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| Please use this form to clearly and concisely report on project progress. The information included should reflect quantifiable results that can be used to evaluate and measure project success. Comments should be limited to the designated boxes. Technical reports, no longer than 4 pages, may be attached to this summary report. |
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| Project Title:  | Spray Application of Double Stranded RNA for Simultaneous Management of Multiple Soybean Fungal and Insect Diseases |
| Organization:  | Louisiana State University Agricultural Center |
| Project Lead Name: | Zhi-Yuan Chen |
| Report Period: | Sep 16, 2022 to Dec 15, 2022 |
| **National Soybean Checkoff Research Database** [**https://www.soybeanresearchdata.com/**](https://www.soybeanresearchdata.com/) **(public website funded by USB). Please include a non-technical project status along with your project status. The non-technical project status will be published to the website. If a non-technical project status is not provided, the contents of this entire report will be published.** |
| Project Status: |
| 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 the third quarter, we mainly focused our research on objectives 2 and 3. For objective 2, we have been having an issue of low yield in dsRNA production from bacterial culture in the past two months. Without enough dsRNA, it was hard to determine the most effective way of delivering dsRNA. After doing a through trouble shooting of all the steps and chemicals used in the production. We think we have solved this problem. The yield has increased greatly and the effectiveness of these new dsRNAs is being tested in the greenhouse. For objective 3, inconsistent fungal infection in greenhouse after artificial inoculation has been a real challenge. In order to get consistent and reproducible infection of soybean plants in greenhouse when inoculating with *C. flagellaris* or *C. sojina*. We have built a mist chamber, which will provide a high humidity environment during the first 48 hrs after the inoculation to ensure proper spore germination and infection. A new batch of soybean plants have been planted and will be used to determine the ideal conditions for dsRNA delivery. For further details, please see the attached technical report. |
| **Non-technical project status:** |
| The objectives of this study in the first year were to produce double stranded RNA (dsRNA) molecules in a bacterial expression system, use the purified dsRNAs to spray on soybean plants under greenhouse and field conditions to see whether they can reduce soybean cercospora leaf blight (CLB), purple seed stains (PPS) and frogeye leaf spot (FLS) diseases. We have cloned and produced dsRNAs targeting four the genes from Cercospora species. Soybean plants grown in greenhouse have been recently treated repeatedly with all four of the dsRNAs and showed clear effect in reducing FLS. Additionally, field grown soybean plants have been used in a small scale study to evaluate the effect of dsRNA in reducing soybean CLB, PSS and FLS under field conditions with natural infection. However, due to raining and harsh weather conditions, the effect of dsRNA in reducing CLB or FLS compared to control is not evident. We plan to repeat this study in next spring. |