Technical report on the progresses of the MSSB project

(June 11, 2026)

TITLE: Spray application of double stranded RNA (dsRNA) for simultaneous management of multiple soybean fungal and insect diseases

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The 2026 objectives of this project year are to: 1) Examine the effect of different nanomaterials (NM) in protecting the dsRNA; 2) Examine different dsRNA+NM on reducing FLS disease under field conditions; and 3) evaluate the formulations with the best adjuvant and NM in reducing dsRNA concentration required for effective management of FLS, CLB, and PSS under field conditions.

In the past quarter, we focused on several studies. The first study was to examine how dsRNA suppresses FLS disease development. In our repeated greenhouse and field studies, we have consistently observed some of the dsRNAs were very effective in reducing the disease symptoms. In order to understand the mechanism of disease suppression by these dsRNAs, we conducted an *in vitro* study to examine the germination and hyphal growth of *C. sojina* spores in

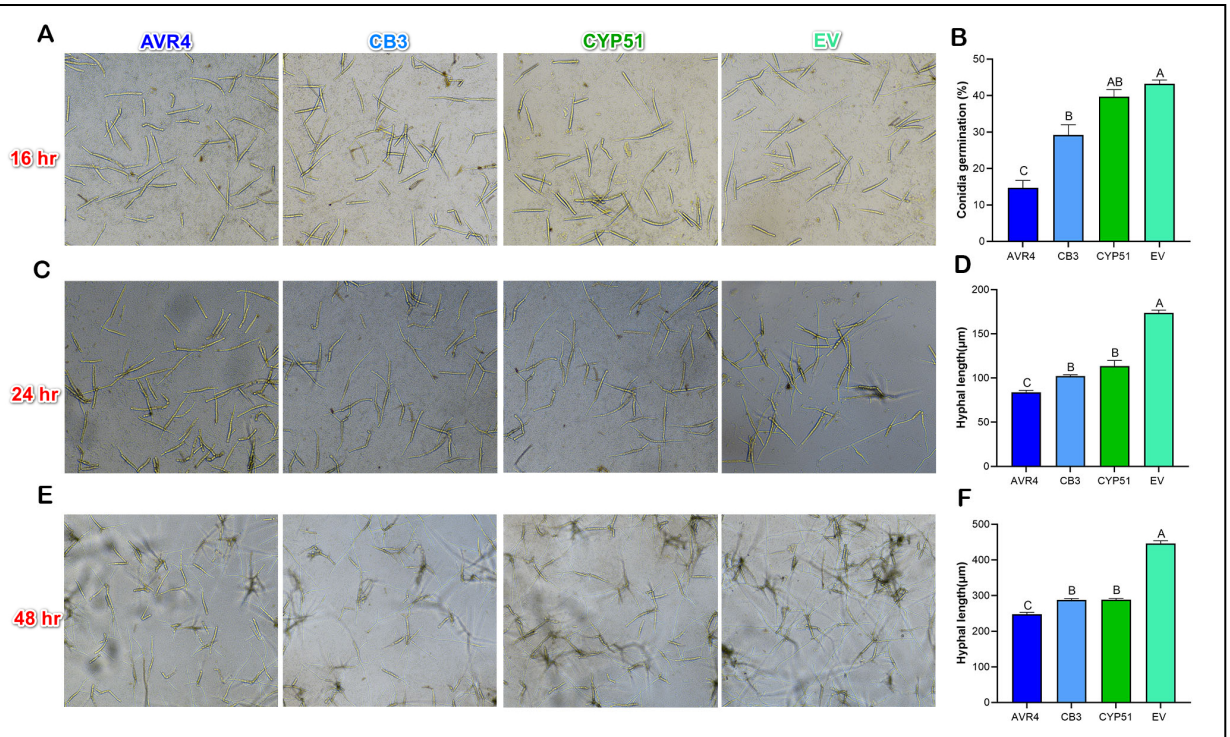

Figure 1. The effect of different dsRNAs in suppressing *Cercospora sojina* spore germination and hyphal growth *in vitro*.

culture in the presence of various dsRNAs for up to 48 hrs. The germination rate of the Avr4 dsRNA treated spores after 16 hrs was only about 1/3 of the control, which was treated with dsRNA from bacterial cultures containing only the empty vector (EV). The hyphal length of Avr4 treated spores was only about 60% of the control at 48 hrs. The other two dsRNAs (CB3 and CYP51 were less effective in suppressing either spore germination or hyphal growth (**Figure 1**).

