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

Cover Page

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Fiscal Year:	2018
USDA-ARS Agreement ID:	N/A
USDA-ARS Agreement Title:	Production of Widely-adapted FHB-resistant Barley Germplasm and Novel Information and Tools via Conventional and Transgenic Breeding Approaches.
FY18 USDA-ARS Award Amount:	$ 51,831

USWBSI Individual Project(s)

USWBSI Research Category*	Project Title	ARS Award Amount
BAR-CP	Identification, Characterization and Development of Widely-Adapted FHB-Resistant Germplasm.	$ 21,831
GDER	Down with DON: Stable Expression of Proven Genes in a Marker-Free Background.	$ 30,000
	FY18 Total ARS Award Amount	**$ 51,831**

Phil Bregitzer 7/12/19

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: *Identification, Characterization and Development of Widely-Adapted FHB-Resistant Germplasm.*

1. **What are the major goals and objectives of the project?**
   1) Identify resistant lines in elite winter germplasm
   2) utilize existing spring resistance sources for new crosses to a) create mapping populations and b) broaden the adaptability of Aberdeen FHB-resistant malting germplasm by introducing broad-spectrum disease resistance.

   Added objective: Investigate qPCR fungal biomass measurements as a proxy for DON

This project supplements the FHB resistance evaluation and breeding (spring malting) by Gongshe Hu

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

Winter germplasm screening

1) Major Activities/Specific Objectives

-Procedures for testing vernalized, transplanted-seedling hills of winter barley were refined and used to screen 200 winter barleys at Aberdeen. This procedure allows late spring planting that results in heading being delayed until warmer, FHB-conducive weather occurs.

--Obtained meaningful screening data on 150 winter lines from 2017/18 trials at Aberdeen and Virginia Tech (VT)

-- We increased our testing capacity by increasing support for, and the number of lines tested by, researchers at Virginia Tech. We initiated a new agreement with Cornell for testing winter barley. We expanded our collaboration with Juliet Marshall (University of Idaho) and assisted with the establishment of a Southern Idaho mist nursery at Kimberly.

--This work enables meeting objectives necessary to accomplish our goal of screening for resistance, namely putting in place new testing infrastructure, securing cooperation for access to existing infrastructure, and using these resources for phenotyping.

2) specific objective(s)

Identify resistant lines in elite winter germplasm

3) Significant Results

--Useful phenotyping data were returned from 2017/18 trials at VT and Aberdeen

--2018/19 trials in progress at VT, Aberdeen, and Kimberly. Cornell trials were destroyed by a harsh winter, which demonstrates the utility of our multi-location effort.

4) Key Outcomes

--The complexity of FHB phenotyping precludes definitive characterizations based on a single year of testing, but the available data indicate that there is variation for FHB resistance within the Aberdeen winter lines and opportunity to select for superior lines.

Mapping and development of new populations

1) Major Activities/Specific Objectives

--Initial crosses completed: 95SR316A/ND Genesis and 95SR316A/Gadsby

--95SR316A/ND Genesis population advanced using the doubled haploid (DH) technique by OSU cooperators. Seed was produced from >200 fully-fertile plants.

--95SR316A/Gadsby was not responsive to DH development. It was advanced instead using "rapid greenhouse cycling" (extended daylength and elevated temperature) to F3:4.

--This work represents successful accomplishment of our objectives for FY2018, which were to establish and advance populations that will allow us to understand the genetics of resistance in Aberdeen spring germplasm, and to produce germplasm that has utility for our program and for other breeding programs.

2) specific objective(s)

utilize existing spring resistance sources for new crosses to a) create mapping populations and b) broaden the adaptability of Aberdeen FHB-resistant malting germplasm by introducing broad-spectrum disease resistance.

3) Significant Results/Key Outcomes

--This work has not advanced to the point where meaningful data can be collected. However, these populations represent useful, new parental combinations. 95SR316A is stripe rust-resistant line with good agronomic and malting characteristics, and potentially useful partial FHB resistance. ND Genesis brings into the mix partial FHB resistance and resistance to foliar pathogens common to the upper Midwest. In particular, we were interested in its resistance to spot blotch and net blotch. Gadsby's reaction to FHB is not known to us, but it possesses resistance to net blotch and scald. These populations therefore represent resources that may contain novel FHB resistance alleles, and the presence of alleles for foliar pathogen resistance will make this germplasm easier to use for breeders outside of the Intermountain West.

