Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Penicillium digitatum* Causing Postharvest Rot of Citron Fruit in Kunming, China

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Citron (*Citrus medica* L.) is a perennial evergreen tree in the Rutaceae grown for economic, medicinal, and ornamental uses in south China (Yang et al. 2015). Fruits are spherical to ovate and 3 to 5 kg (Klein et al. 2016). In June 2021, some postharvest fruits (*Citrus medica* var. *medica*) had decay with a green or greyish mycelium on part or all of the surface in two markets in Kunming, Yunnan Province (25°02' N; 102°42' E), southwest China. Initial symptoms appeared as white or brown, irregular necrotic spots in the pericarp. Lesions gradually grew and developed into green, water-soaked areas that spread rapidly. Fruits became rotten and soft, with green spore masses confined to the surface. The incidence in postharvest fruits was 15 to 35%, which is extremely destructive to Rutaceae fruit (Chen et al. 2019). Pieces (5 mm²) of symptomatic citron fruits were surface disinfected in 75% ethanol and 0.3% NaClO for 30 s and 2 min, respectively, rinsed with distilled water three times, blotted dry, placed onto potato dextrose agar (PDA) medium aseptically, and incubated in a growth chamber at 25 ± 1°C. After 7 days, colonies were isolated and purified on new PDA at 25 ± 1°C for 7 days. By repeated inoculation, six single strains (XY01 to XY06) were obtained and stored in 15% glycerol at -80°C. The strains were inoculated on PDA and incubated at 25 ± 1°C. After 7 days, the obverse of colonies was olive green, with a white margin and grayish-green spores on the surface, and the reverse colorless to cream yellow or pale dull brown. The texture was velutinous, with a special fragrance. The conidia structure was very fragile and broke up easily into many cellular elements. Conidiophores were terverticillate, produced by subsurface or aerial hyphae, irregularly

branched and composed of short stipes with few metulae and branches that terminated in whorls of three to six phialides, which were often solitary, cylindrical, with a short neck. Conidia were hyaline to pale green, smooth walled, aseptate, partially ellipsoidal or obovate (4.9 to 11.9 × 4.3 to 8.9 µm) and partially cylindrical (8.2 to 10.5 × 2.7 to 5.3 µm). There were some small, ellipsoidal or spherical conidia (3.9 to 5.2 × 2.7 to 5.2 µm). Using morphological characteristics, the fungus was identified as *Penicillium digitatum* (Pers.) Sacc. Isolates XY01 and XY02 were used for molecular identification; genomic DNA was extracted using the CTAB method (Aboul-Maaty and Oraby 2019). The universal primers ITS1 and ITS4 were used to amplify and sequence the ITS1, 5.8S, and ITS2 rDNA regions. Using NCBI BLASTn, the sequences of XY01 and XY02 (GenBank nos. MZ976843 and OK513274) showed 100% identity to MK450692 (*P. digitatum* strain CMV010G4). Pathogenicity tests used fruits that were >80% mature. XY01 and XY02 were cultured for 7 days on PDA, washed with sterilized water, and the spore suspensions diluted to 1.0 × 10⁶ spores/ml. Wounds (0.5 × 0.5 cm) were scraped on the surface of fruits with a sterile scalpel and treated with 200 µl of spore suspension (Wild 1994). Control fruits were treated with sterile water. Fruits were incubated at 24 to 26°C. Each treatment was done in triplicate with six fruits. After 3 days, all inoculated fruits had water-stained, pale brown lesions with rapidly formed white hyphae, and a 1.5 to 2.5 × 1 to 2 cm white mold layer. After 7 days, decay developed more quickly, the hyphae rapidly expanded on the pericarp surface, with vague, irregular edges. Then, a green mold layer formed and the whole fruit rotted and softened. When the fruit was cut, the white flesh inside turned black and rotted. Controls had no symptoms. *P. digitatum* was consistently reisolated from the inoculated plants but not the controls. According to Koch's postulates, XY01 and XY02 caused citron decay disease. Based on symptoms, morphological characteristics, rDNA-ITS sequence analysis, and pathogenicity, the fungus was identified as *P. digitatum*. To our knowledge, this is the first report of *P. digitatum* on citron in China.

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