Research

Widespread Occurrence of Quinone Outside Inhibitor Fungicide-Resistant Isolates of *Cercospora sojina*, Causal Agent of Frogeye Leaf Spot of Soybean, in the United States

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Abstract

Isolates of *Cercospora sojina*, causal agent of frogeye leaf spot of soybean (*Glycine max*), were collected across Alabama, Arkansas, Delaware, Illinois, Indiana, Iowa, Kentucky, Louisiana, Mississippi, Missouri, North Carolina, Ohio, Tennessee, and Virginia and were evaluated for quinone outside inhibitor (QoI) fungicide resistance. Collection of these isolates from these 14 states occurred between 2010 and 2017. QoI fungicide-resistant *C. sojina* isolates were detected in all 14 states surveyed and represent a total of 240 counties or parishes. In 2017, these 240 counties and parishes represented approximately 13% of the harvested soybean hectares in the United States. In light of this widespread occurrence of QoI fungicide-resistant *C. sojina* isolates, management of frogeye leaf spot should focus on integrated management practices such as planting resistant soybean cultivars, rotating with nonhost crops, and tilling to speed up decomposition of infested soybean residue. When foliar fungicide application is warranted, fungicide products that contain active ingredients from chemistry classes other than the QoI class should be applied for frogeye leaf spot management.

Frogeye leaf spot of soybean (*Glycine max*), caused by the fungus *Cercospora sojina*, has been reported to reduce soybean yield in most of the major soybean-producing countries in the world (Wrather et al. 2010). In the United States, frogeye leaf spot caused estimated annual yield reductions that ranged between 101,432 and 493,880 metric tons from 2010 to 2014 (Allen et al. 2017). Applications of quinone outside inhibitor (QoI) fungicides had been an effective method of managing frogeye leaf spot (Dorrance et al. 2010; Mengistu et al. 2014; Nelson et al. 2010). Isolates of *C. sojina* highly resistant to QoI fungicides were collected from a soybean field in Tennessee in 2010, for which QoI fungicides were ineffective in managing frogeye leaf spot (Zhang et al. 2012a). This was the first report of QoI fungicide resistance in *C. sojina*, and these isolates were later confirmed to possess the G143A mutation, which confers resistance to QoI fungicides (Zeng et al. 2015). Fungi with the G143A mutation have an amino acid substitution that occurs in the cytochrome b gene, where glycine is substituted with alanine at position 143 (Fungicide Resistance Action Committee 2014). Since the initial observation of QoI fungicide-resistant *C. sojina* isolates in Tennessee in 2010, QoI fungicide-resistant

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C. sojina isolates also have been reported in Arkansas, Illinois, Kentucky, and Mississippi (Standish et al. 2015; Zeng et al. 2015; Zhang 2012). According to a survey conducted by the U.S. Department of Agriculture–National Agricultural Statistics Service, 11% of the 2015 soybean hectares planted in the United States received a fungicide application, and of those hectares, approximately 9% (approximately 2.9 million hectares) received a fungicide application of a product that contained a QoI fungicide as one of the active ingredients (https://www.nass.usda.gov/). Given that QoI fungicides were used on a large number of soybean hectares in the United States, it is important to know where QoI fungicide-resistant isolates of *C. sojina* occur so that effective frogeye leaf spot management guidelines can be developed and implemented. The objectives of this research were to document previously unpublished observations of QoI fungicide-resistant *C. sojina* in the United States and to discuss the importance of both new and previously reported observations on their potential impact on U.S. soybean production and management of frogeye leaf spot.

Sample Collection and Fungal Isolation

Soybean leaves were collected across 14 states (Alabama, Arkansas, Delaware, Illinois, Indiana, Iowa, Kentucky, Louisiana, Mississippi, Missouri, North Carolina, Ohio, Tennessee, and Virginia) using a variety of methods from 2010 to 2017. In some cases, formal surveys were established in states to determine the occurrence of QoI fungicide-resistant isolates of *C. sojina*. In others, soybean leaves were sent to university plant diagnostic laboratories from fields in which QoI fungicide-resistant isolates of *C. sojina* were suspected, or university extension specialists personally visited suspect fields and collected leaf samples. Leaf samples generally were incubated in a high-moisture environment (i.e., sealed plastic box with moistened paper towels or sealed zipper-type plastic bag) at room temperature for at least 24 h to allow for sporulation to occur within lesions. Either resulting conidia were immediately tested for QoI fungicide resistance or a pure culture was obtained by transferring single conidia to microbiological media and was tested for QoI fungicide resistance later.

