Diseases Caused by Fungi and Fungus-Like Organisms

First Report of Anthracnose Caused by *Colletotrichum siamense* and *C. fruticola* of *Camellia chrysantha* in China

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Camellia chrysantha (Hu) Tuyama, belonging to the Theaceae family, is famous for its large size and golden yellow flowers; it has high ornamental and health care functions (Mo et al. 2013). Anthracnose is one of the most important fungal diseases worldwide, causing serious economic losses to many plants. In October 2019, severe anthracnose symptoms were observed on the leaves of *C. chrysantha* in a 0.6-ha field with 15 to 20% disease incidence in Fangchenggang city, Guangxi Zhuang Autonomous Region of China. Diseased leaves initially appeared with irregular chlorotic spots, which afterward enlarged and coalesced. Finally, the spots became dark brown or black, sunken lesions (8 to 22 mm in diameter) and were covered with plenty of acervuli. For pathogen isolation, the leaf lesions were cut into small tissue pieces (5 × 5 mm), disinfected by 0.3% NaOCl for 2 min and 70% ethanol for 40 s, rinsed in sterile distilled water, and then incubated at 28°C on PDA plates. A total of seven fungal isolates with whitish to light gray, dense colonies were recovered at 5 days. These isolates were tentatively identified as belonging to *Colletotrichum gloeosporioides* species complex through morphological and cultural characters (Weir et al. 2012). The conidia were nonseptate, cylindrical with obtuse to rounded ends, 13.9 to 18.3 (average 16.1) × 4.5 to 6.2 (average 5.4) μm (*n* = 50). For further precise identification, the seven *Colletotrichum* isolates were analyzed using partial sequences of genomic loci including the internal transcribed spacer (ITS), β -tubulin (TUB), calmodulin (CAL), actin (ACT), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), glutamine synthetase (GS), and the mating type locus MAT1-2 (ApMat) genes

(Liu et al. 2015). The amplification sequences were compared with the sequences registered in the GenBank database based on nucleotide similarity. The above sequences of four isolates (JZB-PF4232, JZB-PF2231, JZB-PF42, and JZB-PF22) had 99 to 100% identity to the sequences of *Colletotrichum siamense* strains retrieved from GenBank, while the sequences of the other three isolates (JZB-PF3231, JZB-PF32, and JZB-PF41) showed over 99% identity with those of the *C. fruticola* strains. All the sequences were deposited in GenBank as MT708987 to MT709007, MW149430 to MW149433, and MW142259 to MW142282. A multilocus phylogenetic analysis of the concatenated sequences of ITS, TUB, CAL, ACT, GAPDH, GS, and ApMat genes placed the four isolates described above in the *C. siamense* clade, while the other three isolates were attributed to the *C. fruticola* clade. Pathogenicity tests were conducted on seven healthy 2-year-old *C. chrysantha* seedlings (cv. Fangpu), which consisted of 21 wounded leaves made by a sterile needle, with three leaves per seedling. Artificial inoculations were performed by treating each seedling with 20 μl of spore suspension (10⁶ conidia/ml) of each isolate. Leaves of seedlings treated with sterilized water under the same conditions served as controls. The experiment was repeated three times. All the seedlings were covered with plastic bags to maintain high humidity (90% RH) and placed in a greenhouse kept at 25°C with a 16-h light/8-h dark photoperiod. After 8 days, the inoculated leaves of *C. chrysantha* plants developed typical dark brown or black lesions, similar to the symptoms in the field, whereas controls remained symptomless. Koch's postulates were fulfilled by reisolation of the same fungi from symptomatic inoculated leaves, with identification confirmed by morphological and molecular characteristics, respectively. *C. siamense* and *C. fruticola* have been found to cause anthracnose on *Camellia sinensis* (Shi et al. 2018; Wang et al. 2016). *C. fruticola* has also been reported to cause anthracnose on *Citrus sinensis* in China (Hu et al. 2019). To our knowledge, this is the first report of *C. siamense* and *C. fruticola* causing anthracnose on *C. chrysantha* in China.

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