Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Corynespora cassiicola* Causing Black Spot on *Sarcandra glabra* in China

Ni Jiang,^{1,2} Bi-run Lin,^{1,3,†} Li-Sha Song,^{2,†} Guiyu Tan,² Zhan-Jiang Zhang,² and Shu-Gen Wei²

¹ College of Agriculture, Guangxi University, Nanning 530004, China

² Guangxi Botanical Garden of Medicinal Plants, Nanning 530023, China

³ Guangdong Key Laboratory of High Technology for Plant Protection, Institute of Plant Protection, Guangdong Academy of Agricultural Sciences, Guangzhou, China

Funding: This study was supported by the Special Fund for Innovation-Driven Development in Guangxi, P.R. China (AA17204056-4), the Guangxi Science and Technology Base and Talent Special Project (Gui ke AD16380013), Guangxi Science and Technology Plan Project (Gui ke AD17292004), Organic Medicine Cultivation and Evaluation Research Team (Gui yao chuang 2019007), and Youth Science Foundation of Guangxi Botanical Garden of Medicinal Plants (Gui yao ji 201801). Plant Dis. 105:3754, 2021; published online as <https://doi.org/10.1094/PDIS-02-21-0273-PDN>. Accepted for publication 30 April 2021.

Sarcandra glabra is an important Chinese medicinal plant, which was widely cultivated under forest in south China. Guangxi province is the main producing areas of this herb. In June 2019, a serious leaf disease was found causing severe defoliation in the *S. glabra* plantation under bamboo forest in Rongan county, Guangxi province (109°13'E, 24°46'N). About 70% of the plants in the plantation (300 ha) showed similar symptoms. Initially, circular lesions appeared on young leaves as black spots (about 1 to 2 mm). Then, the spots gradually enlarged, usually with an obvious yellowish margin (6 to 8 mm). Finally, the lesions coalesced and formed irregular, black, and large necrotic areas, resulting in leaf abscission. For pathogen isolation, small pieces of tissue (5 × 5 mm) taken from 25 diseased leaves were sterilized with 75% ethanol for 30 s, subsequently soaked in 0.1% HgCl₂ for 2 min, rinsed three times in sterile distilled water, dried, placed aseptically onto potato dextrose agar (PDA) plates, and incubated at 28°C (12 h/12 h light/dark). Three days later, the isolates were placed on a new PDA plate for subsequent purification and sporulation. Twenty pure fungal isolates were obtained from single spores, of which 15 isolates showed similar morphological characteristics. The colonies on PDA were round and dense, with a gray edge and dark gray in the center area. Conidia in culture were appeared light brown, cylindrical in shape, with zero to eight septa, and 55 to 165 × 5.2 to

13.5 µm in size (mean = 106.2 × 8.6 µm, *n* = 30). These morphological characteristics resemble those of *Corynespora* sp. (Berk. & M.A. Curtis) C.T. Wei (Ellis et al. 1971). A single-spore isolate (ZD5) was selected from the 15 fungal isolates for a subsequent molecular identification. The genes of internal transcribed spacer (ITS) of ribosomal DNA, β-tubulin, and actin were amplified with the primer pairs ITS-1/ITS-4 (White et al. 1990), Bt2a/Bt2b (Glass and Donaldson 1995), and ACT-512F/ACT-783R (Carbone and Kohn 1999), respectively. The ITS, β-tubulin, and actin sequences were deposited in the GenBank database with accession numbers MW362446, MW367029, and MW533122. BLAST analysis and neighbor-joining analysis based on ITS, β-tubulin, and actin sequences using MEGA 6 revealed that the isolate was placed in the same clade as *Corynespora cassiicola* with 100% bootstrap support. A pathogenicity test was performed on 2-year-old potted *S. glabra*. Six-millimeter-diameter mycelial plugs were attached to the healthy leaves of *S. glabra* for coculture, whereas the control group was attached with PDA. All plants were covered with plastic bags for 2 days to maintain high humidity and cultured in a greenhouse at 28°C with a 12-h/12-h light/dark cycle. Symptoms that appeared 2 days after coculture were identical to those observed in the field. The same fungus was reisolated from the lesions, and further morphological characterization and molecular assays conducted, as described above. The control leaves remained symptomless during the pathogenicity tests. According to the previous literature, *C. cassiicola* is a plant-pathogenic fungus with a broad host range, which can damage diverse tropical plants including *Salvia miltiorrhiza* (Lu et al. 2019), *Solanum americanum* (Pereira and May-De-Mio 2019), *Vitex rotundifolia* (Yeh and Kirschner 2017), *Cucumis sativus*, *Carica papaya* (Tsai et al. 2015), and so on. To our knowledge, this is the first report of *C. cassiicola* causing leaf spot on *S. glabra* in China.

References:

- Carbone, I., and Kohn, L. 1999. Mycologia 91:553.
- Ellis, M. B., et al. 1971. CMI Descriptions of Pathogen Fungi and Bacteria, No. 303. CMI, Kew, U.K.
- Glass, N. L., and Donaldson, G. C. 1995. Appl. Environ. Microbiol. 61:1323.
- Lu, P. K., et al. 2019. Plant Dis. 103:769.
- Pereira, W. V., and May-De-Mio, L. L. 2019. J. Plant Pathol. 101:755.
- Tsai, J. N., et al. 2015. Plant Dis. 99:1649.
- White, T. J., et al. 1990. Page 315 in: PCR Protocols: A Guide to Methods and Applications. Academic Press, San Diego, CA.
- Yeh, Y. H., and Kirschner, R. 2017. Plant Dis. 101:1550.

The author(s) declare no conflict of interest.

e-Xtra

Keywords: *Corynespora cassiicola*, *Sarcandra glabra*, pathogeny

[†]Indicates the corresponding authors.

B. R. Lin; linbr@126.com, and L. S. Song; lishasong@126.com