Diseases Caused by Fungi and Fungus-Like Organisms

First Report of Leaf Spot Caused by *Colletotrichum fruticicola* on *Myrica rubra* in China

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Myrica rubra is an important fruit tree with a high nutritional and economic value that is widely cultivated in multiple regions of China. In January 2021, an unknown disease which caused leaf spot with approximately 20% ($n = 100$ investigated plants) of incidence was discovered on the leaves of *M. rubra* in Jiujiang City of Jiangxi Province (29.71° N, 115.97° E). The initial symptoms were small pale brown spots (1 to 2 mm in diameter) on the leaves, which gradually expanded into round or irregular dark brown spots with the occurrence of the disease, and the lesion developed necrotic tissues in the center at later stages, eventually leading the leaves to become chlorotic and wilted. Ten diseased leaves with typical symptoms were collected, and leaf tissue (5 × 5 mm) at the junction of diseased and healthy portions was cut. The surfaces were disinfected with 75% ethanol for 45 s, 1% sodium hypochlorite for 1 min, and rinsed in sterile water three times. They were then transferred to potato dextrose agar (PDA) at 28 ± 1°C for 3 days. Five fungal single isolates with similar morphology were purified from single spores. On PDA medium, the colonies initially appeared white with numerous aerial hyphae, and the center of the colony turned gray at later stages with less sporulation. While on modified Czapek Dox medium (peptone 3 g, K₂HPO₄ 1 g, MgSO₄·7H₂O 0.5 g, KCl 0.5 g, FeSO₄ 0.01 g, maltose 30 g, agar 15 g, distilled water 1000 ml, pH = 7.0), the mycelia of the colony were sparse and produced a large number of small bright orange particles (conidial masses). Conidia were single celled, transparent, smooth-walled, had one to two oil globules, cylindrical with slightly blunt rounded ends, and 14.45 to 18.44 × 5.54 to 6.98 μm (average = 16.27 × 6.19 μm, $n = 50$) in size. These morphological characteristics of the pathogen were similar to the descriptions of *Colletotrichum fruticicola* (Ruan et al. 2017; Yang et al. 2021). To further confirm the identity of the pathogen, genomic DNA from a representative isolate was

extracted with a DNA Extraction Kit (Yeasen, Shanghai, China), and the internal transcribed spacer (ITS), glyceraldehyde-3-phosphatedehydrogenase (GAPDH), calmodulin gene (CAL), actin (ACT), and chitin synthase 1 (CHS 1) were amplified by using the primers ITS1/ITS4 (Gardes et al. 1993), GDF/GDR (Templeton et al. 1992), CL1C/CL2C (Weir et al. 2012), ACT-512F/ACT-783R, and CHS-79F/CHS-345R (Carbone et al. 1999), respectively. The PCR amplified sequences were submitted to GenBank (GenBank accession nos. ITS, MW740334; GAPDH, MW759805; CAL, MW759804; ACT, MW812384; and CHS-1, MW759803) and showed 100% identity with *C. fruticicola* (GenBank accession nos. ITS, MT355821.1 [546/546 bp]; GAPDH, MT374664.1 [255/255 bp]; CAL, MK681354.1 [741/741 bp]; ACT, MT364655.1 [262/262 bp]; CHS, MT374618.1 [271/271 bp]). A phylogenetic tree using the maximum likelihood method with a Kimura 2-parameter model, combined with the ITS-ACT-GAPDH-CHS-CAL concatenated sequences, and bootstrap nodal support for 1,000 replicates in MEGA7.0, revealed that the isolate was assigned to *C. fruticicola* strain (ICMP 18581 and CBS 125397) (Yang et al. 2021) with 98% bootstrap support. Pathogenicity was tested on 15 healthy *M. rubra* plants (five for wounded inoculation, five for nonwounded inoculation, and five for controls) in the orchard. Twenty leaves were marked from each plant, and the surface was disinfected with 75% ethanol. A spore suspension (10 μl, 1.0×10^6 conidia/ml) of each isolate from 7-day-old culture was inoculated on the surface of 20 needle-wounded and 20 nonwounded leaves, respectively. Healthy leaves were inoculated with sterile water as controls by the same method. All inoculated leaves were sprayed with sterile water and covered with plastic film to maintain humidification. After 5 days, all the wounded leaves which were inoculated with *C. fruticicola* showed similar symptoms to those observed on the original leaves. Symptoms of nonwounded leaves were milder than the wounded inoculated leaves, while control leaves remained healthy. Finally, *C. fruticicola* was reisolated from the inoculated leaves. *C. fruticicola* has been reported on *Juglans regia*, *Peucedanum praeruptorum*, and *Paris polyphylla* var. *Chinensis* in China (Ma et al. 2020; Wang et al. 2018; Zhou et al. 2020). As far as we know, this is the first report of *C. fruticicola* causing leaf spot on *M. rubra* in China. This result contributes to the better understanding of the pathogens causing diseases of *M. rubra* in this region of China and the development of effective control strategies.

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