Detection of QoI Fungicide-Resistant *C. sojina* Isolates

Determination of QoI fungicide resistance was done by determining the effective concentration at which 50% conidial germination was inhibited (EC_{50}), by using a discriminatory dose assay, or by using a molecular assay. Some *C. sojina* isolates were tested using more than one of these methods. To determine EC_{50} values, methods described by Zhang et al. (2012a, b) were used. Briefly, *C. sojina* conidial suspensions were pipetted onto potato dextrose agar (PDA; Becton, Dickinson, and Company, Franklin Lakes, NJ) that had been amended with different concentrations of technical-grade azoxystrobin (Syngenta Crop Protection,

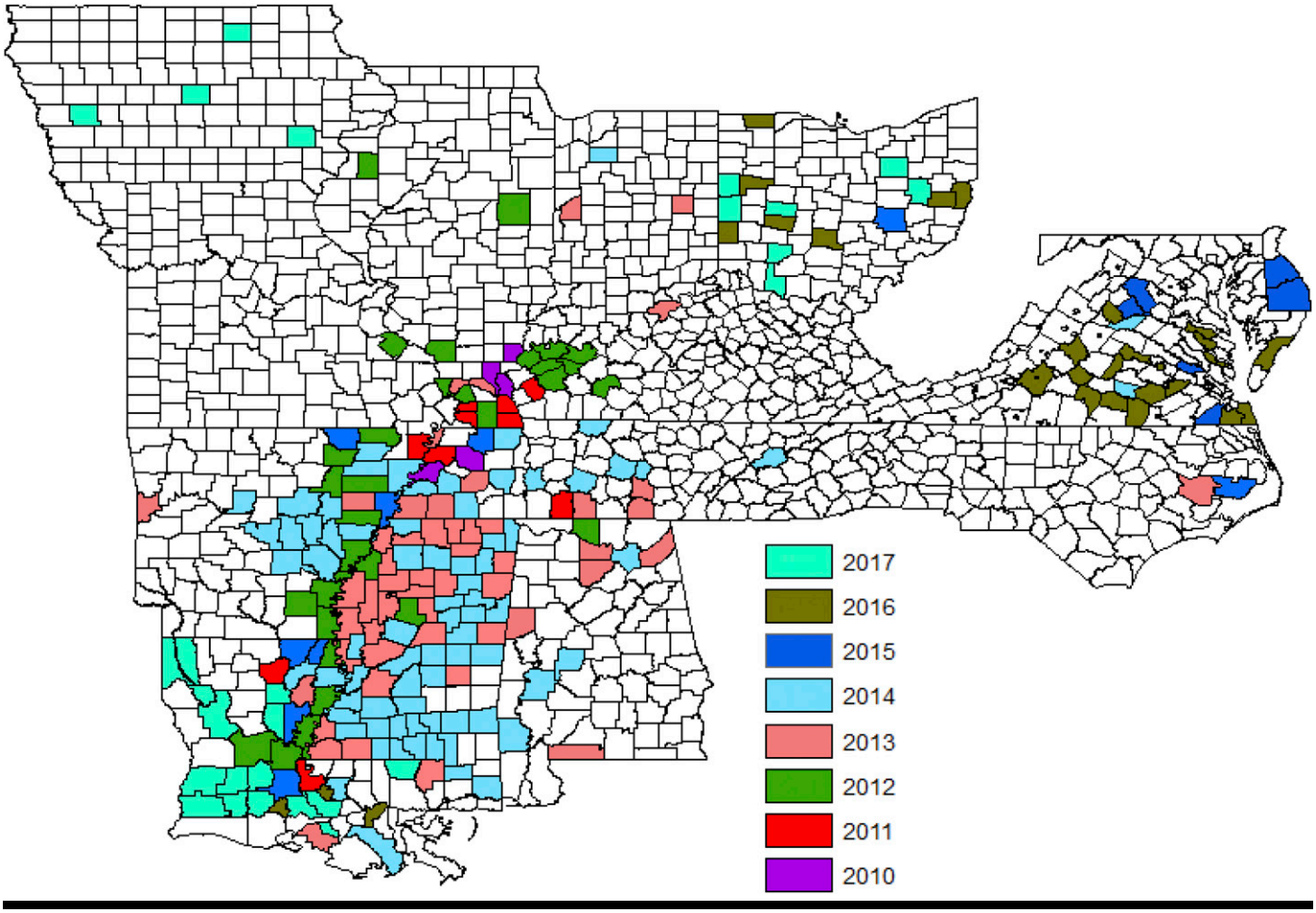

FIGURE 1

Years in which quinone outside inhibitor (QoI) fungicide-resistant *Cercospora sojina* isolates were first confirmed in counties and parishes in the United States from 2010 to 2017.

TABLE 1
Counties and parishes of states in which soybean fields have been confirmed to have quinone outside inhibitor fungicide-resistant isolates of *Cercospora sojina*

State	County or parish	First year observed	Confirming laboratory	Method(s)[a]	Publication(s)[b]
Alabama	Cullman	2013	Bradley	DDA; SSP	None
	DeKalb	2013	Bradley	DDA; SSP	None
	Escambia	2013	Bradley	DDA; SSP	None
	Limestone	2012	Bradley	DDA	None
	Marengo	2014	Bradley	DDA	None
	Marshall	2014	Bradley	DDA; SSP	None
	Morgan	2013	Bradley	DDA; SSP	None
	Perry	2014	Bradley	DDA; SSP	None
	Pickens	2013	Bradley	DDA; SSP	None
	Washington	2014	Bradley	DDA; SSP	None
