Diseases Caused by Fungi and Fungus-Like Organisms

First Report of Leaf Spot Caused by *Botrytis cinerea* on *Cardamine hupingshanensis* in China

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Cardamine hupingshanensis is a selenium (Se) and cadmium (Cd) hyperaccumulator plant distributed in wetlands along the Wuling Mountains of China (Zhou et al. 2018). In March of 2020, a disease with symptoms similar to gray mold was observed on leaves of *C. hupingshanensis* in a nursery located in Changsha, Hunan Province, China. Almost 40% of the *C. hupingshanensis* (200 plants) were infected. Initially, small spots were scattered across the leaf surface or margin. As disease progressed, small spots enlarged to dark brown lesions, with green-gray conidia containing a mold layer under humid conditions. Small leaf pieces were cut from the lesion margins and sterilized with 70% ethanol for 10 s, 2% NaOCl for 2 min, rinsed with sterilized distilled water three times, and placed on potato dextrose agar (PDA) at 22°C in the dark. Seven similar colonies were consistently isolated from seven samples and further purified by single-spore isolation. Strains cultured on PDA were initially white, forming gray-white aerial mycelia, then turned gray and produced sclerotia after incubation for 2 weeks, which were brown to blackish, irregular, and 0.8 to 3.0 × 1.2 to 3.5 mm ($n = 50$). Conidia were unicellular, globose or oval, colorless, and 7.5 to 12.0 × 5.5 to 8.3 μm ($n = 50$). Conidiophores arose singly or in groups, straight or flexuous, septate, brownish to light brown, with enlarged basal cells, 12.5 to 22.1 × 120.7 to 310.3 μm. Based on their morphological characteristics in culture, the isolates were putatively identified as *Botrytis cinerea* (Ellis 1971). Genomic DNA of

four representative isolates, HNSMJ-1 to HNSMJ-4, were extracted by CTAB method. The internal transcribed spacer region (ITS), glyceraldehyde-3-phosphate dehydrogenase gene (*G3PDH*), heat-shock protein 60 gene (*HSP60*), ATP-dependent RNA helicase DBP7 gene (*MS547*), and DNA-dependent RNA polymerase subunit II gene (*RPB2*) were amplified and sequenced using the primers described previously (Aktaruzzaman et al. 2018) (MW820311, MW831620, MW831628, MW831623, and MW831629 for HNSMJ-1; MW314722, MW316616, MW316617, MW316618, and MW316619 for HNSMJ-2; MW820519, MW831621, MW831627, MW831624, and MW831631 for HNSMJ-3; MW820601, MW831622, MW831626, MW831625, and MW831630 for HNSMJ-4). BLAST searches showed 99.43 to 99.90% identity to the corresponding sequences of *B. cinerea* strains, such as HJ-5 (MF426032.1, MN448500.1, MK791187.1, MH727700.1, and KX867998.1). A combined phylogenetic tree using the ITS, *G3PDH*, *HSP60*, and *RPB2* sequences was constructed by neighbor-joining method in MEGA 6. It revealed that HNSMJ-1 to HNSMJ-4 clustered in the *B. cinerea* clade. Pathogenicity tests were performed on healthy pot-grown *C. hupingshanensis* plants. Leaves were surface-sterilized and sprayed with conidial suspension (10^6 conidia/ml), with sterile water as controls. All plants were kept in a growth chamber with 85% humidity at 25°C following a 16 h day/8 h night cycle. The experiment was repeated twice, with three replications each. After 4 to 7 days, symptoms similar to those observed in the field developed on the inoculated leaves, whereas controls remained healthy. The pathogen was reisolated from symptomatic tissues and identified using molecular methods, confirming Koch's postulates. *B. cinerea* has already been reported from China on *C. lyrata* (Zhang 2006), a different species of *C. hupingshanensis*. To the best of our knowledge, this is the first report of *B. cinerea* causing gray mold on *C. hupingshanensis* in China and worldwide. Based on the widespread damage in the nursery, appropriate control strategies should be adopted. This study provides a basis for studying the epidemic and management of the disease.

References:

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