Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Colletotrichum siamense* Causing Anthracnose on *Cornus hongkongensis* in China

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Cornus hongkongensis (Hemsl.) is an excellent ornamental tree species in China and elsewhere. In 2019, *C. hongkongensis* anthracnose was firstly observed at the campus of Jiangxi Agricultural University (JXAU) (28°45'56"N, 115°50'21"E), and then found in parks, in Nanchang, China. In early August, the disease appeared and lasted until the leaves dropped (November). The disease incidence was above 60%, and the diseased leaf rate was above 70%. The lesions mostly appeared along the leaf edges. Some small round to irregular lesions also developed in other parts of the leaves. These diseased leaves had circular or irregularly shaped spots with gray-white color in the center and dark brown on the edge of the lesions. Later, the lesions became necrotic and shriveled. As the disease progressed, the spots coalesced so that affected leaves appeared blighted. To identify the pathogen, leaves with typical symptoms from the campus of JXAU were collected, and small pieces (5 × 5 mm) from the lesion borders were surfaced sterilized in 70% ethanol for 30 s, followed by 1 min in 3% NaOCl, and then rinsed with

sterile distilled water three times. Leaf pieces were placed on potato dextrose agar (PDA) and incubated at 25°C under a 12-h light/dark cycle (3,000 lx). Pure cultures were obtained from individual conidia by single-spore isolation. For studies of microscopic morphology, a representative isolate, JX-S4, was subcultured on PDA. The colony of JX-S4 was white and turning gray and light gray on the reverse side, producing dark-green pigmentation near the center. The conidia were one-celled, straight, hyaline, subcylindrical with rounded ends and $16.9 \pm 1.6 \times 6.0 \pm 0.6 \mu\text{m}$ ($n = 50$) in size. Appressoria were one-celled, pale brown, thick-walled, ellipsoidal, and measured $8.7 \pm 1.7 \times 6.4 \pm 0.8 \mu\text{m}$ ($n = 50$). The morphological characteristics of JX-S4 matched those of the *Colletotrichum siamense* species (Weir et al. 2012). For accurate identification, the internal transcribed spacer (ITS) and the genes encoding glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*), chitin synthase (*CHS-I*), beta-tubulin 2 (*TUB2*), and calmodulin (*CAL*) were respectively amplified with primers ITS1/ITS4, GDF/GDR, CHS-79F/CHS-345R, $\beta\text{t}2\text{a}/\beta\text{t}2\text{b}$, and CL1/CL2. The sequences were deposited in GenBank (accession nos. MT587807, MT628710, MT628711, and MT628708). Phylogenetic analysis was calculated with concatenated sequences (ITS, *GAPDH*, *CHS-I*, *CAL*, and *TUB2*) using MEGA 7. In the maximum likelihood phylogenetic tree, isolate JX-S4 clustered with *C. siamense* with 93% bootstrap support. Based on the morphological characteristics and phylogenetic analysis, JX-S4 was identified as *C. siamense*. Pathogenicity of JX-S4 was verified on 45 attached healthy leaves from three *C. hongkongensis* plants (10 years old) at the campus of JXAU inoculated with mycelial plugs ($\phi = 5 \text{ mm}$) from the culture edge (6 days old) on PDA. An additional 45 healthy leaves were inoculated with PDA plugs as controls. The leaves were wounded with a red-hot needle ($\phi = 0.5 \text{ mm}$). All treatment and control leaves were wrapped up with black plastic bags to keep them moist for 2 days. The pathogenicity tests were repeated twice. Within 7 days, all the inoculated leaves developed lesions, which were similar to those observed in the field. Control leaves were asymptomatic. The same fungus was reisolated from the symptomatic tissues, fulfilling Koch's postulates. To our knowledge, this is the first report of *C. siamense* causing *C. hongkongensis* anthracnose. This finding provides crucial information for managing this disease. For example, when diagnosing *Cornus* anthracnose, *C. siamense* needs to be looked out for and appropriate control measures implemented.

Reference:

Weir, B. S., et al. 2012. Stud. Mycol. 73:115.

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