Arkansas	Arkansas	2014	Faske	DDA; EC_{50}	None
	Ashley	2014	Faske	DDA; EC_{50}	None
	Chicot	2012	Bradley	DDA; EC_{50}	None
	Clay	2012	Faske	DDA; EC_{50}	None
	Conway	2014	Faske	DDA; EC_{50}	None
	Craighead	2014	Faske	DDA; EC_{50}	None
	Crittenden	2015	Faske	DDA; EC_{50}	None
	Cross	2013	Faske	DDA; EC_{50}	None
	Desha	2012	Bradley	DDA; EC_{50}	None
	Drew	2012	Bradley	DDA; EC_{50}	None
	Greene	2014	Faske	DDA; EC_{50}	None
	Jackson	2012	Faske	DDA; EC_{50}	None
	Jefferson	2014	Faske	DDA; EC_{50}	None
	Lawrence	2012	Bradley	DDA; EC_{50}	None
	Lee	2014	Faske	DDA; EC_{50}	None
	Lonoke	2014	Faske	DDA; EC_{50}	None
	Mississippi	2014	Faske	DDA; EC_{50}	None
	Monroe	2014	Faske	DDA; EC_{50}	None
	Phillips	2012	Bradley	EC_{50}; SSP	Zeng et al. (2015)
	Poinsett	2012	Bradley	DDA; EC_{50}	None
	Prairie	2014	Faske	DDA; EC_{50}	None
	Pulaski	2014	Faske	DDA; EC_{50}	None
	Randolph	2015	Faske	DDA; EC_{50}	None
	Saint Francis	2012	Bradley	DDA; EC_{50}	None
	Sebastian	2013	Bradley	DDA; SSP	None
	White	2014	Faske	DDA; EC_{50}	None
	Woodruff	2014	Faske	DDA; EC_{50}	None
Delaware	Kent	2015	Mehl	PYRO	None
	Sussex	2015	Mehl	PYRO	None
Illinois	Alexander	2012	Bradley	EC_{50}; SSP	Zeng et al. (2015)
	Champaign	2012	Bradley	DDA; EC_{50}	None
	Gallatin	2010	Bradley	EC_{50}; SSP	Zeng et al. (2015); Zhang (2012)
	Jackson	2012	Bradley	DDA; SSP	None
	Massac	2013	Bradley	DDA; SSP	None
	Pope	2010	Bradley	EC_{50}	Zhang (2012)
	Pulaski	2013	Bradley	DDA; SSP	None
	Warren	2012	Bradley	DDA; EC_{50}	None
Indiana	Clark	2013	Bradley	DDA; SSP	None

