Diseases Caused by Fungi and Fungus-Like Organisms

First Report of Anthracnose Caused by *Colletotrichum gloeosporioides* on *Akebia trifoliata* in China

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Akebia trifoliata (Thunberg) Koidzumi (three-leaf akebia), a climbing deciduous woody plant, grows wild in mountains of China and Japan. It has long been prized for its delicious sweet taste and medicinal value (Lu et al. 2019). Few pests and diseases reportedly affect this plant (Ye et al. 2013), but with more commercial planting of *A. trifoliata* in China, symptoms of anthracnose on leaves and fruits have increased. Between December 2018 and May 2019, typical anthracnose symptoms were first observed on *A. trifoliata* grown in Wuhan, China, with an incidence up to 15%. Diseased leaves exhibited irregular gray-brown spots with dark brown edges and dark brown undersides, substantially affecting photosynthesis and growth. As disease progressed, white mycelium appeared on stems, causing stem rot and fruit drop. Several round or needle-shaped dark brown spots formed on fruit peel, coalescing into irregular, slightly sunken blotches. Under high humidity, the whole fruit turned brown, and the spots were covered by white mycelia, greatly affecting the fruit's ornamental quality. To isolate the pathogen, 5-mm² pieces of symptomatic tissue from 10 infected leaves and fruits were surface disinfected for 90 s in 1% sodium hypochlorite then 30 s in 75% ethanol, rinsed twice with sterile water, and then incubated on potato dextrose agar (PDA, Oxoid) at 25°C under a 12-h light/dark photoperiod. Pure cultures were obtained from hyphal tips of each colony. Initially, colonies produced white mycelia, turning gray after 5 days. The isolates produced abundant hyaline, single celled, straight and cylindrical

conidia, with mean size 10.35 to 15.58 × 3.46 to 5.69 µm. Morphological characteristics were generally consistent with those of *Colletotrichum gloeosporioides* (Cannon et al. 2012). Genomic DNA of three isolates was extracted for PCR amplification of the internal transcribed spacer (ITS) region and the β-tubulin (TUB2) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) genes (Weir et al. 2012). BLAST search identified all sequences (GenBank accession nos. MT451846 to MT451848 for ITS, MT573957 to MT573959 for TUB2, and MT573960 to MT573962 for GAPDH) as 100% matches to *C. gloeosporioides* (Penz.) Penz. & Sacc. CBS 112999 strain (JQ005152 for ITS, JQ005587 for TUB2, JQ005239 for GAPDH) (Damm et al. 2009). Identification was confirmed by maximum likelihood phylogenetic analysis using MEGA7. To evaluate pathogenicity, isolates were inoculated onto one side of 10 wounded healthy leaves of 1-year-old pot-grown *A. trifoliata* plants and 10 nearly mature fruits, with 10 µl of conidial suspension (10⁶/ml) and colonized PDA pieces (5-mm diameter) from 7-day-old cultures of the fungus in Petri dishes; control sides received 10 µl of sterile distilled water and sterile agar pieces. The test was performed twice. After incubation at 25°C, 70% humidity under 12-h fluorescent illumination/12-h dark for 5 days, similar spots were observed on all inoculated leaves and fruits. Controls remained asymptomatic. The reisolated pathogen was identified as *C. gloeosporioides* by biological characteristics and sequencing analysis, indicating that *C. gloeosporioides* was a causal agent of anthracnose of *A. trifoliata*. Anthracnose caused by *C. acutatum* has been reported on *A. trifoliata* in Japan (Kobayashi et al. 2004). To our knowledge, this is the first report of *C. gloeosporioides* found on *Akebia* species. The new disease primarily reduces the quality and yield of *A. trifoliata*. Effective measures should be taken to manage this disease.

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