Technical report on the progresses of the MSSB project

(March 13, 2026)

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

Investigator: Zhi-Yuan Chen, Professor, Department of Plant Pathology and Crop Physiology, Louisiana University Agricultural Center, Baton Rouge, Tel 225-578-7850; email: zchen@agcenter.lsu.edu

The 2026 objectives of this project year are to: 1) Examine the effect of different nanomaterials (NM) in protecting the dsRNA; Examine different dsRNA+NM on reducing FLS disease under field conditions; and 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 mainly focused on completing the analysis of leaf samples we collected from the two field studies we conducted in 2025 for suppression of Cercospora spp growth by sprayed dsRNA with or without adjuvants. For study I, we have finished scanning the soybean leaves we collected to quantify the disease severity through Image J software analysis. In comparison to untreated or adjuvant only or EV with adjuvant negative controls and Revytek fungicide treated positive control, the target dsRNA with adjuvants can greatly reduce the percentage leaf area (**Figure 1A**) or lesion density (**Figure 1B**) on soybean 15 days after the initial dsRNA application. For study II, we completed extracting RNAs from the collected leaves and finished quantifying fungal growth via real time qPCR. The results were reported in last quarter. Currently, we are analyzing the collected leaf samples for quantitative disease severity assessment through ImageJ analysis. **Figure 2 (page 2)** is a visual assessment of differences of frogeye leaf spot (FLS) symptoms among different treatments 30 days after the initial dsRNA application.

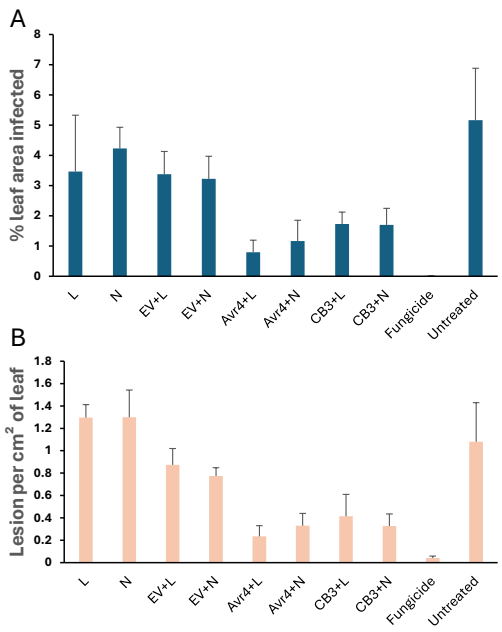

Figure 1. Cercospora disease symptoms (expressed as percentage leaf area, **A** or as lesions per square centimeters, **B**) of soybean leaves 15 days after being sprayed with adjuvants (L, N) or control dsRNA from empty vector (EV) or dsRNA targeting Cercospora pathogens (Avr4, CTB3) compared to non-treated negative control or fungicide Revytek treated positive control.

We also have started synthesizing new nanoparticles and examining their potential in increasing the protection and uptake of dsRNA for

more effective disease management. One of them is chitosan nanoparticles. It was synthesized through three simple steps of mixing tripolyphosphate and chitosan, homogenizing the mixture, and filtration (**Figure 3A, page 3**). Electron microscopic examination of the newly synthesized nanoparticles revealed that they are very homogenous in size of about 60-80 nm (**Figure 3 B and C**), which is ideal for absorption by plants. The chitosan nanoparticles (NP) can bind dsRNA very effectively at a 1 volume of NP with 1.2 volume of dsRNA (**Figure 3 D**). We have conducted a greenhouse study comparing the chitosan NP coated dsRNA compared to non-coated naked dsRNA and to dsRNA coated with layered double hydroxide with palm oil nanoparticles (LDHS). The chitsan NP performs as good as LDHS for ACE dsRNA and better than LDHS for H12 dsRNA in reducing soybean rust fungal growth in our greenhouse study (**Figure 3E and F**).

