Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Lasiodiplodia theobromae* Causing Dieback in Custard Apple (*Annona squamosa*) Tree in China

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Custard apple (*Annona squamosa* L.) is famous for its sweet taste and rich aroma. Hainan Province is the major production area of custard apple in China. In September 2020, wilting of leaves and branches, discoloration of the vascular system and dieback of the plant were observed in Lingao County of Hainan Province, China. This new disease significantly decreased the yield and quality of custard apple. The disease incidence in three orchards was about 19%, and the diseased samples were brought to the laboratory. Fragments of approximately 5 mm in length were obtained from five diseased branches, which were collected from different plants and orchards. Fragment surfaces were sterilized in 75% ethanol for 1 min and 1% mercury chloride for 1 min and then rinsed three times with sterile distilled water. These tissues were placed on potato dextrose agar (PDA) amended with streptomycin and incubated at 28°C for 3 days. Fungal colonies were transferred to fresh PDA plates, and single-spore cultures were obtained. We isolated 26 fungal strains, of which 23 isolates were morphologically identified as *Lasiodiplodia* species (Phillips et al. 2013). The colony morphology was initially round and white, turned to gray or black after 5 to 7 days at 28°C, and formed pycnidia for 20 days. The immature conidia were ellipsoid, colorless, hyaline, and unicellular, becoming brown, bicellular, with longitudinal striations at maturity. Mature conidial size was $26.61 \pm 1.57 \times 14.87 \pm 1.14$ μm ($n = 60$). For molecular identification, genomic DNA of three isolates (HSYF01, HSYF02, and HSYF03) was extracted using an E.Z.N.A. HP Plant DNA kit (Omega Bio-Tek). The internal transcribed spacer of rDNA (ITS), translation elongation factor 1- α (EF1- α), and β -tubulin (TUB) regions were amplified using

the primers ITS1/4, EF1-728F/986R, and Bt2a/Bt2b, respectively (Carbone and Kohn 1999; Glass and Donaldson 1995; White et al. 1990). A BLAST search of ITS, EF1- α , and TUB gene sequences (accession nos. MW625913 to MW625918, MW876481 to MW876483) resulted in 99.8% (493 out of 494 bp), 99.31% (286 out of 288 bp), and 100% (372 out of 372 bp) identity to CBS164.96 of *Lasiodiplodia theobromae* (accession nos. AY640255, AY640258, and KU887532). The identity of the isolate was further confirmed by phylogenetic analysis. Ten healthy custard apples 1 to 2 years old were used for pathogenicity testing. Three 1- to 2-year-old custard apple plants were wounded approximately 15 to 20 cm below the tips with a sterilized scalpel. Each cut was inoculated with a 5-mm agar plug with mycelium. The wound site was moisturized with wet cotton wool and wrapped with laboratory film (Silveira et al. 2018). Two seedlings treated with sterile agar plugs served as a control. The test was repeated twice. All inoculated seedlings showed characteristic discoloration and necrotic lesions starting from the apical branches, after 14 days of incubation in a glasshouse. The stems exhibited browning and vascular streaking of the wood from the inoculation point, whereas the control seedlings remained symptomless. Typical colonies of *L. theobromae* were isolated and identified from all inoculated seedlings, fulfilling Koch's postulates. Although postharvest fruit stem-end rot on custard apple caused by *L. theobromae* was previously described (Hu et al. 2003; Meng et al. 2017), this is the first report of *L. theobromae* causing dieback in mature custard apple trees in China.

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