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[a] Methods of confirmation: discriminatory dose assay (DDA) (Zhang 2012); effective concentration in which 50% conidial germination was inhibited relative to a nonamended control assay (EC_{50}) (Zhang et al. 2012b); polymerase chain reaction (PCR)-restricted fragment length polymorphism method (PCR-RFLP) (Standish et al. 2015); G143A single nucleotide polymorphism detection using pyrosequencing method (PYRO); and sequence specific PCR primers (SSP) (Zeng et al. 2015).

[b] Quinone outside inhibitor fungicide-resistant isolates were reported previously in publication(s) listed in this column, if any.

TABLE 1
(*Continued from previous page*)

State	County or parish	First year observed	Confirming laboratory	Method(s)[a]	Publication(s)[b]
	Delaware	2013	Bradley	DDA; SSP	None
	Fountain	2013	Bradley	DDA; SSP	None
	Pulaski	2014	Bradley	DDA	None
Iowa	Floyd	2017	Bradley	DDA; SSP	None
	Shelby	2017	Bradley	DDA; SSP	None
	Story	2017	Bradley	DDA; SSP	None
	Washington	2017	Bradley	DDA; SSP	None
Kentucky	Ballard	2012	Bradley	DDA; EC_{50}	None
	Butler	2012	Bradley	DDA; EC_{50}	None
	Caldwell	2011	Bradley	EC_{50}	Zhang (2012)
	Calloway	2011	Bradley	EC_{50}	None
	Carlisle	2011	Bradley	EC_{50}	None
	Daviess	2012	Bradley	DDA; EC_{50}	None
	Graves	2012	Bradley	DDA; EC_{50}	None
	Henderson	2012	Bradley	DDA; EC_{50}	None
	Hickman	2011	Bradley	EC_{50}	None
	Hopkins	2012	Bradley	DDA; EC_{50}	None
	Livingston	2010	Bradley	EC_{50}	None
	Marshall	2011	Bradley	EC_{50}	None
	McLean	2012	Bradley	DDA; EC_{50}	None
	Union	2012	Bradley	DDA; EC_{50}	None
	Webster	2012	Bradley	DDA; EC_{50}	None
Louisiana	Acadia	2017	Hollier	EC_{50}	None
	Allen	2017	Hollier	EC_{50}	None
	Avoyelles	2012	Bradley	EC_{50}	None
	Beauregard	2017	Hollier	EC_{50}	None
	Bossier	2017	Hollier	EC_{50}	None
	Caddo	2017	Hollier	EC_{50}	None
	Calcasieu	2017	Hollier	EC_{50}	None
	Caldwell	2017	Hollier	EC_{50}	None
	Catahoula	2015	Price	DDA	None
	Concordia	2012	Bradley	DDA	None
	East Baton Rouge	2014	Tomaso-Peterson	PCR-RFLP	None
	East Carroll	2012	Bradley	DDA	None
	Evangeline	2017	Hollier	EC_{50}	None
	Franklin	2013	Bradley	DDA	None
	Iberville	2017	Hollier	EC_{50}	None
	Jefferson Davis	2017	Hollier	EC_{50}	None
	Lafourche	2014	Tomaso-Peterson	PCR-RFLP	None
	LaSalle	2017	Hollier	EC_{50}	None
	Madison	2014	Bradley	DDA; SSP	None
	Morehouse	2015	Price	DDA	None
	Natchitoches	2017	Hollier	EC_{50}	None
	Ouachita	2011	Bradley	EC_{50}	None
	Pointe Coupee	2011	Bradley	EC_{50}	None
	Rapides	2012	Bradley	DDA; EC_{50}	None
	Red River	2017	Hollier	EC_{50}	None
	Richland	2014	Bradley	DDA; SSP	None
	Saint Landry	2015	Price	DDA	None
	Saint Martin	2017	Hollier	EC_{50}	None
	Saint Mary	2013	Bradley	DDA	None
	Tensas	2012	Bradley	DDA; EC_{50}	None
	Washington	2017	Hollier	EC_{50}	None
	West Carroll	2015	Price	DDA	None

