Technical report of the progresses on the MSSB project

(Quarter 1, 2023)

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 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.

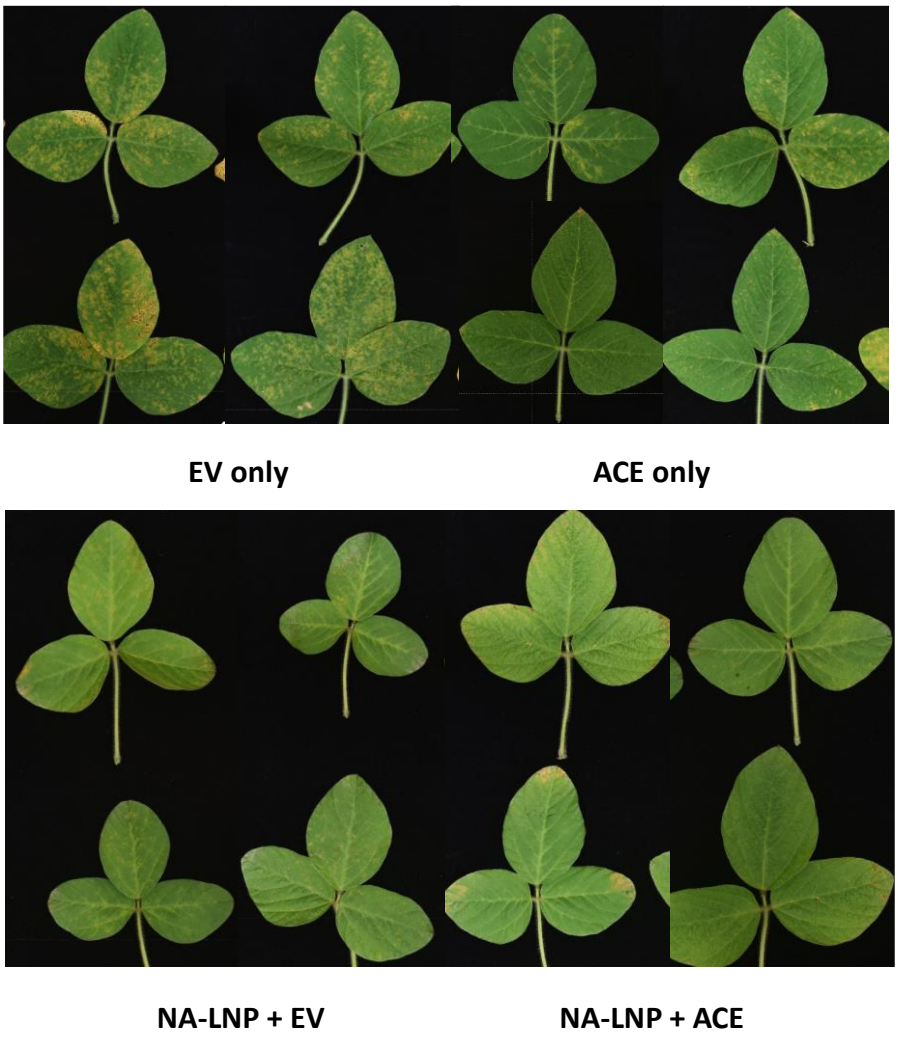

Figure 1. Differences in soybean rust disease symptoms of soybean plants that were treated with dsRNA targeting ACE gene compared to control (dsRNA from Empty Vector) with (bottom) or without (top) being coated with lignin based nanoparticles (NA-LNP).

