Diseases Caused by Fungi and Fungus-Like Organisms

First Report of Leaf Spot Caused by *Colletotrichum fruticicola* on *Dalbergia hupeana* in China

Yang Zhou,^{1,2} Rou Ye,¹ Qin Ying,¹ Yang Zhang,^{1,†} and Linping 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 Natural Science Foundation of China (32160357 and 31660190) and Jiangxi Provincial Department of Science and Technology (20192BAB214019). Plant Dis. 106:1526, 2022; published online as <https://doi.org/10.1094/PDIS-08-21-1842-PDN>. Accepted for publication 10 November 2021.

Dalbergia hupeana is a kind of wood and medicinal tree widely distributed in southern China. Since 2019, a leaf spot disease has been observed on the leaves of *D. hupeana* in Gangxia village, Luoting town in Jiangxi Province, China (28°52'53"N, 115°44'58"E). The disease incidence was estimated to be above 50%. The symptoms began as small spots that gradually expanded, developing a brown center and dark brown to black margin. The spots ranged from 4 to 6 mm in diameter. Leaf pieces (5 × 5 mm) from lesion margins were surface sterilized in 70% ethanol for 30 s followed by 2% NaOCl for 1 min and rinsed three times with sterile water. Tissues were placed on potato dextrose agar (PDA) and incubated at 25°C. Pure cultures were obtained by monospore isolation. Fifteen strains with similar morphological characterizations were isolated, and three representative isolates (JHT-1, JHT-2, and JHT-3) were chosen for further study. Colonies on PDA of three isolates were gray-green with white edges and dark green on the reverse side. Conidia were transparent, cylindrical with rounded ends, and measured 3.6 to 5.3 × 9.5 to 15.2 μm (3.7 ± 0.2 × 13.6 ± 1.1 μm; *n* = 100). Appressoria were dark brown, globose or subcylindrical, and ranged from 6.2 to 9.2 × 5.1 to 6.8 μm (7.9 ± 0.4 × 5.9 ± 0.3 μm; *n* = 100). The morphological characteristics of the three strains were consistent with the description of species in the *Colletotrichum gloeosporioides* complex (Weir et al. 2012). The internal transcribed spacer (ITS) regions, actin (*ACT*), calmodulin (*CAL*), chitin synthase (*CHS-1*), glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*), and beta-tubulin

2 (*TUB2*) were amplified from genomic DNA for the three isolates using primers ITS1/ITS4, ACT-512F/ACT-783R, CL1/CL2, CHS-79F/CHS-345R, GDF/GDR, and T1/Bt2b (Weir et al. 2012), respectively. The sequences were deposited in GenBank (accession nos. MZ482016 to MZ482018 for ITS; MZ463636 to MZ463638 for *ACT*; MZ463648 to MZ463650 for *CAL*; MZ463639 to MZ463641 for *CHS-1*; MZ463642 to MZ463644 for *GAPDH*; MZ463645 to MZ463647 for *TUB2*). A neighbor-joining phylogenetic tree was constructed with MEGA 7.0 using the concatenation of multiple sequences (ITS, *ACT*, *GAPDH*, *TUB2*, *CHS-1*, *CAL*) (Kumar et al. 2016). According to the phylogenetic tree, the three isolates fall within the *Colletotrichum fruticicola* clade (boot support 99%). Based on morphological characteristics and phylogenetic analysis, the isolates were identified as *C. fruticicola*. The pathogenicity of the isolates was studied on 2-year-old seedlings (30-cm tall) of *D. hupeana*. Healthy leaves were wounded with a sterile needle and inoculated with 10 μl of spore suspension (10⁶ conidia per ml). Controls were treated with sterile water. All plants were covered with transparent plastic bags and incubated in a greenhouse at 28°C with a 12 h photoperiod (relative humidity >80%). Within five days, the inoculated leaves developed lesions similar to those observed in the field, and controls were asymptomatic. The experiments were repeated three times and showed similar results. The infection rate was 100%. *C. fruticicola* was reisolated from the lesions, and no fungus was isolated from control leaves. *C. fruticicola* can cause leaf diseases in a variety of hosts, including *Aesculus chinensis* (J.-W. Sun et al. 2020), *Peucedanum praeruptorum* (Ma et al. 2020), and *Mandevilla × amabilis* (M. Sun et al. 2020). *C. brevisporum* and *C. gigasporum* were also reported to infect *D. odorifera* (Chen et al. 2021; Wan et al. 2018). However, this is the first report of *C. fruticicola* associated with leaf spot disease on *D. hupeana* in China. These results will help to develop effective strategies for managing this newly emerging disease.

References:

- Chen, X. Z., et al. 2021. Plant Dis. 105:2255.
- Kumar, S., et al. 2016. Mol. Biol. Evol. 33:1870.
- Ma, J., et al. 2020. Plant Dis. 104:3066.
- Sun, J.-W., et al. 2020. Plant Dis. 104:3065.
- Sun, M., et al. 2020. Plant Dis. 104:3258.
- Wan, Z., et al. 2018. Plant Dis. 102:679.
- Weir, B. S., et al. 2012. Stud. Mycol. 73:115.

The author(s) declare no conflict of interest.

e-Xtra

Keywords: *Colletotrichum*, *Dalbergia hupeana*, identification, multigene phylogeny, pathogen

[†]Indicates the corresponding authors.
Y. Zhang; zhangyang0558@jxau.edu.cn, and
L. Zhang; zlping619@163.com