Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Colletotrichum fructicola* Causing Anthracnose on Cherry (*Prunus avium*) in China

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Cherry (*Prunus avium*) is an economically important fruit in China. In October 2020, a leaf spot disease was found on cherry in the orchard of Taizhou Academy of Agriculture Sciences, Zhejiang, China. Symptoms appeared as small, water-soaked spots on the leaves, which later became larger, dark brown, and necrotic lesions of 1 to 3 × 4 to 8 cm. Disease incidences of ~60% of leaves were observed by sampling five locations. To isolate the causal agent, small fragments from five target symptomatic leaves were surface sterilized with 1.0% NaOCl solution for 1 min and then rinsed three times with sterilized water. Then the leaf fragments were air dried, plated on PDA, and incubated at 25°C in the dark for 2 days. Pure cultures were obtained by transferring hyphal plug of 2 mm diameter onto PDA, which followed single-spore isolation. Colony morphology showed light to dark gray, cottony mycelium, and the underside of the culture became brownish after 7 days. Conidia ($n = 28$) were hyaline, smooth-walled, cylindrical, aseptate, broadly rounded ends, and average size around $3.84 \times 12.82 \mu\text{m}$ (2.99 to 4.87×10.27 to $15.68 \mu\text{m}$). Appressoria ($n = 27$) were mostly brown, ovoid, and slightly irregular in shape, with average size around $8.04 \times 9.68 \mu\text{m}$ (6.29 to 9.67×9.32 to $12.06 \mu\text{m}$). Perithecia average size is $106.25 \mu\text{m}$, textura angularis, thick-walled. Asci 26.35 to 49.18×5.00 to $12.03 \mu\text{m}$ (average size $37.44 \times 7.80 \mu\text{m}$, $n = 17$), unitunicate, thin-walled, clavate or cymbiform. Ascospores 13.69 to 20.93×3.86 to $6.69 \mu\text{m}$ (average size $16.00 \times 5.42 \mu\text{m}$, $n = 30$), one-celled, hyaline, one or two large guttulate at the center, slightly rounded ends. Morphological

characteristics matched well with previous descriptions of *Colletotrichum* species of *C. gloeosporioides* species complex, including *Colletotrichum fructicola* (Fu et al. 2019; Prihastuti et al. 2009). The identity of two representative isolates (cf2-3 and cf4-4) from different leaves was confirmed by multilocus gene sequencing. To this end, genomic DNA was extracted by the Plant Direct PCR kit (Vazyme Biotech, China). Molecular identification was conducted by sequencing the internal transcribed spacer (ITS) rDNA region, partial *glyceraldehyde-3-phosphate dehydrogenase* (*GAPDH*) gene, partial *actin* (*ACT*) gene, partial *beta-tubulin 2* gene (*TUB2*), and partial *chitin synthase* gene (*CHS*). The obtained sequences were deposited in GenBank under accession numbers MW581851 and MW581852 (ITS), MW590586 and MW590587 (*GAPDH*), MW616561 and MW616562 (*ACT*), MW729380 and MW729381 (*TUB2*), and MW729378 and MW729379 (*CHS*). BLAST analysis revealed that the ITS, *GAPDH*, *ACT*, *TUB2*, and *CHS* sequences of both isolates matched with 100% identity to *C. fructicola* culture collection sequences in GenBank database (JX010165, JX009998, JX009491, JX010405, and JX009866, respectively). These morphological characteristics and molecular analyses allowed the identification of the pathogen as *C. fructicola*. Koch's postulates were performed with healthy detached cherry leaves of cultivar 'HongMi' from Taizhou Academy of Agriculture Sciences. Surface-sterilized leaves were inoculated with 5-day-old cultures of *C. fructicola* mycelial discs of 2 mm diameter after being wounded with a needle or nonwounded. Control leaves were inoculated with same size discs of PDA agar. Treated leaves were incubated at 25°C in the dark at high relative humidity. Anthracnose symptoms appeared within 3 days both on nonwounded and wounded inoculation approaches. Mock-inoculated controls remained asymptomatic. Biological repetitions were carried out three times. The fungus was reisolated from infected leaves and confirmed as *C. fructicola* following the methods described above. Until recently, it has been found that *C. fructicola* can infect tea, apple, pear, and *Pouteria campechiana* in China (Fu et al. 2013; Li et al. 2013; Shi et al. 2018; Yang et al. 2021). To the best of our knowledge, this is the first report of *C. fructicola* on cherry in China.

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