PCR estimates of fungal biomass and relationship to DON

1) Major Activities/Specific Objectives

--unprocessed or previously-ground grain samples were obtained from cooperators at Idaho, Minnesota, North Dakota, Virginia Tech, and Cornell (7 locations total) and assessed for fungal biomass based on qPCR for F. graminearum TRI5 normalized against barley actin.

--Based on tests of 2125 samples, the relationship between DON and fungal biomass among locations ranged from r2=0.15 to 0.72, with an average r2=0.62. This

compares favorably to the relationship of visual severity ratings and DON, for which r^2 ranged from 0.1 to 0.47, with an average r^2=0.25.

--Our specific objective is an improved method of FHB infection severity (vs. visual scoring) that gives us a better idea of the level of mycotoxin contamination

2) specific objective(s)

Investigate qPCR fungal biomass measurements as a proxy for DON

3) Significant Results/Key Outcomes

--Despite a wide, location-dependent range of biomass:DON relationships, fungal biomass was a better predictor of mycotoxin contamination than visual severity estimates.

--The source of variability must be investigated. The highest correlations tended to come from samples we received as raw grain and which we extracted DNA from immediately after processing.

--More work is needed to determine the potential for using this method as a selection method. There is potential for using it to identify and eliminate lines that are most susceptible to mycotoxin contamination prior to screening them for DON via GC-MS.

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

At Aberdeen, phenotyping for FHB is a relatively new endeavor. All of us, from the PIs to the technicians, are becoming proficient in the nuances of FHB infection characteristics and methods for phenotyping, including the mechanics of running successful misting systems. The work is providing professional development opportunities for two post-doctoral researchers.

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

Reports of the preliminary field phenotyping data and of the qPCR biomass estimates were presented as posters at the 2018 FHB Forum. Now that the winter phenotyping nurseries in Idaho appear to giving us reliable data, we plan to disseminate the results also directly to producers via the UI Small Grains Extension Report.

Project 2: *Down with DON: Stable Expression of Proven Genes in a Marker-Free Background.*

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

Overall project goals:

1) Reduce FHB and DON in *F. graminearum* (*Fg*)-infected barley via expression of double-stranded (ds) RNA homologous to *Fg* genes for mycotoxin synthesis and/or pathogenicity.
2) Precisely deliver single-copy transgenes via novel methods: direct *Ds* transposition mediated delivery and recombinase mediated cassette exchange (RMCE).

The specific objectives for FY 18 and FY19 were to:

--Demonstrate RMCE functionality
--Produce transgenic barley with antifungal sequences (inverted repeats [IRs]) targeting *TRI6* and *NOXA*

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

1) and 2) Major Activities/Specific Objectives

--transgenic plants with site-specific recombination platforms produced.
--platforms induced to transpose. Plants with single-copy transposed and non-transposed platforms identified.
--a dsRNAi vector shown to have activity against DON accumulation introduced into these plants. Also an EXCH vector designed to exchange selectable markers at the TAG site was introduced.
--if successful, WCIC collaboration will permit completion of USWBSI project goals and contribute a system for barley transformation that promises genotype independence and reduced (or no) somaclonal variation.
--This work addressed both of the specific objectives for FY18 (which continue into FY19)

3) Significant Results

--multiple attempts to produce transgenic barley plants have failed. Various treatment combinations have been assessed based on expression of marker genes, and although robust transient expression of marker genes can be accomplished, transgenic cells are not surviving and proliferating under selection. We are seeking solutions in house. We are pursuing also collaborative solutions with the Wisconsin Crop Innovation Center (Middleton, WI), and have invested significantly in this effort that is designed to permit direct transformation of meristems.

4) Key Outcomes or Other Achievements

The overall rationale for this research is based on prior research by the PI and the co-PI that has shown that transposition and site specific recombination can solve the problem of compromised transgene performance that is based on imprecise insertion of transgenes that are not single, intact copies. Obviously, the failure to obtain transgenic plants at this state prevents the demonstration of the utility of these systems and the production of plants with anti-FHB transgenes. Work will continue.

