Diseases Caused by Fungi and Fungus-Like Organisms

First Report of Anthracnose Caused by *Colletotrichum liaoningense* on *Trichosanthes kirilowii* in China

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Trichosanthes kirilowii Maxim (*T. kirilowii*) is widely grown in central China for its medicinal and edible value. In August 2020, an anthracnose-like disease was observed on fruit of *T. kirilowii* (cv. Wanlou9) in four fields (0.9 ha) located in Taihu county, Anhui Province of China. Approximately 60% of the *T. kirilowii* plants were affected in the fields. The symptoms initially consisted of small off-white necrotic spots, and later became larger, irregular gray necrotic lesions on green fruit, causing fruit rot and sometimes fruit drop. More than 10 symptomatic fruits were sampled, and small pieces of diseased tissue were surface sterilized in 0.1% HgCl₂ for 2 min, 75% ethanol for 45 s, rinsed three times with sterile distilled water, then placed on potato dextrose agar (PDA) and incubated at 25°C in the dark. A fungus was consistently (80%) isolated from symptomatic fruit samples. Aerial mycelia were light gray and radially black with white in reverse medium. Conidia were rarely produced on PDA, but prolific on water agar. The conidia were cylindrical to clavate, both ends rounded, had an obvious circular granule in the center, and ranged from 14.6 to 19.9 µm × 5.4 to 7.3 µm. The morphological characteristics were similar to the descriptions of *Colletotrichum liaoningense* by Diao et al. (2017). For molecular identification, representative isolates LG5-4 and LG9-6 were selected. Genomic DNA was extracted, and

the internal transcribed spacer (ITS) region, actin (*ACT*), glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*), and β-tubulin (*TUB2*) genes were amplified by PCR (Duan et al. 2018; White et al. 1990) and sequenced bidirectionally. A BLAST search of GenBank revealed the *ACT* and *TUB* sequences had 95.83% (KP890097), 99.20%, 95.33% (KP890111), and 99.84% identity, respectively, to *C. liaoningense* CAUOS2. A phylogenetic analysis was conducted using MEGA7 based on concatenated sequences of the four genes, indicating that the isolates were closely clustered with reference strains of *C. liaoningense* (98% bootstrap value). The two strains were deposited in the China General Microbiological Culture Collection Center as CGMCC3.20344 and CGMCC3.20345, and their sequences deposited in GeneBank (accession nos. MW082811–12, MW117926–31, respectively). Pathogenicity tests were conducted on healthy fruit of *T. kirilowii* (cv. Wanlou9) using wound inoculation by pinpricking and droplet (10⁶ conidia/ml) on fruit surface. The experiments were done with three fruit per isolate (LG5-4 and LG9-6) and replicated three times. The controls were inoculated with sterile water. The fruit were covered with plastic bags and kept in a chamber (>90% RH, 28 to 30°C) for 14 days. Typical symptoms of yellow-brown lesions appeared 14 days after inoculation. No symptoms were observed on the controls. The fungus was reisolated from the diseased tissues and identified as *C. liaoningense* by sequencing of the four genes, confirming Koch's postulates. *C. liaoningense* has been reported to cause anthracnose of mango and chili in China (Diao et al. 2017; Li et al. 2019). To our knowledge, this is the first report of *C. liaoningense* causing anthracnose on *T. kirilowii*. Due to cultivation of *T. kirilowii* in the region, further studies are required to develop management strategy of this disease.

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The author(s) declare no conflict of interest.

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