Technical report of the progresses on the MSSB project

(Quarter 1, March 14, 2025)

TITLE: Spray application of double stranded RNA (dsRNA) for simultaneous management of multiple soybean fungal and insect diseases

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The objectives of this proposed study in the third year are to: 1) Continue the effort to fine-tune the conditions to increase the efficacy of dsRNA in disease suppression; 2) Examine the potential of mixing different dsRNA to enhance their effectiveness in reducing disease symptoms under greenhouse conditions; and 3) Perform small scale field studies to determine the effectiveness of these dsRNAs in simultaneous management of CLB, FLS, and PSS through foliar applications.

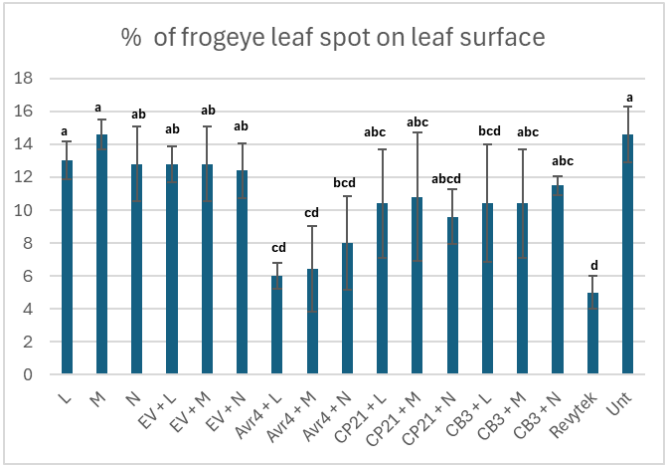

Figure 1. Assessment of frogeye leaf spot (FLS) disease symptoms using imageJ on soybean leaf samples collected 30 days after initial dsRNA treatment.

For this past quarter, we have been focusing on analyzing our soybean leaf samples collected from our field study in the fall at Ben Hur research station. Soybean variety (Syngenta NK43-Y9XFS) planted on June 12, 2024 was treated on a weekly basis for three times on August 19, 26 and Sep 3. Eighty plots consisted of 4 dsRNAs x 4 adjuvants x 5 replicates were used in this study. Each plot consists of 20 soybean plants. Soybean leaf samples were collected at 0 day (Aug 19), 10 day (Aug 29), 20 day (Sep 8) and 30 day after the initial treatment. We have completed the disease symptom assessment using ImageJ on the photos of leaf samples from different treatments we took in the field 30 days after initial treatment on Sept 18 (**Figure 1**).

We have also completed the DNA extraction from the field leaf samples collected at 0, 10 and 20 days after initial treatment. The real time qPCR quantification of the biomass of Cercospora pathogens in leaf samples collected 20 days after initial treatment is shown in **Figure 2**. Based on the data, the dsRNAs targeting Cercospora CB3 gene or CP21 gene were not as

effective in suppressing pathogen growth as targeting fungal Avr4 gene. In addition, among the three adjuvants we tested under field conditions, N and L are much better in enhancing the effectiveness of dsRNA than M.

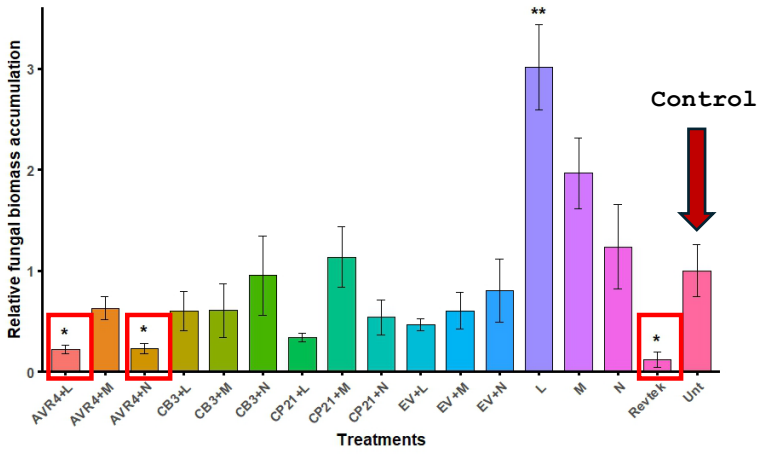

Figure 2. Real time PCR quantification of fungal biomass of Cercospora pathogen from soybean leaf samples collected 20 days after initial treatment with different dsRNA and adjuvant combinations.

In an effect to see whether mix different dsRNA can further boost the effectiveness in disease suppression, we conducted a greenhouse study from late December, however, due to the two unexpected cold freezing weather, which affected our soybean plants in the greenhouse and our visual assessment of the disease symptoms was inconclusive. We have started another batch of soybean plants and will be repeating this study shortly (**Figure 3**).

Figure 3. A new batch of soybean plants has been planted in the greenhouse for repeating the mixed dsRNA study shortly.