Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Colletotrichum fructicola* and *Colletotrichum nymphaeae* Causing Leaf Spot on *Rubus corchorifolius* in Zhejiang Province, China

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Rubus corchorifolius, which is one of the most important economic value fruit trees, belongs to the Rosaceae family (Tian et al. 2021). A severe leaf spot disease on leaves of *R. corchorifolius* was observed in Longquan county, Zhejiang province (118°42'E, 27°42'N) in 2019, with disease incidence of >20% in the area. The investigation was based on the method described by Fang (1998). The symptoms on leaves of naturally affected plants were early necrotic lesions with white centers, surrounded by yellow halos, and later the lesions were expanded, yellowish-brown centers, surrounded by yellow halos. The pathogens were isolated following the method reported by Fang (1998), and two strains (FPZ1 and FPZ2) were selected for further analysis. The fungus was cultured on potato dextrose agar medium for 6 days at 25°C. The colonies showed greyish-green centers, white mycelium, and orange conidial mass. The conidia of FPZ1 were unicellular, smooth-walled, hyaline, cylindrical with obtuse to rounded ends, the size 10.15 to 14.09 µm (mean = 12.95 µm, *n* = 50) × 4.36 to 6.19 µm (mean = 5.19 µm, *n* = 50). Appressoria were single, brown to dark brown, ovoid or irregular in shape, and 5.59 to 12.99 µm (mean = 8.77 µm, *n* = 50) × 4.68 to 10.360 µm (mean = 6.50 µm, *n* = 50). The characteristics of FPZ1 were consistent with species of the *Colletotrichum gloeosporioides* complex (Weir et al. 2012). The conidia of FPZ2 were hyaline, smooth-walled, one-celled, fusiform, the size 9.34 to 14.09 µm (mean = 11.92 µm, *n* = 50) × 3.26 to 6.15 µm (mean = 4.89 µm, *n* = 50). Appressoria were single, darker brown, elliptical or irregular in outline, and 4.49 to 15.06 µm (mean = 9.88 µm, *n* = 50) × 3.23 to 7.42 µm (mean = 5.72 µm, *n* = 50). The characteristics of FPZ2 were consistent with species of the *Colletotrichum acutatum* complex (Damm et al. 2012). For molecular identification of strains, the internal transcribed spacer, glyceraldehyde-3-phosphate

dehydrogenase, beta-tubulin, chitin synthase, and actin genes were sequenced (Weir et al. 2012). For the strain FPZ1, the five sequences were deposited in GenBank (MT846907, MT849313, MT849317, MT849315, and MT849319, respectively). A BLAST search of FPZ1 sequences showed 99% identity with the five loci sequences of type strain *C. fructicola* ICMP 18581 (JX010165, JX010033, JX010405, JX009866, and FJ907426) (Jayawardena et al. 2016). Similarly, for the strain FPZ2, the five sequences were deposited in GenBank (MT846885, MT849314, MT849318, MT849316, and MT849320, respectively). A BLAST search displayed 99% identity with the five loci sequences of type strain *C. nymphaeae* CBS 515.78 (JQ948197, JQ948527, JQ949848, JQ948858, and JQ949518, respectively) (Jayawardena et al. 2016). Based on morphological characteristics and phylogenetic analysis, FPZ1 was identified as *C. fructicola* and FPZ2 as *C. nymphaeae*, respectively. For pathogenicity tests, 10 µl of conidial suspension (1×10^6 conidia/ml) of FPZ1 was dropped on healthy, nonwounded detached leaves. Leaves receiving drops of sterilized water served as a control. The experiment was repeated three times, and each replicate contained five leaves. All leaves were kept on a mist bench at 27°C and 80% relative humidity for 6 days. The pathogenicity determination of FPZ2 referred to the operation of FPZ1. The inoculation sites of both FPZ1 and FPZ2 were brown; however, control leaves showed no symptoms. *C. fructicola* and *C. nymphaeae* were reisolated from the lesions of inoculated leaves, fulfilling Koch's postulates. To our knowledge, this is the first report of *C. fructicola* and *C. nymphaeae* causing leaf spot on *R. corchorifolius* in China. Knowledge about the existence of *C. gloeosporioides* species complexes and *C. acutatum* species complexes will be beneficial to chemical control of anthracnose in *R. corchorifolius*.

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