As an alternative, our investment in research conducted by the WCIC is potentially transformative. In addition to bringing the talents and physical resources of a major, dedicated, and proven transformation facility to this project, we are focusing this effort on transformation of meristematic tissues. This approach promises to alleviate two major problems with barley transformation: 1) severe genotype restrictions, which limit the direct transformation of cultivars most relevant to gene discovery work by USWBSI; and 2) a reduction of somaclonal variation, which essentially is unwanted mutations caused by tissue culture. Extensive prior research by the PI has shown somaclonal variation in barley to be extensive and to cause significant changes to many traits. These changes complicate the assessment of the effects of transgene expression, and having a system for transformation that reduces or eliminates this problem will enhance gene discovery work.

Although no longer funded by the Barley CP, work on a related aspect of this project continues as we continue our research into RNA interference (RNA) in *F. graminearum.* This work was initially conducted as a means to vet the utility of potential barley transformation constructs to reduce FHB symptoms by testing them first in in *F. graminearum*. Over the life of this project, we have found:

--*TRI6* is a good candidate for silencing (Baldwin et al. 2018 PlosONE); *OAH* is not (unpublished*).
--Baldwin et al. 2018 showed small RNA (sRNA) signatures from *TRI6* silencing to be consistent & context-independent.
--This generated two hypotheses (tests in progress): 1) effective, minimal silencing vectors are predictable based on target sequence; and 2) effective silencing vectors are modular—that is, you can "mix and match" multiple individual dsRNA modules to predictively silence multiple *Fusarium* genes.
--*how to distribute "negative" data? (invaluable, unpublishable observations, mutants, and tools for *Fusarium)*: FgMutantDb created (Baldwin et al. 2018 FGB). An internationally-used, web-accessible repository of *Fusarium* wisdom and resources. Also provides annotation cross-referencing across multiple *Fusarium* genome databases.

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

Over the life of this project, there have been several undergraduate summer students from Idaho State University that have contributed to this project and to whom we have provided basic training on molecular procedures such as PCR and selection of plants based on molecular phenotyping. Ann Caspersen, the senior technician working on this project, has gained expertise in various approaches to transformation, and has become proficient in a number of advanced molecular recombinant DNA and PCR techniques, including digital droplet PCR. Post-Doctoral researcher Tom Baldwin has provided novel data to the fungal genetics and FHB research communities as a result of his involvement with this project. This project has been a significant contributor to his professional development as a *Fusarium* expert.

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

Presentations at USWBSI Forums and pee-reviewed publications

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. 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 presented at the FHB Forum:

> 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)

Journal publications.

Baldwin T, Arcibal S, Klos K, Marshall J, Bregitzer P (2019) Deletion of the benzoxazinoid detoxification gene *NAT1* in *Fusarium graminearum* reduces deoxynivalenol in spring wheat. PLOSOne (in press).
Status: Publication date July 12, 2019
Acknowledgement of Federal Support: Yes

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

Nothing to report

Other publications, conference papers and presentations.

Marshall JM, Arcibal SM, Bregitzer P, Baldwin T, Chen J. (2019) FHB now westward bound, and new struggles to keep DON down. In: Proceedings of the 2019 National Fusarium Head Blight Forum, St. Louis, MO p 29.
Status: Abstract published and talk presented
Acknowledgement of Federal Support: YES (talk), NO (abstract)

Baldwin T, Bregitzer P (2019) Information prepublication using FgMutantDB. In: Proceedings of the 2019 National Fusarium Head Blight Forum, St. Louis, MO p 81
Status: Abstract published and poster presented.
Acknowledgement of Federal Support: YES (poster), Yes (abstract)

Balwin T, Arcibal S, Marshall J, Bregitzer P (2019) *Fusarium* biomass measurements evaluated as a selection tool. In: Proceedings of the 2019 National Fusarium Head Blight Forum, St. Louis, MO p 102-105.
Status: Abstract and short paper published and poster presented
Acknowledgement of Federal Support: YES (poster), Yes (abstract)

Hu G, Bregitzer P, Satterfield K, Evans C, Marshall J (2019) FHB resistance of USDA-ARS barley breeding materials in Idaho. In: Proceedings of the 2019 National Fusarium Head Blight Forum, St. Louis, MO p 116.
Status: Abstract published and poster presented
Acknowledgement of Federal Support: YES (poster), Yes (abstract)

Meints B, Filichkin R, Helgerson L, Fisk S, Hayes PM (2019). Fusarium head blight resistance in barley. In: Proceedings of the 2019 National Fusarium Head Blight Forum, St. Louis, MO p 124.
Status: Abstract published and poster presented
Acknowledgement of Federal Support: YES (poster), Yes (abstract)