Resistance to Quinone Outside Inhibitor Fungicides Conferred by the G143A Mutation in *Cercospora sojina* (Causal Agent of Frogeye Leaf Spot) Isolates from South Dakota Soybean Fields

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Frogeye leaf spot of soybean (*Glycine max*), caused by *Cercospora sojina*, is a yield-limiting disease in the United States. From 2010 to 2014, frogeye leaf spot caused estimated annual losses ranging from 101,432 to 493,880 metric tons in the United States and Ontario, Canada (Allen et al. 2017). When quinone outside inhibitor (QoI) fungicides first became registered for use on soybean in the early 2000s in the United States, they were highly effective in managing frogeye leaf spot, but in 2010, QoI-resistant *C. sojina* isolates were detected for the first time in a Tennessee soybean field (Zhang et al. 2012). These QoI-resistant isolates from Tennessee were found to have the G143A mutation that confers resistance to QoI fungicides (Zeng et al. 2015). Since then, QoI-resistant *C. sojina* isolates with the G143A mutation have become widespread across U.S. soybean production regions and have been detected in 240 counties and parishes across 14 states from 2010 to 2017 (Zhang et al. 2018).

In September 2018, soybean leaves exhibiting frogeye leaf spot symptoms (circular to irregular-shaped light-tan to gray lesions with purple margins) were collected from soybean fields in Brookings, Davison, Lincoln, Union, and Yankton Counties in South Dakota, and *C. sojina* isolates were obtained. To isolate *C. sojina*, symptomatic leaves were placed in closed plastic boxes containing moist paper towels to serve as moist chambers, which were left on the lab bench for 24 h to encourage sporulation. Using a micropipette, sterile water was placed onto sporulating lesions, drawn back up to collect conidia, and deposited onto water agar (15 g/liter). The conidial suspensions were spread across water agar with a sterile glass rod and were incubated at room temperature (approximately 23°C). After 12 to 18 h, a single germinating conidium was selected and transferred to soybean stem lima bean agar (Phillips and Boerma 1981). The plates were incubated for 7 days under 12-h light/dark at room temperature. In total, 41 single-spore isolates were obtained and were stored in 15% glycerol solution in sterile cryogenic tubes at –80°C.

A discriminatory dose assay, using 0.1 µg/ml of azoxystrobin and 60 µg/ml of salicylhydroxamic acid, was used to differentiate QoI-resistant and QoI-sensitive isolates (Zhang et al. 2018). Conidia that germinated on the discriminatory dose of azoxystrobin were considered to be resistant to QoI fungicides. Isolates were also tested for the presence of the G143A mutation using a polymerase chain reaction (PCR) assay reported by Zeng et al. (2015). For both the discriminatory dose assay and the PCR assay, known QoI-sensitive and QoI-resistant isolates of *C. sojina* were included as negative and positive controls.

Results of the discriminatory dose assay and the PCR assay showed that 29 out of the 41 South Dakota *C. sojina* isolates were resistant to QoI fungicides. The QoI-resistant isolates came from Davison (four out of six isolates), Lincoln (three out of three isolates), Union (three out of three isolates), and Yankton (19 out of 24 isolates) Counties. No QoI-resistant isolates were detected from Brookings County (zero out of five isolates).

This is the first report of QoI-resistant *C. sojina* isolates occurring in South Dakota. Considering that South Dakota ranked eighth in the United States for soybean production in 2018 with approximately 6.99 million metric tons produced, this finding warrants the need for additional surveys to determine the prevalence of QoI fungicide-resistant *C. sojina* isolates in South Dakota where QoI-resistant isolates have not yet been detected. This finding expands the geographical distribution of QoI-resistant *C. sojina* isolates in the United States, with Iowa being the only adjacent state to South Dakota having reported QoI-resistant isolates (Zhang et al. 2018). This finding is interesting in that only 3% of soybean hectares grown in South Dakota were estimated to have been sprayed with a foliar fungicide in 2017 according to the U.S. Department of Agriculture – National Agricultural Statistics Service (<https://quickstats.nass.usda.gov/>). Soybean growers in South Dakota should consider managing frogeye leaf spot by planting resistant soybean cultivars, rotating soybean with nonhost crops, tilling to speed up decomposition of inoculum, and spraying fungicides that contain active ingredients from chemistry classes other than the QoI class.

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