Diseases Caused by Fungi and Fungus-Like Organisms

First Report of Leaf Blotch of *Salix babylonica* Caused by *Botryosphaeria dothidea* in China

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Salix babylonica L. (weeping willow) is an important ornamental tree commonly planted in China. Since 2018, a new disease with a high incidence has been observed on *S. babylonica* at the campus of Nanjing Forestry University (NFU), Nanjing, Jiangsu, China. The symptoms began as small dark brown lesions formed along the leaf margins and tips, and they later became gray to brown in the center with dark brown borders. Small samples (3 to 4 mm²) from the lesion margins were surface sterilized with 75% ethanol for 30 s and 1% NaClO for 90 s. Subsequently, samples were rinsed with sterile H₂O, plated on potato dextrose agar (PDA), and incubated at 25°C. The same fungus was isolated in 95% of the samples. Pure cultures were obtained by monospore isolation. A representative isolate, NFS1, was used for morphological and molecular characterization and deposited in China's Forestry Culture Collection Center (CFCC 54212). On PDA, colonies were initially white and gradually became grayish-green to dark gray from the center to the edge. After 1 week, colonies turned dark, and after 3 weeks black pycnidia developed on the surface of medium. Conidia were one-celled, hyaline, smooth, and fusoid to ellipsoid. Conidia measurements were $23.0 \pm 1.9 \times 5.8 \pm 0.7 \mu\text{m}$ ($n = 50$). The morphology matched the description of *Botryosphaeria dothidea* (Slippers et al. 2004). For an accurate identification, genomic DNA of NFS1 was extracted to amplify the internal transcribed spacer (ITS) region, the transcription elongation factor (*tefa*-1), beta-tubulin (*β-tub*), the large subunit (*LSU*), and small subunit (*SSU*) genes with the specific primers

ITS1/ITS4 (White et al. 1990), EF1-728F/EF1-986R (Carbone and Kohn 1999), *β*t2a/*β*t2b (Glass and Donaldson 1995), LR0R/LR05 (Schoch et al. 2009), and NS1/NS4 (White et al. 1990), respectively. The sequences were deposited in GenBank (accession nos. MN826233 for ITS, MN855215 for *tefa*-1, MN855216 for *β-tub*, MN886965 for *LSU*, and MN886966 for *SSU*). A BLAST search of GenBank showed that the ITS, *tefa*-1, *β-tub*, *LSU*, and *SSU* sequences of NFS1 were similar to those of *B. dothidea* KY788304 (identity = 527/532; 99%), MG459974 (identity = 247/247; 100%), MH724212 (identity = 404/404; 100%), DQ377850 (identity = 865/867; 99%), and KX091154 (identity = 1,043/1,045; 99%), respectively. A maximum-likelihood and Bayesian posterior probability-based phylogenetic analyses using IQ-TREE version 1.6.8 and MrBayes version 3.2.6 with the concatenated sequences (ITS, *tefa*-1, *β-tub*, *LSU*, and *SSU*) placed NFS1 in the clade of *B. dothidea*. Based on the multigene phylogeny and morphology, NFS1 isolate was identified as *B. dothidea*. To fulfill Koch's postulates, 20 detached and 20 attached healthy 10-week-old leaves from three 30-year-old *S. babylonica* plants at the campus of NFU were inoculated with 5-mm mycelial plugs of isolate NFS1 of 3-day-old cultures grown on PDA. Control leaves were treated with agar plugs. The detached inoculated leaves were placed in Petri dishes on a piece of wet filter paper and incubated at 25°C. The attached leaves were enclosed in a plastic bag along with the branches with a wet cotton ball inside. Sterile H₂O was sprayed into the plastic bags twice daily to keep moisture conditions and incubated for 5 days. The experiment was repeated two times. Within 5 days, all the inoculated points showed lesions similar to those observed in the field, whereas controls were asymptomatic. The same fungus was reisolated from these lesions with a frequency of 100%. *B. dothidea* has been reported to infect a broad range of hosts, including *S. babylonica* in the United States (Grand 1985). This is the first report of *B. dothidea* on *S. babylonica* in China. This finding provides crucial information on this high-risk disease to willow and basis for identifying management strategies.

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