Technical report of the progresses on the MSSB project

(Quarter 2, 2023)

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 first year are to: 1) produce dsRNAs in *E. coli* of 4 different genes from Cercospora species that are important for their infection, growth or toxin production; 2) develop an effective method to deliver the dsRNAs into soybean leaves; and 3) perform various greenhouse and field studies (in Louisiana first, and other states later) to determine the effectiveness of these dsRNAs in simultaneous management of cercospora leaf blight (CLB) and purple seed stains (PSS), which are caused by *C.* cf. *flagellaris* or *C. kikuchii*, and frogeye leaf spot (FLS), which is caused by *C. sojina*, through spray applications.

In this quarter, we mainly focused our research on objectives 2 and 3. For objective 2, we have been testing the efficacy of different adjuvants in enhancing the effects of dsRNA in suppressing disease symptom development. A couple of the adjuvants appear to be able to enhance the effective of dsRNA by 2 fold. These adjuvants will be used in our field studies.

Table 1. Percentage of *Phakopsora pachyrhizi* target gene expression in soybean leaves 2 weeks after being treated with corresponding dsRNA and inoculated with the uredinospores.

Gene	Average
H5	73.67
H6	57.00
H9	56.67
H10	36.75
H12	25.00
NH5	43.75
NH8	46.50
CYP3	31.75
CYP4	22.75
CYTB1	18.33
CYTB2	20.33
ACE	20.00
EV	100.00

For objective 3, we have repeated the greenhouse study on using different dsRNAs to reduce Asian soybean rust (ASR) and soybean flogeye leaf spot (FLS) diseases. We have collected soybean leaf samples and are extracting DNA and RNA samples to quantify the difference in fungal growth between control and dsRNA treated soybean plants. For ASR, several dsRNAs were able to reduce the target gene expression by up to 80%, such as, CYTB1, CYTB2, and CYP4 (**Table 1**). In addition, a small scale field study with the dsRNA has been planned. The soybean plants were planted on May 15, May 30 and June 12, 2023. Some of the soybeans from the second planting were treated with dsRNAs once (**Figure 1**) on July 28,

Figure 1. Soybean plants from second planting were treated with different dsRNAs to suppress the FLS and CLB diseases on July 28, 2023.

Figure 2. Soybean plants from the third planting were treated with 4 different dsRNAs and two controls for three times (Aug 10, 15, and 24) for possible control of CLB and FLS diseases under natural infection.

2023. Some of the soybean plants from the third planting were treated with four dsRNAs and

two controls with 6 replicates per treatment. The soybean plants were sprayed with dsRNAs three times (August 10, 15, and 24) (**Figure 2**). We are currently monitoring the FLS and CLB development to determine whether there is any difference in disease development between dsRNA treated and control soybean plants under natural infection conditions.