Diseases Caused by Fungi and Fungus-Like Organisms

Identification of *Colletotrichum fructicola* Causing Anthracnose on *Peucedanum praeruptorum* in China

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Peucedanum praeruptorum Dunn is a perennial herbaceous plant commonly used in traditional Chinese medicine to dispel wind heat, resolve phlegm, and lower blood pressure. During July 2019, an estimated 30% *P. praeruptorum* plants were observed with anthracnose at two natural fields (approximately 1 ha) in Ningguo city, China. Small brown spots initially appeared on the diseased leaves, which gradually expanded, forming irregular necrotic lesions with a dark brown margin and grayish white center. Over time, the center part of the lesion became thinner and prone to perforation. To identify the pathogen, 30 symptomatic plants were collected. Small pieces of symptomatic leaves (3 × 3 mm) from the margins of diseased tissues were surface sterilized in 2% NaOCl solution for 2 min followed by 70% ethanol for 15 s, rinsed three times with deionized water, and dried on autoclaved filter paper before plating on potato dextrose agar (PDA) medium at 26°C in dark. Fungal colonies with identical morphology developed from diseased tissues after 3 to 4 days. Ten purified isolates were randomly selected from the colonies by single-sporing. Five-day-old colonies of all isolates cultured on PDA showed white fluffy aerial hyphae with small orange masses at the center of the colony. Conidia were one-celled and hyaline, cylindrical, with rounded ends, and sizes ranged from 12.74 to 19.47 µm (mean 15.80 µm) × 3.82 to 6.92 µm (mean 5.34 µm) (*n* = 50). Appressoria were obovoid to ellipsoid, with dimension of 5.64 to

8.74 × 3.64 to 7.46 µm (*n* = 50). The morphological characteristics of these isolates were similar to the descriptions of *Colletotrichum gloeosporioides* species complex (Weir et al. 2012). For molecular identification, fragments of the internal transcribed spacer (ITS), chitin synthase (*CHS*) (Carbone and Kohn 1999), glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*) (Templeton et al. 1992), β-tubulin (*TUB*) (Weir et al. 2012), and *Apn2* (Rojas et al. 2010) genes were sequenced. The sequences from a representative isolate, PPCF-1, were deposited in GenBank with accession numbers MN400080, MN412643, MN412645, MN412644, and MT412407. These sequences shared 100, 98.46, 99.71, 98.93, and 99.89% identity with the corresponding ITS (JX010173), *CHS* (JX009866), *TUB* (JX010405), *GAPDH* (JX010033), and *Apn2* (KJ954570, KJ954628) sequences of *C. fructicola*, respectively (Liu et al. 2015; Lu et al. 2018; Weir et al. 2012). To test pathogenicity, conidial suspensions (3 ml, 2 × 10⁵ spores/ml) of all 10 isolates prepared from 10-day-old culture were sprayed on the leaves of 2-month-old *P. praeruptorum* seedlings, with six plants per isolate. Six healthy *P. praeruptorum* plants treated with sterile water served as controls. Inoculated plants were incubated in a moist chamber at 26°C with a 12-h photoperiod. Small brown spots similar to the lesions observed in the natural field appeared on the inoculated leaves but not on controls at 5 days postinoculation. *C. fructicola* was reisolated from all symptomatic leaves but not controls, fulfilling Koch's postulates. *C. fructicola* was known to cause diseases on *Citrus sinensis* and *Camellia sinensis* in China. To our knowledge, this is the first report of *C. fructicola* causing anthracnose on *P. praeruptorum* in China. Infections of *C. fructicola* severely threaten *P. praeruptorum* production, and effective control strategies are urgently needed.

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