Diseases Caused by Fungi and Fungus-Like Organisms

First Report of Anthracnose Disease on Avocado (*Persea americana*) Caused by *Colletotrichum fruticola* in China

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In September 2019, anthracnose-like symptoms were observed on fresh avocado fruits cv. Hass that were imported from Peru (Mission Produce, Inc.) and purchased at Ganfuyuan store, Guangzhou, China. After being stored for 5 days at room temperature, initial black specks developed into larger brown or black lesions on fruits, and salmon-colored conidial masses were observed in the lesions. To isolate and identify the pathogen, small pieces (5 × 5 mm) were excised from the lesion margins of the fruits, which were surface sterilized by 1% NaOCl (1 min) and 70% ethanol (30 s), and washed twice with sterile distilled water (SDW). Then, tissues were cultured on potato dextrose agar (PDA) to obtain the pure strain NYG. The colonies grown on PDA for 7 days appeared cottony, white to pale gray, and with the presence of a conidial mass. Conidia were 11.7 to 19 × 3.6 to 6.4 μm (*n* = 50), hyaline, aseptate, straight and cylindrical with rounded ends. Appressoria were 6.2 to 11.9 × 4.5 × 7.4 μm (*n* = 50), brown to dark brown in different shapes. Perithecia were thick walled and globose with a prominent, narrow neck. Asci were 31.5 to 55 × 6 to 12.5 μm (*n* = 15), six to eight spored, and clavate to cymbiform. Ascospores were 5 to 18 × 4.5 to 6 μm (*n* = 25), hyaline, large guttulate at the center, and slightly curved with rounded ends. Based on the morphological characteristics, the strain NYG was identified as *Colletotrichum fruticola* (Prihastuti et al. 2009). The identity of the strain was confirmed by means of multilocus gene sequencing. Genomic DNA was extracted using Ezup Column Fungi Genomic DNA Purification Kit (Sangon Biotech Co., China). The internal transcribed spacer (ITS) rDNA region and actin (*ACT*), chitin synthase (*CHS-1*), calmodulin (*CAL*), and glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) partial genes

(Carbone et al. 1999; Templeton et al. 1992) were amplified and sequenced, and sequences were deposited in GenBank (OL413493, OL517766, OL517768, OM141126, and OL517767). BLASTn analysis revealed that DNA sequences of the isolate showed 100% identity with those of *C. fruticola* (MT476840.1, MK208862.1, MZ965245.1, JX009665.1, and MN982434.1, respectively). A phylogenetic tree analysis based on the concatenated sequences confirmed the isolate as *C. fruticola*. Pathogenicity was tested by infecting fresh healthy avocado fruits with the isolated strain NYG. Three unwounded and wounded avocados fruits were surface sterilized and inoculated with 10 μl of conidial suspension (1 × 10⁶ conidia/ml) by the drop inoculation method. Control fruits were inoculated with SDW containing Tween 20 (1 μl/ml of H₂O), respectively. All inoculated fruits were incubated at 25°C in the dark. Anthracnose symptoms were observed on the wounded and unwounded fruits after 3 and 5 days post inoculation, respectively. No symptoms were observed in the control on either wounded or unwounded fruits. The pathogenicity test was performed in duplicate. The inoculated fungus was reisolated from the infected fruits and confirmed as *C. fruticola*, thus confirming Koch's postulates. *C. fruticola* represents an important fungal pathogen in several plantations worldwide (Farr and Rossman 2020), for example, on avocado fruits in Mexico (Fuentes-Aragón et al. 2018) and New Zealand (Hofer et al. 2021). This is the first report of anthracnose caused by *C. fruticola* on imported avocado fruits in China. The results of this study not only help establish effective quarantine measures against anthracnose disease for imported avocado fruits in China, but also provide an important reference to prevent the spread of this disease on China's domestic avocados.

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