**MISSISSIPPI SOYBEAN PROMOTION BOARD**
**PROJECT NO. 61-2015 (YEAR 3)**
**2015 Annual Report**

**Title:** Investigations into Strobilurin Fungicide Resistance of Soybean Pathogens in Mississippi

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## BACKGROUND AND OBJECTIVES

Foliar soybean diseases can result in significant yield loss; therefore, cultural and chemical control practices are necessary. The strobilurin or quinone outside inhibitor (QoI) class of fungicides are primarily applied for late-season disease management as well as for a perceived minor yield benefit.

The two most commonly used fungicides are azoxystrobin (Quadris) and pyraclostrobin (Headline). QoI sensitivity was determined for 634 mono-conidial isolates of the frogeye leaf spot (FLS) pathogen, *Cercospora sojina*, collected from every soybean-producing county in 2013 and 2014. Greater than 93% of *C. sojina* isolates were resistant to QoIs due to a gene mutation, indicating the dominant population is now resistant to QoIs. With the gene mutation conferring QoI resistance, there may be a fitness cost associated with the dominant *C. sojina* population.

Fitness, or the competitive ability of *C. sojina* to survive, develop, and reproduce, is the next step in evaluating the evolutionary shift from QoI-sensitive to QoI-resistant populations of *C. sojina* in Miss. soybean production fields. In general, fungal populations that shift from fungicide sensitive to resistant may have a shift in competitiveness, viability (short- and long-term), reproductive capabilities, and most important from the grower's standpoint, virulence, which is the capacity of a pathogen to cause severe disease. This may manifest itself in the reduction of host-plant resistance to FLS. Efforts in 2015 focused on fitness cost which will provide pertinent information that can be applied to disease management of FLS caused by a new, QoI-resistant population of *C. sojina* in Mississippi soybean production.

### Objectives

1. **Monitor MS soybean production fields and sentinel plots for QoI resistance within the frogeye leaf spot pathogen population.**
2. **Identify the mechanism(s) of resistance and potential proportion within the prevailing fungal community to better understand the dynamics leading to QoI resistance.**
3. **Determine potential fitness costs associated with QoI resistance within the soybean pathogen populations present.**

## 2015 PROGRESS

Objectives 1 and 2 were successfully completed in 2014, culminating with a manuscript published in Plant Disease entitled, "*Occurrence of QoI fungicide resistance in Cercospora sojina* *from Mississippi Soybean*". In 2015, our focus has been on achieving progress toward Objective 3.

Ten representative *C. sojina* isolates, each of QoI-resistant and –sensitive, were selected to conduct fitness studies. To confirm sensitivity or lack thereof, genomic DNA was extracted from each fungal isolate, the partial fragment of cytochrome B (cyt B) gene (QoI target site) was amplified via PCR, and sequence alignments were viewed to determine the amino acid present at the QoI binding site. Multiple studies referred to as fitness measurements were carried out to determine the fitness of QoI-resistant isolates.

The first measurements included colony growth (at 14 days), conidial production (conc. of spores/ml), and viability (18-h conidial germination). To determine genetic stability of selected *C. sojina* isolates, a series of 10 sub-cultures were executed every 14 days. At the end of the 10 days, 14-day sub-cultures from each isolate were evaluated for the same fitness measurements as previously described and was also repeated twice. The results indicated QoI-sensitive and -resistant isolates behaved similarly for all measurements except colony growth at 32 $^{O}$C, where the sensitive isolates produced significantly greater colony growth compared to QoI-resistant isolates. Overall, the QoI-resistant isolates produced 32% more conidia than QoI-sensitive isolates (Fig. 1). These results suggest higher inoculum production in soybean fields dominated by QoI-resistant *C. sojina*, thus resulting in greater potential of frogeye leaf spot epidemics since conidia are the primary inoculum source to initiate disease. Throughout all studies, sub-culturing did not affect the behavior of all selected isolates, indicating genetic stability within each isolate regardless of QoI sensitivity.

