Diseases Caused by Fungi and Fungus-Like Organisms

Stem Rot Disease of *Juglans sigillata* Caused by *Fusarium fujikuroi* in China

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Juglans sigillata Dode is an endemic species in the southwest China and is an important nut and woody oil tree. The shell of its fruit is hard and can be used to make various crafts. From 2016 to 2019, typical stem rot symptoms of 8-year-old *J. sigillata* were observed in cultivated fields in a 600-ha orchard in Zigong, Sichuan province, China. At this orchard, approximately 35% of the trees have been seriously damaged over the past few years. The typical symptoms were water-soaking on the stem, rotting, wilting, and blighting, eventually leading to the death of the plant. In June, 10 diseased tissues were collected and surface sterilized by 3% NaClO and 75% alcohol. Morphological observations were made from the isolates grown on potato dextrose agar (PDA) and incubated at 25°C for 3 to 9 days. Morphological characteristics were determined on pure cultures grown on synthetic low nutrient agar (SNA). Five isolates with similar morphology were isolated from single spores. Colonies on PDA reached 8.3 cm in diameter after 6 days at 25°C; aerial mycelia were white to cream and wool-like, later turning violet and dark purple with age. The hyphae of the strain were colorless and septate. There were two types of conidia on SNA: microconidia and macroconidia. Microconidia ($n = 50$) were oval, elliptic or clavate, zero septate, and 2.2 to 3.8×7.6 to $11.7 \mu\text{m}$. Conidiophores were branched or unbranched, solitary or in groups, phialides cylindrical to flask-shaped,

monophialidic and polyphialidic. Macroconidia ($n = 50$) were long and slender with a curved apical cell and foot-like basal cell, three to four septate, and 2.1 to 3.9×26.2 to $53.4 \mu\text{m}$. For molecular identification, the internal transcribed spacer (ITS), β -tubulin (TUB2), translation elongation factor (TEF1), and large subunit (LSU) were amplified with the corresponding primer pairs ITS1/ITS4 (White et al. 1990), BT2A/BT2B, EF1/EF2 (O'Donnell et al. 1997), and LROR/LR5 (Rehner and Samuels 1994), respectively. BLAST search results indicated that the ITS, TUB2, TEF1, and LSU sequences (GenBank nos. MT791384, MT786729, MN853324, and MT705246) showed 99 to 100% identity with *Fusarium fujikuroi* sequences at NCBI (GenBank nos. MG798789, MH398245, MK604519, and KJ954504). The results were confirmed by multilocus phylogenetic analysis. Based on the morphological characteristics and molecular analysis of the isolates, the fungus was identified as *F. fujikuroi* (Leslie and Summerell 2006). Koch's postulates were checked under controlled conditions. Fifteen 2-year-old healthy potted *J. sigillata* were inoculated by pricking the epidermis of the stem with a needle and applying 150 μl of a microconidial suspension (1×10^6 spores/ml) to the wounded surface with a brush. Sterilized distilled water was used as the control. The experiment was repeated three times. All the plants were incubated at $25 \pm 2^\circ\text{C}$ after inoculation for daily observation of disease development. After 12 days, the inoculated plants showed the same symptoms as observed in the original diseased plants, whereas the control plants were asymptomatic. The fungus was reisolated from the symptomatic stems and was completely identical to the isolates used to inoculate the plants. Thus, we confirmed that *F. fujikuroi* caused the stem rot of *J. sigillata*. To our knowledge, this is the first report of this fungus causing stem rot in *J. sigillata* in China. Our results can help identify stem rot disease of *J. sigillata* and develop control measures for the disease.

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