Research

Distribution of G143A Mutations Conferring Fungicide Resistance in Virginia Populations of *Parastagonospora nodorum* Infecting Wheat

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Abstract

Stagonospora nodorum blotch (SNB) caused by *Parastagonospora nodorum* is an important leaf spot disease in the mid-Atlantic United States. Disease management approaches include use of resistant varieties, cultural control, and foliar fungicides. Frequent use of foliar fungicides can select for fungicide resistance within pathogen populations. Recently, the first report of quinone outside inhibitor (QoI) fungicide resistance in the United States was made based on a relatively small collection of *P. nodorum* isolates from Virginia. The objective of this study was to conduct a statewide, 2-year survey of *P. nodorum* populations in Virginia wheat and quantify frequencies of the target-site mutation that confers QoI resistance. A total of 318 isolates of *P. nodorum* were obtained from wheat collected at seven locations distributed throughout the wheat-growing regions of Virginia in 2018 and 2019. A previously designed pyrosequencing assay that detects the G143A substitution in the cytochrome *b* gene of *P. nodorum* was used to screen isolates for the presence or absence of the target site mutation. The G143A substitution was detected in all sampled fields. Among locations and years, frequencies of the mutation in *P. nodorum* populations ranged from 5 to 32% (mean = 19%). Thus, the QoI-resistance-conferring G143A mutation was widespread in *P. nodorum* populations in Virginia, and it occurred at a relatively high frequency. Results suggest that fungicides containing QoI active ingredients may not be effective for controlling SNB in Virginia and the surrounding region, and application of standalone QoI fungicides for disease control in wheat is not recommended.

Keywords: *Parastagonospora nodorum*, Stagonospora nodorum blotch, quinone outside inhibitors, pyrosequencing

Stagonospora nodorum blotch (SNB), also known as Stagonospora nodorum glume and leaf blotch of wheat, is caused by the fungal pathogen *Parastagonospora nodorum* ([Berk.] Quaedvlieg, Verkley & Crous) (teleomorph: *Phaeosphaeria nodorum* [Hedjar.] syn. *Leptosphaeria nodorum* [Müll.], syn. *Septoria nodorum* [Berk.], syn. *Stagonospora nodorum* [Berk.]). Yield losses up to 50% have been attributed to SNB in Europe and the United States (Bhathal et al. 2003; King et al. 1983). Management recommendations for SNB include cultural practices such as tillage and crop rotation that reduce sources of primary inoculum, planting of moderately resistant wheat varieties, and application of foliar fungicides. As the soil conservation practice of reduced tillage has become more common in certain regions including the mid-Atlantic United States, earlier onset and increased severity of SNB have been observed in association with increasing levels of crop residues (Mehra et al. 2015). Thus, more intensive disease management practices including earlier, more frequent fungicide applications are needed for adequate control of SNB in some fields. Although wheat cultivars with moderate resistance to SNB are available, none have complete resistance, so growers rely on use of foliar fungicides to manage SNB.

Fungicide chemistries recommended for control of SNB include quinone outside inhibitors (QoIs), demethylation inhibitors (DMIs), and succinate dehydrogenase inhibitors (SDHIs). Due to the availability of generic formulations of QoI fungicides and QoI active ingredients being included in several fungicide premixes labeled for wheat, a large proportion of the wheat crop is exposed to this class of fungicides (Sylvester and Kleczewski 2018; Sylvester et al. 2018). QoI fungicides inhibit mitochondrial respiration by blocking the transfer of electrons between the cytochrome *b* and cytochrome c_1 complex, which slows down the growth of the fungus (Esser et al. 2004). Due to this site-specific mode of action, QoI fungicides are considered high risk for fungicide resistance development (Brent and Hollomon 2007). Several point mutations in the amino acid sequences of the cytochrome *b* gene including G143A, F129L, and G137R have been identified that confer moderate to complete resistance to the QoI fungicides in different fungal pathogens (Grasso et al. 2006; Sierotzki et al. 2007). However, G143A is the only mutation that has been reported for QoI resistance in *P. nodorum* (Blixt et al. 2009; Kaur et al. 2021).

