Diseases Caused by Fungi and Fungus-Like Organisms

First Report of Leaf Spot Caused by *Colletotrichum siamense* on *Michelia alba* in China

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Michelia alba (common name: white champaca), native to Indonesia, is a preciously ornamental and medicinal plant in the west and southeast of China and widely distributed in Nanning, Guangxi, China (Hou et al. 2018). In May 2020, a foliar disease of *M. alba* was observed in Nanning (22°51' N; 108°17' E), Guangxi, China, present on ~20 to 30% of the leaves. The disease began to develop from the margins of leaves in most cases. The symptoms recorded were light yellow spots, which gradually developed into ellipsoidal to irregular brown spots, surrounded by a wide yellow halo. The spots gradually enlarged in size and became gray-brown, with the dimension of 3.5 × 2.8 to 11.0 × 3.5 cm, even more than half of leaf area. In the later stage of infection, these spots coalesced, resulting in necrosis and early shedding of the leaves. Sometimes black acervuli were observed on some lesions. For isolation of the fungus, 10 symptomatic leaves were randomly sampled from five trees and washed with sterile water. Small pieces of infected tissue (about 4 mm²) were surface disinfested in 75% alcohol for 30 s and in 0.1% aqueous solution of mercury chloride for 1 min. Finally, these tissue pieces were rinsed three times with sterile water, plated on potato dextrose agar (PDA), and then incubated for 7 days at 28°C with a photoperiod of 12 h. Fifteen strains with similar morphological characterizations were isolated, and five representative isolates (BL-1 to BL-5) were purified. These cultures gave rise to gray-white colonies with a few bright orange conidial masses that contained one-celled, hyaline, guttulate conidia, measuring 12.68 to 20.70 × 4.27 to 7.84 μm (average 15.36 × 5.35 μm, *n* = 100). Appressoria formed from conidia were brown, ellipsoidal or inverted trapezoid, and measured 6.36 to 12.13 × 5.07 to 7.39 μm

(average 8.29 × 6.36 μm, *n* = 30). These morphological characteristics were similar to those of the *Colletotrichum gloeosporioides* species complex (Weir et al. 2012). To confirm identification, genomic DNA from mycelium of these five isolates was extracted, and the sequences of internal transcribed spacer (ITS), chitin synthase (*CHS-1*), glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*), actin (*ACT*), calmodulin (*CAL*), and β-tubulin (*TUB2*) were amplified (Zhang et al. 2020); the GenBank accession numbers for the sequences were MW186173 to MW186177 (ITS), MW161290 to 161294 (*CHS-1*), MW161295 to MW161299 (*GAPDH*), MW161285 to 161289 (*ACT*), MW084710 to 084714 (*CAL*), and MW161300 to MW161304 (*TUB2*). The phylogenetic tree of six combined genes of the five isolates clustered with *Colletotrichum siamense* strains (CBS 125378, ICMP 17795, and ICMP 18121). Therefore, the pathogen was identified as *C. siamense*. Five isolates (BL-1 to BL-5) were tested for pathogenicity. Wounded and unwounded detached healthy leaves were inoculated using mycelial discs (5 mm in diameter) and conidial suspensions (with the concentration of 1 × 10⁵ conidia/ml) at the same time, incubated in a growth chamber at 25 to 30°C (85 to 90% relative humidity, with a photoperiod of 12 h). Three leaves (wounded left half blade and unwounded right half blade) were inoculated with different methods for each isolate, and the tests were repeated three times. Four days after inoculation, leaf spots were observed on all wounded leaves, and 5 to 10% of the unwounded leaves showed lesions. Control leaves inoculated with PDA discs and sterile water remained symptomless. *C. siamense* was reisolated from the lesions, confirming Koch's postulates. At least 60 plant species have been reported to be infected by *C. siamense* worldwide (Ji et al. 2019). To our knowledge, this is the first report of *C. siamense* causing leaf spot on *M. alba* in China.

References:

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