Diseases Caused by Fungi and Fungus-Like Organisms

First Report of Leaf Spot Caused by *Colletotrichum fructicola* and *C. siamense* on *Ziziphus mauritiana* in Guangxi, China

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Ziziphus mauritiana Lam. is an important tropical fruit tree and has significant economic value. It is widely planted in Hainan, Guangdong, Guangxi, and Fujian provinces in China (Yang et al. 2017). In March 2019, leaf spot was observed on leaves of *Z. mauritiana* at Bagui fields in Nanning, Guangxi, China, with incidence exceeding 50%. Symptomatic leaves developed a yellow to tan-brown sunken lesions and finally abscised. To isolate the pathogen causing the symptoms, small pieces (5 × 5 mm) of infected leaves were surface sterilized by exposure to 75% ethanol for 10 s followed by 1% sodium hypochlorite for 1 min and rinsed three times in sterile water. Fifty pieces were isolated and surface sterilized, and pieces were plated onto potato dextrose agar (PDA) and grown at 28°C for 7 days. The isolation rate of *Colletotrichum* species was 100%. Three representative isolates (DQZ3-1, DQZ3-2, and DQZ3-3) were selected for further study. Mycelia were greyish-white for all three isolates, with isolate DQZ3-1 also appearing dark green in the center of the colony. Conidia were elliptical, aseptate and hyaline, with sizes of $13.4 \pm 0.12 \times 5.7 \pm 0.1$ µm, $14.8 \pm 0.1 \times 5.8 \pm 0.1$ µm, and $15.1 \pm 0.1 \times 5.5 \pm 0.1$ µm for DQZ3-1, DQZ3-2, and DQZ3-3, respectively. Genomic DNA was extracted using the DNAsecure Plant Kit (Tiangen Biotech, Beijing, China) and the internal transcribed

spacer (ITS), partial actin (ACT), calmodulin (CAL), chitin synthase (CHS-1), beta-tubulin (TUB2), and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) genes were sequenced (Weir et al. 2012). Phylogenetic analysis of the three isolates was performed with MEGA-X (version 10.0) based on sequences of multiple loci (ITS, ACT, CAL, CHS-1, TUB2, and GAPDH) using maximum likelihood analysis. Isolate DQZ3-1 was identified as *Colletotrichum fructicola*, and the other two isolates, DQZ3-2 and DQZ3-3, were identified as *C. siamense* (accessions MT039396 to MT039410, for ACT, CAL, CHS-1, GAPDH, and TUB2 of DQZ3-1, DQZ3-2, and DQZ3-3; MT041651 to MT041653 for ITS of DQZ3-1, DQZ3-2, and DQZ3-3). Pathogenicity tests were conducted on 1-year-old plants. Young, healthy leaves were artificially wounded by gently scratching with a sterile needle, and 10-µl droplets of conidial suspension (10^6 spores/ml) were applied per wound site for each isolate. Some wounded leaves were inoculated with 10-µl droplets of water as controls. Each isolate was inoculated onto three plants, with 15 leaves at least for each plant, the same as the controls. All inoculated plants were sprayed with water and covered with plastic bags to maintain high humidity. Symptomatic lesions were observed on the inoculated leaves after 7 days at 28°C, whereas no symptoms were observed on the control leaves. To fulfill Koch's postulates, fungi were reisolated from 50 symptomatic leaf pieces, and fungi reisolated from each leaf piece were morphologically identical to the inoculated isolates, for a 100% isolation frequency. To our knowledge, this is the first report of leaf spot caused by *C. fructicola* and *C. siamense* on *Z. mauritiana* worldwide. This research may accelerate the development of future epidemiological studies and management strategies for anthracnose caused by *C. fructicola* and *C. siamense* on *Z. mauritiana*.

References:

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