Diseases Caused by Fungi and Fungus-Like Organisms

First Report of Leaf Spot of *Weigela florida* Caused by *Epicoccum layuense* in China

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Weigela florida (Bunge) A. DC. is a dense, rounded, deciduous shrub commonly planted in landscapes. It is also used in Chinese medicine to treat sore throat, erysipelas, cold, and fever (Zheng et al. 2019). In May 2019, leaf spots were observed on approximately 50% of *W. florida* plants grown in the Wisdom Plaza Park of Anhui Agricultural University in Hefei, Anhui Province, China. Leaf spots began as small light brown and irregular lesions, enlarged, turned reddish brown, coalesced to form large blighted areas, and eventually covered the entire leaf surface. Five pieces of tissues were removed from the lesion margins of each diseased leaf (five leaves from five different plants), chopped into several 3- to 4-mm² pieces, disinfected with 1.5% NaOCl for 2 min, rinsed three times with sterile distilled water for 1 min, plated onto potato dextrose agar (PDA) medium containing 50 µg/ml of ampicillin and kanamycin, and incubated at 25°C with a 12-h photoperiod for 5 days. One segment of the fungal growth from the growing edge of the colony was transferred onto a fresh PDA plate for purification and incubated under the same conditions for another 5 days. The colony morphology of one representative isolate (AAU0519) was characterized by a pale orange cushion in the center surrounded by irregular pink margin, diffusing red-orange pigments into the PDA medium. Isolate AAU0519 was cultured on PDA medium for 7 days at 25°C in the dark to induce sporulation. The produced conidia were globose, subglobose to pyriform, golden brown to brown, and with a diameter of 7.7 to 23.8 µm. Both cultural and morphological characteristics suggested that isolate AAU0519 was an *Epicoccum* species, according to the description by Chen et al. (2017). Amplification and sequencing of the internal transcribed spacer (ITS), beta-tubulin, and 28S large subunit ribosomal RNA (LSU) gene fragments from the extracted

genomic DNA of AAU0519 were performed using primer sets ITS1/ITS4 (White et al. 1990), Bt2a/Bt2b (Glass and Donaldson 1995), and LSU1Fd/LR5 (Crous et al. 2009; Vilgalys and Hester 1990), respectively. A phylogenetic tree was constructed by the maximum-likelihood method with 1,000 bootstrap replications based on the concatenated ITS, beta-tubulin, and LSU sequences from isolate AAU0519 and representative strains of 22 species of the genus *Epicoccum* (Chen et al. 2017). Isolate AAU0519 clustered with ex-holotype CGMCC 3.18362 of *Epicoccum layuense* Q. Chen, Crous & L. Cai (Chen et al. 2017). All obtained sequences were deposited into GenBank under accession numbers MK983497 (ITS), MN328723 (beta-tubulin), and MN328724 (LSU). A pathogenicity test was conducted on leaves of five 3-year-old *W. florida* cultivar 'Red Prince' planted in the field (five leaves for each treatment and control per plant) by spraying 30 ml of a spore suspension (10⁶ spores/ml) of isolate AAU0519 as the treatment or sterilized distilled water as the control. Before the inoculation, the leaves were disinfected with 70% ethanol. After inoculation, the leaves were wrapped with a plastic bag to keep high relative humidity. The average air temperature was about 28°C during the period of the pathogenicity test. The experiment was repeated once. Ten days after inoculation, the fungal-inoculated leaves developed light brown lesions resembling those of naturally infected leaves, and control leaves did not develop any symptoms. *E. layuense* was recovered from leaf lesions, and its identity was confirmed by morphological and sequence analyses as described above. To our knowledge, *E. layuense* has been previously reported as a pathogen of *Perilla* sp. (Chen et al. 2017), oat (*Avena sativa*) (Chen et al. 2020a), and tea (*Camellia sinensis*) plants (Chen et al. 2020b), but this is the first report of *E. layuense* causing leaf spot on *W. florida* in China. This pathogen could pose a threat to the ornamental value of *W. florida* plants. Thus, it is necessary to adopt effective management strategies against leaf spot on *W. florida*.

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