Diseases Caused by Fungi and Fungus-Like Organisms

First Report of Stem Dieback Caused by *Lasiodiplodia parva* on *Styphnolobium japonicum* in China

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Styphnolobium japonicum (L.) Schott is a variant of *Robinia pseudoacacia* and is a popular Asian tree widely used in traditional medicine. From March 2019 to 2021, a disease was found on the campus of Nanjing Forestry University and several landscape sites of Xuanwuhu Park, causing dieback. Approximately 40% of the trees had rotted branches. On average, 60% of the branches per individual tree were affected. The initial round lesions were grayish brown. In the later stage, the whole branch became black and produced spherical fruiting bodies. Twenty diseased branches were picked from three random trees. Small tissues (3 to 4 mm²) were surface sterilized in 75% ethanol for 30 s followed by 1% NaClO for 90 s, placed on PDA, and incubated in the dark at 25°C for 3 days. Hyphae emerged from 70% of the samples. Three representative isolates (Lth-soj1 to 3) were obtained and deposited in the China Forestry Culture Collection Center (Lth-soj1: cfcc55896; Lth-soj2: cfcc55897; and Lth-soj3: cfcc55898). The colonies of the three isolates on PDA were fast growing and white, then turned gray to dark gray after 3 days of incubation in the dark at 28°C. Two-week-old colonies were black and fluffy on PDA, with abundant aerial mycelium, and the reverse side of the mycelium was also black. The fungus usually grew well on PDA and produced pycnidia and conidia within 3 to 4 weeks. Conidia were initially hyaline and aseptate, ellipsoid to ovoid, with granular content, apex broadly rounded, remaining hyaline and later becoming dark brown, one septate, thick walled, base truncate or round, and longitudinally striate. The conidia ($n = 90$) of three representative isolates measured $24.3 \pm 0.3 \times 13.3 \pm 0.5 \mu\text{m}$. Morphological characteristics of the three isolates matched those of *Lasiodiplodia parva*

(Alves et al. 2008). For accurate identification, the DNA of the three isolates was extracted. The ITS region, *EF1- α* , and *TUB2* genes were amplified using the primer pairs ITS1/ITS4 (White et al. 1990), EF1-728F/EF1-986R (Slippers et al. 2004), and Bt2a/Bt2b (Glass and Donaldson 1995), respectively. The sequences were deposited in GenBank under MZ613154, MZ643245, and MZ643242 for Lth-soj1; MZ613155, MZ643246, and MZ643244 for Lth-soj2; and MZ613157, MZ643247, and MZ643243 for Lth-soj3. The ITS, *EF1- α* , and *TUB2* sequences of isolate Lth-soj1 (MZ613154, MZ643245, and MZ643242) were 100% (519 of 519 nt), 99.34% (299 of 301 nt), and 99.77% (436 of 437 nt) identical to those of MZ182360, EF622063, and MK294119, respectively. Interspecific differences were observed in a maximum-likelihood tree of *Lasiodiplodia* spp. using the concatenated dataset (ITS, *EF1- α* , and *TUB2*). Based on the morphological and molecular evidence, the isolates were identified as *L. parva* (Adesemoye et al. 2014). The pathogenicity of three isolates were tested on potted 3-year-old seedlings (100 cm tall) of *S. japonicum* maintained in a greenhouse. Healthy stems were wounded with a sterile needle, then inoculated with 10 μl of conidial suspension (2×10^5 conidia/ml). Control plants were treated with double-distilled H₂O. In total, 12 seedlings were inoculated, including three controls. Three seedlings per isolate and 10 stems per seedling were used for each treatment. The plants were kept inside sealed polythene bags for the first 24 h and sterilized H₂O was sprayed into the bags twice a day to maintain humidity; then, plants were kept in a greenhouse at the day and night temperatures at 25 and 16°C. Within 7 days, all the inoculated points showed lesions similar to those observed in the field and the conidiomata growing on the surface of the branches, whereas controls were asymptomatic. The infection rate of each of the three isolates was 100%. The strain was reisolated from the lesions (whereas not from control stems) and sequenced as *L. parva*. This is the first report of *L. parva* causing rotten branches of *S. japonicum* worldwide. These data will help to develop effective strategies for managing this newly emerging disease.

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