We also conducted several other studies: one is to identify new gene targets involved in the biosynthesis of host-non-specific phytotoxin cercosporin for suppressing Cercospora leaf blight or frogeye leaf spot diseases. We identified two proteins that were induced in *C.* cf. *flagellaris* (causal agent of CLB) cultures grown under light compared to grown under dark conditions (**Figure 4, page 4**). We have sequenced these two protein spots and identified them as HNR and AHCY proteins based on their peptide sequences. We are trying to determine whether suppressing the expression of these two proteins via dsRNA treatment can reduce cercosporin production and CLB or FLS disease severity on soybean plants.

In addition, we just published a manuscript in peer-reviewed journal "Plants" summarizing our recent findings on identifying additional gene targets that can effectively suppress Asian soybean rust disease when their corresponding dsRNAs were sprayed onto soybean plants and the funding support from MSSB was properly acknowledged.

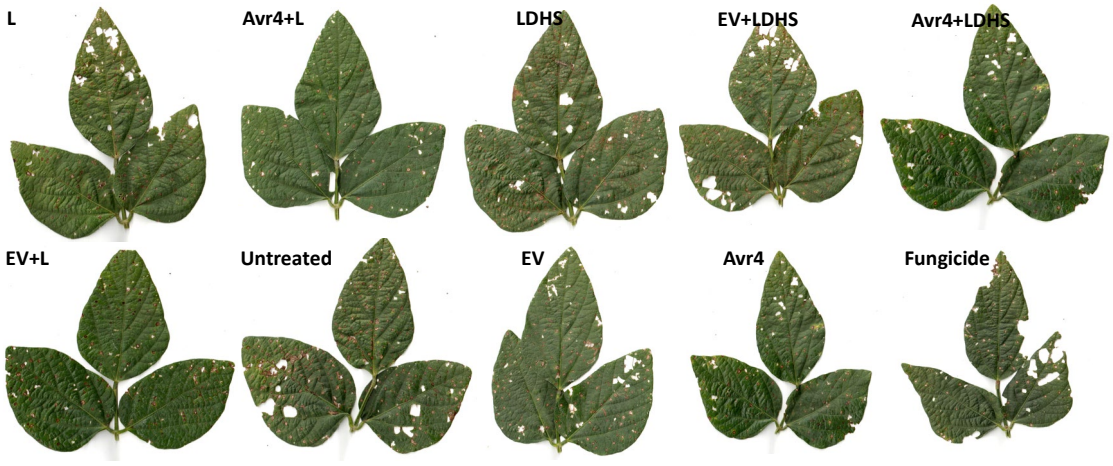

Figure 2. Visual assessment of differences of frogeye leaf spot (FLS) symptoms among different treatments 30 days after the initial dsRNA application.

