Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Botrytis cinerea* Causing Gray Mold on *Sanchezia nobilis* in Brazil

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Sanchezia nobilis (Acanthaceae) is an evergreen shrub, native to the forests of Ecuador and Peru, widely used in urban landscaping due to its colorful leaves and inflorescences in addition to year-round flowering and being an attractive trophic resource for the hummingbird community (Leonard and Smith 1964). From 2016 to 2018, dark brown to black lesions with abundant fungal sporulation on flowers, inflorescence bending, and decayed flowers were observed on *S. nobilis* in the gardens of the Universidade Federal de Viçosa (UFV), municipality of Viçosa (Minas Gerais, Brazil). Samples of infected inflorescences were collected, dried in a plant press, and deposited in the Herbarium of the UFV under the accession number VIC 47363. From symptomatic tissues, conidia were transferred to PDA dishes using a sterile needle. Two single-spore isolates were obtained and deposited in the Otávio de Almeida Drumond cultures collection at UFV as COAD 2842 and COAD 2843. After 7 days of growth on PDA, mycelia with abundant sporulation were produced. Fungal structures were mounted on slides in colorless lactoglycerol and were visualized with a light microscope (Olympus BX 53) equipped with a digital camera (Olympus Q-COLOR3). The measures of 35 conidiophores and 50 conidia were obtained for each isolate. The size of conidiophores ranged from 295 to 1,555 × 9.0 to 26.5 µm (average = 1,033 ± 280 × 15.5 ± 2.8 µm, *n* = 70). Conidia were single, aseptate, pale brown, and globose to subglobose, 6.5 to 17.0 × 4.5 to 10.5 µm (average = 12 ± 1.8 × 7.5 ± 0.9 µm, *n* = 100). Morphological characterization matched *Botrytis cinerea* description (Ellis and Walker 1974). Fungal genomic DNA was obtained with the Wizard Genomic DNA Purification Kit (Promega Corporation, U.S.A.). A multilocus phylogeny was performed using partial sequences of the nuclear genes encoding glyceraldehyde-3-phosphate dehydrogenase (G3PDH), heat-shock protein 60 (HSP60), and DNA-dependent RNA polymerase subunit II (RPB2), which were amplified by PCR using the

respective primer pairs G3PDHfor/G3PDHrev, HSP60for/HSP60rev, and RPB2for/RPB2rev (Staats et al. 2005). Each PCR reaction was prepared using 12.5 µl of GoTaq G2 Colorless Master Mix (Promega), 1.5 µl of each primer forward and reverse (10 µM) synthesized by Sigma Aldrich, 2 µl of genomic DNA (15 ng/µl), and 7.5 µl of nuclease-free water to make up the total volume of 25 µl. PCR conditions were the same described by Staats et al. (2005). Sequences of *Botrytis* species were retrieved from GenBank to perform maximum likelihood and bayesian inference phylogenetic analyses. Newly generated sequences, obtained by Sanger sequencing, were deposited in GenBank as accession numbers MN547171, MN561080, MN561136, MN547189, MN561099, MN561155, MN547193, MN561103, and MN561159. The phylogenetic analyses confirmed the studied isolates as belonging to the species *B. cinerea*. A pathogenicity test was performed by spraying 2 ml of conidial suspension (1 × 10⁶ conidia/ml) from a 7-day culture of the isolate COAD 2842 onto five healthy inflorescences of a living shrub of *S. nobilis*. Sterilized water was used as the non-inoculated control treatment. A humid chamber was made with plastic bags for 24 h. After 7 days, two inoculated inflorescences developed similar symptoms to those of the naturally occurring disease, whereas control subjects remained healthy. Isolates with identical morphological and molecular characteristics were recovered from the diseased inflorescences, thus fulfilling Koch's postulates. *B. cinerea* has one of the widest known host ranges (Ngugi and Scherm 2006). In Brazil, more than 100 hosts of this fungus have been reported (EMBRAPA Recursos Genéticos 2019). This is the first report of *B. cinerea* causing gray mold on *S. nobilis*, as well in the genus *Sanchezia*, worldwide. The knowledge of pathogens present in the country and their host range are pillars for the elaboration and planning of disease management strategies.

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