Diseases Caused by Fungi and Fungus-Like Organisms

First Report of Red-Fleshed Apple Anthracnose Caused by *Colletotrichum siamense*

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Red-fleshed apple (*Malus niedzwetzkyana*) is a special germplasm with colored fruit and rich anthocyanins in its fruit, and it has become more and more popular among consumers (Yang et al. 2020). In recent years, red-fleshed apple anthracnose disease occurred in most orchards and germplasm gardens in Shandong Province, China, and the incidences were above 50%, even greater than 90% in some orchards. Initially, the spots on fruit were subcircular shaped, sunken, and pale brown. The black lesions enlarged and amalgamated into large necrotic areas. The older spots in the center became blackish and oozed slimy pink conidial masses. In September 2015, 15 fruit with anthracnose symptoms were collected, and 11 isolates were obtained. Representative isolates (JNTW11, JNTW2, and JNTW33) were used for morphological and molecular characterization. On PDA, the colonies were initially white and turned to pale brown in 3-day-old cultures, and orange-brown pigmentation was produced near the center on the reverse. Aerial mycelium was cottony, dense, and pale white to pale gray. Acervuli developed visible orange conidial masses. Conidiophores were colorless, septate, not branched, or branched at the base. Conidia were one celled, hyaline, subcylindrical, oblong, attenuated with blunt ends, and the average size was

16.7 ± 1.5 by 6.1 ± 0.9 µm (*n* = 50). Appressoria were midbrown, obovoid or irregular, and 9.2 ± 1.6 by 8.0 ± 1.8 µm (*n* = 20). The morphological characteristics matched the descriptions of *Colletotrichum gloeosporioides* sensu lato (Cannon et al. 2008). Isolates JNTW11, JNTW2, and JNTW33 were further studied by phylogenetic analysis of the partial sequence of six loci, including the ribosomal internal transcribed spacer (ITS), actin (*ACT*), β-tubulin 2 (*TUB2*), calmodulin (*CAL*), chitin synthase (*CHS-1*), and glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) (Weir et al. 2012). The ITS (MT577037, MT577040, and MT577042), *ACT* (MT767712, MT767715, and MT767717), *TUB2* (MT767723, MT767726, and MT767728), *CAL* (MT767689, MT767692, and MT767694), *CHS-1* (MT767700, MT767703, and MT767705), and *GAPDH* (MT767734, MT767737, and MT767739) sequences were deposited in GenBank. The six sets of sequence data were concatenated in an order of ITS-GAPDH-ACT-CHS-1-TUB2-CAL, and the aligned sequences (2,007 bp) had 99.0% similarity to ex-type *C. siamense* ICMP18578. In the maximum-likelihood phylogenetic tree, the highest log likelihood was -9,148.55, and the isolates tested were in the *C. siamense* cluster, with 96% bootstrap support. Thus, the isolates were identified as *C. siamense* according to multilocus phylogenetic analyses and morphological observations. To complete Koch's postulates, healthy red-fleshed apple fruit ('Jiuhong', 1 month preharvest) were inoculated using a point inoculation with hyphal plugs and a blank agar as a control. All inoculated fruit were placed in sterile tissue culture bottles containing two layers of wet paper towels at 28°C under a cycle of 12 h of light and 12 h of darkness. All fruits developed anthracnose symptoms whereas the controls did not show any symptoms in 7 days. The symptoms were similar to the disease observed in the field. The same fungus was reisolated from the lesions. Up to now, *C. acutatum*, *C. asianum*, *C. chrysophilum*, *C. cuscatae*, *C. fioriniae*, *C. fragariae*, *C. fruticola*, *C. gloeosporioides*, *C. godetiae*, *C. kahawae*, *C. karstii*, *C. limeticola*, *C. melonis*, *C. noveboracense*, *C. nymphaeae*, *C. paranaense*, *C. rhombiforme*, *C. salicis*, and *C. theobromicola* could infect *M. coronaria*, *M. domestica*, *M. prunifolia*, *M. pumila*, and *M. sylvestris* worldwide. To our knowledge, this is the first report of *C. siamense* as a pathogen of *M. niedzwetzkyana*. This finding provides crucial information for the appropriate management of this disease.

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