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TABLE 1
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State	County or parish	First year observed	Confirming laboratory	Method(s)[a]	Publication(s)[b]
Mississippi	Adams	2013	Tomaso-Peterson	PCR-RFLP	Standish et al. (2015)
	Alcorn	2013	Tomaso-Peterson	PCR-RFLP	Standish et al. (2015)
	Amite	2013	Tomaso-Peterson	PCR-RFLP	Standish et al. (2015)
	Attala	2013	Tomaso-Peterson	PCR-RFLP	Standish et al. (2015)
	Benton	2013	Tomaso-Peterson	PCR-RFLP	Standish et al. (2015)
	Bolivar	2013	Tomaso-Peterson	PCR-RFLP	Standish et al. (2015)
	Calhoun	2013	Tomaso-Peterson	PCR-RFLP	Standish et al. (2015)
	Carroll	2012	Bradley	DDA; EC_{50}	None
	Chickasaw	2013	Tomaso-Peterson	PCR-RFLP	Standish et al. (2015)
	Choctaw	2014	Tomaso-Peterson	PCR-RFLP	Standish et al. (2015)
	Claiborne	2014	Tomaso-Peterson	PCR-RFLP	Standish et al. (2015)
	Clay	2014	Tomaso-Peterson	PCR-RFLP	Standish et al. (2015)
	Coahoma	2012	Bradley	DDA; EC_{50}	None
	Copiah	2014	Tomaso-Peterson	PCR-RFLP	Standish et al. (2015)
	Covington	2014	Tomaso-Peterson	PCR-RFLP	Standish et al. (2015)
	DeSoto	2013	Tomaso-Peterson	PCR-RFLP	Standish et al. (2015)
	Forrest	2014	Tomaso-Peterson	PCR-RFLP	Standish et al. (2015)
	Franklin	2014	Tomaso-Peterson	PCR-RFLP	Standish et al. (2015)
	Grenada	2013	Tomaso-Peterson	PCR-RFLP	Standish et al. (2015)
	Hinds	2013	Tomaso-Peterson	PCR-RFLP	Standish et al. (2015)
	Holmes	2014	Tomaso-Peterson	PCR-RFLP	Standish et al. (2015)
	Humphreys	2013	Tomaso-Peterson	PCR-RFLP	Standish et al. (2015)
	Issaquena	2013	Tomaso-Peterson	PCR-RFLP	Standish et al. (2015)
	Itawamba	2013	Tomaso-Peterson	PCR-RFLP	Standish et al. (2015)
	Jackson	2014	Tomaso-Peterson	PCR-RFLP	Standish et al. (2015)
	Jefferson	2014	Tomaso-Peterson	PCR-RFLP	Standish et al. (2015)
	Jefferson Davis	2014	Tomaso-Peterson	PCR-RFLP	Standish et al. (2015)
	Jones	2014	Tomaso-Peterson	PCR-RFLP	Standish et al. (2015)
	Kemper	2014	Tomaso-Peterson	PCR-RFLP	Standish et al. (2015)
	Lafayette	2014	Tomaso-Peterson	PCR-RFLP	Standish et al. (2015)
	Lamar	2014	Tomaso-Peterson	PCR-RFLP	Standish et al. (2015)
	Lawrence	2014	Tomaso-Peterson	PCR-RFLP	Standish et al. (2015)
	Leake	2013	Tomaso-Peterson	PCR-RFLP	Standish et al. (2015)
	Lee	2013	Tomaso-Peterson	PCR-RFLP	Standish et al. (2015)
	Leflore	2013	Tomaso-Peterson	PCR-RFLP	Standish et al. (2015)
	Lincoln	2014	Tomaso-Peterson	PCR-RFLP	Standish et al. (2015)
	Lowndes	2013	Tomaso-Peterson	PCR-RFLP	Standish et al. (2015)
	Madison	2013	Tomaso-Peterson	PCR-RFLP	Standish et al. (2015)
	Marion	2014	Tomaso-Peterson	PCR-RFLP	Standish et al. (2015)
	Marshall	2013	Tomaso-Peterson	PCR-RFLP	Standish et al. (2015)
	Monroe	2013	Tomaso-Peterson	PCR-RFLP	Standish et al. (2015)
	Montgomery	2013	Tomaso-Peterson	PCR-RFLP	Standish et al. (2015)
	Neshoba	2014	Tomaso-Peterson	PCR-RFLP	Standish et al. (2015)
	Newton	2013	Tomaso-Peterson	PCR-RFLP	Standish et al. (2015)
	Noxubee	2013	Tomaso-Peterson	PCR-RFLP	Standish et al. (2015)
	Oktibbeha	2013	Tomaso-Peterson	PCR-RFLP	Standish et al. (2015)
	Panola	2013	Tomaso-Peterson	PCR-RFLP	Standish et al. (2015)
	Pearl River	2013	Tomaso-Peterson	PCR-RFLP	Standish et al. (2015)
	Pike	2014	Tomaso-Peterson	PCR-RFLP	Standish et al. (2015)
	Pontotoc	2013	Tomaso-Peterson	PCR-RFLP	Standish et al. (2015)
	Prentiss	2014	Tomaso-Peterson	PCR-RFLP	Standish et al. (2015)
	Quitman	2013	Tomaso-Peterson	PCR-RFLP	Standish et al. (2015)
	Rankin	2014	Tomaso-Peterson	PCR-RFLP	Standish et al. (2015)
	Scott	2014	Tomaso-Peterson	PCR-RFLP	Standish et al. (2015)
	Sharkey	2014	Tomaso-Peterson	PCR-RFLP	Standish et al. (2015)