Two additional fungicide classes were tested in sensitivity assays to determine whether dual resistance occurred among QoI-resistant isolates. The selected *C. sojina* isolates were exposed to varying concentrations of Topsin-M [thiophanate-methyl (TM)], a benzimidazole, and Domark [tetraconazole (TC)], a triazole. The results indicated all isolates were sensitive to TM with a range of $EC_{50}$ values of 0.96 to 12.67 ppm with an average of 6.34 ppm.

The target site of TM, β-tubulin 2 (TUB2), was sequenced to confirm the presence of amino acids that bind the TM molecule, resulting in fungal inhibition. The range of $EC_{50}$ values for TC was 0.33 to 3.11 ppm with an average of 0.75 ppm. Of the 20 isolates exposed to TC, only one showed reduced sensitivity based on *in vitro* assays. This same isolate is also QoI-resistant, indicating cross resistance to triazoles may be developing within the QoI-resistant *C. sojina* population in Mississippi soybean.

Two virulence studies were conducted in December 2015 and February 2016. Soybean seedlings (V2) were inoculated with selected *C. sojina* isolates, resulting in excellent disease pressure; however, no differences were noted in virulence between the QoI-sensitive and –resistance isolates (Fig. 2). No disease was present on the second pathogenicity study in February 2016. Additional studies are currently underway to complete the virulence portion of Objective 3.

A five-gene phylogenetic analysis is currently underway to compare the lineage of QoI-sensitive and -resistant *C. sojina* populations. Research studies suggest divergence within fungal populations expressing a loss of fungicide sensitivity such as the FLS pathogen we collected throughout Mississippi.

**IMPACTS/BENEFITS TO MISSISSIPPI SOYBEAN PRODUCERS**

This research project has and will continue to have a positive impact on Mississippi soybean production. Information generated on the behavior of QoI-resistant *C. sojina* is vital in future frogeye leaf spot disease management programs. The fact that QoI-resistant isolates have the tendency to produce greater numbers of conidia could lead to greater disease pressure under ideal conditions.

Those isolates causing FLS will not respond to QoI fungicides. This reality may fundamentally change the growers' approach for disease management. In soybean fields where practical resistance (fungal population is resistant to a class of fungicides) occurs, QoI fungicides will not be effective. **Frogeye leaf spot-resistant soybean varieties should be considered a tool in managing fungicide resistance**, along with crop rotation, fungicide applications using the label rate, and alternating fungicide classes.

**END PRODUCTS**

**Oral presentations:**

Standish, J., Tomaso-Peterson, M., Allen, T., Sabanadzovic, S., Aboughanem-Sabanadzovic, N. An investigation into QoI resistance in isolates of *Cercospora sojina* throughout Mississippi soybean production fields. Southern Division-American Phytopathological Society. Atlanta, GA 2 Feb 2015.

Standish, J., Tomaso-Peterson, M., Allen, T. Moore, B., Sabanadzovic, S., Aboughanem-Sabanadzovic, N. Quinone outside inhibitor resistance in *Cercospora sojina* throughout Mississippi soybean. Future of Agriculture Graduate Student Competition. Mississippi State University. 5 Feb 2015.

Brochard, N., Tomaso-Peterson, M., Allen, T. Determining fitness cost in QoI-resistant isolates of the frogeye leaf spot pathogen. Future of Agriculture Graduate Student Competition. Mississippi State University. 5 Feb 2015.

Standish, J., Tomaso-Peterson, M., Allen, T. Moore, B., Sabanadzovic, S., Aboughanem-Sabanadzovic, N. Investigating fungi sensitivities beyond the QoIs in *Cercospora sojina* from Mississippi. Southern Soybean Disease Workshop. Pensacola Beach, FL. 11-12 March 2015.

