Diseases Caused by Fungi and Fungus-Like Organisms

Brown Leaf Spot on *Juglans sigillata* Caused by *Ophiognomonia leptostyla* in Sichuan, China

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Juglans sigillata Dode (iron walnut) is mostly distributed in southwestern China and valued for wood and nuts (Feng et al. 2018). In April 2020, we surveyed a walnut garden located in Baisha Town, Wanyuan City, (Sichuan, China), where brown spot symptoms were observed on leaves of 10 trees among 100 plants, and this disease can result in a reduced growth potential when trees are severely infected. Necrotic and subcircular lesions with conidiomata were observed on diseased leaves. Symptomatic leaves were collected and taken back to the laboratory for further analysis. Using the single-spore isolation technique developed by Chomnunti et al. (2014), five isolates were grown from the infected leaves on potato dextrose agar medium (PDA). The five isolates had similar colony morphology, which was initially white, suborbicular, gradually turning yellowish with black spots, developing fluffy aerial mycelium. Morphological characteristics were examined using light microscopy on the PDA. Conidiogenous cells were subcylindrical to cylindrical, or ampulliform, hyaline, rarely branched. Macroconidia were lunate, reniform, hyaline, one- to three-septate, mostly one-septate, distinctly constricted at the septum, the basal cell was bluntly rounded, the apical cell had an acute end, and the basal cell was equal to or larger than the apical cell, measuring 22 to 40.5 × 2.5 to 8.3 μm (mean = 32 × 6.2 μm, n = 50).

Microconidia were botuliform, or subfusiform, hyaline, both ends were rounded, straight or curved, aseptate, and measured 10 to 28.5 × 1.9 to 3.7 μm (mean = 17.2 × 2.7 μm, n = 20). A multilocus approach was conducted for precise identification of a representative isolate SICAUCC 20-0012. The internal transcribed spacer regions (ITS), guanine nucleotide-binding protein subunit beta gene (*MS204*), and translation elongation factor 1-alpha (*tefl-α*) of isolate SICAUCC 20-0012 were amplified and sequenced as described by Sogonov et al. (2008) and Walker et al. (2012a). GenBank accession numbers for ITS, *MS204*, and *tefl-α* are MW250303, MW246773, and MW246775, respectively. Phylogenetic analyses showed 100% support with *Ophiognomonia leptostyla* (Fr.) Sogonov, and the morphology was consistent with the asexual stage of *O. leptostyla* documented by Walker et al. (2012b). To test Koch's postulates, five healthy plants of *J. sigillata* (2 to 3 years old) with five to eight leaves per plant were inoculated with conidial suspensions (10⁴ conidia/ml) after being wounded with a small pin as described by Desai et al. (2019), and the same number of healthy plants were wounded and sprayed with sterile distilled water as controls. Plants were sprayed regularly with distilled water every day and placed in a growth chamber at 25°C with a 12-h fluorescent light/dark regimen. After 15 days, typical brown spot symptoms developed on inoculated leaves but not on the controls. The fungus *O. leptostyla* was reisolated from the lesion as described above but not from noninoculated leaves. *O. leptostyla* has been reported on some walnut trees: for example, *J. ailantifolia*, *J. californica*, *J. cinerea*, *J. hindsii*, *J. major*, *J. mandshurica*, *J. nigra*, and *J. regia* (Farr and Rossman 2020). However, to our knowledge, this is the first report of *O. leptostyla* causing brown leaf spot on *J. sigillata*. *J. sigillata* is an economically important tree in southwest China, and fungicide treatments should be considered to prevent the spread of this fungus before it becomes more widespread.

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