The second study was to examine whether chitosan nanoparticles can enhance the effectiveness of dsRNA in suppressing soybean fungal diseases. We have completed our first greenhouse study with chitosan nanoparticles and dsRNA for suppressing Asian soybean rust. In the last report, we showed that chitosan NP was as good or even better than LDHS in enhancing the effect of dsRNA. Here are some of the representative scanned leaf samples showing the difference in disease severity with different dsRNA and NP treatments (**Figure 2**). We are in the process of quantifying the disease severity and fungal growth inside the soybean leaves among different treatments.

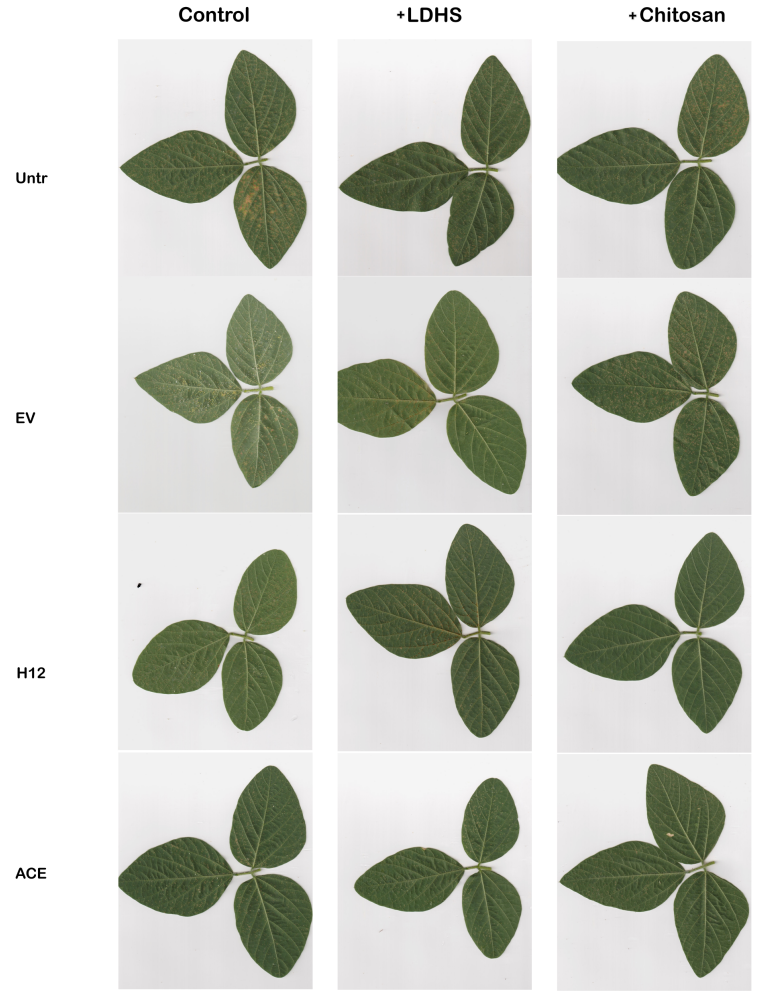

Figure 2. Visual ASR disease symptoms of soybean plants treated with different dsRNA with or without LDHS or chitosan nanoparticles (NP).

We have also extracted RNA from these treatments for RNA sequencing. The purpose is to determine whether the expression of the specific *P. pachyrhizi* genes was reduced when soybean plants were sprayed with the corresponding dsRNA as well as whether coating of dsRNA with NP can further suppress the target gene expression. So far, 20 transcriptome libraries have been sequenced and a total of 1,857,873,076 raw reads or a total of 1,788,501,920 clean reads were generated. We are currently still in the process of mining the data.

In order to determine how stable our dsRNA with or without coating with nanoparticles is in the natural environment, we have conducted a series of *in vitro* studies. First, we exposed our dsRNA to UV radiation at a strength similar to the sunlight, we noticed the majority of our dsRNA is degraded after exposing to UV for over 3 days (**Figure 3A**). We also found out that dsRNA solutions are very stable under room temperature. The dsRNA integrity did not change significantly after being left at room temperature for 4 months (**Figure 3B**). In a more fine-tuned study, which the quality of dsRNA was checked every 3 hours after being continuously exposed to UV. It is clear that the gene specific dsRNA is almost completely degraded around 48 hours (**Figure 3C**). Coating the dsRNA with chitosan nanoparticles can clearly extend the integrity of dsRNA to over 81 hours (from 2 days to almost 4 days) (**Figure 3D**). In addition, dsRNA when being mixed with microbes extracted from leaf tissue or soil did not seem to significantly affect its stability. The majority of the dsRNA remained intact up to 6-7 days (**Figure 3E and 3F**). This study will allow us to be more precisely deciding how frequent the soybean plants need to be sprayed with dsRNA in order to obtain a satisfactory control of soybean fungal diseases.

