**Technical report of the progresses on the MSSB project**

**(Quarter 2, September 12, 2024)**

**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.

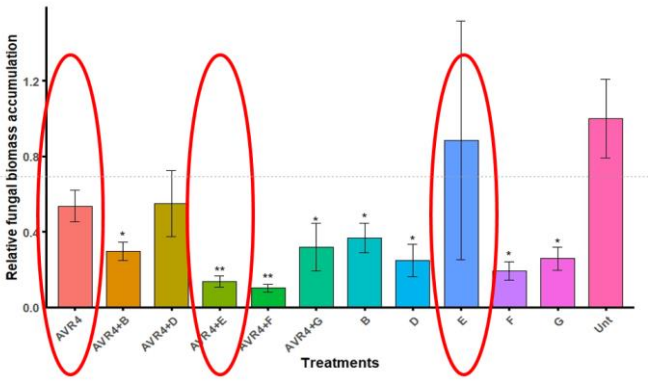


**Figure 1.** Evaluating the effect of different adjuvants on enhancing the dsRNA effectiveness in reducing soybean frogeye leaf spot (FLS) disease in greenhouse conditions.

For objective 1, in this quarter, we screened 5 different adjuvants for their effectiveness in enhancing the dsRNA ability in suppressing soybean frogeye leaf spot (FLS) disease in the greenhouse. After analyzing the collected soybean leaf samples for the fungal growth through real-time PCR, adjuvant E

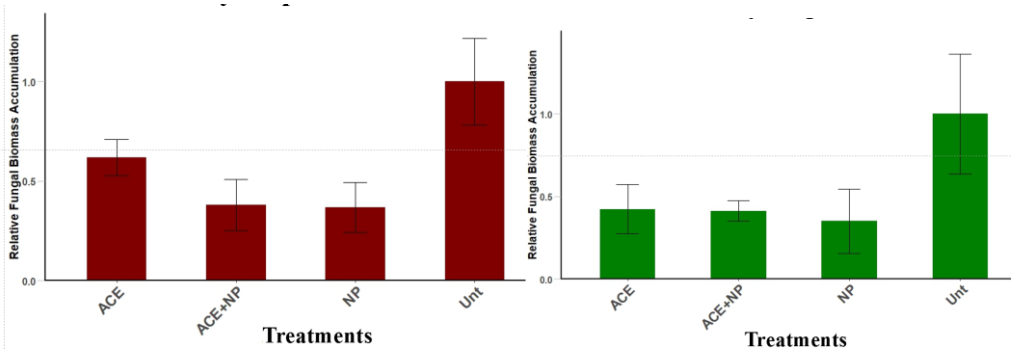


**Figure 2.** Differences in relative fungal growth among soybean plants that had been sprayed with dsRNA alone (ACE) or with dsRNA coated with nanoparticles (ACE+NP) compared to two controls (nanoparticles alone, NP and untreated plants, Unt) and exposed to direct sunlight for 7 days (left) and 10 days (right) before the plants were moved to the inside of greenhouse and inoculated with *Phakopsora pachyrhizi* (soybean rust). The leaf samples were collected two weeks after fungal inoculation and analyzed for fungal biomass using real time PCR.

was found to be very effective in enhancing the effect of Avr4 dsRNA on suppressing FLS (Figure 1).

For objective 2, we also finished analyzing the samples we collected in the winter on the ability of nano-particles in protecting the dsRNA and extending its effect on suppressing soybean rust disease development (Figure 2). The data did not show a clear protection of dsRNA+NP (nanoparticles with dsRNA) under sunlight for 5 or 7 days in comparison to dsRNA alone. We will try to repeat this experiment with more replications and also look into testing other nano materials.


**Figure 3.** Graduate student Sunira was spraying the soybean plants with various dsRNAs/adjuvants and evaluate for FLS symptom differences one week after each sprays (top). Clear reduction of FLS symptoms was observed after two sprays for plants treated with two of the dsRNAs. The commercial fungicide Revytek at the recommended rate was used as a positive control.

For objective 3, soybean variety (Syngenta NK43-Y9XFS) was planted three times (on May 22, 2024; June 12, 2024; and July 5, 2024). Soybean plants in the second planting were treated on a weekly basis for three times starting on August 19. Eighty plots consisted of 4 dsRNAs x 4 adjuvants x 5 replicates were used. Each plot consists of 20 soybean plants. Visual assessment showed that at least two dsRNAs with formulation L showed a clear reduction in FLS symptoms when the FLS symptoms were evaluated after two sprays on Sep 3 (Figure 3).