Technical report on the progresses of the MSSB project

(Quarter 4, December 12, 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 project 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.

For the fourth quarter, we mainly focused on analyzing the leaf samples we collected from the field. Two separate field studies were conducted in the field in 2025: one for evaluating additional dsRNAs for their efficacy in suppressing Frogeye leaf spot and Cercospora leaf blight and one for assessing the nanoparticles and adjuvants in enhancing dsRNA stability and uptake by soybean plants (**Figure 1**). Based on our greenhouse study of three different nanoparticles in the last year, we selected the best one (LDH) and adjuvant L to continue their evaluation under field conditions in 2025. Soybean (Syngenta NK43-Y9XFS) were planted on three separate dates: May 19th, June 2nd, and June 16th, 2025.

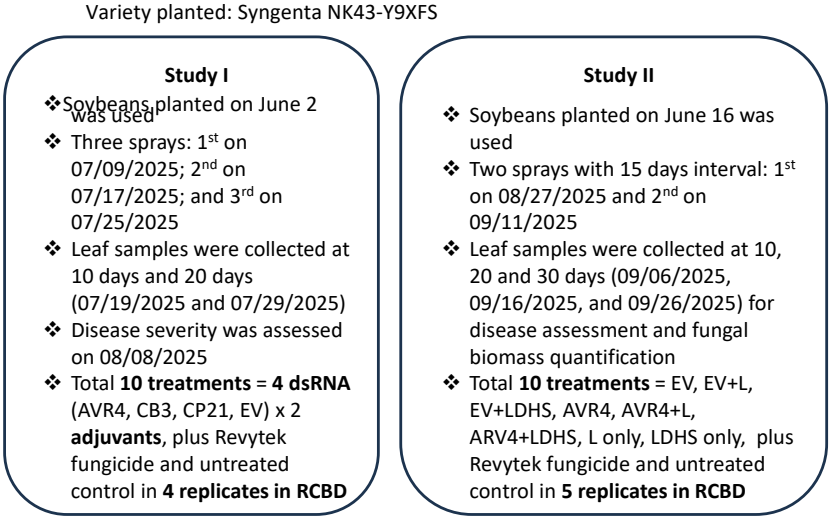

Figure 1. Two separated field studies were planned in 2025. The effort to evaluate additional dsRNA for managing FLS under field conditions through **study I** was not conclusive due to daily afternoon rains in early to mid-July that made the dsRNA treatment did not significantly reduce the FLS severity based on visual assessment. The **study II** using later planted soybean on evaluating the effect of different nanomaterials was successful.

Leaf samples were collected at 10, 20 and 30 days after the initial dsRNA application from Study II. We have just finished analyzing the 10-day leaf samples and both adjuvant L and

nanoparticles enhanced the effect of dsRNA in suppressing fungal growth in treated soybean leaves (**Figure 2**). We have finished scanning the soybean leaves we collected to quantify the disease severity through Image J software analysis. We are also continuing working extracting RNAs from the collected leaves and perform quantification of fungal growth in the collected samples.

We also conducted several other studies: one is to identify additional targets for suppressing soybean rust and Cercospora leaf blight or frogeye leaf spot diseases. We screened 11 additional gene targets and identified that three of these targets were very sensitive to suppression by foliar sprayed dsRNAs, such as S10, S12, CYTB1 and CYTB2. They are as effective or even better as the previous reported gene target ACE in our growth chamber and greenhouse studies (**Figure 3, next page**). Through similar studies, we have also identified two more gene targets for suppression of FLS disease via dsRNA: CTB1 and CB3.

In addition, we just finished preparing a manuscript summarizing our recent findings, which we plan to get it submitted shortly.

