Diseases Caused by Fungi and Fungus-Like Organisms

First Report of Anthracnose Caused by *Colletotrichum fruticicola* on *Loropetalum chinense* in China

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Loropetalum chinense Oliv. (Hamamelidaceae), an evergreen shrub or small tree, is widely used as a bonsai and landscape plant because of its aesthetic value. It also has medicinal value; its leaves, flowers, and roots can be used to treat diarrhea, coughs, hemorrhaging, and burns (Zhang et al. 2013). In October 2020, leaf spot symptoms were observed on *L. chinense* in Meiling Scenic Spot of Nanchang, Jiangxi Province, China (28.78° N, 115.83° E). We surveyed ~500 m² of the mountain area, which has ~60 trees of *L. chinense*, with an incidence of up to 20%. Initially, small gray-brown spots with dark brown edges appeared on the leaf surface, becoming large circular or irregular brown necrotic lesions over time. To isolate the pathogen, 10 leaves of infected tissues were cut into 4-mm² pieces, surface disinfected with 75% ethanol for 30 s and 1% NaClO for 1 min, rinsed three times with sterile water, plated on potato dextrose agar (PDA), and incubated at 25°C in the dark for 5 to 7 days. Five isolates with similar morphological characteristics were obtained. Conidia were hyaline, aseptate, smooth walled, and cylindrical with obtuse to slightly rounded ends, and dimensions were 8.57 to 15.80 × 1.56 to 4.65 μm (*n* = 20). The morphological characteristics of the isolates matched those of *Colletotrichum gloeosporioides* species complex (Cai et al. 2009; Liu et al. 2015; Weir et al. 2012). For molecular identification, two representative isolates (JAUCC L003 and JAUCC L004) were selected for genomic DNA extraction, and the internal transcribed spacer (ITS), partial calmodulin (CAL), chitin synthase (CHS-1), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), and actin (ACT) were amplified using primer pairs ITS1/ITS4, CL1C/CL2C, CHS-79F/CHS-345R, GDF1/GDR1, and ACT-512F/ACT-783R, respectively (Weir et al. 2012; White et al. 1990; Yang et al. 2021). The sequenced loci (GenBank accession nos. ITS: MZ054401, MZ054400; CAL: MZ056750, MZ056754; CHS-1: MZ056751,

MZ056755; GAPDH: MZ056752, MZ056756; ACT: MZ056753, MZ056757) exhibited up to 99% homology with corresponding sequences of *Colletotrichum fruticicola* Prihast., L. Cai & K.D. Hyde strains (GenBank accession nos. LC494271, MN525833, MN525854, MN525875, and MN525814). Concatenated sequences of the five genes were used to conduct a phylogenetic analysis using the maximum likelihood method in MEGA7. The isolates were identified as *C. fruticicola* based on morphology and a multigene phylogenetic analysis. Pathogenicity of representative isolate JAUCC L003 was tested indoors by inoculating the top wounded leaves of six healthy *L. chinense* plants, rather than inoculating unwounded leaves due to the low incidence. Three leaves from each of three plants were punctured with flamed needles and sprayed with a conidial suspension (1 × 10⁶ conidia/ml), and three leaves from each of the other three plants were wounded and inoculated with mycelial plugs (5 × 5 mm). Mock inoculations were used as controls with sterile water and PDA plugs on three leaves each. Treated plants were incubated in an artificial climate box at 25°C and 90% relative humidity, with a 12-h photoperiod. All leaves inoculated with conidial suspension and mycelial plugs produced similar symptoms as described above 10 days postinoculation, whereas the mock inoculated plants remained asymptomatic. The fungus isolated from inoculated leaves was identical to the original pathogen based on morphological and molecular data, confirming Koch's postulates, but not from the mock inoculated plants. Anthracnose disease caused by *C. fruticicola* affects numerous plants worldwide, including cotton, coffee, grape, citrus, mango, apple, pear, and cassava (Guarnaccia et al. 2017; Oliveira et al. 2018). To our knowledge, this is the first report of *C. fruticicola* causing anthracnose on *L. chinense* in China. This disease is a significant concern in horticulture due to its impact on the aesthetics of ornamentals used in landscape plantings.

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