Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Davidia involucrata* Leaf Blight Caused by *Nigrospora oryzae* in Sichuan, China

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Davidia involucrata Baill., known as the dove tree, is listed as a first-class national protected plant in China and is the only extant member of the Davidiaceae family (Fu and Jin 1992). Referred to as a “living fossil” and “giant panda” owing to its evolutionary status as a Tertiary relic and its native distribution, *D. involucrata* has substantial ornamental and academic value (Fang and Song 1975; Wu et al. 2004). Its small, rounded head inflorescence beneath big white bracts has unique charm that catches people’s attention, thus they were cultivated in many areas of the world as an ornamental plant (Claßen-Bockhoff and Arndt 2018). In September 2021, *D. involucrata* in Meigu country (N 28°33′, E 103°14′), Sichuan Province, China, were found with symptoms of leaf blight of unknown origin. The blight disease incidence was 90% in a survey of 30 *D. involucrata* trees. Early symptoms appeared as circular, necrotic tissue that developed into circular or irregular spots. Five leaves exhibiting typical symptoms of this form of leaf blight were excised from the margin between diseased and healthy tissue. These pieces were treated for 40 s with 75% ethanol for surface sterilization, treated with 5% NaClO for 2 min, rinsed, plated on potato dextrose agar (PDA) medium supplemented with carbenicillin and ampicillin (each 50 µg/ml), and incubated in the dark for 4 days at 28°C. Pure cultures were prepared by transferring hyphal tips from the edges of these colonies onto fresh PDA plates, with isolate LW11 being selected as a representative isolate for causal pathogen characterization. These cultured colonies were initially white before turning

grayish-black over time. Conidia were single-celled, black, spherical or oblate, and 10 to 16.0 µm in diameter (mean = 12.5 ± 0.43 µm; *n* = 40), with conidia being present at the tip of conidiophores on hyaline vesicles. These morphological traits were found to align well with those of *Nigrospora oryzae* (Wang et al. 2017). To confirm this tentative identification, gDNA was extracted from isolate LW11, followed by the amplification of the internal transcribed spacer (ITS) region, beta-tubulin (*TUB2*), and translation elongation factor 1- α (*TEF1- α*) sequences with the respective ITS1/ITS4 (White et al. 1990), Bt-2a/Bt-2b (Glass and Donaldson 1995), and TEF1-728F/EF1-986R primer pairs (Carbone and Kohn 1999). Fragments of 577, 442, and 309 bp were obtained. A maximum likelihood bootstrapping approach (1,000 bootstrap replicates) was used to construct a phylogenetic tree based on a combination of the ITS, *TUB*, and *TEF1- α* sequences, which indicated that isolate LW11 clustered with other *N. oryzae* isolates. The ITS, *TUB*, and *TEF1- α* sequences from isolate LW11 were deposited in GenBank with accession numbers OL659284, OL685345, and OL685347, respectively. A pathogenicity test was performed by inoculating detached *D. involucrata* leaves with mycelial plugs, with the other half being inoculated using pure agar plugs as a negative control. After incubation for 5 days, black lesions were evident on leaves inoculated with mycelial plugs but not on control leaves. To our knowledge, this is the first report of *D. involucrata* leaf blight by *N. oryzae* in China or anywhere else in the world. Further research is needed to better manage the spread of this disease with the goal of protecting this living fossil species.

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