Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Alternaria alternata* Causing Fruit Rot on *Tetradium ruticarpum* in China

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Tetradium ruticarpum, previously and commonly known as *Evodia rutaecarpa*, is a tree that produces a fruit that is one of the most important traditional Chinese medicine herbs in China (Zhao et al. 2015). In July 2019, an investigation of diseases of *T. ruticarpum* was conducted in the farmland of Ruichang County (29.68° N, 115.65° E), Jiujiang City, China. An unknown fruit rot disease was observed, and the incidence rate was estimated to be 60 to 70% within a 5,000-m² area. The early symptoms appeared as small circular to irregular dark brown or black spots on the fruit, which gradually coalesced to a light brown to black discoloration and caused fruit rot. To identify the causal agent of the disease, 10 diseased fruits were collected and surface disinfected with 2% sodium hypochlorite for 2 min followed by 70% ethanol for 30 s, rinsed in sterile water, and dried on filter paper. Tissues from nonsymptomatic tissue as well as from the margin between healthy and affected edge were incubated on potato dextrose agar (PDA) at 25 ± 1°C (12 h light/dark) with 90% relative humidity for 5 days. The colonies were brown to black with abundant whitish margins. Conidiophores were brown and measured 20.40 to 43.10 × 1.30 to 4.20 µm (25.47 × 2.35 µm on average, *n* = 50). Conidia, produced in single or branched chains, were obclavate or ovoid, approximately 9.90 to 32.80 × 6.50 to 14.50 µm (28.75 × 12.57 µm on average, *n* = 50) with two to five transverse septa and zero to three longitudinal septa. The colonies were

consistent with *Alternaria alternata* (Simmons 2007). For molecular identification, the partial internal transcribed spacer (ITS) regions, glyceraldehyde-3-phosphate dehydrogenase (gapdh) gene, translation elongation factor 1- α (TEF), and *Alternaria* major allergen (Alt a1) gene of the isolate were amplified using primers ITS1/ITS4 (White et al. 1990), GDF/GDR (Templeton et al. 1992), EF1-728F/EF1-986R (Carbone and Kohn 1999), and Alt-for/Alt-rev (Hong et al. 2005). Sequence data showed 100% homology to *A. alternata* (GenBank accessions MN625176.1 [570/570 bp], MK683866.1 [618/618 bp], MK637432.1 [281/281 bp], KT315515.1 [488/488 bp]), respectively, and the sequence data were deposited into GenBank with accession numbers MN897753 (ITS), MT041998 (gapdh), MT041999 (TEF), and MT042000 (Alt a1). Based on both morphological and molecular characteristics, the pathogen was identified as *A. alternata*. To confirm pathogenicity, 10 µl of a spore suspension (1.0×10^6 conidia/ml) obtained from 5-day-old PDA cultures of the strain were inoculated on 20 wounded (using a sterile needle) and 20 nonwounded healthy *T. ruticarpum* fruits previously disinfected in 75% ethanol. Control fruits including 20 wounded fruits and 20 nonwounded fruits were inoculated with sterilized water. All fruits were incubated at 25 ± 1°C (12 h light/dark) with 90% relative humidity. Four days later, all the wounded and nonwounded fruits showed the initial symptoms of black rot, which were similar to those observed in the field, whereas the wounded and nonwounded fruits treated with sterile water remained healthy. The same pathogen was again isolated from the inoculated fruits. The pathogenicity experiment was repeated three times with the same results. As far as we know, this is the first report of *A. alternata* causing fruit rot on *T. ruticarpum* in China, and the identification of the pathogen will provide useful information for developing effective control strategies.

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