Although there are widespread reports of QoI fungicide resistance in other pathogens in the United States, there are only a few reports of QoI resistance in *P. nodorum* worldwide. The first report of *P. nodorum* QoI insensitivity was reported from Sweden over a decade ago (Blixt et al. 2009), but the first report of

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QoI resistance in the United States was not documented until recently in Virginia. Kaur et al. (2021) characterized QoI resistance in a relatively small collection of *P. nodorum* isolates and determined that the G143A mutation was associated with insensitivity to QoI fungicides. Thus, as part of this previous study, a pyrosequencing assay targeting the single nucleotide polymorphism (SNP) that results in the G143A substitution was developed as a rapid method to screen *P. nodorum* for the QoI-resistance-conferring mutation. Except the two reports mentioned above, widespread surveys of potential QoI resistance in *P. nodorum* populations are lacking.

As a follow-up on the first report of QoI fungicide resistance in *P. nodorum* in the United States (Kaur et al. 2021), which was limited in sample size and geographic scope, there was a need for a more detailed study of distributions of QoI-resistant *P. nodorum* in Virginia in order to better understand the potential impacts of fungicide resistance on disease management. Thus, the objective of this study was to conduct a statewide, 2-year survey of *P. nodorum* populations in Virginia wheat and quantify frequencies of the target-site mutation that confers QoI resistance.

Wheat Leaf Sample Collection and Fungal Isolation

Wheat leaf samples were collected in April to May 2018 and 2019 from soft red winter wheat variety trials at seven locations representing major wheat-growing areas throughout Virginia (Fig. 1). From each plot, 20 to 25 leaf samples with signs and symptoms of SNB were collected from five different wheat varieties (Hilliard, Shirley, USG3316, MAS#61, and Bullet), which varied in susceptibility to SNB. The samples were collected from different varieties to increase the chance of isolating a diversity of genotypes that may vary in fungicide sensitivity and the presence or absence of the G143A mutation. Leaf samples were collected at the Feekes 11.3 stage (hard dough), and samples were stored in paper bags at 4°C until further use for isolation. No fungicide was applied to the plots from which samples were collected.

Leaf samples collected from three replicate plots of the same variety at each location were combined, and 30 random lesions were plated for isolations. Four to five isolates of *P. nodorum* were obtained from each sample as described previously (Eyal et al. 1987; Jordahl and Francl 1992). Briefly, each leaf with lesions of SNB was cut into 4- to 5-cm-long pieces with only a single lesion on each piece. Leaf pieces were surface disinfested by dipping in 70% ethanol for 10 s, rinsing in sterilized distilled water for 60 s, soaking in 0.5% commercial bleach for 60 s, and rinsing a final time with distilled water. Leaf tissue was blotted dry on filter paper, and surface-disinfested leaves were placed on moistened filter paper in a Petri dish. Petri dishes were incubated under white, fluorescent light at 25°C for 48 h. Leaves were then examined under a stereoscope for pycnidia oozing pycnidiospores. A sterilized needle was used to transfer oozing spore masses from the leaf, and spores were observed under the microscope to confirm the pathogen, *P. nodorum*, based on conidial morphology (Eyal et al. 1987).

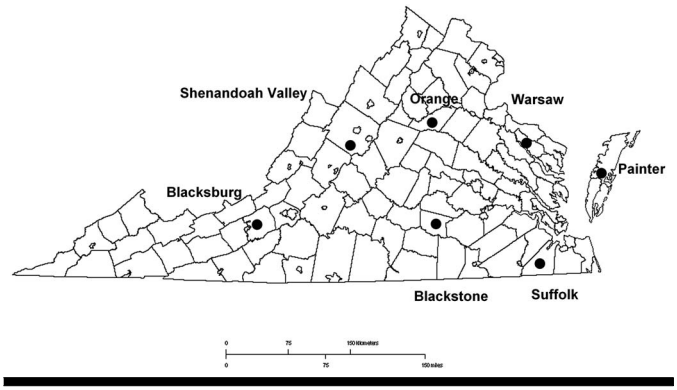

FIGURE 1

Field locations where wheat leaf samples infected with *Parastagonospora nodorum* were collected in Virginia in spring 2018 and 2019.