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TABLE 1
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State	County or parish	First year observed	Confirming laboratory	Method(s)[a]	Publication(s)[b]
	Simpson	2014	Tomaso-Peterson	PCR-RFLP	Standish et al. (2015)
	Stone	2014	Tomaso-Peterson	PCR-RFLP	Standish et al. (2015)
	Sunflower	2013	Tomaso-Peterson	PCR-RFLP	Standish et al. (2015)
	Tallahatchie	2013	Tomaso-Peterson	PCR-RFLP	Standish et al. (2015)
	Tate	2013	Tomaso-Peterson	PCR-RFLP	Standish et al. (2015)
	Tippah	2013	Tomaso-Peterson	PCR-RFLP	Standish et al. (2015)
	Tishomingo	2013	Tomaso-Peterson	PCR-RFLP	Standish et al. (2015)
	Tunica	2013	Tomaso-Peterson	PCR-RFLP	Standish et al. (2015)
	Union	2013	Tomaso-Peterson	PCR-RFLP	Standish et al. (2015)
	Walthall	2014	Tomaso-Peterson	PCR-RFLP	Standish et al. (2015)
	Warren	2013	Tomaso-Peterson	PCR-RFLP	Standish et al. (2015)
	Washington	2013	Tomaso-Peterson	PCR-RFLP	Standish et al. (2015)
	Wayne	2014	Tomaso-Peterson	PCR-RFLP	Standish et al. (2015)
	Webster	2013	Tomaso-Peterson	PCR-RFLP	Standish et al. (2015)
	Wilkinson	2013	Tomaso-Peterson	PCR-RFLP	Standish et al. (2015)
	Winston	2014	Tomaso-Peterson	PCR-RFLP	Standish et al. (2015)
	Yalobusha	2013	Tomaso-Peterson	PCR-RFLP	Standish et al. (2015)
	Yazoo	2013	Tomaso-Peterson	PCR-RFLP	Standish et al. (2015)
Missouri	Pemiscot	2011	Bradley	EC_{50}	None
	Ste. Genevieve	2012	Bradley	DDA; EC_{50}	None
North Carolina	Beaufort	2013	Bradley	DDA; SSP	None
	Hyde	2015	Mehl	PYRO	None
Ohio	Auglaize	2016	Dorrance	SSP	None
	Brown	2017	Dorrance	SSP	None
	Champaign	2017	Dorrance	SSP	None
	Clark	2016	Dorrance	SSP	None
	Clinton	2017	Dorrance	SSP	None
	Darke	2017	Dorrance	SSP	None
	Fulton	2016	Dorrance	SSP	None
	Harrison	2016	Dorrance	SSP	None
	Jefferson	2016	Dorrance	SSP	None
	Mercer	2017	Dorrance	SSP	None
	Muskingum	2015	Dorrance	SSP	None
	Pickaway	2016	Dorrance	SSP	None
	Preble	2016	Dorrance	SSP	None
	Tuscarawas	2017	Dorrance	SSP	None
	Wayne	2017	Dorrance	SSP	None
Tennessee	Cannon	2014	Kelly	DDA	None
	Coffee	2013	Kelly	DDA	None
	Dyer	2011	Bradley	EC_{50}	None
	Fayette	2013	Kelly	DDA	None
	Franklin	2013	Kelly	DDA	None
	Gibson	2010	Bradley	EC_{50}	Zhang (2012)
	Giles	2013	Kelly	DDA	None
	Hardeman	2014	Kelly	DDA	None
	Haywood	2014	Kelly	DDA	None
	Henderson	2014	Kelly	DDA	None
	Henry	2014	Kelly	DDA	None
	Knox	2014	Kelly	DDA	None
	Lake	2013	Kelly	DDA	None
	Lauderdale	2010	Bradley	EC_{50}; DDA; SSP	Zeng et al. (2015); Zhang (2012); Zhang et al. (2012a)
	Lawrence	2011	Bradley	EC_{50}	None
	Madison	2013	Kelly	DDA	None

