

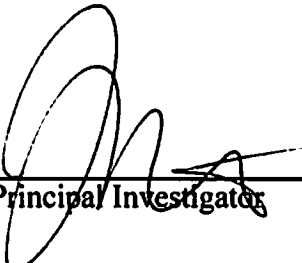
**USDA-ARS/  
U.S. Wheat and Barley Scab Initiative  
FY14 Final Performance Report  
July 15, 2015**

**Cover Page**

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<b>Fiscal Year:</b>	FY14
<b>USDA-ARS Agreement ID:</b>	NA
<b>USDA-ARS Agreement Title:</b>	Down with DON: Stable Expression of Proven Genes in a Marker-free Background.
<b>FY14 USDA-ARS Award Amount:</b>	\$ 5,000

**USWBSI Individual Project(s)**

<b>USWBSI Research Category*</b>	<b>Project Title</b>	<b>ARS Award Amount</b>
GDER	Down with DON: Stable Expression of Proven Genes in a Marker-free Background.	\$ 5,000
	<b>FY14 Total ARS Award Amount</b>	<b>\$ 5,000</b>

  
Principal Investigator

7/14/15  
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?**

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 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, which involves delivery of transgenes as synthetic *Ds* transposons. This method involves the transposition, or movement, of the transgene of interest from the original locus created by *Agrobacterium*-mediated transformation, to a new location. After segregation, this produces barley plants with single-copy transgenes free of the vector DNA and undesirable transgene arrangements that typically exist at the original locus. 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, which is introduced via hybridization with the primary transgenic plant (produced via *Agrobacterium*-mediated transformation). Progeny are then screened for single-copy, vector-free transgene loci that have transposed from the original, complex locus, and segregated away from the transposase to produce a plant with a relocated, stable, and useful transgene insertion.

The second method we are developing 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 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 once Founder lines are created there is a distinct advantage: any given Founder line can be used repeatedly, enabling the analysis of different transgenes without expression variability caused by variability in the sites of insertion. Furthermore, multiple transgenes can be stacked at the TAG locus.

The goal of our research is to use both of these methods to introduce transgenes conferring FHB resistance. Our choice of transgenes has evolved with our understanding of RNA

interference, and our approach will involve interfering with fungal development and mycotoxin production by suppressing the expression of key fungal genes via *in planta* production of double-stranded RNA that targets key gene transcripts for degradation

- 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:**

Recombinase mediate cassette exchange (RMCE) portion – James Thomson

Vectors have been assembled with components specific for use in monocot species. This includes multiple promoters and terminators for monocot expression so as to avoid duplication with commonly used genetic elements. Unique promoters have been obtained from a series of crop plants including rice, switchgrass, brachypodium and wheat. The synthesized, codon optimized and restriction site ablated Bxb1, CinH and phiC31 recombinase coding sequences have been cloned and are currently being ‘loaded’ into our novel EXCH vector system. The novel EXCH system is designed to allow large or multiple gene cassette cloning into a large capacity T-DNA, which in turn is compatible with the recombinase cassette exchange (RMCE) system. Cargo being loaded into the EXCH system includes an RNAi anti-fusarium knockout gene (provided by Dr. Bregitzer), selectable and visual markers for testing the system as well as the RMCE targeting system. Finally, a modified AcTransposase gene has been generated – to ease cloning difficulties – and is ready for EXCH loading and Barley founder line ‘TAG’ testing. Assuming no problems, both the EXCH and AcTransposase systems will be available for testing within three months.

**Impact:**

These critical steps of the RMCE (site-specific recombination) process enables development of Founder lines that will be used for insertion of anti-FHB transgenes into appropriate locations. Introduction into Conlon is especially desirable because of its relevance to the North American malting and brewing industry. Use of transpose-mediated transposition will allow production of more (and clean up of genetically complex lines) through a breeding process rather than a tissue culture. Thus one ‘good’ starting line could produce many more Founder lines with unique characteristics such as tissue specificity and or expression levels.

### **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?**

None

**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?**

None

**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?**

FY14 (approx. May 14 – May 15)

FY14 Final Performance Report

PI: Thomson, James J.

USDA-ARS Agreement #: NA

**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.**

**None.**

**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.**

**None.**