In this quarter, we mainly focused our

research on objectives 2 and 3. For objective 2, we have tested two different ways to increase the effectiveness of dsRNA on protecting soybean plants against fungal diseases. One was to coat our dsRNA with lignin-based nanoparticles (LGNP). Due to difficulty in producing spores from *C. sojina* and getting good FLS disease symptoms, we used soybean rust pathogen instead for this part of study. Based on the preliminary results we obtained in the first study (see report in the previous quarter), we observed that the pH of the solution used to resuspend the lignin nanoparticles (NA-LNP) was too low and caused leaf damage. In this second trial, the pH was raised from 4.0 to around 6.5. The experiment was conducted in the same way as before: 4 soybean plants in 2 pots were used in each treatment. Only the two fully opened tri-foliate leaves in each plant at V4 stage were used in the treatment. Soybean leaves were treated with either dsRNA from empty vector (EV) or from ACE only (500 µg dsRNA per pot), or with NA-LNP + EV, or NA-LNP + ACE dsRNA. The soybean plants were inoculated with spore suspensions from *Phakopsora pachyrhizi* (1 x 10^6 spores/mL in sterile water containing 0.01% Tween 20) one day later. The rust disease symptoms were evaluated two weeks later. It is clear the our dsRNA targeting rust pathogen ACE gene can clearly reduce soybean rust disease symptoms compared to the control (dsRNA prepared from empty vector) without the help of lignin-based nanoparticles (NA-LNP) (**Figure 1,** top). It is also clear that NA-LNP has some suppressive effect on soybean rust disease development (**Figure 1**, left side top to bottom). This is likely due to the fact that lignin itself can trigger plant defense mechanism. In order to accurately assess the effect of NA-LNP in enhancing ACE's effect in reducing soybean rust disease, the rust disease severity was rated according to the scale developed by Franceschi et al. (2020) (**Figure 2**). Due to suppressive effect of lignin nanoparticles on soybean rust disease, it is hard to determine whether lignin nanoparticles enhanced the ACE effect on soybean rust disease control (**Figure 3,** next page).

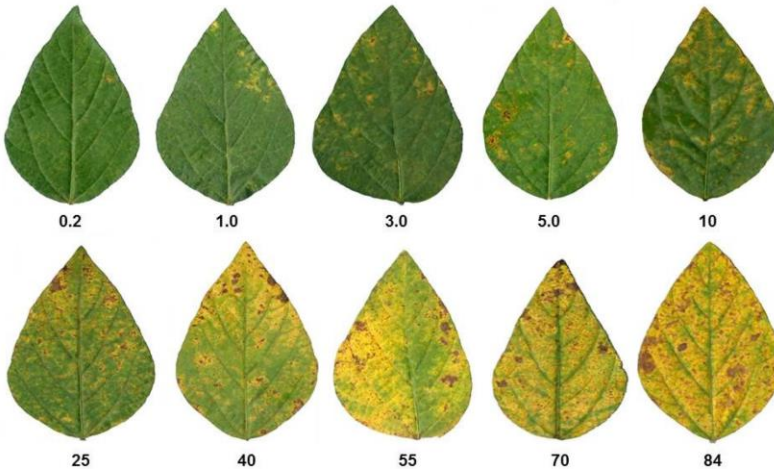

Figure 2. Soybean rust disease rating scale. This scale was developed by Franceschi et al. 2020 (Plant Pathology 69: 495-505).

For objective 3, we have repeated the greenhouse study on using different dsRNAs to reduce soybean rust 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. In addition, a small scale field study with the dsRNA has been planned. The soybean plants were first planted in May 15, and second planting was in June 1. There will be one more planting in the coming week. The soybean plants

are looking good despite under some drought weather (**Figure 4**).

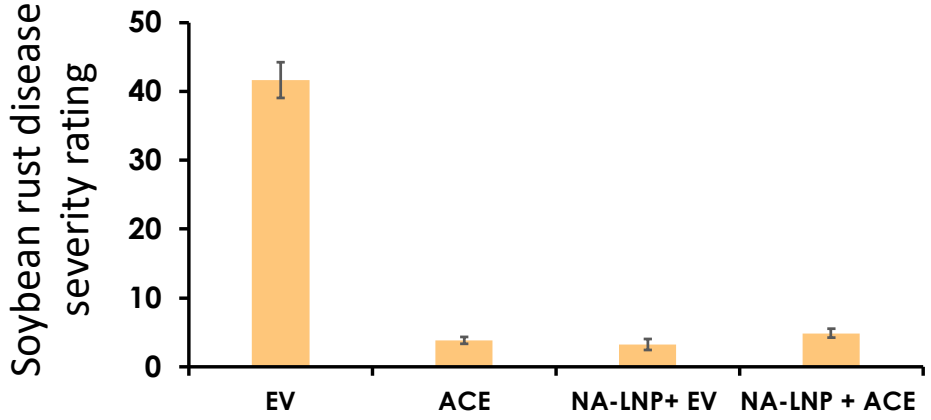

Figure 3. The percentage severity scale of soybean rust on soybean leaves (cultivar S42-B9XS) after dsRNA (Ace and EV) treatment with or without nanoparticles (NA-LNP) and followed by inoculation with *Phakopsora pachyrhizi* spores. The disease symptoms were rated 14 days after inoculation. Bars represent standard error.

Figure 4. Soybean have been planted in Ben Hur research station for a small scale study of dsRNA treatment on reducing CLS and FLS diseases. The photo was taken on June 8, 2023.