Plant Health Brief

The G143A Mutation is Responsible for Strobilurin Fungicide Resistance in *Cercospora* cf. *flagellaris*, a Leaf Blight and Purple Seed Stain Pathogen of Louisiana Soybean

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Cercospora leaf blight (CLB) and purple seed stain (PSS) are economically important diseases of soybean worldwide. In the United States, these diseases are among the most damaging in the Lower Mississippi River Valley and southeastern states along the Gulf of Mexico, causing estimated losses of $45 to $50 million over the last two years (Allen et al. 2016). For many years, farmers in these areas have consistently applied strobilurin fungicides during soybean reproductive stages in an attempt to manage CLB and PSS. As a result, fungicide resistance in the pathogen population was recently documented using radial growth assays (Price et al. 2015).

Strobilurin fungicides are in a class of chemicals known as quinone outside inhibitors (QoI) that inhibit mitochondrial respiration in fungi by disrupting the third (cytochrome *b*) complex in the electron transport chain. Fungi are known to overcome this sensitivity by two alternate point mutations in the cytochrome *b* gene: a single gene mutation from glycine to alanine at the 143rd codon position (G143A) and/or, less commonly, an amino acid change from a phenylalanine to leucine at position 129 (F129L).

The objective of this study was to determine if the G143A or F129L mutations were responsible for QoI resistance in isolates of *Cercospora* cf. *flagellaris*, which recently was determined to be associated with CLB/PSS in Louisiana (Albu et al. 2016).

Resistant and sensitive isolates of *C.* cf. *flagellaris* (98) from the radial growth assay study (Price et al. 2015) were selected for molecular characterization along with 13 isolates from pokeweed from Illinois, Arkansas, and Louisiana, and two isolates from Louisiana cotton with unknown sensitivities. Culture preparation, DNA isolation, and PCR amplification were performed as described in Albu et al. (2016). A portion of the cytochrome *b* gene of approximately 650 to 700 bp, containing the 129th and 143rd codon positions, was sequenced using the primers cyt-f2/cytb-r1 (Imazaki et al. 2006) on the Applied Biosystems platform at Beckman Coulter Genomics (Danver, MA) using Big Dye Terminator chemistry.

In 97% of the cases, the G143A mutation was associated with strobilurin resistance, while none of the screened isolates carried the F129L mutation. The 13 *C.* cf. *flagellaris* isolates from pokeweed were sensitive, while the two isolates from cotton were resistant. Of the 113 isolates in this study, three did not correspond with radial growth assays (Price et al. 2015), where two isolates were determined to be sensitive and one resistant. This inconsistency may be explained by mitochondrial heteroplasmy, a condition where populations of strobilurin-resistant and sensitive mitochondria can exist simultaneously in a single fungal isolate. Further research is needed to explain this discrepancy. The strength of the correlation between the G143A mutation and strobilurin resistance suggests that molecular screening may be used instead of radial growth assays to define resistance in the CLB/PSS pathogen population; however, a minority of resistant isolates (<3%) may be misassigned.

LITERATURE CITED

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