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:	N/A
USDA-ARS Agreement Title:	Down with DON: Stable Expression of Proven Genes in a Marker-free Background.
FY17 USDA-ARS Award Amount:	$ 51,246

USWBSI Individual Project(s)

USWBSI Research Category*	Project Title	ARS Award Amount
BAR-CP	Small RNAs in Response to RNAi Down Regulation in F. graminearum.	$ 26,246
GDER	Down with DON: Stable Expression of RNAi Contructs in a Marker-free Plant.	$ 25,000
	FY17 Total ARS Award Amount	**$ 51,246**

Phil Bregitzer 7/31/2018
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: *Small RNAs in Response to RNAi Down Regulation in F. graminearum.*

1. **What are the major goals and objectives of the project?**
   To enhance understanding of the process of RNA interference (RNAi) in *F. graminearum* in support of transgenic efforts to enhance FHB resistance (project 2). Key objectives are vector construction, fungal transformation with RNAi constructs that encode inverted repeats (IRs) that will produce double stranded (ds) RNA targeting fungal genes, and characterizations of fungal phenotypes and the small RNA (sRNA) profiles that result from degradation of dsRNA.

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

   1) Major activities:
   - Additional investigation of sRNA profiles generated from dsRNA vectors targeting *TRI6.* In FY17, a set of vectors encoding dsRNA encoding very short (50-150 bp) regions of *TRI6* were designed and constructed. Four of these vectors have been used for the production of new fungal mutant strains, and work to produce strains from an additional three very short vectors is in progress. This work will address the potential for predicting the potential silencing effects of short dsRNA vectors based on examination of the sRNA profiles generated from longer dsRNA vectors.
   - New candidate fungal genes for inclusion in RNAi targets designed to target multiple genes for silencing have been selected. Vector construction has been planned for several variants that will target *TRI6, NOXA,* and two novel pathogenicity factors that are expressed in cushion cells during the initial infection process. This work will address the modularity of individual short dsRNA vector components—that is, whether the function of individual vector components will retain their function when combined in a single vector.

   2) Specific objectives:
   These activities continued work which addressed objective 1 (construct and transform *Fg* with fungal vectors with variable IRs and different HR sites, targeting single genes as well as multiple genes); objective 2 (characterize transformants for their sRNA profile resulting from IR expression); and objective 3 (explore novel candidate pathogenicity factors for FHB and for pyramiding IR traits.

   3) Significant results:
   In FY17, we concentrated on the publication of the work with full-length and short IR vectors (see below), and on the development of new mutant strains transformed with very-short IR. We now have additional strains that will allow us to address questions raised by previous work. Experiments are in progress that we expect will generate additional sRNA profile data in the third quarter of 2018.

   4) Key outcomes or other achievements:
   The ability to silence genes using RNAi vectors is well-established, including the use of vectors designed to silence genes in an invading disease organism (aka host-induced gene

suppression). However, there is very little in the literature regarding rational, predictive design of RNAi vectors, and thus certain aspects of the failure or success of specific experiments are difficult to understand. This work is providing useful information that will lead to a more complete understanding of what is important for an effective RNAi vector. We have shown that specific, discontinuous, and repeatable sRNA profiles result from degradation of dsRNA produced by vectors targeting full-length *TRI6* (which has a regulatory role in mycotoxin synthesis) transcripts. This process was associated with the desired result of drastic reductions of mycotoxin synthesis. This result suggested that specific regions of *TRI6* could be targeted by shorter dsRNA-generating vectors, thus enabling the construction of shorter vectors that retained silencing activity but which would be easier to combine with dsRNA targeting additional genes and that would have less propensity for undesired off-target effects. Next, a set of RNAi vectors encoding IRs targeting shorter (200-350 bp) regions of *TRI6* were assembled and introduced into new fungal mutant strains. The targeted regions corresponded to regions of *TRI6* that generated specific peaks of sense or antisense sRNA, and sRNA profiling of these strains showed that the sRNA profiles of each short-IR strain. This suggested that dsRNA constructs might have "modularity", meaning that specific dsRNA sequences with silencing effects could be identified and assembled that would have a predictable effect, which would be particularly useful for the assembly of silencing vectors designed to target multiple fungal genes and pathways.

