of transgenics to engineer the plant to silence genes in the pathogen. We are searching for good targets in the pathogen that can be used for host expressed gene silencing (HIGS) as a means of engineering resistance to the head blight pathogen. Genes that we have found to be important in initial ingress into the plant are the aquaporin genes, the water channels.

2. List the most important accomplishments and their impact (i.e. how are they being used) to minimize the threat of Fusarium Head Blight or to reduce mycotoxins. Complete both sections; repeat sections for each major accomplishment:

Accomplishment: Following point inoculation, the mutants are severely compromised in the ability to spread beyond the site of inoculation in the head of the susceptible wheat. The mutants are able to grow on individual excised barley florets in a high humidity environment, but the aquaporin mutants grow at a slower rate than wild type accumulating less mycelial growth and less disease. Excised floret culture allows much of the resistance to break down, so any inhibition usually shows much greater strength when tested on intact plants. Generation and analysis of double mutant combinations is ongoing. In light of this result, we are generating host-induced gene silencing (HIGS) constructs to transform into barley to silence the fungal aquaporin genes. Generation of one construct to silence all three genes in transgenic barley is in progress.

Impact: We have identified a set of targets in the fungus that would likely increase host resistance using HIGS.

Training of Next Generation Scientists

Instructions: Please answer the following questions as it pertains to the FY14 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 FY14 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 FY14 award period? No

If yes, how many?

3. Have any post docs who worked for you during the FY14 award period and were supported by funding from your USWBSI grant taken faculty positions with **universities**? None

If yes, how many?

4. Have any post docs who worked for you during the FY14 award period and were supported by funding from your USWBSI grant gone on to take positions with private ag-related companies or federal agencies? None

If yes, how many?

Include below a list of all germplasm or cultivars released with full or partial support of the USWBSI during the FY14 award period. List the release notice or publication. Briefly describe the level of FHB resistance. *If not applicable because your grant did NOT include any VDHR-related projects, enter N/A below.*

N/A

Include below a list of the publications, presentations, peer-reviewed articles, and non-peer reviewed articles written about your work that resulted from all of the projects included in the FY14 grant. Please reference each item using an accepted journal format. If you need more space, continue the list on the next page.

Presentations:

Trail, F. 2015. Research on *Fusarium graminearum* on wheat. Presentation to the Michigan Wheat Growers. January 2015.

Trail, F. 2015. Research at MSU on Fusarium Head Blight. Presentation at the Wheat Field Day at Michigan State University, June.

Trail, F. and Roze, L.2014. Spread and colonization of *Fusarium graminearum* during infection in a resistant wheat cultivar carrying FHB1 resistance. Poster presented at the Fusarium Forum in St. Louis Mo, December.

Imboden, L. and Trail, F. 2014. *Fusarium graminearum* interaction with the epidermis of the barley palea. Poster presented at the Fusarium Forum in St. Louis Mo, December.

Results from this project were used obtain a USDA-NIFA grant:

• 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.