Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Pseudonectria buxi* Causing Volutella Blight on Boxwood (*Buxus microphylla*) in the Republic of Korea

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Funding: Funding was provided by the National Research Foundation of Korea (2021R1A2C1004673) and Ministry of Agriculture, Food and Rural Affairs (321101-03, 321107-03). Plant Dis. 106:2518, 2022; published online as <https://doi.org/10.1094/PDIS-01-22-0042-PDN>. Accepted for publication 3 March 2022.

Boxwoods (*Buxus* spp.) are evergreen landscaping plants commonly used as hedges and fresh greenery. In July and August 2020, boxwood (*Buxus microphylla* Sieb. et Zucc) samples with blight symptoms were collected from the parterres of Seoul National University (Seoul, Republic of Korea). Diseased leaves and stem tissues were soaked in 1% sodium hypochlorite for 1 min, rinsed with sterile water, cultured on potato dextrose agar (PDA; Difco, Sparks, MD, USA) and incubated at 25°C for 5 days. Four isolates (B2S72-1, B2S72-3, B3B2, and B4L3-3) were pure-cultured using the single-spore isolation method. Light pink sporodochia containing one-celled, fusoid conidia were observed on PDA. Mean conidial size was $9.11 \times 3.79 \mu\text{m}$ and ranged from 7.68 to 10.71×3.18 to $4.92 \mu\text{m}$. Morphological features suggested that these isolates possessed the same characteristics as previously described for *Pseudonectria buxi* (Bezerra, 1963; Yang et al. 2021). Genomic DNA was extracted from each isolate and the internal transcribed spacer (ITS) region of the ribosomal DNA (rDNA), β -tubulin coding sequence, and D1/D2 region of the large subunit of the

rDNA gene cluster (LSU) were amplified using the primer pairs ITS4/ITS5 (White et al. 1990), Btub4Rd/Btub2Fd (Woudenberg et al. 2009), and LR0R/LR5 (Rehner and Samuels 1994; Vilgalys and Hester 1990), respectively. The sequences were deposited in GenBank (GenBank accession nos. OM169273 to OM169276, OM176571 to OM176574, and OM176566 to OM176569). A BLAST search showed high similarity to GenBank sequences of the ex-type strain of *P. buxi* for ITS ($\geq 99.50\%$, MH892589.1 and MH892583.1). In addition, neighbor-joining (NJ) phylogenetic analysis was performed using MEGA-X based on the combined sequences. The four isolates were clustered in the same clade with *P. buxi* reference strains with a high bootstrap value (100%). For the pathogenicity test, leaves of 1-year-old boxwood were given a single cut with a razor blade and a 5-mm PDA mycelial plug was placed on the wound site. Plants were kept in a transparent box at 25°C with a 12-h photoperiod. After 21 days, inoculated leaves had turned yellow and orange to pink sporodochia were observed. *P. buxi* was successfully reisolated from the symptomatic tissues but not from the control leaves, therefore Koch's postulates were completed. To our knowledge, this is the first report of Volutella blight caused by *P. buxi* in the Republic of Korea.

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The author(s) declare no conflict of interest.

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Keywords: boxwood, *Pseudonectria buxi*, Volutella blight

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