For single-spore purification of isolates, a sterilized needle was dipped into the spore mass oozing out from one single pycnidium and placed on a 1.5% water agar medium. A sterilized coffee stick was used to spread the spore mass on the water agar medium in the Petri dish. These plates were incubated under continuous fluorescent light at 25°C for 24 h, and plates were then examined under the compound microscope for germinated spores. A thin needle was flamed and used to transfer at least three germinated spores onto antibiotic-amended V8 potato dextrose agar (V8PDA) medium (150 ml of V8 juice, 10 g of PDA, 10 g of agar, 3 g of $CaCO_3$, 250 mg of chloramphenicol succinate, and 50 mg of streptomycin per liter). Plates were incubated for 3 to 4 days (continuous light, 25°C), and single-spore purified cultures were transferred to V8PDA and incubated at 20°C for 7 days. Agar plugs were then cut from plates and left under the laminar flow hood for drying overnight followed by storage at –20°C until further use. Overall, a total of 318 isolates were obtained over 2 years and seven locations (Table 1).

Detection of the G143A Mutation in *P. nodorum* Isolates Using Pyrosequencing

Genomic DNA was extracted from 318 isolates grown on V8PDA using the Fast DNA SPIN Kit (MP Biomedicals, Solon, OH). Fungal mycelia and spores were scraped from the plates with a sterilized wooden stick into lysing matrix tubes, followed by the addition of 1 ml of lysis buffer. The fungal tissue was macerated in a bead-beater (BioSpec Products, Bartlesville, OK) for 40 s, and the remaining steps were performed as described in the manufacturer's protocol. DNA concentrations were measured using a NanoVue Plus spectrophotometer (GE Healthcare, Chicago, IL) and adjusted to a concentration of 2 ng/µl.

A previously designed pyrosequencing assay that targets the G143A mutation in the cytochrome *b* gene of *P. nodorum* was used to screen isolates for the presence or absence of the mutation that confers QoI resistance (Kaur et al. 2021). The region surrounding the G/C SNP was amplified using HotStart PCR Pre-Mix (Bioneer, Alameda, CA) with 0.2 µM each of forward (5′-TACGTCCTTCCTTATGGGCAAATG-3′) and reverse (5′-TCACCGTAATGTGGTCCAACTG-3′) primers and 2 ng template DNA in a total reaction volume of 20 µl. PCR conditions were an initial denaturation step at 94°C for 5 min; followed by 30 cycles of 94°C for 30 s, 62°C for 30 s, and 72°C for 45 s; followed by a final extension step at 72°C for 10 min. PCR products were run on 1.5% agarose gel (IBI Scientific, Dubuque, IA), 1× TBE, at 85V for 150 min to confirm amplification of the target region before running it on the pyrosequencer. GelRed Nucleic Acid Gel Stain (Biotium, Fremont, CA) was added directly to the molten agarose gel prior to pouring, and amplicons were visualized under UV light (Bio-Rad Molecular Image Gel DOC XR+ with Image Lab software). The pyrosequencing reaction was performed on a PyroMark Q48 Autoprep Pyrosequencer (Qiagen, Germantown, MD) according to the manufacturer's protocol using the sequencing primer 5′-TGGGCAAATGTCACTAT-3′ (Kaur et al.

2021). Previously characterized isolates (Kaur et al. 2021) with and without the G143A mutation were included as controls.

Frequencies and Distributions of the G143A Mutation in Virginia Populations of *P. nodorum*

A total of 318 isolates of *P. nodorum* collected from seven Virginia locations over 2 years were screened for the presence of the QoI-resistance-conferring G143A mutation using pyrosequencing. Among locations and years, the frequency of the mutation ranged from 5 to 32%. Among the 159 isolates that were collected each year from wheat fields, 19% (n = 31) and 18% (n = 28) had the G143A mutation in 2018 and 2019, respectively. Frequencies of the mutation did not differ between the 2 years of the survey (X^2 = 0.19, P = 0.67). Isolates with the G143A mutation were recovered from all seven locations in both years (Table 1), indicating that the mutation is widespread in Virginia populations of *P. nodorum*. Even though the sampled fields were distributed across three distinct regions (Coastal Plain, Piedmont, and Valley and Ridge) that vary in agronomic factors and cropping practices, the frequency of the G143A mutation was not statistically different among locations (X^2 = 6.31, P = 0.41). Among the locations, Warsaw had the numerically greatest frequency of the G143A mutation followed by Blacksburg, Painter, Suffolk, and Blackstone (Table 1). The G143A mutation was detected less frequently in the northwestern portion of the state (Orange and Shenandoah Valley, Fig. 1) compared with other locations (Table 1).

