Diseases Caused by Fungi and Fungus-Like Organisms

First Report of Leaf Spot Caused by *Colletotrichum siamense* on *Salix matsudana* in China

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Salix matsudana Koidz. (Chinese willow) is an important landscaping tree widely grown in China (Zhang et al. 2017). In October 2019, a characteristic leaf spot disease of *S. matsudana* was found at Nanjing Forestry University. Most 25-year-old *S. matsudana* trees (13 out of 21, approximately 62%) on campus showed the leaf spot disease. On average, 70% of the leaves per individual tree were affected by this disease. Foliar symptoms began as dark brown, irregular spots with gray-white centers, gradually enlarging with time. Leaf spot symptomatic leaves were collected from three infected *S. matsudana* trees (10 leaves/tree), and small infected tissues (3 to 4 mm²) were surface-sterilized in 75% ethanol for 30 s, 1% NaClO for 90 s, rinsed in ddH₂O, dried on sterilized filter paper, plated on potato dextrose agar (PDA), and then incubated at 25°C. Three isolates (NH1-1, NH1-2, and NH1-3) of the same fungus were obtained in 85% of the samples and deposited in China's Forestry Culture Collection Center (NH1-1: cfcc55354, NH1-2: cfcc55355, NH1-3: cfcc55359). The colonies of three isolates were white, but the reverse side was grayish white. The conidia of NH1-1 were one-celled, straight, subcylindrical, hyaline, $14.4 \pm 0.9 \times 5.4 \pm 0.4 \mu\text{m}$ ($n = 50$), with a rounded end. Conidiophores were hyaline to pale brown, septate, and branched. Appressoria were one-celled, ellipsoidal, brown or dark brown, thick-walled, and $8.0 \pm 0.9 \times 5.9 \pm 0.5 \mu\text{m}$ ($n = 50$). The conidia and appressoria of the other two isolates were almost identical to NH1-1. The morphological characters of the three isolates were matched with those of the *Colletotrichum gloeosporioides* complex (Weir et al. 2012). For accurate identification, the DNA of the three isolates was extracted. The internal transcribed spacer region (ITS), actin (*ACT*), calmodulin (*CAL*), chitin synthase

(*CHS-1*), glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*), superoxide dismutase (*SOD2*), and β -tubulin 2 (*TUB2*) genes were amplified using the primer pairs ITS1/ITS4, ACT-512F/ACT-783R, CL1C/CL2C, CHS-79F/CHS-345R, GDF1/GDR1, SODglo2-F/SODglo2-R, and Bt2a/Bt2b, respectively (Weir et al. 2012). The sequences were deposited in GenBank (accession nos. MW784679 and MW808959–MW808964 for NH1-1; MW784726 and MW808965–MW808970 for NH1-2; MW784729 and MW808971–MW808976 for NH1-3). A BLAST search showed that ITS, *ACT*, *CAL*, *GAPDH*, *SOD2*, and *TUB2* sequences of the three isolates were identical to *C. siamense* at a high level (>99%), and *CHS-1* sequences of three isolates were consistent with *C. fructicola* at a high level (>99%). Maximum likelihood and Bayesian posterior probability analyses using IQtree v. 1.6.8 and Mr. Bayes v. 3.2.6 with the concatenated sequences (ITS, *ACT*, *CAL*, *CHS-1*, *GAPDH*, *SOD2*, and *TUB2*) placed NH1-1, NH1-2, and NH1-3 in the clade of *C. siamense* with high bootstrap support values (ML/BI = 93/1). The pathogenicity of three isolates were tested on potted 2-year-old seedlings (50-cm tall) of *S. matsudana*, which were grown in a greenhouse. Healthy leaves were wounded with a sterile needle and then inoculated with 10 μl of conidial suspension (10^6 conidia ml⁻¹). Controls were treated with ddH₂O (Zhu et al. 2019). In total, 12 seedlings were inoculated including controls. Three seedlings/isolate and 10 leaves/seedling were used for each treatment. The plants were covered with plastic bags after inoculation and sterilized H₂O was sprayed into the bags twice per day to maintain humidity and kept in a greenhouse at day/night temperatures of $25 \pm 2^\circ\text{C}$ and $16 \pm 2^\circ\text{C}$. Within 7 days, all the inoculated points showed lesions similar to those observed in field, whereas controls were asymptomatic. The infection rate of each of the three isolates was 100%. *C. siamense* was reisolated from the lesions, whereas no fungus was isolated from control leaves. The diseases caused by *C. siamense* often occur in tropical and subtropical regions of China, with a wide range of hosts, such as *Hevea brasiliensis* and *Coffea arabica* (Cao et al. 2019; Liu et al. 2018). This is the first report of *C. siamense* causing leaf spot of *S. matsudana* in China and the world. These data will help to develop effective strategies for managing this newly emerging disease.

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