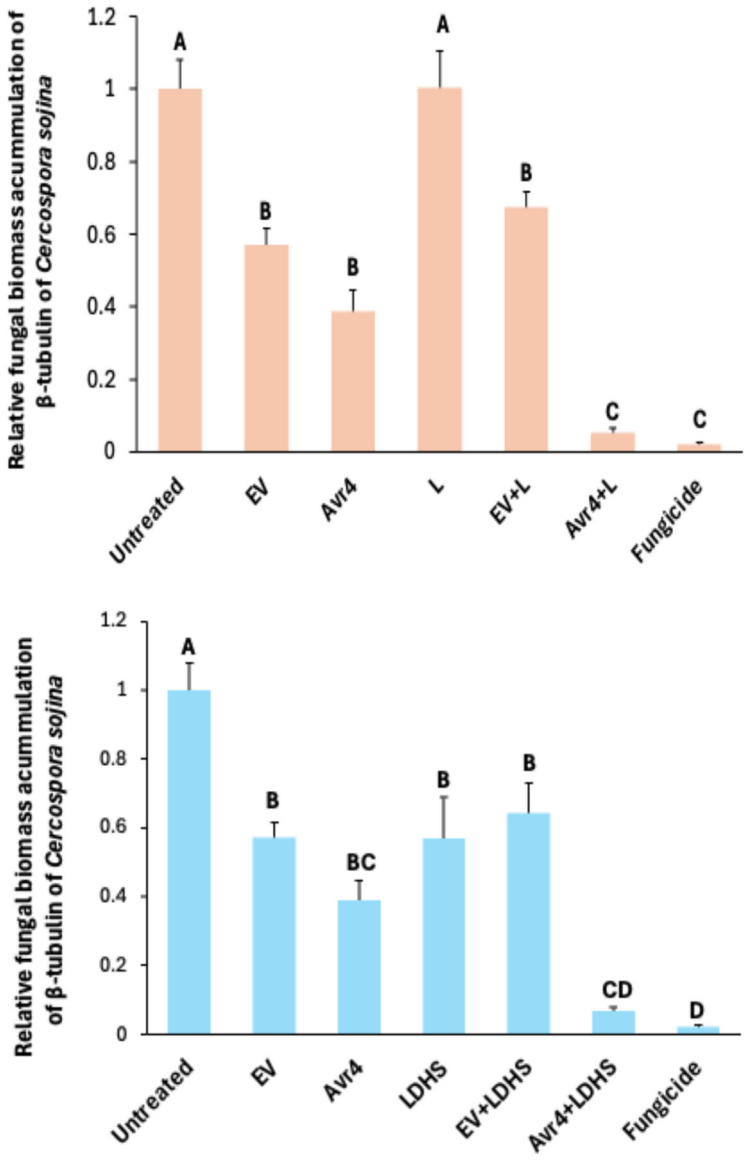

Figure 2. Top: The real time PCR quantification of fungal growth in the soybean leaves 10 days after being treated with Avr4 dsRNA only or with Adjuvant L in comparison to control leaves treated with EV dsRNA with or without Adjuvant L or not treated or to fungicide Revytek treated control. Bottom: Fungal growth in the soybean leaves treated with Avr4 dsRNA with or without nanoparticle LDHS in comparison to controls 10 days after initial dsRNA application.

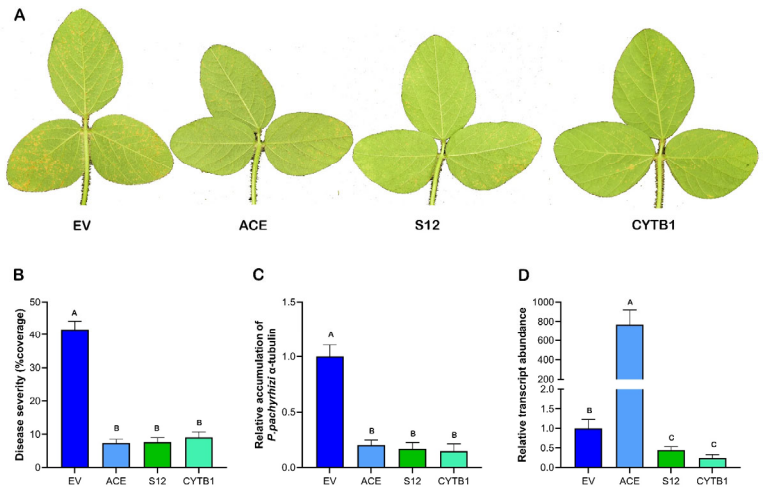

Figure 3. Effect of different dsRNAs on suppression of Asian soybean rust in greenhouse conditions. **A.** Visual soybean rust disease symptoms of representative leaves that had been treated with dsRNAs of target genes: ACE, S12 and CYTB1 with empty vector (EV) dsRNA as a negative control. Photos were taken 14 days after total RNA (250 ng/µL) treatments and inoculation with urediniospores of *P. pachyrhizi*. **B.** The rust disease severity of soybean leaves was quantified using ImageJ (Fiji). **C.** Relative accumulation of *P. pachyrhizi* α-tubulin gene (as an indicator of fungal growth or biomass) to soybean ubiquitin gene (as an indicator of soybean biomass) was quantified by real time PCR with the EV set at 1. **D.** Relative levels of target gene expression in soybean leaves 2 weeks post-inoculation with *P. pachyrhizi.* Values are expressed relative to the endogenous *P. pachyrhizi* α-tubulin gene with the EV set at 1. Data are presented as means ± standard error of the mean (SEM). One-way ANOVA followed by Tukey's multiple comparison test was used to separate the differences. Bars with different letters are significantly different at $P \leqslant 0.05$.