N. Brochard, M. Tomaso-Peterson, T.W. Allen, and R. Melanson. Comparison of QoI resistant and sensitive isolates of the frogeye leaf spot pathogen. Mississippi Plant Pathologists and Nematologists Annual Meeting. Starkville, MS. 20 October 2015.

**Poster presentations:**

N. Brochard, M. Tomaso-Peterson, T. Allen, J. R. Standish. Thiophanate-methyl sensitivity of the frogeye leaf spot pathogen in Mississippi. American Phytopathological Society. Pasadena, CA. 1-5 August 2015.

**Referreed manuscripts:**

J. Standish, M. Tomaso-Peterson, T. W. Allen, S. Sabanadzovic, N. Aboughanem-Sabanadzovic. 2015. Occurrence of QoI fungicide resistance in *Cercospora sojina* from Mississippi soybean. Plant Disease. 99:1347-1352.

**Thesis:**

A comprehensive study into quinone outside inhibitor resistance in *Cercospora sojina* from Mississippi Soybean. M.Sc. Thesis. Jeffrey Russell Standish. Mississippi State University. May 2015.

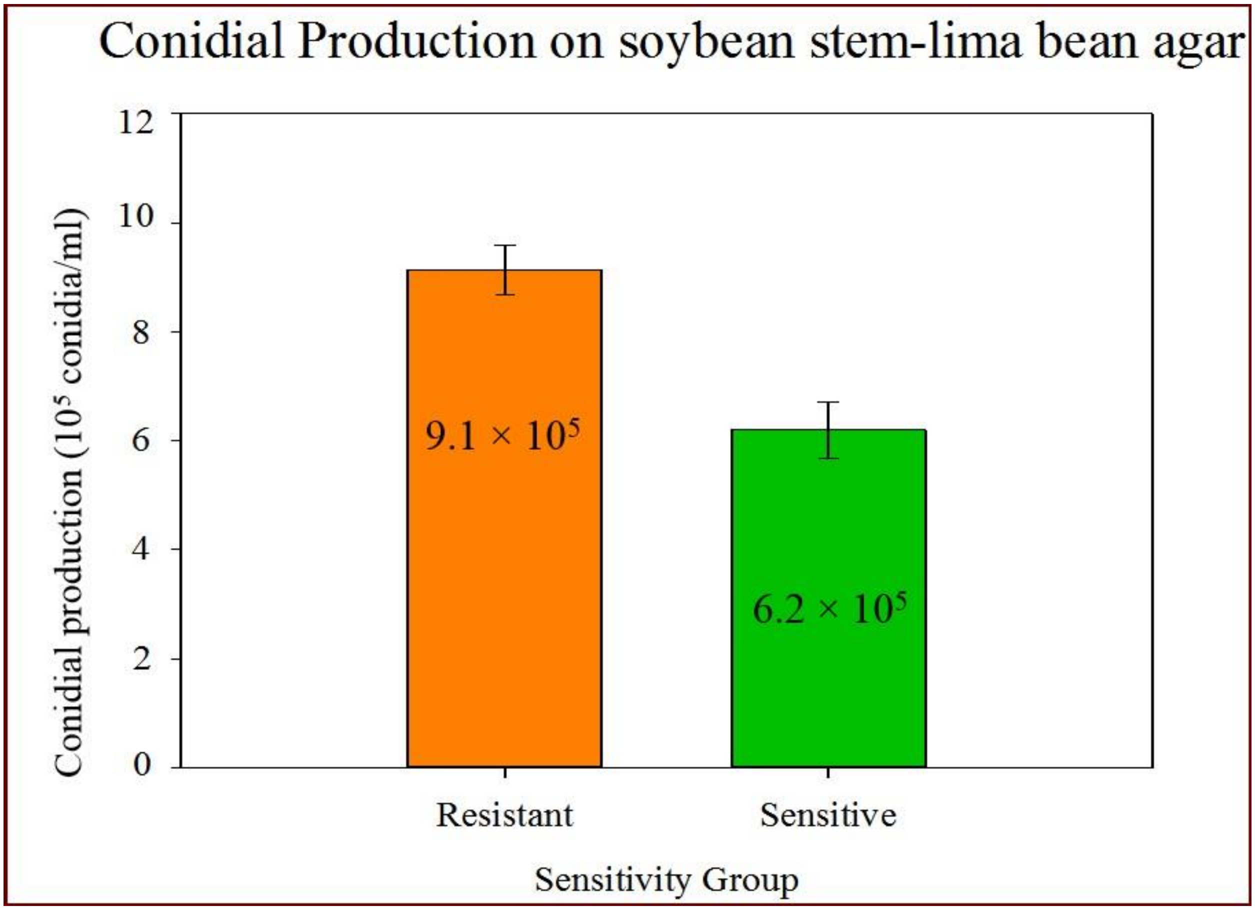


**Figure 1.** Conidia production of selected QoI-resistant and -sensitive *Cercospora sojina* isolates. Conidia serve as the primary inoculum in frogeye leaf spot epidemics.