Other achievements over the life of this project include the determination that silencing the candidate target gene *OAH,* which is involved in oxalate production, did not reduce pathogenicity and thus would not have utility in a host-induced silencing approach to producing FHB resistance in barley. This is new information, but publication would be difficult because of its negative nature. To facilitate dissemination of this and other negative and preliminary results within the *Fusarium* research community, and to provide information on the existence and sources of useful mutants, a database (FgMutantDb) was developed, implemented, and described in the literature. Multiple researchers worldwide have contributed to and are participating in the use of this database. We have investigated also the utility of targeting *FDB2,* which detoxifies BOA, which is a defense compound produced by plants including members of the Triticieae. However, we discovered that silencing *FDB2* target would not be applicable to barley, but it may be useful to wheat, and we are working with Juliet Marshall to take this work forward on wheat. Related to this, we have shown that exogenous BOA application—and a related compound, gramene, which is present in barley--reduces DON production. This suggests a potential control method applicable to both barley and wheat.

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

All work involving the *F. graminearum* system is the responsibility of Tom Baldwin, post-doc with specific expertise in fungal genetics, and this work has enabled him to learn from and contribute to the community of FHB researchers. His work provides training in the most up-to-date concepts, procedures, and analytical techniques relevant to this field of genetic research. In addition, the communication of the PI's expertise in barley genetics and

transformation have expanded Dr. Baldwin's beyond fungal genetics. In addition, we employed a student assistant on this project, Cole Morrison, and involved him in these experiments. He now has a position with the Idaho State Dept. of Agriculture, where he will be dealing with invasive species.

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

Poster presentations at the 2016 and 2017 USWBSI Forums; at the 2017 Asilomar 29th annual Fungal Genetics conference; by preparing manuscripts for peer-reviewed publications (see below); and via personal communication with various members of the barley and fungal genetics communities. The development and deployment of FgMutantDB has involved substantial personal communication with curators of other fungal databases and *Fusarium* researchers.

Publications:

Baldwin, T. T. Basenko, E., Harb, O., Brown, N. A., Urban, M., Hammond-Kosack, K., and P. P. Bregitzer. Sharing mutants and experimental information prepublication using FgMutantDb (https://scabusa.org/FgMutantDb). **Fungal Genet. Biol. 115:90-93.** 2018

Thomas T. Baldwin, Emir Islamovic, Kathy Esvelt Klos, Paul Schwartz, James Gillespie, Samuel Hunter and Phil Bregitzer.Silencing efficiency of dsRNA fragments targeting *Fusarium graminearum TRI6* and patterns of small interfering RNA associated with reduced virulence and mycotoxin production. **PLOS ONE** (final revision under review).

Project 2: *Down with DON: Stable Expression of RNAi Contructs in a Marker-free Plant.*

1. **What are the major goals and objectives of the project?**
   This project is concentrated on the development of transgene delivery systems based on *Ds*-mediated transposition and recombinase-mediated cassette exchange (RMCE), and the delivery of transgenes that will reduce FHB and the accumulation of DON in barley.

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

   1) Major activities:
   - New barley *Ds* (transposition competent) direct-transformation vectors encoding dsRNA against *TRI6* are being introduced into barley.
   - *EXCH vectors encoding alternative selection genes are being introduced into barley lines previously transformed with TAG site-specific recombination platforms. This is "proof-of-concept" work that, if successful, will be followed up by introduction of antifungal genes.
   - *Assessment of progeny of crosses of plants with TAG site-specific recombination platforms to plants expressing *Ac transposase* were conducted with the objective of identifying plants that have a single-copy TAG platform segregated from the original transgene insertion site. Distribution of TAG sites to various genomic locations via transposition has been accomplished. Multiple lines with TAG transpositions have been identified and characterized for the location of the TAG site. Several lines have been identified where transposed TAG sites have segregated from the original, "empty-donor site" of the TAG transgene T-DNA insertion. Homozygous lines have been identified via progeny testing, and digital droplet PCR is being used to identify lines that have single TAG loci.

