Technical report of the progresses on the MSSB project

(Quarter 3, December 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.

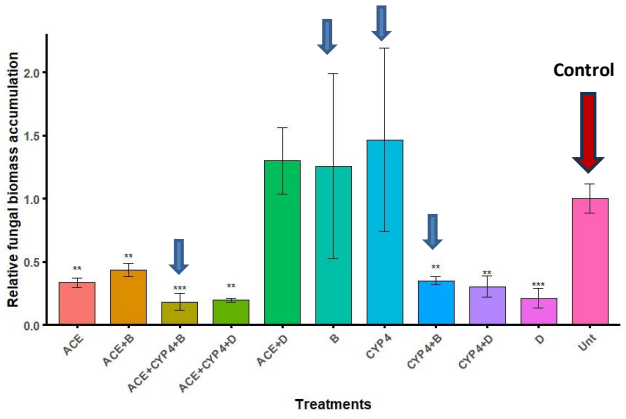

Figure 1. Evaluating the effect of adjuvant B and D on enhancing the dsRNA effectiveness in reducing Asian soybean rust (ASR) disease in greenhouse conditions. Adjuvant B (blue arrow) clearly increased the effect of CYP4 dsRNA.

For objective 1, in our study reported in the previous quarter, we identified adjuvant E was the best among the 5 different adjuvants we screened for its ability in enhancing the effectiveness of Avr4 dsRNA in suppressing frogeye leaf spot (FLS) disease. In this quarter, two of the above adjuvants were also screened for their effectiveness in enhancing the dsRNA ability in suppressing soybean rust disease in the greenhouse. After analyzing the collected soybean leaf samples for the fungal growth through real-time PCR, adjuvant B was found to more effective than D in enhancing the effect of CYP4 dsRNA on suppressing soybean rust (Figure 1). Without adjuvant B, CYP4 was ineffective in suppressing ASR.

For objective 2, we reported our findings on nanoparticles in protecting dsRNA under natural weather conditions in last quarter. We need to repeat this experiment with more replications and also look into testing other nano materials.

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 on August 19, 26 and Sep 3. Eighty plots consisted of 4 dsRNAs x 4 adjuvants x 5 replicates were used. Each plot consists of 20 soybean plants. Soybean leaf samples were collected at 0 day (Aug 19), 10 day (Aug 29) and 20 day (Sep 8) after the

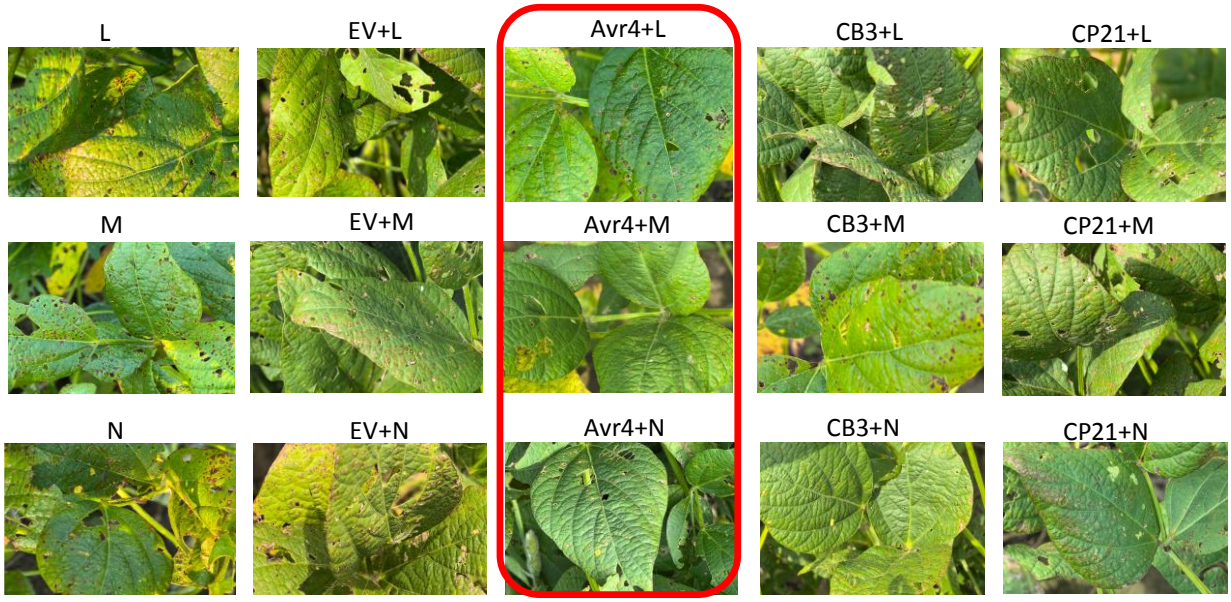

Figure 2. Representative images of soybean leaves treated with different adjuvants or dsRNA plus adjuvants from five replicated field plots to show the differences in FLS disease severity. The visual assessment was done one month after the initial dsRNA treatment of soybean plants (Syngenta NK43-Y9XFS) at R3 stage on August 19, 2024.

initial treatment. Visual assessment of FLS disease severity conducted on 30 days after initial treatment on Sept 18 showed that Avr4 dsRNA is the most effective in reducing FLS severity (Figure 2, Table 1). Among the three different adjuvants we used in the field, they all seemed effective in enhancing dsRNA uptake.

Table 1. FLS disease severity rating from five replicated trial plots for each treatment based on a 1 to 10 scale*.

	Average Disease Severity		
	L	M	N
Adjuvant only	7	8.2	7.4
EV	7.4	7.4	7.2
Avr4	4	4.2	5
CB3	6.2	6.2	6.2
CP21	6.2	6.4	5.8

*: The Revetek treated soybean leaves have a disease rating of 3 or 3-4.

Our field evaluation also indicated that one application Revtek (8 oz/acre) at R3 effectively reduced FLS disease symptoms even after one month when compared to untreated control soybeans (data not shown).

In addition, we have completed the DNA extraction from the field collected leaf samples and are conducting qPCR to quantify the biomass of Cercospora pathogens. We should have our results on biomass of Cercospora pathogens detected in the collected soybean leaf samples in the next several weeks.