

Technical report of the progresses on the MSSB project

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

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1. *Conducted greenhouse evaluation of the effect of different dsRNAs in reducing soybean frog eye leaf spot disease.*

Soybean plants grown in pots in greenhouse were sprayed with various dsRNAs at V5 stages (**Figure 1**). For each treatment, 8 plants in 4 pots were used. Only two fully developed trifoliolate leaves per plant were sprayed with dsRNA one day before the whole plants were inoculated with spores of *Cercospora sojina*, the causal agent of frog eye leaf spot (FLS) disease. Disease symptoms were evaluated 2 weeks after inoculation (**Figure 2**). We are in the process of repeating this study.



Figure 1. Soybean plants grown in the greenhouse that had been treated with two of the dsRNAs for controlling *Cercospora* diseases before inoculation with *C. sojina* spores that cause *Cercospora* frog eye leaf spot disease.

2. *Conducted small scale field evaluation of the effect of dsRNA in reducing soybean CLB and FLS diseases.*

Soybeans have been planted three different times (left to right, first planting on May 4, second on May 25, and the last planting was on June 8) in the field for evaluating the effect of topically applied dsRNA on reducing soybean *Cercospora* diseases, such as *Cercospora* leaf blight (CLB), frog eye leaf spot (FLS) and purple seed stains (PSS). We started treating soybean plants in the field in mid-August for the 2nd and 3rd plantings (**Figure 3**). The soybeans in the first plant were too old and already showing clear CLB and FLS symptoms. Each plot was 5-ft row and was replicated four times. However, no clear visual differences on disease symptoms were observed between treated and control soybean plants due to frequent rains in every afternoon. We

will try to repeated this part of study in year 2.

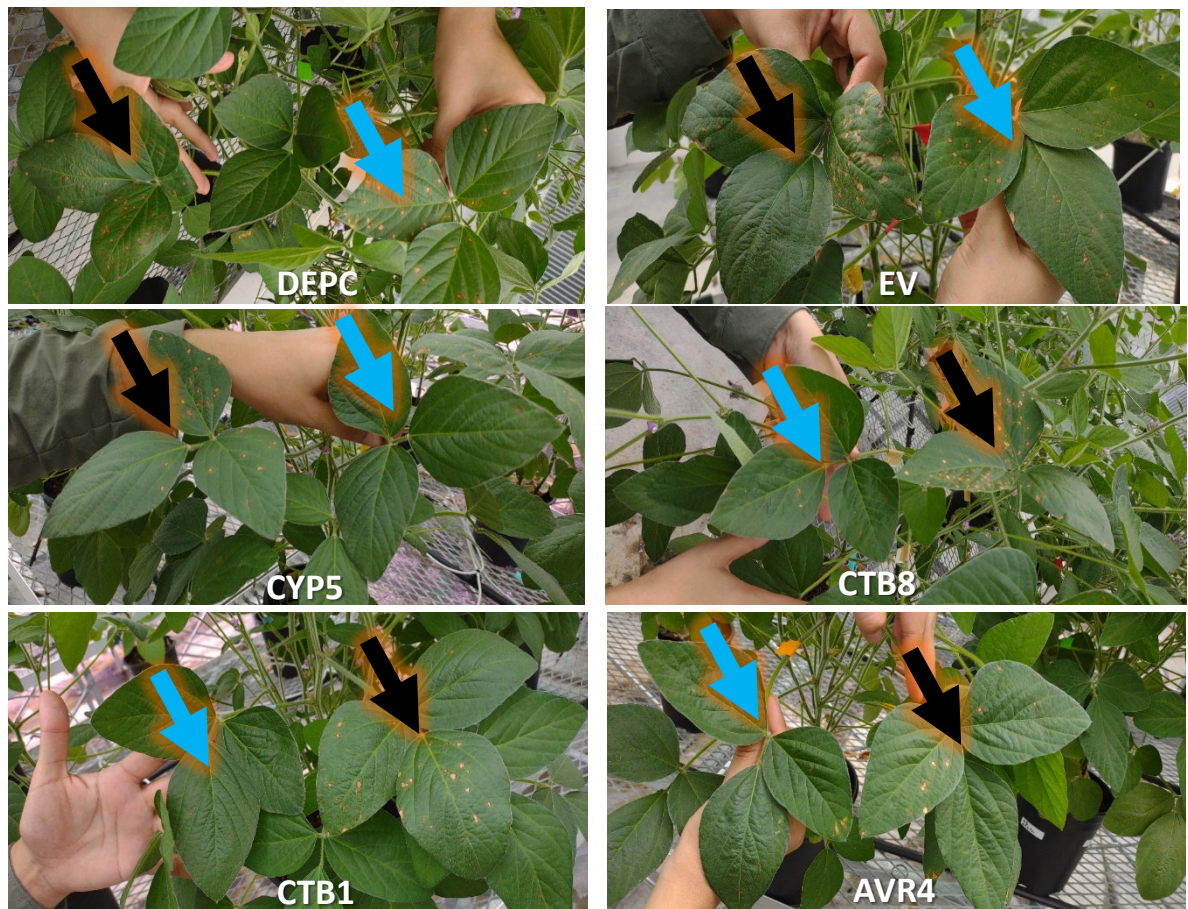


Figure 2. Soybean plants grown in greenhouse were pre-treated with various dsRNAs one day before being inoculated with *Cercospora soja*. The differences of leaves treated (indicated with blue arrows) with dsRNAs targeting fungal CYP5, CTB8, CTB1 and AVR4 genes compared to control leaves treated with DEPC water or empty vector (EV) (indicated with black arrows) are very clear, indicating the effectiveness of the dsRNAs in reducing disease symptoms.



Figure 3. Soybean variety Syngenta S42-B9XS was planted three times and was treated with dsRNA in mid-August by spraying with battery operated air-compressor.