In addition, we have planted soybeans in the field for examining how effective nanoparticles and adjuvants in enhancing dsRNA suppression of soybean diseases under field conditions (Objective 2 of the 2026 project). The first planting was on 4/28/26 and the second planting was on 5/23/26 (**Figure 4**). We will have another planting in the coming week.

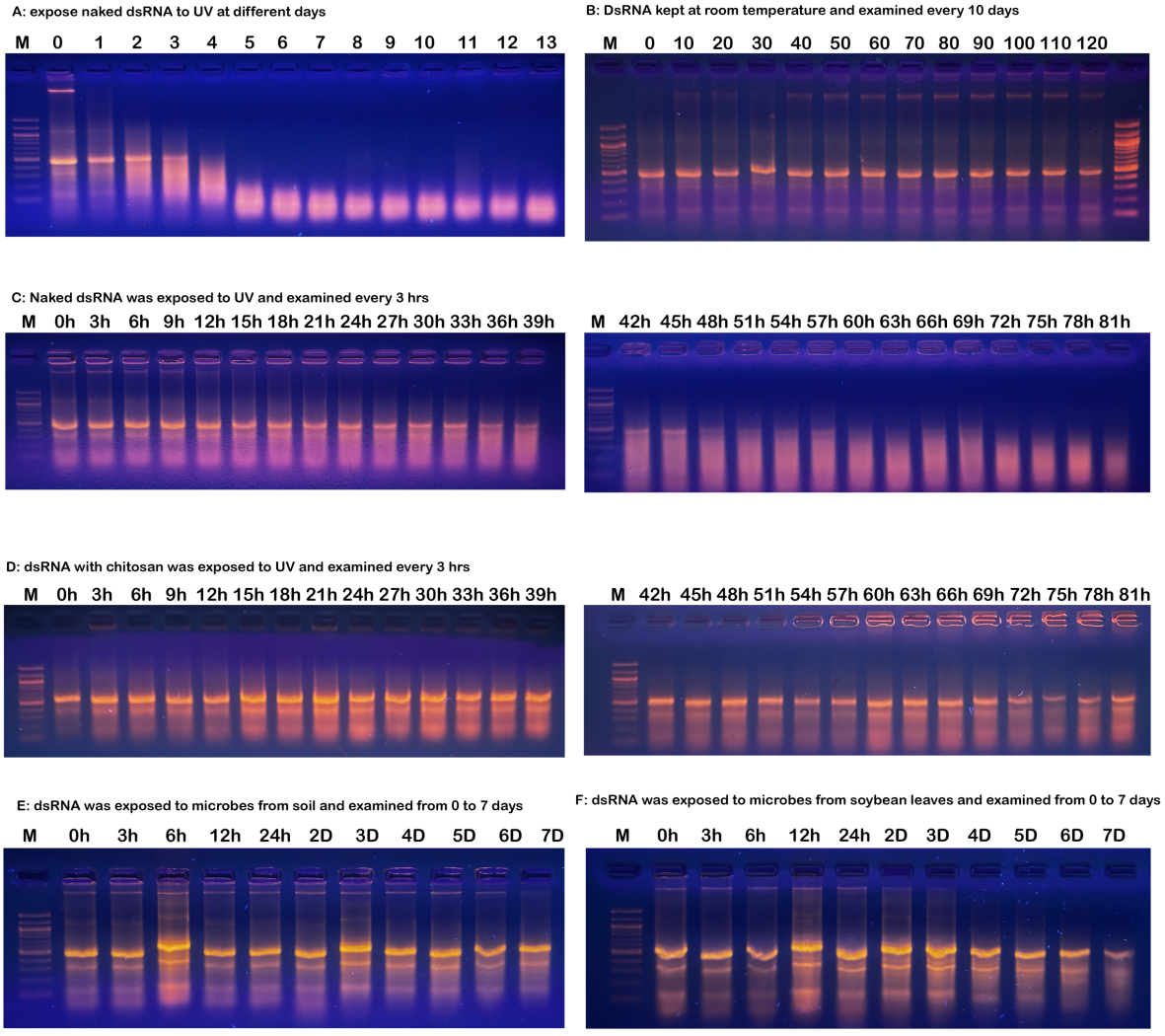

Figure 3. Examining the stability of dsRNA under different environmental conditions and the potential of using chitosan nanoparticles (NP) to protect the dsRNA from damage caused by UV radiation.

Figure 4. Soybeans have been planted at Ben Hur Research Station for examining the effect of dsRNA with nanoparticles, adjuvants, and a combination of both in managing Cercospora Leaf Blight and Frogeye Leaf Spot diseases under field conditions.