Diseases Caused by Fungi and Fungus-Like Organisms

First Report of Leaf Spot Caused by *Diaporthe tulliensis* on Boston Ivy (*Parthenocissus tricuspidata*) in Taiwan

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Author Contribution: C.C.H. isolated the fungal pathogens, completed the Koch's postulates to confirm the pathogenicity, and drafted the manuscript. C.C.H. and H.H.L. completed the morphological characterization and the molecular identity for the pathogen. P.H.W. verified the pathogenicity. H.X.C. discovered the disease, revised the manuscript, and supervised the project.

The ITS, EF1 α , β -tubulin, and calmodulin sequences of *Diaporthe tulliensis* were deposited in NCBI GenBank under accession numbers MT974186, MT982963, MT982962, and MW759801, respectively.

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From May to August 2020 (average humidity 76.6% and temperature 25.2°C in Taipei), Boston ivy (*Parthenocissus tricuspidata*) plants on the campus of National Taiwan University (25°01'05.4"N 121°32'36.6"E) exhibited leaf rusts caused by *Phakopsora ampelopsidis* (Tzean et al. 2019) and leaf spots caused by an unknown pathogen. The leaf spots appeared reddish to brown color and mostly irregular to round shape on the simple and trifoliate leaflets. The leaf spots were surface disinfected with 1% NaOCl for 30 s, and the margin of healthy and infected tissues was cut and placed onto water agar and then incubated at room temperature. Hyphae grown out from leaf spots were subcultured on potato dextrose agar (PDA), and the majority of isolates exhibited white colony with black pycnidial conidiomata embedded in PDA. The pycnidial conidiomata of 2-week-old culture has an average diameter of 463 $\pm$ 193 μ m (n = 30), and the sizes of α -conidia were 5.71 $\pm$ 0.49 μ m in length and 2.42 $\pm$ 0.32 μ m in width (n = 50), similar to the previous records (Crous et al. 2015). The α -conidium was one-celled, hyaline, and ovoid with two droplets. This putative pathogen was reinoculated to confirm its pathogenicity on the leaves of Boston ivy plants. A PDA block with actively growing fungal edge was placed on the tiny needle-wounded leaves of detached branches and the whole plants in pots in a moist chamber at 28°C

in dark. Reddish to brown leaf spots were observed by 2 days postinoculation (dpi), and the leaf spots expanded by 5 dpi. To complete Koch's postulates, the pathogen was reisolated from inoculated leaves, and the reisolated pathogen exhibited identical morphology to the original isolate. The internal transcribed spacer (ITS), translational elongation factor subunit 1- α gene (EF1 α), β -tubulin (BT), and calmodulin (CAL) were amplified using the primers ITS1/ITS4 (Martin and Rygiewicz 2005), EF1-728F/EF1-986R, Bt2a/Bt2b, and CAL-228F/CAL-737R, respectively (Manawasinghe et al. 2019). Using BLAST in the NCBI database, the ITS (MT974186), EF1 α (MT982963), and β -tubulin (MT982962) sequences showed 98.57% (NR_147574.1, 553 out of 561 bp), 98.04% (KR936133.1, 350 out of 357 bp), and 99.23% (KR936132.1, 518 out of 522 bp) identity to the *Diaporthe tulliensis* ex-type BRIP 62248a, respectively (Dissanayake et al. 2017). Phylogenetic analysis using concatenated sequences of ITS, EF1 α , and β -tubulin grouped the *D. tulliensis* isolated from Boston ivy leaf spots with the *D. tulliensis* ex-type. In summary, the morphological and molecular characterizations supported the causal pathogen of Boston ivy leaf spot as *D. tulliensis*. Although *Diaporthe ampelopsidis* was reported to infect *Parthenocissus quinquefolia* and *P. tricuspidata* (Anonymous 1960; Wehmeyer 1933), there is no record for *D. tulliensis* infecting Boston ivy according to the U.S. National Fungus Collections (Farr and Rossman 2020). Because pathogens of Boston ivy such as *P. ampelopsidis* may also infect close-related crops like grape (*Vitis vinifera* L.) and *D. tulliensis* has been known to infect kiwifruits (*Actinidia chinensis*) and cocoa (*Theobroma cacao*) (Bai et al. 2017; Yang et al. 2018), the emergence of *D. tulliensis* should be noted to avoid potential damage to economic crops.

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The author(s) declare no conflict of interest.

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