Diseases Caused by Fungi and Fungus-Like Organisms

First Report of Leaf Spot Caused by *Colletotrichum siamense* on *Magnolia grandiflora* in China

Yang Zhou,^{1,2} Dou Yang,¹ Lin-Ping Zhang,¹ Yong-Chun Zeng,¹ and Yang Zhang^{1,†}

¹ Key Laboratory of National Forestry and Grassland Administration for the Protection and Restoration of Forest Ecosystem in Poyang Lake Basin, Jiangxi Agricultural University, Nanchang 330045, Jiangxi, China

² Co-Innovation Center for Sustainable Forestry in Southern China, College of Forest, Nanjing Forestry University, Nanjing 210037, Jiangsu, China

Funding: This work was supported by the National Natural Science Foundation of China (31660190) and Jiangxi Provincial Department of Science and Technology (20192BAB214019). Plant Dis. 106:769, 2022; published online as <https://doi.org/10.1094/PDIS-08-21-1698-PDN>. Accepted for publication 23 August 2021.

Magnolia grandiflora (Southern magnolia) is a popular evergreen tree, planted especially as an ornamental for landscaping. In September 2019, leaf spots were observed on *M. grandiflora* at the campus of Jiangxi Agricultural University (28°45'56"N, 115°50'21"E). Approximately 64% (23 out of 36) of *M. grandiflora* trees on the campus (most 24 years old) had leaf spot disease. On average, 40% of the leaves per individual tree were affected. Foliar symptoms began as small dark brown lesions formed along the leaf margins. As the disease developed, the lesion's center was sunken with a dark brown border. Symptomatic leaves were collected and cut into 5 × 5 mm pieces. Leaf pieces from the margin of the necrotic tissue were surface sterilized in 70% ethanol for 30 s followed by 2% NaOCl for 1 min and then rinsed in sterile water three times. Tissues were plated on potato dextrose agar (PDA) and incubated at 25°C. Of more than 35 isolates, most shared a similar morphology, with an isolation rate of 85%. Three isolates (JNG-1, JNG-2, and JNG-3) were chosen for single-spore purification and used for morphological characterization and identification. Colonies on PDA of the three isolates were white, cottony, and grayish-white on the undersides of the culture. Conidia were single-celled, straight, hyaline, cylindrical, clavate, and measured 4.4 to 5.6 × 13.2 to 17.8 μm (4.7 ± 0.3 × 14.6 ± 1.0 μm, *n* = 100).

Appressoria were brown to dark brown, ovoid to clavate, slightly irregular to irregular, and ranged from 5.5 to 9.2 × 4.6 to 6.5 μm (7.3 ± 0.4 × 5.4 ± 0.3 μm, *n* = 100). Morphological features were similar to *Colletotrichum siamense* as previously described (Weir et al. 2012). The internal transcribed spacer (ITS) regions, actin (*ACT*), calmodulin (*CAL*), beta-tubulin 2 (*TUB2*), chitin synthase (*CHS-1*), and glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) were amplified from genomic DNA for the three isolates using primers ITS1/ITS4, ACT-512F/ACT-783R, CL1/CL2, T1/Bt2b, CHS-79F/CHS-345R, and GDF/GDR (Weir et al. 2012), respectively, and sequenced. All sequences were deposited into GenBank (ITS, MZ325948 to MZ325950; *ACT*, MZ461477 to MZ461479; *GAPDH*, MZ461483 to MZ461485; *TUB2*, MZ461486 to MZ461488; *CHS-1*, MZ441182 to MZ441184; *CAL*, MZ461480 to MZ461482). A neighbor-joining phylogenetic tree was constructed with MEGA 7.0 using the concatenation of multiple sequences (Kumar et al. 2016). According to the phylogenetic tree, all three isolates fall within the *C. siamense* clade (bootstrap support 96%). The pathogenicity of three isolates were tested on *M. grandiflora* plants grown in the field. Healthy leaves were wounded with a sterile needle and then inoculated with 10 μl of spore suspension (10⁶ conidia/ml). Controls were treated with ddH₂O (Zhu et al. 2019). All the inoculated leaves were covered with black plastic bags to keep a high-humidity environment for 2 days. All inoculated leaves showed similar symptoms to those observed in field, whereas control leaves were asymptomatic for 10 days. The infection rate of was 100%. *C. siamense* was reisolated from the lesions, whereas no fungus was isolated from control leaves. It was confirmed that *C. gloeosporioides* is the causal agent of leaf spot on *Magnolia virginiana* in the U.S. (Xiao et al. 2004). However, this is the first report of *C. siamense* causing leaf spot on *M. grandiflora* in China. This study provides crucial information for epidemiologic studies and appropriate control strategies for this newly emerging disease.

References:

- Kumar, S., et al. 2016. Mol. Biol. Evol. 33:1870.
- Weir, B. S., et al. 2012. Stud. Mycol. 73:115.
- Xiao, C. L., et al. 2004. Phytopathology 94:446.
- Zhu, L. H., et al. 2019. Plant Dis. 103:1431.

The author(s) declare no conflict of interest.

e-Xtra

Keywords: Coelomycete, pathogen identification, multi-gene phylogeny, species complex

[†]Indicates the corresponding author.
Y. Zhang; zhangyang0558@jxau.edu.cn