   2) Specific objectives:
   This work addressed objective 3 (Introduce dsRNA sequences effective against *Fg* into barley *Ds* and EXCH vectors), objective 4 (Produce transgenic Conlon plants with *Ds*-bordered *Ds*-vectors or TAG sites), objective 6 (Select plants with *Ds*-vectors or TAG sites segregated from *AcT* and the original insertion site). Objective 7 (Introduce EXCH vectors carrying antifungal transgenes that will be incorporated into TAG sites via site-specific recombination) has been addressed also, except that proof-of-concept work is using a test vector that does not incorporate and antifungal gene.

   3) Significant results:
   Distribution of TAG sites to various genomic locations via transposition has been accomplished. Multiple lines with TAG transpositions have been identified and characterized for the location of the TAG site. Several lines have been identified where transposed TAG sites have segregated from the original, "empty-donor site" of the TAG transgene T-DNA insertion. Homozygous lines have been identified via progeny testing, and digital droplet PCR is being used to identify lines that have single TAG loci.

   4) Key outcomes or other achievements:
   We now have the complete suite of tools (direct-*Ds*, TAG, EXCH vectors; transgenic plants with transposed TAG platforms) to demonstrate and/or utilize precise systems for

producing transgenic barley plants in which the transgenes are in suitable places for sustained expression, and in which extraneous sequences are not included.

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

Dr. Tom Baldwin, who brought a high level of expertise in fungal genetics and pathology to this project, has been given significant mentorship in practical aspects of barley and wheat production and in plant breeding, and in the uses of transformation for genetic studies and germplasm development. Dr. Bregitzer, his ARS colleagues Drs. Gongshe Hu and Kathy Klos, have worked closely with him, as has Dr. Juliet Marshall of the University of Idaho. As a result of these interactions, Tom has acquired a significant understanding how molecular genetics/pathology tools can be applied to selection techniques and cultivar development that directly address the needs of stakeholders in the agribusiness community.

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

Poster presentations at the 2016 and 2017 USWBSI Forums, and via personal communication with various members of the barley and fungal genetics communities. Publications related to certain aspects of this work have prepared (see project 1).

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. 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.

Baldwin, T. T. Basenko, E., Harb, O., Brown, N. A., Urban, M., Hammond-Kosack, K., and P. P. Bregitzer. Sharing mutants and experimental information prepublication using FgMutantDb (https://scabusa.org/FgMutantDb). **Fungal Genet. Biol. 115:90-93.** 2018

Thomas T. Baldwin, Emir Islamovic, Kathy Esvelt Klos, Paul Schwartz, James Gillespie, Samuel Hunter, and Phil Bregitzer.Silencing efficiency of dsRNA fragments targeting *Fusarium graminearum TRI6* and patterns of small interfering RNA associated with reduced virulence and mycotoxin production. **PLOS ONE** (final revision under review).

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

Other publications, conference papers and presentations.

Baldwin, T. T., Arcibal S. S., Bregitzer, P. P., and J. M. Marshall. Deletion of *FgNAT1* reveals a potential role of benzoxazinoids in suppressing DON accumulation. *Proceedings of the 2017 National Fusarium Head Blight Forum* (p. 43). East Lansing, MI/Lexington, KY: U.S.

Baldwin, T. T., and P. P. Bregitzer. Silencing efficiencies of RNAi vectors targeting specific 50 bp regions of *TRI6. Proceedings of the 2017 National Fusarium Head Blight Forum* (p. 44). East Lansing, MI/Lexington, KY: U.S.