Project FY24-PB-001: Explore RNAi to control FHB and mycotoxin contamination

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1. What are the major goals and objectives of the research project?

The goal of this project is to develop an endophytic fungal RNAi delivery platform to reduce FHB and mycotoxin contamination.

The objectives of this proposal are:

- **Objective 1:** Design and generate *F. graminearum* RNAi mutants targeting genes that are essential for its pathogenesis, and trichothecene and zearalenone biosynthesis, and determine their effects on toxin production and FHB severity.
- **Objective 2**: Build, evaluate, and optimize the *S. zeae*-mediated RNAi delivery system. We will generate *S. zeae* GFP-RNAi strains, examine RNAi molecule production and the transferring of RNAi signals from *S. zeae* to plants and *F. graminearum*, and determine gene silencing efficacy.

2. What was accomplished under these goals or objectives? *(For each major goal/objective, address these three items below.)*

What were the major activities?

Objective1

- We performed wheat heads treatment with *S. zeae* strain with different concentrations and examined disease and mycotoxin reduction efficacy.
- We performed wheat seeds treatment with *S. zeae* TRI5 strain with different concentrations and determined RNAi efficacy.

Objective 2:

- In collaboration with Dr. Innes from Indiana University, we isolated dsRNA and sReNA from cultures and media grown *S. zeae* GFP RNAi strain, performed Northern Blot. As a control, we also transformed Fusarium graminearum PH-1 strain with GFP RNAi construct, isolated sRNA and conducted Northern blot.

What were the significant results?

We confirmed that *S. zeae* GFP RNAi strain can produce and secret dsRNA. Using PH-1 strain expressing GFP RNAi as a control, we confirmed *S. zeae* can produce dsRNA but could not process dsRNA to sRNA.

List key outcomes or other achievements.

While we worked on *S. zeae* RNAi strain. We discovered that *S. zeae* alone can suppress 15-ADON production in cultures. In collaboration with Dr. Susan McCormick, we identified a secondary metabolite that can suppress Fusarium growth and toxin production. Further investigations are underway to check its ability to reduce fungal growth and toxin production by seed treatments.

3. **What opportunities for training and professional development has the project provided?**
   One ORISE fellow, Nick Rhodes, has been trained in molecular biology, such as RNA and sRNA isolation, generation GFP-labelled fungal strain, confocal microscope to investigate fungal colonization. He was also trained in seed treatment, fungal inoculation and FHB virulence assays.

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

   Poster presentation at the 2024 National Fusarium Head Blight Forum, Dec. 7-11, 2024. Austin, TX

5. **What do you plan to do during the next reporting period to accomplish the goals and objectives?**
   Funding for this project was terminated. We will summarize the data and prepare a manuscript for publication. In addition, we will do some follow up studies on the secondary metabolite we identified from *S. zeae* for potential FHB and toxin control.