Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Cinnamomum japonicum* Anthracnose Caused by *Colletotrichum fioriniae* in Sichuan, China

Jie Chen, Shan Han, Shujiang Li, Tianmin Qiao, Yujue Zhou, and Tianhui Zhu[†]

College of Forestry, Sichuan Agricultural University, Chengdu, Sichuan Province, 611130, Sichuan, P.R. China

J. Chen and S. Han contributed equally to this work.

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Cinnamomum japonicum Sieb is widely cultivated in urban in China. It is used to make essential oil, lubricant, soap, and waterproof timber. In September 2019, this new leaf spot was discovered in Chengdu city (30°05' to 31°26'N, 102°54' to 104°53'E), with approximately 61.20% disease incidence. The symptoms started to occur from May to June, with the worst from August to September. First, the typical symptom showed round or oval, brown, and slightly sunken necrotic lesions. Gradually, the necrotic lesions increased in number, and expanded; under humid conditions, the central part of the spots became black and ruptured, and orange conidial masses emerged at the margin of lesions. Finally, the leaves turned yellow and fell off. Infected tissues from 10 samples were cut into small pieces (2 by 2 mm), surface sterilized for 30 s in 3% sodium hypochlorite and 60 s in 75% ethanol, rinsed three times in sterile water, placed onto potato dextrose agar (PDA) amended with streptomycin sulfate (50 µg/ml), and incubated at 25°C in a dark. Finally, eight typical isolates exhibited the morphology described as *C. fioriniae* (Grammen et al. 2019). After 5 days, the colony diameter reached 28.6 to 41.2 mm and had white to light-gray aerial mycelium, but was pink at the base. Orange conidia masses formed after 6 to 7 days; conidia were oval, slender, and fusiform with acute ends, measuring 8.3 to 19.6 by 2.9 to 7.1 µm (average: 13.9 by 4.8 µm) (Chechi et al. 2019; Tashiro et al. 2018). For molecular identification, DNA was extracted from eight fungal colonies using a plant genomic DNA extraction kit (Solarbio, Beijing, China). The 5.8S nuclear ribosomal genes with the two flanking regions internal transcribed spacer (ITS) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and

partial sequences of the *actin* (*ACT*), *chitin synthase 1* (*CHS-1*), *β-tubulin* (*TUB2*), *histone3* (*HIS3*), *calmodulin* (*CAL*), and *glutamine synthetase* (*GS*) genes were amplified and sequenced using the primer pairs ITS1/ITS4 (White et al. 1990), GDF/GDR (Templeton et al. 1992), ACT-512F/ACT-783R, CAL 228F/CAL 737R (Carbone et al. 1999), CHS-354R/CHS-79F (Weir et al. 2012), TUB1F/Bt2bR, CYLH3F/CYLH3R (Crous et al. 2004), and GSF1/GSR1 (Liu et al. 2015), respectively. Sequences were deposited in GenBank (ITS: MT466533, GAPDH: MT460415, ACT: MT460414, CAL: MT954332, CHS-1: MT954330, TUB2: MT460416, HIS3: MT954331, and GS: MT460417). BLAST analysis showed >99.4% identity with several reference sequences of *Colletotrichum fioriniae* strain CBS 128517 and strain EHS58 (teleomorph of *Glomerella fioriniae*) previously deposited in GenBank. The conidial suspension (1×10^7 conidia/ml) collected from PDA cultures with 0.05% Tween 80 buffer was used for inoculation by spraying leaves of 5-year-old *Cinnamomum japonicum* plants for pathogenicity tests. Ten leaves of each plant (10 pots in total) were inoculated with spore suspensions (approximately 500 µl per leaf). An equal number of control leaves were sprayed with 0.05% Tween 80 buffer to serve as a control. Twenty days later, the inoculated plants showed similar symptoms to those of the original diseased plants but the controls remained asymptomatic. *Colletotrichum fioriniae* was reisolated from the infected leaves and identified by morphological characteristics and DNA sequence analysis. The pathogenicity test was repeated three times with similar results, confirming Koch's postulates. To our knowledge, this is the first report of *Cinnamomum japonicum* anthracnose caused by *C. fioriniae* in China.

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[†]Indicates the corresponding author.
T. Zhu; zhuth1227@126.com