Technical report of the progresses on the MSSB project

(Quarter 3, 2023)

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 second year are to: 1) Continue the effort to fine-tune the conditions to effectively deliver dsRNA into soybean plants; 2) Examine the potential of nano-particles in enhancing dsRNA stability on leaf surface; and 3) Perform various greenhouse or field studies to determine the effectiveness of these dsRNAs in simultaneous management of CLB, FLS, and PSS through folia applications.

In this quarter, we mainly focused our research on objectives 1 and 3. For objective 1, we have been testing different adjuvants to determine their potential in enhancing dsRNA delivery. In last quarter, a couple of them were found to enhance the efficacy of applied dsRNA by about 2 fold. In this quarter, we are testing several more adjuvants and we are still analyzing the data for their effectiveness (**Figure 1**). For objective 3, besides repeating the dsRNA treatments in greenhouse, we setup a field study in 2023. The soybean plants were planted on May 15, May 30 and June 12, 2023. The soybean plants from the second planting that were treated with different dsRNAs once on July 28, 2023 did not show a clear difference in FLS symptoms among different treatments with dsRNAs against Avr4, CP21, CTB1, CTB8 and EV. The soybean plants from the third planting were treated with four dsRNAs and two controls with 6 replicates per treatment. Ten soybean plants were used in

Figure 1. Adjuvants were found to be able to increase the effectiveness of ACE dsRNA in reducing soybean rust disease.

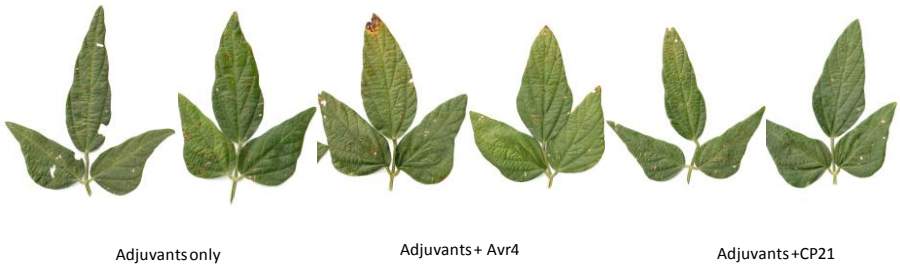

Figure 2. Differences in FLS symptoms of samples of soybean leaves collected from the field study that had been treated with adjuvants or adiuvants with dsRNAs.

each treatment. The soybean plants were sprayed with dsRNAs three times (August 10, 15, and 24). Preliminary assessment of the FLS symptoms showed some reduction in FLS when treated with CP21 dsRNA (**Figure 2**).