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State	County or parish	First year observed	Confirming laboratory	Method(s)[a]	Publication(s)[b]
	Maury	2014	Kelly	DDA	None
	Perry	2014	Kelly	DDA	None
	Robertson	2014	Kelly	DDA	None
	Rutherford	2014	Kelly	DDA	None
	Shelby	2013	Kelly	DDA	None
	Tipton	2014	Kelly	DDA	None
	Weakley	2015	Kelly	DDA	None
Virginia	Accomack	2016	Mehl	PYRO	None
	Appomattox	2016	Mehl	PYRO	None
	Bedford	2016	Mehl	PYRO	None
	Brunswick	2016	Mehl	PYRO	None
	Charlotte	2016	Mehl	PYRO	None
	Chesapeake	2016	Mehl	PYRO	None
	Culpeper	2015	Mehl	PYRO	None
	Cumberland	2016	Mehl	PYRO	None
	Dinwiddie	2016	Mehl	PYRO	None
	Fauquier	2015	Mehl	PYRO	None
	Goochland	2016	Mehl	PYRO	None
	Lunenburg	2016	Mehl	PYRO	None
	Madison	2016	Mehl	PYRO	None
	Middlesex	2016	Mehl	PYRO	None
	Nelson	2016	Mehl	PYRO	None
	New Kent	2015	Mehl	PYRO	None
	Nottoway	2014	Mehl	PYRO	None
	Orange	2014	Mehl	PYRO	None
	Prince George	2016	Mehl	PYRO	None
	Richmond	2016	Mehl	PYRO	None
	Suffolk	2015	Mehl	PYRO	None
	Virginia Beach	2016	Mehl	PYRO	None
	Westmoreland	2016	Mehl	PYRO	None

Greensboro, NC), pyraclostrobin (BASF Corp., Research Triangle Park, NC), or trifloxystrobin (Bayer CropScience, Research Triangle Park, NC). Nonamended PDA was included as a control, and salicylhydroxamic acid (Sigma-Aldrich, St. Louis, MO) was added to PDA (60 μg/ml) to prevent alternative respiration (Wood and Hollomon 2003). After 18 h, conidial germination was evaluated through a compound microscope, and EC_{50} values were determined.

The discriminatory dose assay was developed and described by Zhang (2012). The methods used for this assay were the same as described above for EC_{50} determination, except that only a single concentration of a fungicide was used, along with a nonamended control. Generally, azoxystrobin was the only fungicide used in the discriminatory dose assay (1 μg/ml), but pyraclostrobin (0.1 μg/ml) or trifloxystrobin (1 μg/ml) were used occasionally. Conidia that germinated on these discriminatory doses of these fungicides were considered to be resistant to QoI fungicides. For each assay conducted, known QoI fungicide-resistant (isolate CS 1036, from Lauderdale County, TN [Zhang and Bradley 2017; Zhang et al. 2012a]) and fungicide-sensitive (isolate S9, from Georgia [Zhang et al. 2012b]) *C. sojina* isolates were included as internal controls.