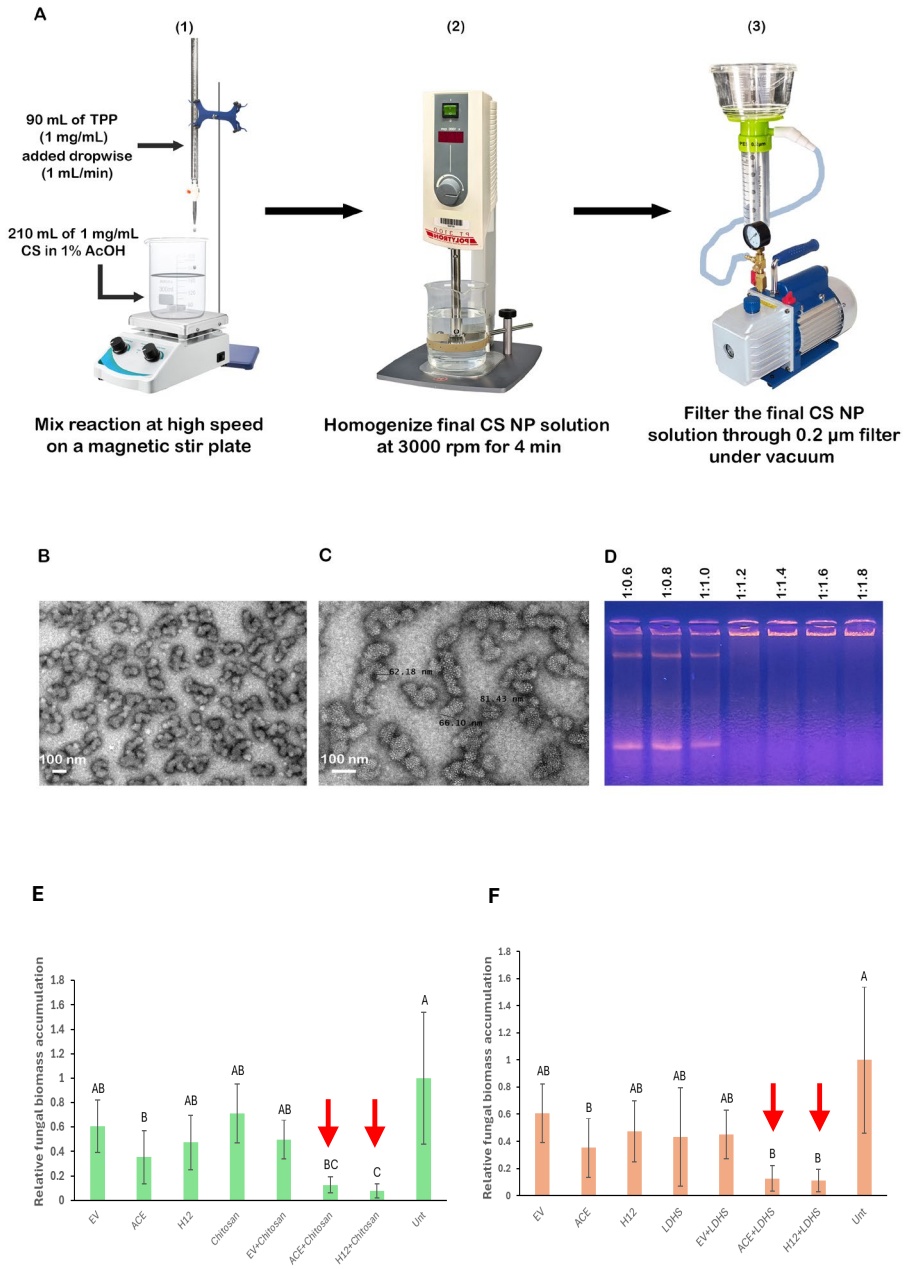

Figure 3. Examining the potential of chitosan nanoparticles (NP) in increasing the protection and uptake of dsRNA by soybean plants for a more efficient management of soybean fungal diseases. **A**. The three steps for the synthesis of chitosan NP. **B** and **C**: Transmission Electron Microscope images showing the shape and size of the synthesized chitosan NP. **D**. Binding assay to show that dsRNA can be easily loaded onto chitosan NP. **E** and **F**, showing the effectiveness of dsRNA coated chitosan NP in protecting soybean plants against rust diseases in comparison to naked dsRNA or dsRNA with LDHS. Please refer to the text in report for more details.

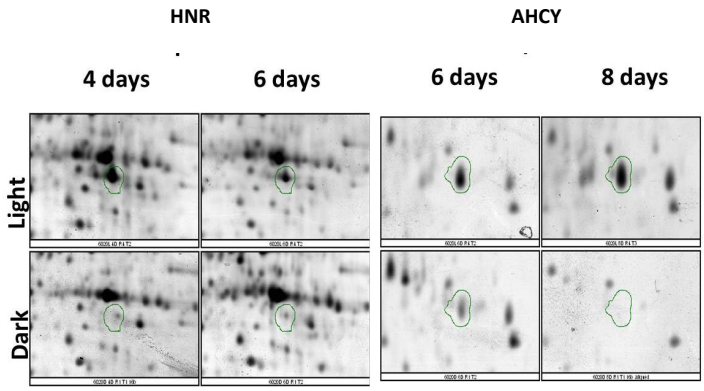

Figure 4. Identification of two light induced proteins (HNR and AHCY) in *Cercospora* cf *flagellaris* cultures that are potentially involved in cercosporin synthesis and can be targeted by dsRNA for managing CLB and FLS diseases on soybean.