Corynespora cassiicola Isolates from Soybean in Alabama Detected with G143A Mutation in the Cytochrome *b* Gene

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Corynespora cassiicola (Berk. & M.A. Curtis) C.T. Wei is a widespread plant pathogenic fungus causing target necrotic spots on plant leaves, stems, and fruits, is worldwide on up to 400 plant species, and is known as *Corynespora* leaf spot or target spot on cotton and soybean. In the southeastern United States genetically distinct populations of *C. cassiicola* were found causing target spot epidemics on cotton and soybean (Sumabat et al. 2018).

Disease management has been a concern due to increasing occurrence of target spot (Godoy 2015). Severe disease symptoms and significant yield losses occur with this pathogen, especially during rainy seasons. Yield losses of 18 to 32% have been documented on soybeans in the United States (Godoy 2015). Fungicides have been a crucial tool in disease management; however, there are reported cases of *C. cassiicola* isolates from tomato, cucumber, and soybean resistant to systemic fungicides (Fungicide Resistance Action Committee [FRAC], <https://www.frac.info/publications>).

C. cassiicola is considered a high-risk pathogen for development of fungicide resistance (FRAC 2019), and mutations associated with quinone outside inhibitor (QoI) resistance have been detected in the *cytochrome b* (*cytb*) gene based on three amino acid substitutions: G143A, F129L, and G137R (Duan et al. 2019). G143A mutation has been characterized for *Cercospora soja* (Mathew et al. 2019) in the United States but not for *C. cassiicola*.

Fungicides are used to control cotton and soybean diseases; QoI-resistant populations of *C. cassiicola* might be present in the United States. For this reason, the objective was to assess the occurrence of point mutations in the *cytb* gene associated with QoI resistance from Alabama isolates of *C. cassiicola*.

Symptomatic leaves were randomly collected from cotton and soybean plants (Fig. 1A to B) across Alabama in 2017/2018. Isolates of *C. cassiicola* were obtained by direct isolation on PDA containing 0.005% kanamycin. PDA plates were incubated at

room temperature ($25 \pm 2^\circ\text{C}$), and then pure colonies were obtained to establish the *C. cassiicola* Alabama collection (Fig. 1C to D). All isolates were identified as *C. cassiicola* based on conidiophore and conidia morphology (Fig. 1E to F) and internal transcribed spacer sequencing (ITS1/ITS4).

Total DNA was extracted using a ZR Fungal/Bacterial MiniPrep kit. To identify nucleotide point mutation on *cytb* gene, fragments were amplified from total DNA using PCR primers described by Duan et al. (2019). Purified PCR products were Sanger sequenced by Eurofins MWG Operon LLC (Louisville, KY), and nucleotide sequences were edited and aligned using BioEdit Alignment Editor (Tom Hall, Ibis Biosciences). Sequences were deposited in GenBank under accession numbers MN564884 to MN564895. QoI-sensitive (C6-2) and QoI-resistant (ST-20S-1) sequences of *C. cassiicola* (Ishii et al. 2007) were included to illustrate the nucleotide point mutation.

A single DNA fragment (600 bp) was amplified using described primers, and based on *cytb* nucleotide sequences, four out of 12 isolates of *C. cassiicola* were found to have a mutation that replaces the codon for amino acid 143 from GGT to GCT, resulting in an amino acid change from glycine to alanine (G143A) (Fig. 2). These isolates originated from soybeans located in north (LIM14) and central (ELM04, ELM06, and ELM07) Alabama (Fig. 3). The G143A mutation was not found on isolates of *C. cassiicola* from cotton plants. No other point mutations on *cytb*, such as F129L and G137R, were found in our isolates.

According to FRAC (2019), the short development of resistance to a different fungicide class by *C. cassiicola* on soybean is one example of a pathogen that must be classified as threatening. The amino acid change on *cytb* from glycine to alanine at position 143 (G143A) is known to confer high levels of resistance to QoI fungicides, whereas F129L and G137R mutations are responsible for low to moderate levels of resistance (Duan et al. 2019).

QoI fungicides have a single-site mode of action and are extensively applied to manage multiple diseases in field crops. Given the high risk of *C. cassiicola* to develop fungicide resistance (Duan et al. 2019; Ishii et al. 2007), the management of fungicide resistance will be a major challenge. Knowing that field populations of *C. cassiicola* have mutations associated with QoI resistance, it will be necessary to monitor the spread of resistant isolates to manage resistance development. To the best of our knowledge, this study is the first to report G143A mutants in *C. cassiicola* from field populations from soybean in the United States.

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FIGURE 1

Target spot and its causal agent, *Corynespora cassiicola*. Disease symptoms on cotton (A) and soybean (B) leaves. Top and bottom of PDA plates with pure colonies of *C. cassiicola* obtained from cotton (C) and soybean (D) to establish a new collection from Alabama. Detail of the *C. cassiicola* conidiophore (E) and conidia (F) morphology.