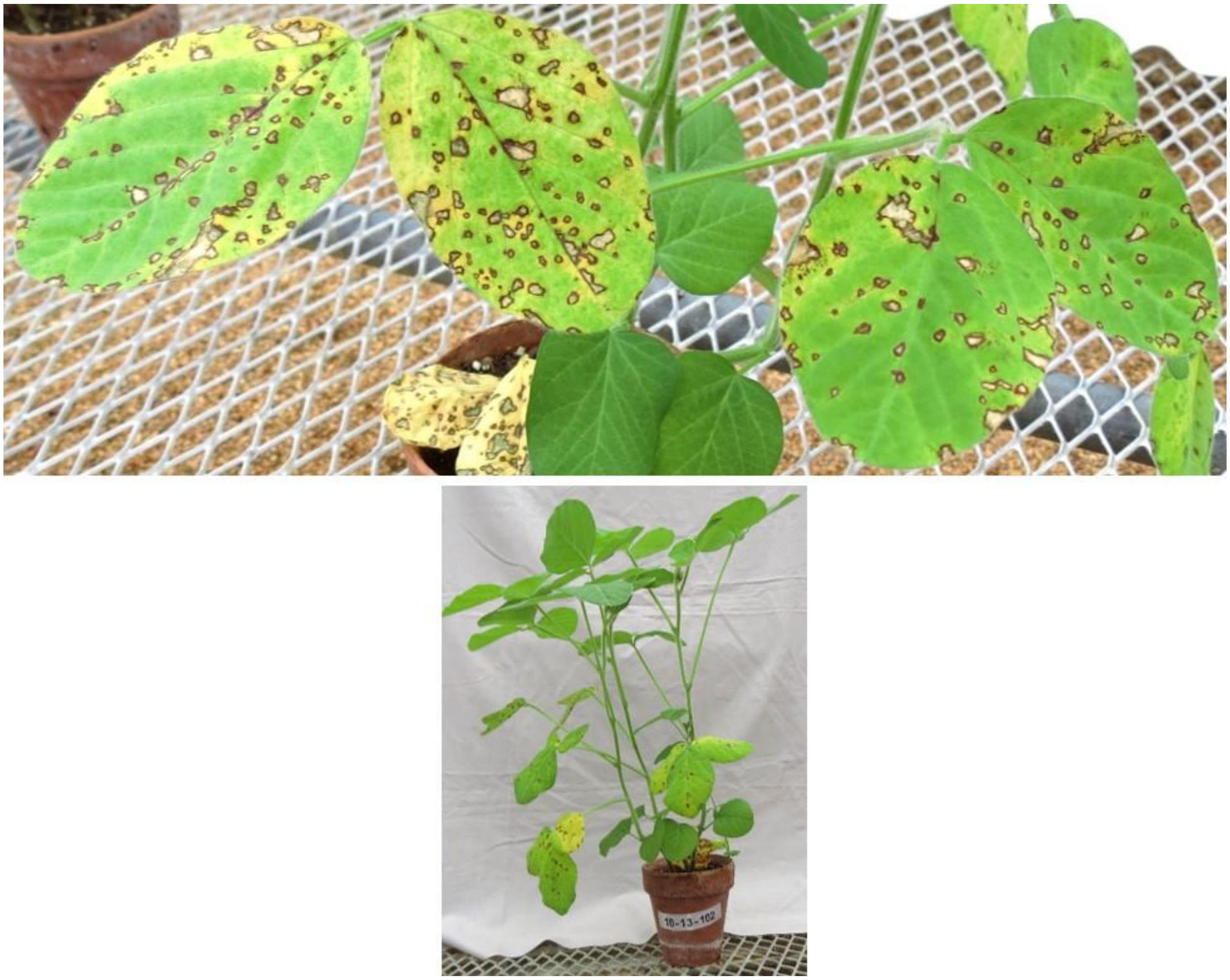


**Figure 2.** Frogeye leaf spot occurrence on soybean seedlings inoculated with a QoI-resistant *Cercospora sojina* isolate.

