Technical report of the progresses on the MSSB project

(Quarter 2, June 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.

For this quarter, we have conducted several studies to increase the dsRNA efficacy. One of the studies was to understand how dsRNA suppresses fungal disease development on soybean by examining the dsRNA treatment on fungal spore germination and hyphal growth under in *vitro* conditions. This study tested five different dsRNAs and a control dsRNA from empty vector (EV). Most of the dsRNAs we examined significantly reduced fungal growth by over 70% (**Figure 1, bottom**) and one of the dsRNA (H12) also has the most effect on suppressing fungal spore germination, which reduced the spore germination rate from ~75% to less than 5% (**Figure 1, top**). The other study we conducted was to assess the

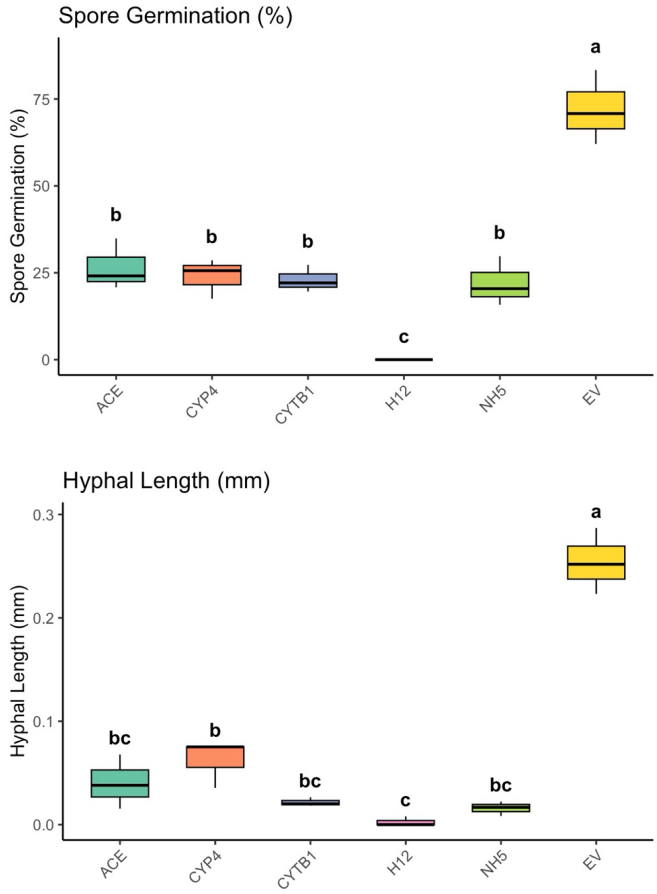

Figure 1. The effect of dsRNA treatment *in vitro* on soybean rust spore germination (top) and hyphal growth (bottom). The spore germination was measured 4.5 hrs after dsRNA treatment. The hyphal growth was measured at 9 hrs after dsRNA treatment. Treatments not sharing the same letter are significantly different based on Tukey's Honest Significant Difference (HSD) post hoc test following one-way ANOVA ($p < 0.05$).

effect of different nanoparticles in protecting the dsRNA. We examined the effect of three different dsRNAs with or without being coated with one of the three different nanoparticles in protecting soybean plants from fungal diseases in the greenhouse conditions (**Figure 2, top**). Based on our real time PCR quantification of fungal growth in inoculated soybean leaves, all three dsRNAs provided significant protection against fungal infection in comparison to the untreated control or soybean plants treated with dsRNA against green fluorescent protein (GFP) (another control) (**Figure 2, bottom**). Currently, this study is being repeated under field conditions. For performing small scale field studies to determine the effectiveness of dsRNA in managing soybean Cercospora diseases, the first batch of soybean (Syngenta NK43-Y9XFS) was planted on May 19, 2025 (**Figure 3, next page**) and second batch was planted on June 2, 2025. The third batch will be planted next week. These soybean plants will be treated with dsRNA with different adjuvants and or nanomaterials before and after Cercospora diseases start to show up in the field.

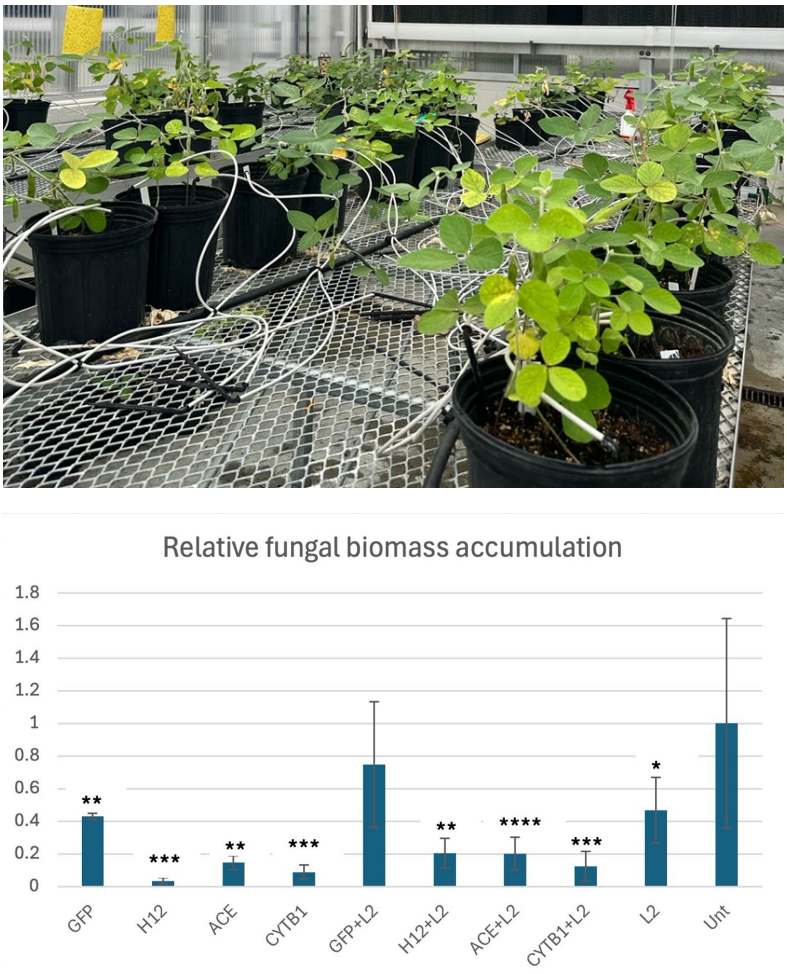

Figure 2. Top: Greenhouse study of the effect of different dsRNAs with or without nanomaterials in suppressing Asian Soybean Rust. **Bottom:** Real time PCR quantification of relative fungal biomass of Asian Soybean Rust pathogen in soybean leaf samples collected two weeks after initial treatment with different dsRNAs with or without nanoparticle L2 in comparison to that in the dsRNA untreated soybean leaf samples (control), which is normalized to 1. Student's t test was performed to compare the differences among different treatments to the Unt control. The error bars represent mean values ± SEM four biological replicates. Asterisks indicate statistical significance: *:p <0.05, **:p <0.01, ***p<0.001, ****p<0.0001 in comparison to untreated control.

Figure 3. Soybean seeds have been planted in Ben Hur Research Station in separate batches since mid-May for the planned small scale field studies in the late summer with our dsRNA, adjuvants and nanomaterials to assess their effectiveness in managing soybean Cercospora diseases.