A molecular assay described by Zeng et al. (2015) also was used to confirm QoI fungicide resistance in collected *C. sojina* isolates. Methods described by Zeng et al. (2015) were used. Briefly, DNA from single-spored pure cultures was extracted using FastDNA kits (Qbiogene, Carlsbad, CA), and the polymerase chain reaction (PCR) primer pairs Cs-1F/Cs-1R-2 and Cs-2F/Cs-5R-2 were used to detect *C. sojina* isolates with and without the G143A mutation, respectively. For some isolates from Delaware, North Carolina, and Virginia, a pyrosequencing assay was designed to detect the presence of the G143A mutation (Zhou and Mehl 2016). PCR and pyrosequencing primers targeting the cytochrome b gene were designed using PyroMark Assay Design 2.0 software (Qiagen, Germantown, MD). Pyrosequencing reactions were run on a PyroMark Q24 (Qiagen). Previously published QoI fungicide-resistant isolates from Mississippi that are also reported here were confirmed using a PCR restricted fragment length polymorphism method described by Standish et al. (2015).

Confirmations of QoI fungicide-resistant *C. sojina* isolates by year of first detection in a county or parish are presented in Figure 1. In addition, the methods used to identify these QoI fungicide-resistant isolates and the laboratory in which the confirmations were completed are shown in Table 1. When multiple methods were used to determine QoI fungicide resistance, all methods were in agreement every time. These confirmations reported here include previously reported findings from counties in Arkansas, Illinois, Kentucky, and Mississippi (Standish et al. 2015; Zeng et al. 2015; Zhang 2012; Zhang et al. 2012a). From 2010 to 2017, QoI fungicide-resistant *C. sojina* isolates were detected in 240 counties or parishes from 14 states (Alabama, Arkansas, Delaware, Illinois, Indiana, Iowa, Kentucky, Louisiana, Mississippi, Missouri, North Carolina, Ohio, Tennessee, and Virginia). These 240 counties and parishes represent approximately 13% of the harvested soybean hectares in

the United States in 2017 (https://www.nass.usda.gov/). We are only reporting counties or parishes in which QoI fungicide-resistant isolates were detected and are not reporting counties or parishes in which QoI fungicide-resistant isolates were not detected. In addition, detection of only one QoI fungicide-resistant isolate in a county or parish was the threshold for reporting that county or parish in Figure 1 and Table 1.

Conclusions and Implications

Our research has determined that QoI fungicide-resistant isolates of *C. sojina* are widespread across many soybean-producing states. Based on the widespread occurrence, counties or parishes within these and other states that have not yet been confirmed likely have QoI fungicide-resistant isolates. It is also important to note that not all counties and parishes in these and other states have been surveyed for QoI fungicide-resistant *C. sojina* isolates. QoI fungicide-resistant isolates likely will persist in these areas, because no fitness costs associated with the G143A mutation have been previously reported in *C. sojina* (Zhang and Bradley 2017).

In light of the widespread occurrence of QoI fungicide-resistant *C. sojina* isolates, management of frogeye leaf spot may become more complex. Zhang and Bradley (2017) reported that, compared with sensitive isolates, QoI fungicide-resistant *C. sojina* isolates were more aggressive in causing frogeye leaf spot on soybean within the first 8 days after inoculation in the greenhouse. An integrated management approach that includes planting frogeye leaf spot-resistant soybean cultivars, rotating soybean with nonhost crops, and tilling to help increase decomposition of soybean residue may be required to reduce frogeye leaf spot severity below economically damaging levels. When foliar fungicide application is warranted, fungicide products that contain active ingredients from chemistry classes other than the QoI class should be applied for frogeye leaf spot management, which will help reduce additional selection for QoI fungicide-resistant *C. sojina* isolates. Fungicides in the demethylation inhibitor and methyl benzimidazole carbamate chemistry classes have been reported to reduce frogeye leaf spot severity (Akem 1995; Akem and Dashiell 1994; Backman et al. 1979; Dashiell and Akem 1991; Dorrance et al. 2010; Galloway 2008) compared with nontreated controls, and they should be considered as alternatives or supplements to QoI fungicides for management of frogeye leaf spot.

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