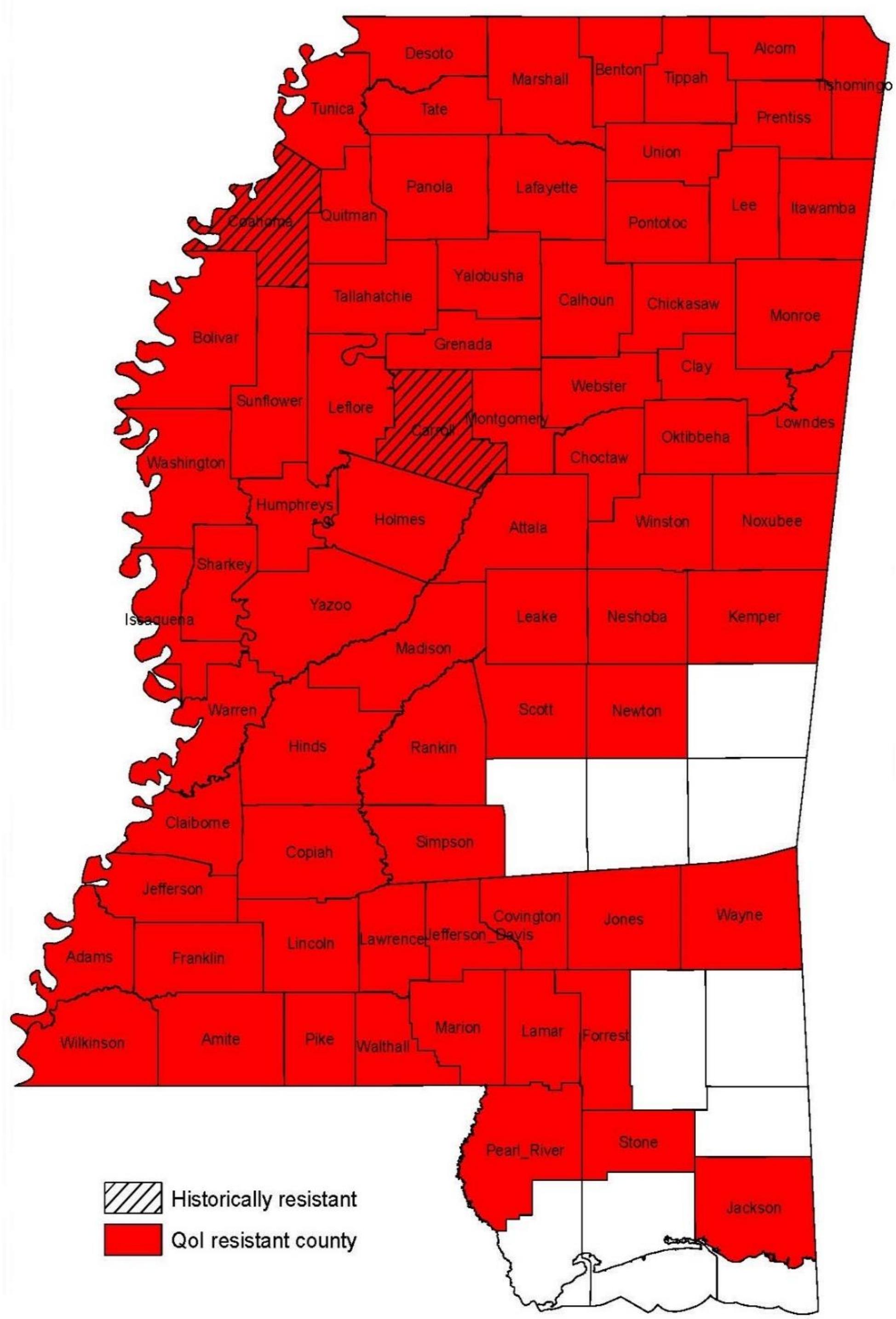


**Figure 3.** Distribution of QoI- or strobilurin-resistant *Cercospora sojina*, causal organism of frogeye leaf spot. Red-shaded areas represent counties where at least one isolate was found to carry the G143A amino acid substitution conferring resistance to the QoI fungicides. (*Courtesy T.W. Allen*)

**Table 1.** Frogeye leaf spot severity on soybean resulting from infection by QoI-sensitive and QoI-resistant isolates of *Cercospora sojina* following an application of foliar fungicide in a greenhouse.

| | Severity (%) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Treatment and rate/ha[x] | 13-11-R[y] | | 13-31-S | 13-34-R | | 13-36-S | 13-85-S | 13-104-R | |
| Untreated Control | 14.5 | a[z] | 3.9 | 5.4 | a | 0.8 | 0.6 | 1.1 | a |
| Headline (0.877 L/ha) | 4.2 | b | 0.3 | 2.3 | b | 0.0 | 0.3 | 0.3 | b |
| Quadris (1.133 L/ha) | 3.1 | b | 1.4 | 1.1 | b | 0.6 | 0.0 | 0.1 | b |
| Topguard (1.023 L/ha) | 0.0 | b | 0.0 | 0.0 | b | 0.0 | 0.0 | 0.0 | b |

[x] Fungicides were applied at the highest label rate at the V2 growth stage.
[y] R = Resistant isolates; S = Sensitive isolates.
