ISRC Project Report, January 2024

1) **About the project**
   - ***Project Title*: Seed Treatment Effects on the Seed and Soil Microbiomes**
   - ***Lead PI***: Gary Munkvold, Dept. of Plant Pathology, Entomology, and Microbiology
     - **Co-PIs**: Larry Halverson, Dept. of Plant Pathology, Entomology, and Microbiology
   - ***Projects year(s)*:** October 1, 2022 to September 30, 2025
   - ***Total amount of funding*:** $200,000
   - ***Leveraged/Additional Funding, including federal or private organizations*:**

2) **Project Summary**

   - ***Objectives***: Determine the effects a chemical and a biological seed treatment on the spermosphere, rhizosphere, and endosphere microbiomes of soybean when planted in field soil that has and has not been inoculated with *Fusarium graminearum*.
   - ***Benefit to Soybean Farmers***: This project will help soybean farmers make more informed decisions when assessing the potential impact of seed treatments on their crops.

3) **Progress Report**: In the first year of this project, our primary task was to identify a graduate student to work on the project and establish an experimental system that would allow reliable microbiome sampling of spermosphere, rhizosphere, and endosphere. We successfully recruited a first-year student in the Interdepartmental Microbiology Graduate program after she had completed a rotation in our lab, joining the project May 2023. Here we outline progress made in establishing our experimental system. First, we decided to use soil from a typically managed corn-soybean rotation at the Marsden Long-term cropping system experimental site. This site was selected because the management history is well documented and our prior experience using this site for studies examining how crop diversification influences the soybean rhizosphere and endosphere microbiome. Second, we selected Williams82 soybean as the genotype to be used in these studies given its history and its well-characterized genetics. Third, preliminary experiments were conducted to identify a reasonable growth temperature for these experiments. We selected growth at 20 °C in order to mimic cool soil conditions during the planting season, while still allowing for relatively rapid germination and uniform emergence, facilitating collection of microbiome samples from plants at similar developmental stages. From this finding we were able to establish a timeline for collecting spermosphere and then rhizosphere and endosphere microbiome samples. Frequency of sampling will decrease over time since we anticipate seed treatments to have the greatest influence on the spermosphere microbiome assembly and the influence of the seed treatment on the rhizosphere and endosphere microbiome will diminish as the

root develops (See Figure 1). We will grow plants in containers packed to the same bulk density. Fourth, in ongoing experiments we are exploring two approaches (Figure 2) for inoculating soil with *F. graminearum* that would result in increased pathogen pressure without killing all the plants or making them too sickly. This will allow us to assess potential interactions between seed treatments and pathogen pressure on the soybean spermosphere and rhizosphere/endosphere microbiomes. Lastly, we are working to optimize DNA extraction from rhizosphere/spermosphere samples and plant endosphere for microbiome analyses and for assessing the extent of *F. graminearum* infection of plants.

4) **Supporting attachments**:

