Diseases Caused by Fungi and Fungus-Like Organisms

Leaf Spot Disease Caused by *Pestalotiopsis kenyana* on *Zanthoxylum schinifolium* in Sichuan Province, China

Chang Liu,¹ Fengying Luo,¹ Tianhui Zhu,¹ Shan Han,¹ and Shujiang Li^{1,2,*}

¹ College of Forestry, Sichuan Agricultural University, Chengdu, Sichuan Province, 611130, Sichuan, P.R. China

² National Forestry and Grassland Administration Key Laboratory of Forest Resources Conservation and Ecological Safety on the Upper Reaches of the Yangtze River, College of Forestry, Sichuan Agricultural University, Chengdu, Sichuan Province, 611130, Sichuan, P.R. China

F. Y. Luo and C. Liu contributed equally to this work.

Funding: Funding was provided by General Program of Science and Technology Department of Sichuan (2019YFN0156) and National Natural Science Foundation of China (31700568). Plant Dis. 105:3747, 2021; published online as <https://doi.org/10.1094/PDIS-10-20-2247-PDN>. Accepted for publication 20 May 2021.

Zanthoxylum schinifolium Sieb. et Zucc. a species of prickly ash, is a main economic plant in China and mainly grown in southwest China. The planting area of *Z. schinifolium* accounts for >70% of the total area of prickly ash, and one of the largest plantings of *Z. schinifolium* is in Jianyang City (Sichuan) with an area of 6.67 km². Since 2018, *Z. schinifolium* in Jianyang City have developed leaf spot disease, with ~50% showing disease symptoms. At the beginning of the occurrence, yellow-brown lesions formed on the leaves; in the later stages, the area of the lesions expanded. At the severe stage, multiple lesions merged into one large, dead spot, and the plants failed to blossom and bear fruit. The samples were collected from typical symptomatic *Z. schinifolium* leaves in Jianyang City (30°16'26"N, 104°26'30"E). A total of 20 leaf samples were collected from five *Z. schinifolium* plants (four leaves per plant) and were cut into 2 × 2-mm pieces at the junction of infected and healthy tissues. These tissues were surface disinfested for 30 s in 3% NaOCl and for 60 s in 75% ethanol, rinsed three times in sterile water, placed onto PDA (Solarbio, Beijing) amended with streptomycin sulfate (50 µg/ml), and incubated in a dark incubator at 25°C. Morphological observation was performed on 18 recovered isolates, 15 of which were described as *Pestalotiopsis* sp. (Keith et al. 2006). Colonies were incubated on PDA at 25°C for 7 days and reached a diameter of 80 to 90 mm. Colonies were white with undulate edges and were similar in color on the reverse side. After colony culture at 25°C for 10 days, gregarious black conidiomata were scattered on the mycelial mats. The conidia and appendages of the samples were measured by Leica Application Suite X 3.4.1.17822 (20 conidia per isolate), and the sizes were consistent with the description from Maharachchikumbura et al.

(2014). Based on morphological observations, the isolates were identified as *Pestalotiopsis kenyana* Maharachch., K.D. Hyde & Crous. PCR was performed with primers ITS1/ITS4 for the ITS region (White et al. 1990), D1/D2 for the LSU, 5f2/7cr for the RPB2 (Liu et al. 1999), Bt2a/Bt2b for the TUB (Glass and Donaldson 1995), and EF1-526F/EF2-567R for the TEF (Rehner 2001). The Sanger-sequenced PCR products were blasted in GenBank, and the sequences showed high identities (ITS, 99.17% [594 of 599 bp]; LSU, 100% [909 of 909 bp]; RPB2, 99.17% [832 of 832 bp]; TUB, 100% [774 of 774 bp]; TEF, 100% [485 of 485 bp]) with the type specimen of *P. kenyana* CBS 442.67 (ITS, NR147549.1; LSU, MH870724.1; RPB2, MH554958.1; TUB, KM199395.1; TEF, KM199502.1). Representative sequences were deposited in GenBank (ITS, MT509798; LSU, MT509800; RPB2, MT522448; TUB, MT522450; TEF, MT522449). To fulfill Koch's postulates, leaves on 15 healthy 1-year-old potted *Z. schinifolium* plants were sterilized by 75% ethanol cotton balls and were rinsed by sterile water three times. Then each leaf was punctured with sterile needles for two wounds (five leaves per plant). The wounds were inoculated by placing 8-mm mycelial plugs obtained from the periphery of 7-day-old single-spore cultures. An equal number of plants were wounded with the same method and were respectively inoculated with sterile water and PDA plugs without mycelium as controls. All plants were placed in a growth chamber at 25°C under 90% relative humidity. After 7 days, all mycelial-inoculated leaves showed symptoms identical to those described above, whereas control plants remained symptom free. *P. kenyana* was reisolated from infected leaves and confirmed to be the same as the inoculated pathogen through analyses of morphological characteristics and molecular techniques. The pathogenicity test was repeated three times with similar results. To our knowledge, this is the first report of *P. kenyana* as a causal agent of leaf spot disease on *Z. schinifolium* in China. These findings will aid the development of better preventive measures in accordance with the emergence of this new pathogen.

References:

- Glass, N. L., and Donaldson, G. C. 1995. Appl. Environ. Microbiol. 61:1323.
- Keith, L. M., et al. 2006. Plant Dis. 90:16.
- Liu, Y. J., et al. 1999. Mol. Biol. Evol. 16:1799.
- Maharachchikumbura, S. S. N., et al. 2014. Stud. Mycol. 79:121.
- Rehner, S. A. 2001. Primers for elongation factor 1- α (EF1- α). <https://web.archive.org/web/20080514151555/http://ocid.NACSE.ORG/research/deephyphae/EF1primer.pdf>.
- White, T. J., et al. 1990. Page 315 in: PCR Protocols: A Guide to Methods and Applications. Academic Press, San Diego, CA.

The author(s) declare no conflict of interest.

e-Xtra

Keywords: *Pestalotiopsis kenyana*, *Zanthoxylum schinifolium*, leaf spot disease

*Indicates the corresponding author.
S. Li; 14087@sicau.edu.cn