Technical report on the progresses of the MSSB project

(Quarter 3, September 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 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 this quarter, we mainly focused on conducting field study to evaluate the effectiveness of different dsRNAs in suppressing cercospora diseases. This is a repeated study of the one we conducted in 2024 to ensure the consistency and reproducibility of the dsRNAs in reducing soybean cercospora diseases. For performing these small scale field studies, soybeans (Syngenta NK43-Y9XFS) were planted on three separate dates: May 19th, June 2nd, and June 16th, 2025. Two separate studies were conducted: one is the repeat of last year's study with 4 dsRNAs (Avr4, CB3, CP21, and EV) with two adjuvants, plus a Revytek fungicide (positive control) and an untreated control (negative control) for a total of 10 treatments with four replicates per treatment in a randomized complete block design. The dsRNAs were applied three times with 10 days apart in between the applications (July 9th, July 17th, and July 25th, the dates were slightly adjusted due to raining weather conditions at the planned application date) (Figure 1). The leaf samples were collected at 10 days and 20 days after the initial dsRNA application (July 19th and 29th). We are currently analyzing the collected leaf samples for fungal growth and disease severity. Also, disease severity was visually assessed for each of plots on August 08. A second study was conducted on the soybeans planted on June 16th. This study is to evaluate the

Figure 1. Field trials to examine the effectiveness of foliar applied dsRNA in reducing soybean cercospora diseases. Left, graduate student Sunira was spraying dsRNA onto soybean plants on July 9th, 2025. Right, visiting scholar Emily was collecting soybean leaf samples 10 days after initial dsRNA application for quantifying disease severity and fungal growth.

effectiveness of layered double hydroxide (LDH) nanoparticles in enhancing the dsRNA protection of soybean plants. This is the continuation of our last quarter's effort in assessing whether dsRNA coated with nanoparticles is more effective in suppressing fungal growth. Based on our greenhouse study of three different nanoparticles in the last quarter, we selected the best one (LDH) to continue its evaluation under field conditions.

We also conducted several other studies to understand how each of the specific dsRNAs suppresses soybean disease development. For example, we demonstrated that dsRNA ACE and H12 suppress soybean rust disease by significantly slowing down the spore germination and hyphal growth through *in vitro* cultural assays (Figure 2).

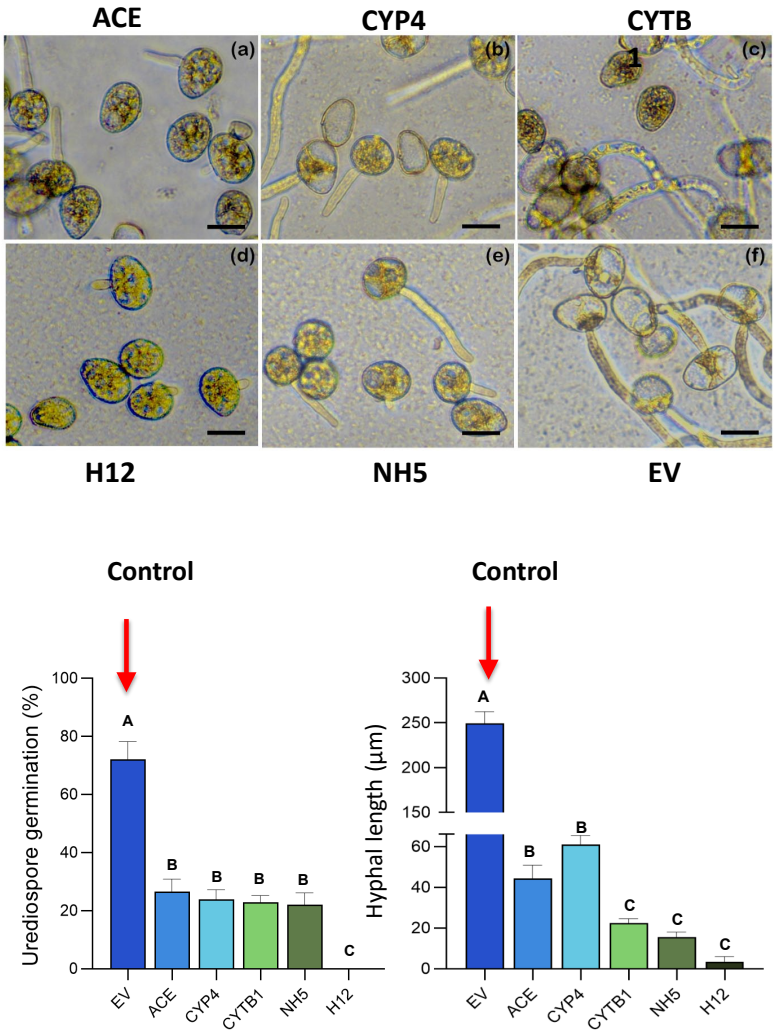

Figure 2. Top: The visual effect of different dsRNAs in suppressing *Phakopsora pachyrhizi* spore germination and hyphal growth in comparison to the control spores treated with dsRNA from empty vector (EV) when examined under a Nikon Ti2e microscope 4.5 hrs after treatment with dsRNA. The scale bar represents 20 µm. **Bottom:** The spore germination rate at 4.5 hrs (left) and hyphal growth determined at 9 hrs (right) after treatment with different dsRNAs.