USDA-ARS/
U.S. Wheat and Barley Scab Initiative
FY14 Final Performance Report
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Cover Page

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Fiscal Year:	FY14
USDA-ARS Agreement ID:	NA
USDA-ARS Agreement Title:	Down with DON: Stable Expression of Proven Genes in a Marker-free Background.
FY14 USDA-ARS Award Amount:	$ 25,000

USWBSI Individual Project(s)

USWBSI Research Category*	Project Title	ARS Award Amount
GDER	Down with DON: Stable Expression of Proven Genes in a Marker-free Background.	$ 25,000
	FY14 Total ARS Award Amount	**$ 25,000**

Principal Investigator Date

* 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
WES-CP – Western 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: *Down with DON: Stable Expression of Proven Genes in a Marker-free Background.*

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

We are investigating improved methods for transgene delivery, and for identifying genetic constructs (genes) with efficacy against *F. graminearum* growth and/or mycotoxin production. Genetic engineering can create valuable germplasm for genetic investigations of the host-pathogen interaction and for breeding programs. This technology is limited by our ability to rapidly identify effective transgenes, to produce plants with single-copy transgene insertions, to maintain robust and heritable expression of the transgene, and by transgene linkage to undesirable sequences derived from bacterial cloning vectors. Tools to solve these problems are needed, and our research is directed at developing and deploying these tools in barley.

We are using two methods for improving transgene delivery. One is direct *Ds* delivery, *i.e.* the delivery of transgenes as recombinant *Ds* transposons. Transgene transposition from the original locus to a new location can result, after segregation, in plants with single-copy transgenes free of vector DNA and undesirable transgene arrangements. Transposed loci typically have high and heritable levels of transgene expression. Application of this system is simple, requiring only conversion of a transgene to a synthetic *Ds* transposon via attachment of short sequences to each end of the transgene. These terminal sequences are recognized by a transposase enzyme (AcT), which is introduced via hybridization with the primary transgenic plant. Progeny are then screened for single-copy, vector-free, transposed transgene loci.

The second method is recombinase mediated cassette exchange (RMCE), also known as site-specific recombination. This method involves the production of Founder lines that contain a TAG locus possessing selectable markers bordered by specific recombination sites. Suitable Founder lines have single-copy TAG loci in areas supporting good transgene expression. We have engineered TAG loci as synthetic Ds transposons (*Ds*-TAG) to efficiently produce multiple novel Founder lines from a single transformation event. Transgenes of interest can then be incorporated into this locus by introducing an EXCH vector possessing the desired transgene bordered by recombinase recognition sites that interact specifically with the TAG recombination sites. The result is the exchange of the selectable markers for the transgene of interest, and a plant with a single copy of the desired transgene. This system has additional steps relative to direct Ds delivery, but Founder lines can be used repeatedly, enabling "stacking" multiple transgenes or analysis of different transgenes without site-of-insertion-based expression.

Identifying transgenes with activity against *F. graminearum* is complicated by the years-long process creating transgenic lines and making them ready for analysis. Therefore, we are investigating several methods of vetting construct components based on assessing their effects directly on *F. graminearum*. All current experiments involve the downregulation of key *F. graminearum* genes via RNA interference (RNAi), a system that is invoked by the presence of double stranded RNA (dsRNA). Two approaches are being used: direct delivery of synthetic dsRNA; and transformation of *F. graminearum* with dsRNA-encoding constructs. Transgenes currently being assayed are *TRI6* and *TRI10*, both transcription factors

for *TRI5* (trichothecene synthase = the first step in mycotoxin production). Other genes are being investigated but are not discussed here.

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: Production of *Ds*-TAG plants in Golden Promise, Conlon, and Pinnacle background, and making crosses of these plants to Golden Promise- or Conlon-background AcT-expressing plants.
Impact: These steps have created the raw materials for introducing RMCE into barley and the production of Founder lines. Introduction into Conlon and Pinnacle is noteworthy as these varieties have commercial relevance to the North American malting and brewing industry.

Accomplishment: Completion of the direct-delivery Ds vector, and insertion of an inverted repeat (IR) of *TRI6* into it. This vector includes a multicloning site between the maize ubiquitin promoter complex and a NOS terminus, all of which is flanked by Ds termini. Insertion of *TRI6* IR into the multicloning site enables a transposition-competent expression cassette that will express dsRNA and invoke RNAi against *TRI6*.
Impact: This vector allows immediate application of the direct Ds-delivery method using a transgene known to be a key component of the mycotoxin production pathway.

Accomplishment: Demonstration that synthetic, 21-mer dsRNAs (aka small interfering RNAs, siRNAs) designed against *TRI10* and *TRI6* can alter the expression of TRI5. However, effective concentrations were higher than reported in the literature. Furthermore, longer (200—850 bp) synthetic dsRNAs were not effective, even at 100X that reported in the literature.
Impact: Designing and synthesizing these molecules is easy. If they can be taken up by the fungus and processed by RNAi machinery, extremely rapid vector-component testing can take place. However, conflicting reports of efficacy exist. Our results suggest problems with dsRNA uptake that may be specific to our assay system. These results provide insight into the lack of consensus about this approach and suggest that more robust methodology should be used. Thus, these results validate the fungal transformation with dsRNA-encoding constructs as the most robust approach available to us..

Accomplishment: Demonstration that *F. graminearum* transformed with *TRI6* IR shows reduced *TRI5* expression (confirmation of a previous report); and that siRNA targeting TRI6 is produced as a result of *TRI6* IR expression (new information). Preliminary data suggests mycotoxin levels are associated with *TRI5* expression alterations.
Impact: Repeating prior work is of great importance, as the RNAi literature is rife with reports that have not been repeatable. Demonstration of sRNA production shows that the RNAi machinery appears to working as expected in *F. graminearum*, a conclusion that is still under investigation. Overall, the results show that our strategy of testing construct components directly in the fungus is valid. This will facilitate discovery of genes with

efficacy against *F. graminearum*, and enable concentration of barley transformation efforts on the introduction of proven construct components. This will increase the efficacy and reduce the costs of transgenic approaches to *F. graminiearum* resistance.

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?**

   If yes, how many? None.

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?**

   If yes, how many? None

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?**

   If yes, how many? None.

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?**

   If yes, how many? None.

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.

Baldwin, T., and P. Bregitzer. 2014. A rapid assay for synthetic siRNA activity against *TRI5*. Proceedings of the 2014 National Fusarium Head Blight Forum, p. 51, St. Louis, MO, Dec. 7-9, 2014.

Baldwin, T., and P. Bregitzer. 2015. A rapid assay for synthetic siRNA activity against *TRI5*. Proceeding of the 28th Fungal Genetics Conference, Genetics Society of America, p. 496, Pacific Grove, CA, March 17-22, 2015.

Bregitzer, P. 2014. Engineering FHB resistance in barley using precise transgene delivery methods. Invited presentation given at the Western Barley Growers Association, 37th Annual Convention, Calgary, AB, Canada, February 12-14. 2014.

Vence, T. 2014. Barf-less brews. TheScientist, published June 1, 2014, http://www.the-scientist.com/?articles.view/articleNo/40021/title/Barf-Less-Brews/.