- ***Photos/graphs/other graphics***

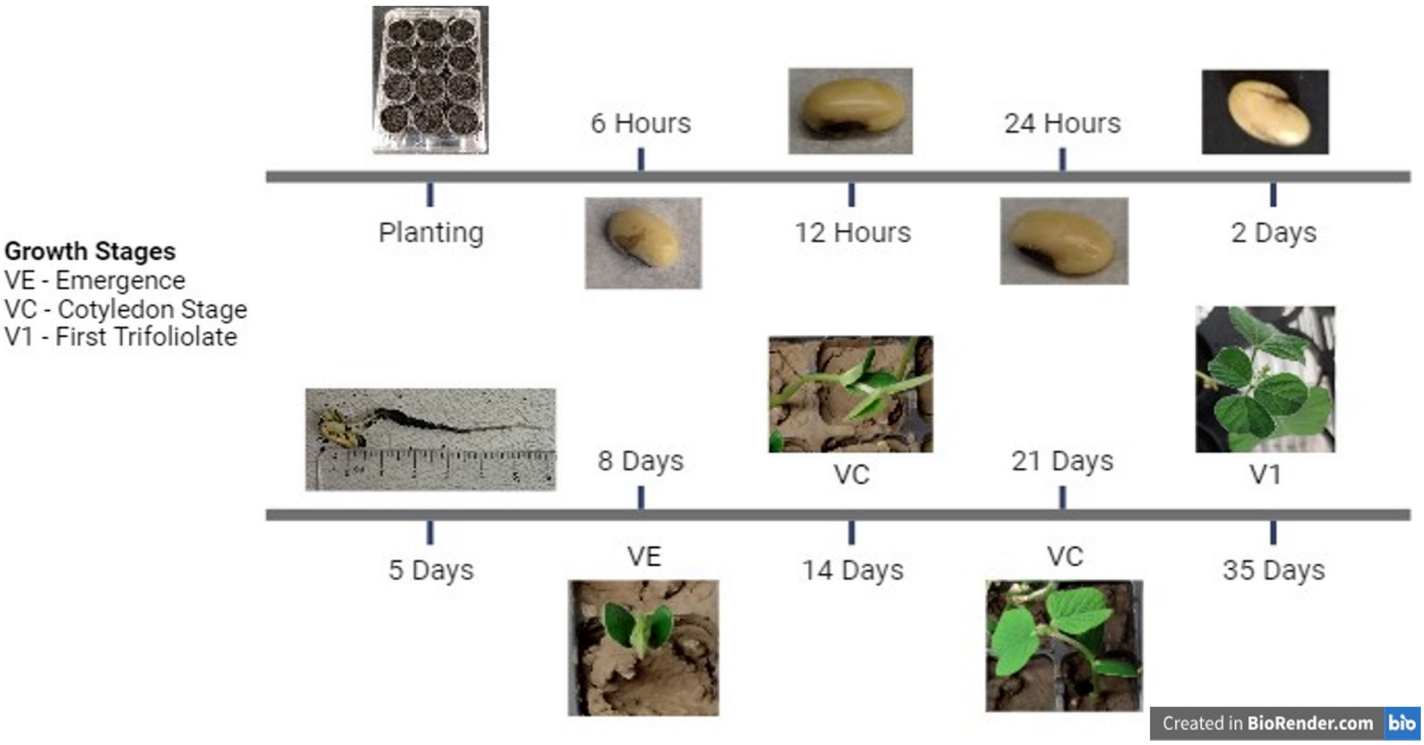

Figure 1. Sampling Timeline

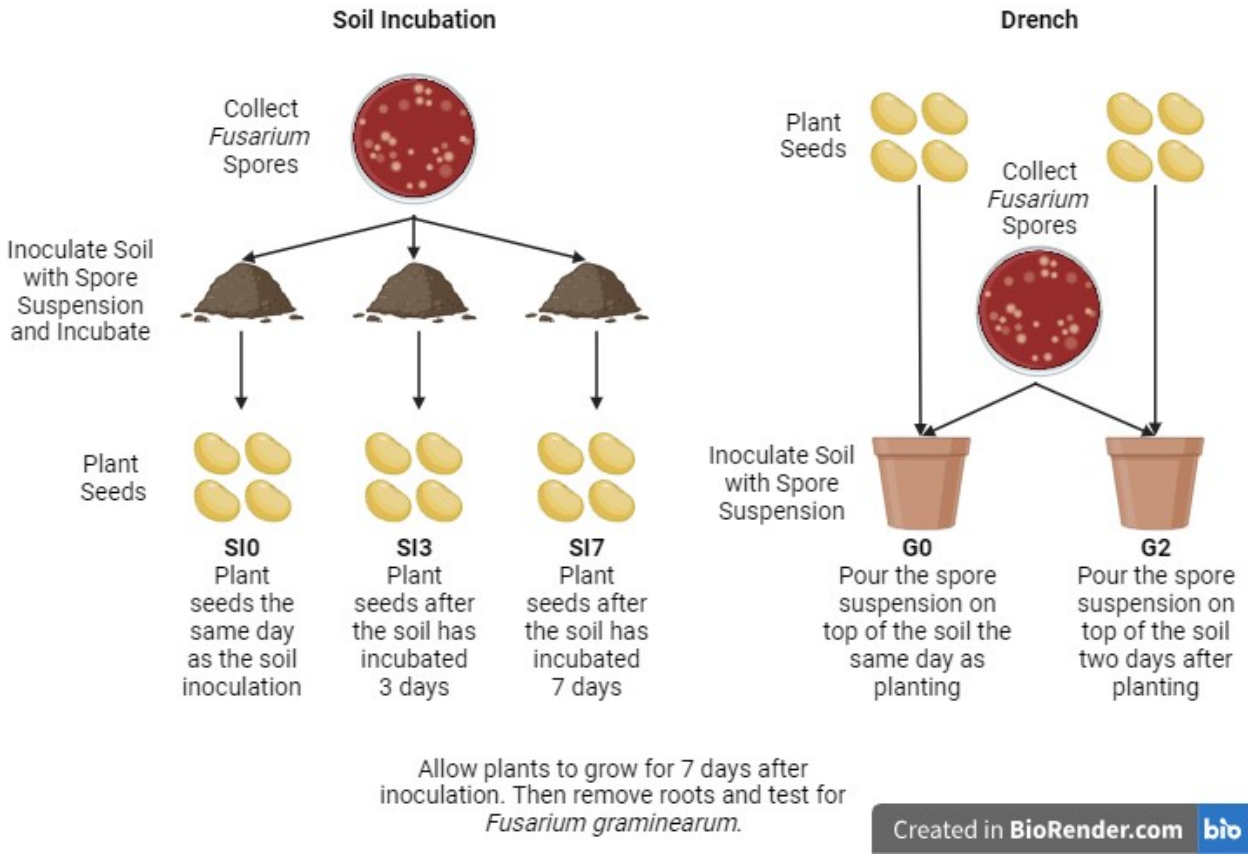

Figure 2. Assessment of *F. graminearum* soil inoculation protocols.