Diseases Caused by Fungi and Fungus-Like Organisms

First Report of Anthracnose of *Mandevilla × amabilis* Caused by *Colletotrichum fruticola* in Guangxi, Southern China

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Mandevilla × amabilis is an ornamental plant widely used in southern China, appreciated for its large flowers, bright color, and heat tolerance (Oder et al. 2016). In June to August 2019, an anthracnose disease was observed on *M. × amabilis* plants in a nursery in Nanning, Guangxi, China (108°14'30"E, 22°51'02"N). Symptoms began as red-brown spots, which gradually enlarged (0.2 to 1.1 cm in width and 0.3 to 1.5 cm in length), overlapped, and extended until the leaves wilted. Of the 70 plants surveyed, more than 80% of leaves showed these symptoms. To determine the causal agent, samples were gathered from symptomatic leaves, cut to 3 × 3 mm, moistened with 75% ethanol for 10 s, sterilized with 2% NaClO for 2 min, and rinsed three times in sterile water. Sterilized samples were placed onto potato dextrose agar (PDA) and incubated at 25°C in the dark. After 6 days, hyphal margins were subcultured onto fresh PDA and incubated at 25°C. Cultures grown on PDA started as gray-white, turned dark gray after 9 days of growth, and conidia were produced after 13 days. Conidia were hyaline, guttulate, unicellular, cylindrical, and averaged 15.74 × 5.84 µm. Three single-spore isolates, PXT1-1, PXT1-5, and PXT2-1, were selected as representatives for molecular identification. The rDNA internal transcribed

spacer region (ITS), a partial sequence of the actin gene (ACT), the chitin synthase 1 gene (CHS), glyceraldehyde-3-phosphate dehydrogenase gene (GAPDH), beta-tubulin (TUB2), and intergenic region of apn2 and MAT1-2-1 genes (ApMat) of isolates PXT1-1, PXT1-5, and PXT2-1 were amplified with the primer pairs ITS1/ITS4, ACT-512F/ACT-783R, CHS-79F/CHS-354R, GDF1/GDR1, T1/Bt-2b (Weir et al. 2012), and AM-F/AM-R (Silva et al. 2012), respectively. The sequences were compared with the GenBank nucleotide sequence database and had more than 99% similarity to ITS (JX010165), ACT (FJ907426), CHS (JX009866), GAPDH (JX010033), TUB2 (JX010405), and ApMat (JQ807838) of *Colletotrichum fruticola* isolate ICMP18581. The sequences were deposited into GenBank (accessions MT193419 to MT193421 for ITS, MT193101 to MT193103 for ACT, MT193098 to MT193100 for CHS, MT193095 to MT193097 for GAPDH, MT193092 to MT193094 for TUB2, and MT193089 to MT193091 for ApMat, respectively). Based on genetic and morphological results, the isolates were identified as *C. fruticola*. Pathogenicity was tested on 72 healthy leaves from nine 2-year-old plants (*M. × amabilis*, eight leaves per plant) in November 2019 in a greenhouse in Nanning, Guangxi, China using isolates PXT1-1, PXT1-5, and PXT2-1. Two wounds were made slightly on either side of the vein on each leaf using a sterilized toothpick, and each wounded site was inoculated with 10 µl of a 1 × 10⁶ conidia/ml suspension. Leaves of three plants were treated with sterile water for the control. Plants were covered with plastic bags to maintain high humidity and placed in a greenhouse kept at 28°C with a 12-h photoperiod. At 5 days postinoculation, all the inoculated leaves developed dark brown lesions (0.1 to 1.4 cm in width and 0.2 to 1.6 cm in length), whereas controls remained disease free. The fungus was reisolated from symptomatic inoculated leaves and identification confirmed by morphology and sequencing, fulfilling Koch's postulates. *C. fruticola* can cause leaf diseases in a variety of crops and ornamental plants, including apple, tea plants, sage, and orchid (Guarnaccia et al. 2019; Kim et al. 2018). To our knowledge, this is the first report of *C. fruticola* causing anthracnose on *M. × amabilis* in China. These findings provide a foundation for future studies on the epidemiology and control of anthracnose caused by *C. fruticola* on *M. × amabilis*.

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