Diseases Caused by Fungi and Fungus-Like Organisms

First Report of Anthracnose Fruit Rot Caused by *Colletotrichum fioriniae* on Litchi in China

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Anthracnose fruit rot of litchi (*Litchi chinensis* Sonn.), caused by *Colletotrichum* spp., has been mainly associated with the *C. acutatum* species complex and *C. gloeosporioides* species complex (Farr and Rossman 2020). In June 2010, isolates of the *C. acutatum* species complex were isolated together with the *C. gloeosporioides* species complex from anthracnose lesions on litchi fruits (cv. Nuomici) obtained from a litchi orchard in Shenzhen (N 22.36°, E 113.58°), China. Symptoms typically appeared as brown lesions up to 25 mm in diameter, causing total fruit rot and sometimes fruit cracking. Based on the number of isolates collected, the *C. acutatum* species complex appears less frequently on infected fruit compared with the *C. gloeosporioides* species complex. Because only the *C. gloeosporioides* species complex has been reported in China (Ann et al. 2004; Qi 2000), we focused on the *C. acutatum* species complex in this study. Pure cultures of fungal isolates were obtained by single-spore isolation. Isolate GBLZ10CO-001 was used for morphological characterization, molecular and phylogenetic analysis, and pathogenicity testing. Colonies cultured on potato dextrose agar (PDA) at 25°C for 7 days were circular, raised, cottony, gray or pale orange, with reverse carmine, and 39.6 to 44.7 mm in diameter. Conidia were 13.5 to 19 × 4 to 6 µm (mean ± SD = 15.9 ± 1.1 × 5.2 ± 0.3 µm, *n* = 50), hyaline, smooth-walled, aseptate, straight, and fusiform to cylindrical with both ends acute. Appressoria were 5.5 to 13.5 × 4.5 to 7.5 µm (mean ± SD = 7.6 ± 1.6 × 6.0 ± 0.7 µm, *n* = 50), subglobose to elliptical, sometimes clavate or irregular, smooth-walled,

with entire edge, sometimes undulate, and pale to medium brown. Morphological characteristics were consistent with the descriptions of several *Colletotrichum* species belonging to the *C. acutatum* species complex, including *C. fioriniae* (Damm et al. 2012; Shivas and Tan 2009). For molecular identification, genomic DNA was extracted, and the ribosomal internal transcribed spacer (ITS) and partial sequences of the β-tubulin (*TUB2*), actin (*ACT*), glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*), chitin synthase 1 (*CHS-1*), and histone3 (*HIS3*) genes were amplified and sequenced using the primer pairs ITS4/ITS5, T1/Bt2b, ACT512F/ACT783R, GDF1/GDR1, CHS-79F/CHS-354R, and CYLH3F/CYLH3R, respectively (Damm et al. 2012; White et al. 1990). The resulting sequences were submitted to GenBank (ITS, MN527186; *TUB2*, MT740310; *ACT*, MN532321; *GAPDH*, MN532427; *CHS-1*, MT740311; *HIS3*, MT740312). BLAST searches showed 98.70 to 100% identity to the sequences of the *C. fioriniae* ex-holotype culture CBS 128517. The phylogram reconstructed from the combined dataset using MrBayes 3.2.6 (Ronquist et al. 2012) showed that isolate GBLZ10CO-001 clustered with *C. fioriniae* with high posterior probability. Koch's postulates were performed in the field to confirm pathogenicity. Isolate GBLZ10CO-001 was grown on PDA (25°C for 7 days) to produce conidia. In June 2014, litchi fruits (cv. Nuomici) were sprayed with conidial suspensions (10⁶ conidia/ml), with sterile water as blank controls, and each treatment inoculated at least 15 fruits. Inoculated fruits were covered by an adhesive-bonded fabric bag until the trial ended. After 31 days, typical symptoms were observed, whereas control fruits remained asymptomatic. The fungus was reisolated from diseased fruits and identified as *C. fioriniae* according to the methods described above. To our knowledge, this is the first report of anthracnose fruit rot on litchi caused by *C. fioriniae*, one species of the *C. acutatum* species complex, in China. For the difficulty in distinguishing anthracnose caused by *C. fioriniae* from the *C. gloeosporioides* species complex just by the symptoms, and mixed infection usually occurring in the field, further investigations are required to reliably assess the potential threat posed by *C. fioriniae* for litchi production in China.

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