The G143A Mutation Confers Azoxystrobin Resistance to Soybean *Cercospora* Leaf Blight in Bolivia

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In Bolivia, two soybean crops are produced per year in the same field plot without crop rotation. Since 2003, the frequency of fungicide applications for the control of Asian soybean rust (ASR) in soybean fields in Bolivia and Brazil has been increasing, with the total number of applications approximating 70 to 104 uninterrupted strobilurin applications per field plot. Although these applications target ASR, nontarget pathogens, including *Cercospora* spp., are also exposed to fungicides. Quinone outside inhibitor (QoI) fungicide resistance in *Cercospora* cf. *flagellaris* and *C. cf. sigesbeckiae*, causal agents of *Cercospora* leaf blight (CLB) of soybean (*Glycine max*) in North America, were first reported in 2015 and 2016 (Albu et al. 2016a; Price et al. 2015) and known to be conferred by the G143A mutation in cytochrome *b*. Although *Cercospora* species are present in South America in association with CLB (Soares et al. 2015), G143A mutants have not been reported outside of North America. The objectives of this study were to determine the identity of *Cercospora* species associated with CLB in Bolivia, the prevalence of QoI resistance in these populations, and, if present, the mutations conferring resistance.

Thirty-eight *Cercospora* spp. isolates were collected from soybean leaves displaying symptoms of CLB from commercial soybean fields at three locations in Santa Cruz, Bolivia (16.84948889 S, 63.43944167 W; 16.469625 S, 63.44434167 W; and 16.47700556 S, 63.424625 W). Isolates were collected in February 2017 from soybean plants sprayed with fungicides and originated from cultivars ‘Milagrosa’, ‘NA8009’, and ‘SEMW-EST4863’. The same isolation technique previously described in Price et al. (2015) was used, and DNA extraction was carried out according to the method of Albu et al. (2016b). Portions of cytochrome *b* ($N = 38$) and calmodulin ($N = 37$) were amplified, sequenced, edited, and assembled as previously described (Albu et al. 2016a, b). The calmodulin sequences represented three distinct species based on a preliminary multilocus phylogenetic analysis incorporating all known species represented by publicly available sequence data from Groenewald et al. (2013). Two representative isolates from each lineage (with distinct calmodulin haplotypes, if

present) were selected for sequencing of two additional loci, histone 3 and elongation factor 1- α , for species identification as previously described (Albu et al. 2016b). Based on the corresponding multilocus phylogenetic analysis, the 37 isolates belong to three species: *C. nicotianae* ($N = 14$); *C. sp. “P”* ($N = 16$), a lineage delimited but not formally described by Groenewald et al. (2013); and *C. kikuchii* ($N = 7$).

All 38 isolates (100%) possessed the G143A mutation in cytochrome *b*, and none carried the F129L mutation, another known to confer resistance to QoI fungicides. Results of phenotypic assays on a subset of 15 isolates (five from each species) supported a high level of resistance to azoxystrobin. Each isolate was plated in duplicate on concentrations of azoxystrobin (ChemService, West Chester, PA) of 0.001, 0.01, 0.1, 1, and 10 ppm made from serial dilutions of a concentrated stock solution. Three radial measurements were taken from each colony and averaged to estimate growth rates per replicate plate. All G143A mutants developed colonies on potato dextrose agar (PDA) amended at 10 ppm, so an additional concentration of 50 ppm was tested on all isolates. All isolates grew at 50 ppm after 5 days of incubation, and none were inhibited more than 48% at this concentration. The effective concentration to inhibit 50% radial growth (EC_{50}) values estimated from a nonlinear regression (least squares fit) of log concentration by percent inhibition (GraphPad Prism 7.04, La Jolla, CA) ranged from 1.7 to 60 μ g/ml. Both the largest and smallest EC_{50} values were estimated from isolates of *C. nicotianae* at 60 and 1.7 μ g/ml, respectively. All G143A mutants also developed colonies after 5 days of growth on PDA plates amended with salicylhydroxamic acid (SHAM) and 10 ppm of azoxystrobin. Isolates of both *C. kikuchii* and *C. sp. “P”* exhibited less inhibition in the presence of SHAM plus azoxystrobin than solely exposed to azoxystrobin (10 ppm); however, isolates of *C. nicotianae* were all inhibited more than those only challenged with azoxystrobin.

This is the first report of the G143A mutation in *Cercospora* species associated with CLB in South America and the first report of *C. nicotianae* associated with CLB. Future monitoring for G143A mutants of *Cercospora* spp. in South America will be necessary to assess whether strobilurin resistance is widespread.

Literature Cited

- Albu, S., Price, P. P., III, Doyle, V., Padgett, G. B., and Schneider, R. W. 2016a. The G143A mutation is responsible for strobilurin fungicide resistance in *Cercospora* cf. *flagellaris*, a leaf blight and purple seed stain pathogen of Louisiana soybean. *Plant Health Prog.* 17:197.

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- Albu, S., Schneider, R. W., Price, P. P., III, and Doyle, V. P. 2016b. *Cercospora* cf. *flagellaris* and *Cercospora* cf. *sigesbeckiae* are associated with *Cercospora* leaf blight and purple seed stain on soybean in North America. *Phytopathology* 106:1376-1385.
- Groenewald, J. Z., Nakashima, C., Nishikawa, J., Shin, H.-D., Park, J.-H., Jama, A. N., Groenewald, M., Braun, U., and Crous, P. W. 2013. Species concepts in *Cercospora*: Spotting the weeds among the roses. *Stud. Mycol.* 75:115-170.
- Price, P. P., III, Purvis, M. A., Cai, G., Padgett, G. B., Robertson, C. L., Schneider, R. W., and Albu, S. 2015. Fungicide resistance in *Cercospora kikuchii*, a soybean pathogen. *Plant Dis.* 99:1596-1603.
- Soares, A. P. G., Guillin, E. A., Borges, L. L., da Silva, A. C. T., de Almeida, Á. M. R., Grijalba, P. E., Gottlieb, A. M., Bluhm, B. H., and de Oliveira, L. O. 2015. More *Cercospora* species infect soybeans across the Americas than meets the eye. *PLoS One* 10:e0133495.