Although specific reports of QoI fungicides losing their field efficacy against *P. nodorum* in the region are lacking, there have been observations of increased SNB disease incidence and severity in the mid-Atlantic United States (Sylvester and Kleczewski 2018). This may be at least in part due to reduced sensitivity of *P. nodorum* populations to fungicides. Although baseline fungicide sensitivity data for Virginia populations of *P. nodorum* are not available, the first report of QoI-resistant *P. nodorum* in the United States was for isolates (n = 74) collected from two varieties that were either nonfungicide treated or sprayed with a DMI fungicide in spring 2017 from four Virginia wheat fields that included three of the locations that were sampled in the current study (Kaur et al. 2021). In 2017, 10% of isolates (n = 2/20 total isolates from location) collected from Suffolk had the G143A mutation, 7% of isolates (n = 1/15) from Orange had the mutation, and none of the isolates from Warsaw (n = 0/19) had the mutation. Although differences can be attributed to sampling error, the overall G143A mutation frequency detected at these three locations in 2017 (6%) was less than the 19% frequency that was detected in 2018 and 2019 (X^2 = 6.29, P = 0.04). Isolates in the current study originated from wheat that was not treated with a QoI fungicide, but it is possible that application of QoI fungicides to wheat in previous years in or near the sampled fields may have selected for higher frequencies of the G143A mutation in the *P. nodorum* population.

Sensitivity of *P. nodorum* isolates to QoI fungicides was not evaluated in the current study, but previous studies have documented that the G143A substitution confers high levels of fungicide resistance in this fungal pathogen. Pyraclostrobin effective concentration (EC_{50}) values previously determined for Virginia isolates of *P. nodorum* were between 0.11 and 7.3 ppm for QoI-sensitive (without G143A mutation) isolates, whereas EC_{50} values for insensitive isolates with the G143A mutation ranged from 19 to 407 ppm (Kaur et al. 2021). Furthermore, isolates with the G143A mutation were up to 351 and 771 times less sensitive to pyraclostrobin and azoxystrobin, respectively, than the most sensitive *P. nodorum* isolate identified in the study. In a similar study of *P. nodorum* populations in Sweden, 98% of the isolates from four fields had the G143A substitution, and this mutation was associated with EC_{50} values exceeding 1,000 ppm (Blixt et al. 2009). Thus, these two studies indicate that QoI resistance is increasing in *P. nodorum* populations in association with the emergence of the G143A mutation. However, it should be noted that based on the previous study of Virginia *P. nodorum* populations (Kaur et al. 2021), there are isolates without the G143A mutation that are insensitive to azoxystrobin. This indicates that there are other mechanisms of resistance to older QoI fungicides to which fungal populations have been exposed over longer periods of time, and low frequencies of the G143A mutation do not necessarily mean all QoI-based fungicides will be effective for control of SNB. However, resistance to pyraclostrobin is associated with the G143A mutation, so increasing frequencies of G143A are likely to be associated with reduced efficacy of fungicides containing this active ingredient and possibly other newer QoI chemistries.

TABLE 1
Frequencies of the G143A mutation in populations of *Parastagonospora nodorum* isolated from wheat in Virginia, 2018 to 2019

			No. of isolates		G143A mutation (%)	
Region	Location	Year	G143A mutation	Total	By location and year	Average for location
Coastal Plain	Painter	2018	8	25	32	21
		2019	2	22	9	
	Suffolk	2018	5	23	22	20
		2019	4	23	16	
	Warsaw	2018	5	25	20	26
		2019	8	25	32	
Piedmont	Blackstone	2018	5	25	20	20
		2019	5	24	21	
	Orange	2018	3	20	15	10
		2019	1	22	5	
Valley and Ridge	Blacksburg	2018	4	24	16	20
		2019	5	21	24	
	Shenandoah Valley	2018	1	17	6	10
		2019	3	22	14	