[z] Means (n=9) within columns followed by the same letter are not significantly different according to Fisher's protected least significant difference test ($P$ = 0.05).

**Table 2.** Frogeye leaf spot lesion count on soybean resulting from infection by QoI-sensitive and QoI-resistant isolates of *Cercospora sojina* following an application of foliar fungicide in a greenhouse.

| | Lesion count[w] | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Treatment and rate/ha[x] | 13-11-R[y] | | 13-31-S | 13-34-R | | 13-36-S | 13-85-S | 13-104-R | |
| Untreated Control | 37 | a[z] | 1 | 6 | a | 0 | 1 | 8 | a |
| Headline (876.9 ml/ha) | 7 | b | 0 | 0 | b | 0 | 0 | 3 | b |
| Quadris (1132.7 ml/ha) | 3 | bc | 0 | 1 | b | 0 | 0 | 1 | b |
| Topguard (1023.0 ml/ha) | 0 | c | 0 | 0 | b | 0 | 0 | 0 | b |

[w] Frogeye leaf spot rated as number of lesions per pot (two soybean plants/pot).
[x] Fungicides were applied at the highest label rate at the V2 growth stage.
[y] R = Resistant isolates; S = Sensitive isolates.
[z] Means (n=9) within columns followed by the same letter are not significantly different according to Fisher's protected least significant difference test ($P$ = 0.05).