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Project ID: FY18-BR-029 | **ARS Agreement #:** *N/A*

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
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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).

Project objectives:
1. Construct a) *Ds*, b) RMCE, and c) EXCH barley vectors (completed)
2. Construct and test in *Fg* RNAi vectors targeting *TRI6* (completed) & NOXA (in progress)
3. Produce transgenic barley with *Ds*-bordered, transposed, single-copy TAG sites (completed)
4. Demonstrate RMCE functionality
5. Produce transgenic barley with antifungal sequences (inverted repeats [IRs]) targeting *TRI6* and *NOXA*
6. Characterize transgene expression, FHB severity/DON, plant performance, and develop resistant lines.

For FY18-19, objectives 4 and 5 will be addressed, and work may start on objective 6 depending on the success of initial efforts to create transgenic barley containing antifungal IR sequences.

Plans to accomplish project goals: IR sequences that induce RNA interference (RNAi) and silence genes for mycotoxin development and growth have been or will be identified in a fungal model that supports rapid assessment of efficacy. RMCE efficacy will be demonstrated by exchanging a *Ds*-transposed TAG site containing a hygromycin resistance cassette (now present in transgenic barley plants) with a cassette encoding glufosinate-ammonium resistance (carried on the EXCH vector). If successful, one or more effective IRs will be introduced into EXCH vector for introduction into barley. Concurrently, effective IRs will be incorporated into *Ds*-bordered vectors that are capable of transposition. These vectors can function regardless of the success of RMCE. Both approaches are expected to introduce transgenes into regions capable of supporting good expression (a characteristic of *Ds* transposition). Direct *Ds* delivery is simpler but the specific location of transposition is not controllable. RMCE, by producing lines (Founder lines) with a TAG site in a known location will enable multiple introductions of different sequences to a specific, known location, and will allow "stacking" multiple genes in the same location.

Statement of mutual interest: Transgenic strategies may enable higher levels of resistance more rapidly than conventional breeding, and enable rapid and complex queries of the host-pathogen interaction that can inform breeding strategies. Precise introduction will enable repeatable and meaningful results. Application of RNAi against FHB in the context of methodological improvements makes the research useful to all stakeholders from basic researchers to producers and users of barley.