Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Colletotrichum fructicola* Causing Anthracnose on *Camellia yuhsienensis* in China

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Camellia yuhsienensis Hu is an endemic species from China, where is the predominant oilseed crop due to its anthracnose resistance (Kuang 2015; Li et al. 2020; Nie et al. 2020). In April 2019, anthracnose symptoms were observed on *C. yuhsienensis* in a plantation in Youxian, Zhuzhou, Hunan Province, China (113.32°E, 26.79°N). Approximately 10% anthracnose incidence was detected in 500 two-year-old plants in a 5,000-m² cultivated area. Diseased leaves showed irregular grayish brown spots with dark brown edges and dark brown undersides. Symptomatic tissues (4 to 5 mm²) were surface disinfected for 90 s in 75% ethanol, rinsed twice with sterile water, and finally incubated on potato dextrose agar (PDA) at 28°C (Jiang et al. 2018). Pure cultures were obtained by the single-spore isolation method. A total of 100 fungal isolates were obtained from 85 symptomatic leaves, from which 81 had similar colony morphology. Colonies on PDA were white, fluffy, and cottony, becoming dark gray after 5 days. The character of the reverse of the colony was similar to that of the upper of the colony, but the color was darker at the same time. The isolates produced a large number of single-celled, hyaline, straight, and cylindrical conidia, with 10.35 to 17.58 µm length × 3.46 to 5.69 µm width ($\bar{x}$ = 13.61 × 4.63 µm, n = 30). The isolates were preliminarily identified as *Colletotrichum* spp. according to morphological features (Weir et al. 2012). Representative isolate YX2-5-2 was used for

molecular identification: internal transcribed spacer (ITS), partial actin (ACT), chitin synthase (CHS-1), and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) genomic DNA regions were amplified by PCR (Weir et al. 2012). Gene sequences were deposited in GenBank (accession nos. MW398863 for ACT, MW886232 for CHS-1, MW398864 for GAPDH, and MW398865 for ITS). BLAST analysis revealed that DNA sequences of YX2-5-2 at the ITS, GAPDH, ACT, and CHS-1 loci showed 100, 99.25, 100, and 99.33% sequence identity, respectively, to their corresponding loci in strains ZH6 (GenBank accession no. MT476840.1), ICKP18B4 (LC494274.1), YN17 (MN525804.1), and ICKG4 (LC469131.1) of *Colletotrichum fructicola*. A maximum likelihood phylogenetic tree based on the combined ACT, CHS-1, ITS, and GAPDH sequences revealed that the representative isolate YX2-5-2 clustered with *C. fructicola*. In addition, the morphological features of YX2-5-2 were similar to *C. fructicola*, which has been reported (Weir et al. 2012). Pathogenicity was tested using isolate YX2-5-2 by inoculating leaves of 2-year-old *C. yuhsienensis*. Four leaves of each healthy *C. yuhsienensis* were sprayed with a conidial suspension (10⁵ conidia/ml) of isolate YX2-5-2, and the above steps were repeated three times. Two additional mock-inoculated control plants were sprayed with sterilized liquid potato dextrose medium. The plants were incubated in a greenhouse at 28°C and 90% humidity with a 12-h photoperiod. Anthracnose-like symptoms were observed 5 days postinoculation. The control plant tissues remained healthy. *C. fructicola* was reisolated on PDA from lesions, and the morphological features were consistent with YX2-5-2, confirming Koch's postulates. To our knowledge, this is the first report of anthracnose of *C. yuhsienensis* caused by *C. fructicola* in China. Anthracnose of *C. oleifera* has been reported for a long time (Li et al. 2016). *C. yuhsienensis*, as a wild relative of *C. oleifera* (commonly known as tea-oil tree), has been of concern about its resistance to anthracnose. Therefore, the occurrence of *C. yuhsienensis* anthracnose hindered the control of anthracnose of tea-oil tree. This finding will lay the foundation for studying the pathogenesis of anthracnose of tea-oil tree and developing effective prevention methods.

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