PI: Phil Bregitzer **PI's E-mail: Phil.Bregitzer@ARS.USDA.GOV**

Project ID: FY14-BR-014 **ARS Agreement #: NA**

Research Category: **GDER** **Duration of Award: 1 Year**

Project Title: Down with DON: Stable Expression of Proven Genes in a Marker-free Background.

PROJECT 2 ABSTRACT

(1 Page Limit)

The goal of the proposed research is to reduce FHB severity and DON in FHB-infected transgenic Conlon plants via expression of *Tri12* and *tlp*, and by RNA interference (RNAi)-based silencing of key genes in the trichothecene pathway. Initial transgene delivery will be by *Agrobacterium* infection, followed by secondary delivery via transposition. Transposition is enabled by flanking transgenes with short, maize-derived *Ds* terminal sequences which interact with *Ac transposase* (*AcT*). *AcT* will be introduced via hybridization with *AcT*-expressing plants. Transposition will deliver single-copy *Ds*-flanked transgenes (or *Ds*-flanked TAG vectors; see below) to regions that support stable transgene expression, and de-link them from vector backbone and selectable markers. This will enable production of transgenic plants without vector backbone. For recombinase-mediated cassette exchange (RMCE), *Ds*-flanked TAG sites, which enable site-specific recombination, will be introduced into Conlon. Transposition will move TAG sites to favorable genomic locations, and then EXCH vectors carrying antifungal transgenes will be introduced, enabling their incorporation into TAG sites via site-specific recombination.

An important new element of this proposal is that inverted repeats (IRs) of RNAi vectors will be tested for efficacy in *F. graminearum* (*Fg*) prior to their introduction into plants. This will enable rapid screening and optimization of potential RNAi vectors. We have secured special funding from ARS equivalent to ~90% of the salary of a post-doctoral researcher to support this aspect of our proposed research. Our objectives are:

1. Construct barley vectors for *Ds*/RMCE-based delivery of proven genes (UDP-glucosyltransferase, *Tri12*, *tlp*); (FY13).
2. Construct and transform *Fg* with fungal RNAi vectors against *Tri5* and *Tri6*; use *in vitro* assays to assess growth and DON reduction; and construct barley *Ds*/RMCE vectors based on the results.
3. Produce transgenic Conlon plants with *Ds*-bordered antifungal transgenes or TAG sites.
4. Initiate transposition of *Ds*-bordered antifungal transgenes and TAG sites by crossing to *AcT* plants.
5. Select plants with antifungal transgenes or TAG sites segregated from *AcT* and the original insertion site.
6. For RMCE only: Introduce EXCH vectors carrying antifungal transgenes that will be incorporated into TAG sites via site-specific recombination.
7. Characterize transgene expression, FHB severity/DON, plant performance, and develop resistant lines.

This proposal addresses the Gene Discovery and Engineering Resistance Research Area by: 1) Identifying candidate genes for resistance against FHB and/or reduced DON accumulation; 2) Developing effective FHB resistance and/or reduced DON accumulation through transgenic strategies; and 3) Developing improved methods for the generation of transgenic wheat and/or barley.