Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Fusarium incarnatum-equiseti* Species Complex Associated with Stalk Rot Disease of *Eucommia ulmoides* in China

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Eucommia ulmoides Oliver is an important economic tree species in China, mainly distributed in the southwestern mountainous areas. It is used in ecological, medical, agricultural, and manufacturing industries (Li et al. 2016). Its wood is used in furniture and its bark in medicine (Yahara et al. 1990). Stalk rot (SR) is a harmful disease of many plants, notably maize. Infected stalks become hollow tubes; rotted, weakened stalks are prone to lodging; and SR diseases reduce yield both directly and indirectly (Stack 1999). *Fusarium*, *Pseudomonas lapsa*, *Erwinia carotovora*, and other pathogens cause SR (Cristina et al. 2013; Rangarajan and Chakravarti 1970). In July 2017, SR was found on 1-year-old *E. ulmoides* in a nursery in Yutang Town, Dujiangyan City, Sichuan Province, China. After 2 weeks, the stalk base was brown, and the leaves were chlorotic, withered, and wilted. After 4 weeks, the stalk cortex atrophied, the endothelial tissue of the diseased area became spongy, and the xylem and pith of severely damaged seedlings turned brown. Edges of the diseased tissues were collected from stalk cortex by dissecting with a scalpel; 100 5-mm pieces of necrotic tissue were surface decontaminated for 60 s in 3% NaOCl and 60 s in 75% ethanol, rinsed three times in sterile water, and placed onto PDA (Solarbio, Beijing) amended with streptomycin sulfate (Solarbio, 50 µg/ml) to isolate the pathogenic fungi. A total of 26 isolates were obtained, including 20 morphologically similar isolates. Single-spore cultures from the colony produced aerial hyphae and yellow spore powder after 7 days of growth on Spezieller Nährstoffarmer agar (Nirenberg 1976) at 25°C under a 12-h day/night cycle. Microconidia were single-celled, hyaline, non-septate, ovoid, and 8.5 to 11.3 × 1.6 to 5.2 µm, and the numerous macroconidia were long and slender. Macroconidia were sickle-shaped, tapering at both ends, with three to five septa and 21.95 to 37.84 × 2.99 to 3.29 µm (Leica Application Suite X 3.4.1.17822). The 20 isolates were identified morphologically as *Fusarium incarnatum-equiseti* species complex (FIESC) (Leslie and Summerell 2006). For molecular identification, a

HiPure Fungal DNA Mini Kit (Magen Biotech, China) was used to extract DNA from a representative isolate (FI01). PCR was performed with primers ITS1/ITS4 and ITS4/ITS5 for the ITS region (White et al. 1990), RPB2AM-1bf/RPB2AM-7R for RPB2 genes (Miller and Huhndorf 2005), and EF1-728F/EF1-986R for EF-1α (Carbone and Kohn 1999). Using a BLASTn search of the ITS, EF-1α, and RPB2 sequences in GenBank and *FUSARIUM-ID*, all showed the highest similarity to members of the FIESC: ITS1/4 showed 99.06% similarity to KY496923 and 99.8% to FD.0162; ITS4/5 showed 100% similarity to MT012109 and FD_01695; EF-1α had 99.55% similarity to MN961601 and 100% to FD_01664; RPB2 had 99.70% similarity to MN657400 and 99.11% to FD_08556. GenBank numbers for FI01 were ITS1/4, MN317335; ITS4/5, MN317336; TEF, MN328057; and RPB2, MN383188. The internode cortexes of ten 1-year-old potted *E. ulmoides* plants were inoculated by injecting 100 µl of conidial suspension (1×10^5 conidia/ml) into the stalk with polypropylene syringes. Sixty 1-year-old potted *E. ulmoides* plants were inoculated with the six other isolated fungi (10 for each) that were different from the 20 morphologically similar isolates, and ten 1-year-old potted *E. ulmoides* plants inoculated with sterile water were controls. The inoculation site was wrapped with wet gauze to maintain moisture. All plants were maintained in the greenhouse (20 to 25°C, 80% RH). One month later, FIESC-inoculated plants showed the same symptoms as originally observed. Plants inoculated with other fungi or sterile water were asymptomatic. FIESC was reisolated from stalk cortexes of diseased *E. ulmoides* and identified by morphological characteristics and DNA sequence analysis. The pathogenicity test was repeated three times with similar results, confirming Koch's postulates. This is the first report of FIESC as an SR disease on *E. ulmoides* in China.

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