Diseases Caused by Fungi and Fungus-Like Organisms

Round Spot Disease of *Zanthoxylum armatum* Caused by *Fusarium fujikuroi* in China

Y. R. Jiang,¹ J. M. Yi,¹ T. H. Zhu,¹ S. J. Li,¹ T. M. Qiao,¹ Y. G. Liu,¹ C. L. Yang,¹ T. T. Lin,¹ Y. L. Wang,¹ T. Li,² J. Li,² N. Wu,² and S. Han^{1,†}

¹ College of Forestry, Sichuan Agricultural University, Chengdu, Sichuan 61130, China

² Station of Forest Pest Control and Quarantine, Sichuan Forestry and Grassland Bureau, Chengdu, Sichuan 61000, China

Y. R. Jiang and J. M. Yi contributed equally.

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Zanthoxylum armatum DC, a deciduous tree in Rutaceae, has significant economic value as an important food condiment, spice, and medicine (Cao et al. 2019). Recently, an unknown round leaf spot disease was found on *Z. armatum* in Meishan and surrounding areas of Sichuan province. The disease mainly affected the leaves, mostly on seedlings, with incidence of approximately 50%. Isolate HJYB-4 was isolated from typical diseased leaves and purified on potato dextrose agar (PDA). The isolate produced floccose white, magenta, or gray aerial mycelium. On the reverse side of the culture, the colony had the pigment of pale gray or magenta, with concentric rings of dark red and pale brown in the center. Morphological characteristics were recorded using a pure culture grown on PDA and synthetic low nutrient agar (SNA). The hyphae of the isolate were colorless and septate. There were two types of conidia on SNA: microconidia and macroconidia. Macroconidia were long and slender with parallel dorsoventral sides, usually three to five septate, and 21.89 to 47.21×3.33 to 4.73 μm . Microconidia were oval, melon-shaped, ovate, zero to one septate, mostly no septate, and 5.35 to 11.22×2.24 to 3.79 μm . A few pyriform microconidia and chlamydospores were observed. DNA of the isolate was extracted using the Column Fungal DNAout 2.0 (Tiandz, Beijing, China). PCR was performed using primers ITS1/ITS4, TEF1/TEF2, TUBT1/TUBT2, fRPB2-5f2/fRPB2-7cr, LR0R/LR5, and NL1/NL4 to amplify the loci

of the representative isolate in the ribosomal internal transcribed spacers (ITS), translation elongation factor EF-1 α (TEF1), β -tubulin 2 (TUB2), RNA polymerase II largest subunit (RPB2), the large subunit (LSU) region of rDNA, and the 26S rDNA D1/D2 domain (NL). The products were sequenced and blasted. BLAST analysis of the ITS, TEF1, TUB2, RPB2, LSU, and NL amplicons revealed more than 99% sequence identity with *Fusarium fujikuroi*. These sequences were submitted to GenBank, and the accession numbers were as follows: MT864359 (ITS), MT864358 (LSU), MT877222 (NL), MT902141 (RPB2), MT902140 (TEF1), and MT902139 (TUB2). The phylogenetic tree was inferred from the combined datasets (TEF1, TUB, and RPB2) from members of the *F. fujikuroi* species complex analyzed in this study (Jayawardena et al. 2019). The phylogenetic tree revealed isolate HJYB-4 matched *F. fujikuroi* with a clade credibility value of 100%. According to the morphological characteristics and multigene phylogenetic analysis, the isolate was identified as *F. fujikuroi*. To complete pathogenicity tests, healthy leaves were needle-wound inoculated with mycelial plugs. The leaves that were inoculated with PDA-only plugs served as the control. After 3 days incubation at $26 \pm 2^\circ\text{C}$ and 100% relative humidity, brown lesions that developed from inoculated leaves were similar to those in the field. No symptoms developed in the controlled leaves. Typical fungal cultures were consistently isolated from symptomatic leaves, indicating that the fungus was responsible for the development of the disease. *F. fujikuroi* has been reported to cause root rot on *Reineckia carnea* (Sun et al. 2018), black rot on *Macleaya cordata* (Yu et al. 2019), and a wilt disease on sugarcane (Bao et al. 2020). This is the first report of *F. fujikuroi* attacking leaves of *Z. armatum* in China. The identification of this disease could provide the basis for the prevention and control of the disease at the seedling stage of *Z. armatum*.

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[†]Indicates the corresponding author.
S. Han; hanshan6618@163.com