Although QoI resistance has only been reported recently for *P. nodorum* in the United States, widespread occurrence of QoI resistance conferred by the G143A substitution has been documented in populations of *Cercospora sojina*, the causal agent of soybean frogeye leaf spot, in Virginia (Zhou and Mehl 2020) and throughout the United States (Zhang et al. 2018). In Virginia, *C. sojina* populations from 80% of sampled fields had the G143A substitution, and within individual fields, the frequency of the mutation ranged from <1 to 100%, indicating that selection pressure for QoI resistance was high in some areas (Zhou and Mehl 2020). Although this represents a different fungal pathogen and crop than the current study, soybean and wheat are grown in rotation in Virginia and other production areas in the United States, and many of the same fungicide formulations and active ingredients are applied to both crops. For example, 11/18 and 20/33 of the most commonly used foliar-applied fungicides for both wheat and soybean contain either a standalone QoI active ingredient or a QoI active ingredient as part of a fungicide premix, and 9 out of 11 of the QoI-containing fungicides labeled for control of foliar diseases in wheat are also labeled for soybean (Crop Protection Network 2021a, 2021b). To reduce the risk of fungicide resistance development, fungicide labels restrict the total quantity and number of applications that can be made to a crop per acre per year for fungicides containing the same mode of action (Brent and Hollomon 2007). However, in some growing regions including Virginia, "double crop" soybean is frequently planted after winter wheat is harvested in the spring (Parvej et al. 2020). Thus, there are two consecutive crops planted to the same field in a single year to which QoI-containing fungicides are potentially applied. Thus, even if fungicide active ingredients with different modes of action are being rotated or mixed as recommended for fungicide resistance management, there is a high level of selection pressure on *P. nodorum* populations due to QoI-containing fungicides being applied to multiple crops that are grown in the same field.

In the current study, a rapid pyrosequencing method was used to detect the presence of a target site point mutation that is associated with reduced sensitivity to QoI fungicides in *P. nodorum* and other fungal pathogens. Although this DNA-based method cannot give the relative measure of the fungicide insensitivity, it does allow for more rapid screening of populations for potential fungicide resistance compared with culture-based assays using fungicide-amended media. Pyrosequencing has been used to detect fungicide-resistance-conferring mutations in other pathogens including the G143A substitution in the soybean foliar pathogen *C. sojina* (Zhou and Mehl 2020) and SDHI fungicide resistance conferring mutations in *Botrytis* spp. (Gobeil-Richard et al. 2016). Although in this study the pyrosequencing assay was used to screen individual isolates for the presence/absence of the G143A SNP, this method can be adapted to quantify proportions of SNPs from pools of fungal DNA isolated directly from plant tissues as described previously for *C. sojina* infecting soybean leaves (Zhou and Mehl 2020) and *Aspergillus flavus* infecting corn grain (Mehl and Cotty 2010). Thus, the pyrosequencing assay described here can be used to rapidly screen field populations of *P. nodorum* infecting wheat leaves for frequencies of the G143A mutation, and this has the potential to be a useful tool for long-term, area-wide monitoring of the emergence of QoI resistance in *P. nodorum* populations.

Conclusions and Implications for SNB Management

Based on a 2-year, statewide survey, this study demonstrated that the QoI-resistance-conferring G143A mutation is widespread in *P. nodorum* populations in Virginia. Relatively high frequencies of this mutation indicate that QoI-based fungicides may not be effective for controlling SNB in the region. Continued application of fungicides containing QoI active ingredients may select for increased frequencies of this mutation and associated decreases in fungicide efficacy, so G143A mutation frequencies should be monitored in *P. nodorum* populations not only in Virginia but in other states where SNB is a major foliar disease of wheat. Integrated fungicide resistance management practices should be implemented such as using moderately resistant varieties to minimize the need for fungicide applications, minimizing crop residues that serve as sources of inoculum, and mixing and rotating fungicide modes of action when fungicide applications are needed for disease control.

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Literature Cited

Bhathal, J., Loughman, R., and Speijers, J. 2003. Yield reduction in wheat in relation to leaf disease from yellow (tan) spot and Septoria nodorum blotch. Eur. J. Plant Pathol. 109:435-443.

Blixt, E., Djurle, A., Yuen, J., and Olson, Å. 2009. Fungicide sensitivity in Swedish isolates of *Phaeosphaeria nodorum.* Plant Pathol. 58:655-664.

Brent, K. J., and Hollomon, D. W. 2007. Fungicide Resistance: The Assessment of Risk. FRAC Monograph No. 2, 2nd rev. ed. Crop Life International, Brussels, Belgium.

Crop Protection Network. 2021a. Fungicide efficacy for control of soybean foliar diseases. CPN-1019.

Crop Protection Network. 2021b. Fungicide efficacy for control of wheat diseases. CPN-3002.

