Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Botryosphaeria dothidea* Causing Leaf Spot and Wilt on *Celtis sinensis* in China

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Celtis sinensis Pers. (Chinese hackberry), belonging to the family Ulmaceae, is widely used as a street tree or landscape plant because of its long life and beautiful posture. In addition, *C. sinensis* has high economic and medicinal values; its roots and bark can be used for the treatment of lumbago, measles, tumors, and so on (Zhang et al. 2016). In July 2019, symptoms of leaf spot were observed on *C. sinensis* in Yunshan National Forest Park of Zibo, Shandong Province, China (36.48°N, 117.84°E). We surveyed more than 500 m² of forest area, and the disease incidence was over 80%, which seriously affected the ornamental effect. Symptoms on infected leaves appeared as regular round or oval spots, yellow colored with brown borders, and the areas of the lesion were connected into an irregular spot as the disease progressed. Twenty leaves of infected tissues were cut into ~2-mm pieces and surface disinfected with 75% ethanol for 30 s, rinsed three times with sterile deionized water, and air dried, and then the segments were cultured on potato dextrose agar (PDA) and incubated at 25°C for 5 to 7 days. More than 15 isolates were obtained, and cultures were initially white, gradually becoming gray green to dark after 1 week, producing copious gray aerial mycelium. Three representative single isolates were used for molecular identification, which were verified based on the amplification of DNA sequences of internal transcribed spacer

region, translation elongation factor 1 alpha, and beta-tubulin genes, using the primers ITS1/ITS4 (White et al. 1990), EF1-728F/EF1-986R (Carbone and Kohn 1999), and BT-2a/BT-2b (Glass and Donaldson 1995), respectively. The sequenced genes (GenBank accession nos. MT367874, MT385087, MT374083) exhibited 99.63% (identity = 545/547), 99.00% (identity = 297/300), and 100.00% (identity = 451/451) homology with the corresponding genes of type specimen of *Botryosphaeria dothidea* strain CBS110302 (GenBank accession nos. AY259092, AY573218, EU673106), respectively. Morphological and molecular results showed that the isolates were *B. dothidea* (Slippers et al. 2014; Zhai et al. 2014). A pathogenicity test was confirmed using five living, healthy *C. sinensis* plants with three leaves wound inoculated with mycelial plugs (about 4 mm in diameter) of *B. dothidea* from a 7-day-old culture grown on PDA, and inoculation with sterile PDA plugs on the same leaves served as negative controls. All the treated plants were covered by plastic sheeting and kept at high relative humidity by adding water regularly. Seven days later, all inoculated leaves displayed round, dark brown spots which were larger than those discovered outdoors, whereas no symptoms appeared on negative controls. Pathogens reisolated from inoculated leaves were confirmed as *B. dothidea* on account of morphological and molecular results, and the pathogenicity test was repeated three times to confirm Koch's postulates, which showed similar results. Based on the above descriptions and characteristics, the causal agent on *C. sinensis* was confirmed as *B. dothidea*. To our knowledge, this is the first report on the presence of *B. dothidea* affecting *C. sinensis* plants in China. This finding is beneficial to the protection and application of *C. sinensis*, a very common greening tree species.

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