Diseases Caused by Fungi and Fungus-Like Organisms

Brown Leaf Spot of *Juglans hybrid* Caused by *Ophiognomonia leptostyla* in China

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Plant Dis. 105:3740, 2021; published online as <https://doi.org/10.1094/PDIS-05-21-0981-PDN>. Accepted for publication 4 June 2021.

‘Chuanzao 2’ is a walnut variety derived from the hybridization of *Juglans regia* L. and *J. sigillata* Dode distributed in southwest China, where it is an economically important tree species in rural regions (Xiao et al. 2012). In April 2020, the variety in a walnut garden showed symptoms of brown leaf spot in Beishan Town (107°21′43.93″E, 31°28′12.34″N), Dazhou City in Sichuan, China, with 5 to 10% of leaves per plant affected (five plants). Symptomatic leaves showed brown to dark brown spots (2 to 5 mm) with a dark brown to black halo and grayish-tan center. The spots were subcircular to irregular in shape and gradually expanded and formed necrotic spots. A single conidium isolation was performed (Senanayake et al. 2020) and transferred to potato dextrose agar. Five isolates were obtained from five different infected leaves. Colonies of five isolates were subcircular, erose or dentate, flat or effuse, white initially, gradually becoming yellowish with white margins, developed and fluffy aerial mycelia, and conidiogenesis was produced underneath mycelia after 25 days of incubation. Conidiogenous cells were subcylindrical to cylindrical, or irregular in shape, and hyaline. Macroconidia were lunate, reniform, hyaline, basal cell bluntly rounded, apical cell with acute end, one-septate, rarely aseptate, sometimes slightly constricted at septum, basal cell equal or larger than apical cell, and measured 16.5 to 30.5 × 5

to 8.5 µm (mean = 23.2 × 6.3 µm, *n* = 50). Microconidia were not observed. These morphological characteristics resembled those of *Ophiognomonia leptostyla* (Fr.) Sogonov (Walker et al. 2012a). For molecular identification, genomic DNA (isolates SICAUCC 21-0008 and SICAUCC 21-0010) was extracted, and the internal transcribed spacer (ITS) region, guanine nucleotide-binding protein subunit beta (*MS204*) gene, and translation elongation factor 1- α (*tef1- α*) were amplified and sequenced by using the primers ITS5/ITS4 (White et al. 1990), E1F1/E5R1a (Walker et al. 2012a), and EF1-728F/EF1-1567R (Walker et al. 2012b), respectively. Phylogenetic analyses (maximum likelihood) based on a combined dataset showed 100% bootstrap support values in a clade with *O. leptostyla*. The sequences of ITS, *MS204*, and *tef1- α* genes were deposited in GenBank with accession numbers MW493111/MZ026300, MW495270/MZ031975, and MW495271/MZ031974, respectively. To fulfill Koch’s postulates, five healthy hybrid plants (2 to 3 years old) with five to eight leaves per plant were spray inoculated with conidium suspensions (10⁴ conidia/ml; isolate SICAUCC 21-0008) prepared from 40-day-old cultures onto the wounded sites via pinprick inoculation. Similarly, five noninoculated plants sprayed with sterile water served as controls. Plants were placed in a growth chamber at 25°C on a 12-h fluorescent light/dark regime and sprayed daily with sterile distilled water. After 2 weeks, observed symptoms were similar to those from natural infections. No disease symptoms were found on control plants. The fungus *O. leptostyla* was reisolated from the diseased leaves and characterized morphologically. *O. leptostyla* is a global pathogen and has been reported to cause the leaf spot in many walnut trees, namely, *J. ailantifolia*, *J. californica*, *J. cinerea*, *J. major*, and so on. To our knowledge, this is the first report of *O. leptostyla* causing brown leaf spot on *Juglans hybrid* (*J. regia* × *J. sigillata*) in China. The increasing risk of this pathogen in the walnut-growing areas of Sichuan Province of China needs a further exploration and outreach effort to develop effective control measures.

References:

- Senanayake, I. C., et al. 2020. Mycosphere 11:2678.
- Walker, D. M., et al. 2012a. Mol. Phylogenet. Evol. 64:500.
- Walker, D. M., et al. 2012b. Fungal Divers. 57:85.
- White, T. J., et al. 1990. Page 315 in: PCR Protocols: A Guide to Methods and Applications. Academic Press, San Diego, CA.
- Xiao, Q.-W., et al. 2012. Acta Hortic. Sin. 39:2317.

The author(s) declare no conflict of interest.

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Keywords: fungi, trees, tree nuts, *Ophiognomonia leptostyla*

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