Esser, L., Quinn, B., Li, Y.-F., Zhang, M., Elberry, M., Yu, L., Yi, C.-A., and Xia, D. 2004. Crystallographic studies of quinol oxidation site inhibitors: A modified classification of inhibitors for the cytochrome bc_1 complex. J. Mol. Biol. 341:281-302.

Eyal, Z., Scharen, A. L., Prescott, J. M., and van Ginkel, M. 1987. The Septoria Diseases of Wheat: Concepts and Methods of Disease Management. CIMMYT, Mexico City, Mexico.

Gobeil-Richard, M., Tremblay, D.-M., Beaulieu, C., Van der Heyden, H., and Carisse, O. 2016. A pyrosequencing-based method to quantify genetic substitutions associated with resistance to succinate dehydrogenase inhibitor fungicides in *Botrytis* spp. populations. Pest Manag. Sci. 72:566-573.

Grasso, V., Palermo, S., Sierotzki, H., Garibaldi, A., and Gisi, U. 2006. Cytochrome *b* gene structure and consequences for resistance to Qo inhibitor fungicides in plant pathogens. Pest Manag. Sci. 62:465-472.

Jordahl, J. G., and Francl, L. J. 1992. Increase and storage of cultures of *Pyrenophora tritici-repentis*. Page 109 in: Advances in Tan Spot Research. L. J. Francl, J. M. Krupinsky, and M. P. McMullen, eds. North Dakota Agricultural Experiment Station, Fargo, ND.

Kaur, N., Mullins, C., Kleczewski, N. M., and Mehl, H. L. 2021. Occurrence of quinone outside inhibitor (QoI) resistance in Virginia populations of *Parastagonospora nodorum* infecting wheat. Plant Dis.

King, J., Cook, R., and Melville, S. 1983. A review of Septoria diseases of wheat and barley. Ann. Appl. Biol. 103:345-373.

Mehl, H. L., and Cotty, P. J. 2010. Variation in competitive ability among isolates of *Aspergillus flavus* from different vegetative compatibility groups during maize infection. Phytopathology 100:150-159.

Mehra, L. K., Cowger, C., Weisz, R., and Ojiambo, P. S. 2015. Quantifying the effects of wheat residue on severity of Stagonospora nodorum blotch and yield in winter wheat. Phytopathology 105:1417-1426.

Parvej, M. R., Holshouser, D. L., Kratochvil, R. J., Whaley, C. M., Dunphy, E. J., Roth, G. W., and Faé, G. S. 2020. Early high-moisture wheat harvest improves double-crop system: I. Wheat yield and quality. Crop Sci. 60: 2633-2649.

Sierotzki, H., Frey, R., Wullschleger, J., Palermo, S., Karlin, S., Godwin, J., and Gisi, U. 2007. Cytochrome *b* gene sequence and structure of *Pyrenophora teres* and *P. tritici-repentis* and implications for QoI resistance. Pest Manag. Sci. 63:225-233.

Sylvester, P. N., and Kleczewski, N. M. 2018. Evaluation of foliar fungicide programs in mid-Atlantic winter wheat production systems. Crop Prot. 103:103-110.

Sylvester, P. N., Lana, F. D., Mehl, H. L., Collins, A. A., Paul, P. A., and Kleczewski, N. M. 2018. Evaluating the profitability of foliar fungicide programs in Mid-Atlantic soft-red winter wheat production. Plant Dis. 102:1627-1637.

Zhang, G., Allen, T. W., Bond, J. P., Fakhoury, A. M., Dorrance, A. E., Weber, L., Faske, T. R., Giesler, L. J., Hershman, D. E., Kennedy, G. S., Neves, D. L., Hollier, C. A., Kelly, H. M., Newman, M. A., Kleczewski, N. M., Koenning, S. R., Thiessen, L. D., Mehl, H. L., Zhou, T., Meyer, M. D., Mueller, D. S., Kandel, Y. R., Rupe, J. C., Sikora, E. J., Standish, J. R., Tomaso-Peterson, M., Wise, K. A., and Bradley, C. A. 2018. Widespread occurrence of quinone outside inhibitor fungicide-resistant isolates of *Cercospora sojina*, causal agent of frogeye leaf spot of soybean, in the United States. Plant Health Prog. 19:295-302.

Zhou, T., and Mehl, H. L. 2020. Rapid quantification of the G143A mutation conferring fungicide resistance in Virginia populations of *Cercospora sojina* using pyrosequencing. Crop Prot. 127:104942.