Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Botryosphaeria dothidea* Causing Leaf Spot on *Kadsura coccinea* in China

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Kadsura coccinea (Lem.) A. C. Smith, belonging to Schisandraceae, is an evergreen, woody climbing plant that is distributed widely in southwest China. Additionally, *K. coccinea* is used as an ethnic medicine and its main chemical components are lignin and terpenoids. The roots of the plant have been effectively used for treatment of cancer and dermatosis and as an anodyne to relieve pain (Song et al. 2010). In June 2019, a leaf spot disease on *K. coccinea* was first observed in a greenhouse in Qiandongnan Miao and Dong Autonomous Prefecture, Guizhou Province, China. Over 300 plants were surveyed in the three greenhouses, and nearly 70% of the plants were infected. The diseased plants grew poorly and appeared stunted, and severely affected plants died. The symptoms occurred on leaves as small brown spots initially and then developed into suborbicular or irregular-shaped brown necrotic lesions, which often displayed irregular concentric rings. Four diseased leaves from four symptomatic *K. coccinea* plants were randomly collected for pathogen isolation. Diseased tissues were cut into about 2 mm diameter fragments, surface sterilized with 75% ethanol for 15 s and 1% NaClO for 2 min, and then rinsed twice in sterilized distilled water. After being dried on sterilized filter paper, the fragments were placed on potato dextrose agar (PDA) and incubated at

25°C in the dark for 5 days. The same fungus was isolated in 95% of the samples. A representative isolate, F2020003, was used for morphological and molecular characterization. The colonies were initially white, gradually turning gray-green to dark gray after 7 days, with abundant gray aerial mycelium. Conidia were one-celled, hyaline, fusoid to ellipsoid, and measured $24.3 \pm 1.9 \times 4.8 \pm 0.7 \mu\text{m}$ ($n = 50$). The conidial morphology matched the description of *Botryosphaeria dothidea* (Slippers et al. 2004). To verify identity, the partial sequences of the internal transcribed spacer region, translation elongation factor 1 alpha genes, and beta-tubulin, were amplified from isolate F2020003 with primers ITS1/ITS4 (GenBank accession no. MW111267), EF1-728F/EF1-986R (GenBank accession no. MW196739), and BT-2a/BT-2b (GenBank accession no. MW206378), respectively (Sun et al. 2014). The isolates were confirmed as *B. dothidea* based on morphological comparisons and BLAST searches (Zhai et al. 2014). To assess pathogenicity, five healthy leaves on each of the three 6-month-old healthy *K. coccinea* plants were wound-inoculated with a sterilized needle. Mycelium plugs (4 mm in diameter) taken from a 5-day-old culture on PDA were inoculated on surface-sterilized leaves (sprayed with 75% ethanol). PDA plugs with no mycelium were used as a control. Plants with treated leaves were covered with plastic bags and incubated in a greenhouse at 25°C. The pathogenicity test was repeated three times. Within 4 days, all the inoculated points showed lesions similar to those previously observed in the greenhouse, whereas controls were asymptomatic. Fungi reisolated from inoculated leaves were confirmed as *B. dothidea* on the basis of morphological and molecular characterization as described above. *B. dothidea* is a member of Botryosphaeriaceae, and it has been reported to cause leaf spot on *Celtis sinensis* (Wang et al. 2021) and branch canker on *Malosma laurina* (Aguirre et al. 2018) in China. To our knowledge, this is the first report of *B. dothidea* causing leaf spot on *K. coccinea* in China. The identification of this pathogen will be helpful to prevent and control this disease in the future.

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