Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Alternaria* spp. Causing Leaf Spot on Sweet Viburnum in China

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Funding: Funding was provided by the Anhui Provincial Natural Science Foundation Sponsored by Department of Science and Technology of Anhui Province, China (1808085MC67), the High-level Talent Subsidy Project Supported by Education Department of Anhui Province of China, the Startup Fund for High-level Talents Sponsored by Anhui Agricultural University of China (0601510), and the Graduate Innovation Fund of Anhui Agricultural University, China (XJDCY2019378). Plant Dis. 105:2253, 2021; published online as <https://doi.org/10.1094/PDIS-06-20-1407-PDN>. Accepted for publication 14 February 2021.

Sweet viburnum (*Viburnum odoratissimum* [L.] Ker Gawl) is an evergreen shrub mainly cultivated along roadsides in urban landscapes and in parks and residential areas. A foliar disease occurred on ~40% of sweet viburnum plants near Anhui Grand Theater, Anhui Province, China, in June 2019. Early symptoms appeared as small brown spots 2 to 3 mm in length on the leaves. Spots developed on the upper, middle, and lower leaves; however, upper leaves were more severely affected. As the disease developed, spots enlarged and became rectangular or oval, brown to dark brown, and their centers became ashen gray. In later stages, diseased leaves wilted. Diseased leaves were surface disinfested, and three 2- to 3-mm² sections were cut from lesion margins. Sections were placed in 1.5% NaClO for 2 min, submerged in three changes of sterilized distilled water (SDW) for 1 min each, placed onto PDA amended with 50 µg/ml of ampicillin and kanamycin, and incubated at 25°C for 3 days. The mycelium from the leading edge of colonies growing from the tissue was subcultured onto a PDA plate for 3 days, followed by spore induction (Simmons 2007) and single-spore isolation to obtain a pure culture. Colonies of single-spore isolate HF0719 were rounded and grayish white with dense aerial mycelium viewed from above and dark brown viewed from below. On potato carrot agar, conidiophores were branched or occasionally unbranched. On branched conidiophores, conidia were in dwarf tree-like branched chains of 2 to 5 conidia. On unbranched conidiophores, conidia were simple or in chains of 2 to 8 conidia. Conidia were light brown or dark brown, ovoid, ellipsoidal to fusiform, and 7 to 26.5 × 4.5 to 11 µm (average 16 × 7 µm, *n* = 500 spore observations), with one beak and 1 to 7 transverse, 0 to 3 longitudinal, and 0 to 3 oblique septa. Beaks were (1.5 to) 2 to 10 (to

16) µm long. Based on cultural and morphological characteristics, HF0719 was identified as *Alternaria* spp. (Simmons 2007). For molecular identification, total genomic DNA was isolated from mycelia collected from 7-day-old colonies of HF0719 using the fungal genomic DNA extraction kit (Solarbio, Beijing, China). Fragments of five genes, including those encoding GAPDH (*gpd*), plasma membrane ATPase, actin, calmodulin, and the *Alternaria* major allergen (*Alt a1*) regions of HF0719 were amplified and sequenced using primer pairs *gpd1/gpd2* (Berbee et al. 1999), *ATPDF1/ATPDR1*, *ACTDF1/ACTDR1*, *CALDF1/CALDR1* (Lawrence et al. 2013), and *Alt-for/Alt-rev* (Hong et al. 2005), respectively. The obtained nucleotide sequences were deposited in GenBank: *gpd*, MT614365; *ATPase*, MT614364; *actin*, MT614363; *calmodulin*, MN706159; and *Alt a1*, MN304720. A phylogenetic tree using a maximum likelihood bootstrapping method based on the five-gene combined dataset (*gpd*, *ATPase*, *actin*, *calmodulin*, and *Alt a1*) of HF0719 and standard strains of 120 *Alternaria* species (Lawrence et al. 2013) was constructed. HF0719 formed a separate branch. Based on morphology and phylogenetic pattern, HF0719 was identified as *Alternaria* spp. Pathogenicity was tested by rubbing 32 healthy leaves of six 5-year-old sweet viburnum plants with a cotton swab dipped in spore suspension containing 2.6 × 10⁶ spores/ml, following leaf surface disinfection with 70% ethanol in the open field. SDW was used as a control. Average air temperature was ~28°C. Eleven days after inoculation, 100% of inoculated leaves showed symptoms identical to those in the field. Control leaves were symptomless. The experiment was done three times. The reisolated pathogen from the leaf lesion had the same morphological and molecular characteristics as HF0719, satisfying Koch's postulates. *Alternaria* has been reported to cause leaf spot on sweet viburnum in Florida, U.S.A. (Alfieri et al. 1984). This is the first report of *Alternaria* spp. causing leaf spot on sweet viburnum in China. Our findings will contribute to monitoring and adopting strategies to manage leaf spot disease on sweet viburnum.

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The author(s) declare no conflict of interest.

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Keywords: leaf spot, sweet viburnum, *Alternaria* spp.

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