Diseases Caused by Fungi and Fungus-Like Organisms

First Report of Leaf Spot Caused by *Corynespora cassiicola* on *Viburnum odoratissimum* var. *awabuki* (Sweet Viburnum) in China

Tianning Zhang, Huanhuan Liu, Qingni Song, Jun Liu, Qingpei Yang, Fenggang Luan,[†] and Dong Li[†]

Key Laboratory of State Forestry Administration on Forest Ecosystem Protection and Restoration of Poyang Lake Watershed (JXAU), Nanchang 330045, China

Funding: Funding was provided by the Educational Commission of Jiangxi Province of China (GJJ170974 and GJJ190199) and the Innovative Research Program for Graduates of Jiangxi Agricultural University (NDYC2019-S010). Plant Dis. 106:1062, 2022; published online as <https://doi.org/10.1094/PDIS-04-21-0849-PDN>. Accepted for publication 7 September 2021.

Sweet viburnum [*Viburnum odoratissimum* Ker-Gawl. var. *awabuki* (K. Koch) Zabel ex Ruml., family Adoxaceae, is a medical and landscape plant native to Korea (Jeju Island), Taiwan, and Japan. In June and September 2019, leaf spots were observed on ~65 to 80% of sweet viburnum plants in a hedgerow located in Fenghe Xincheng District (28°41'52.9"N 115°52'14.3"E) in Nanchang, China. Initial symptoms appeared as dark brown spots surrounded by red halos, which expanded irregularly. Finally, the center of the lesions desiccated and became light-brown, surrounded by deep red halos. Ten leaf samples with typical symptoms were collected and washed with tap water for 15 min. The tissue between the healthy and necrotic area (~4 × 4 mm) was cut with a sterile scalpel and surface sterilized with 70% alcohol for 45 s, 2% NaClO for 2 min, washed in sterile deionized water three times, dried on sterilized filter paper, then placed in Petri dishes and incubated at 25°C in the dark. After 3 to 5 days, the hyphal tips from the edges of growing colonies were transferred to fresh PDA dishes. Eventually, 54 fungal isolates were obtained, and of these, 39 were identical in their morphological characteristics. Morphological analysis was performed according to Ellis (1971). Representative isolate S18 formed a gray to grayish brown colony with concentric circles on PDA, and a diameter of 8.5 to 9 cm after 7 days incubation at 25°C. Conidia were hyaline, straight or slightly curved, needle shaped, truncate at the base, and acuminate at the tip, with 2 to 6 pseudosepta, 18.90 to 38.38 µm (avg. = 27.51 µm) × 1.64 to 4.50 µm (avg. = 2.60 µm) (*n* = 36). The genes of fungal isolates (i.e., ITS, tub2, and ACT) were amplified with ITS4/ITS5 for ITS (White et al. 1990), Bt2a/Bt2b for tub2 (Glass and Donaldson 1995), and ACT783R/ACT512F for ACT (Carbone and Kohn 1999) and sequenced. The sequences were deposited in GenBank (MW165772 for ITS, MW175900

for ACT, and MW168659 for tub2), and showed more than 99.1% similarity with ITS accession MK212952, ACT accession FJ853005, and tub2 accession MN887509. Pathogenicity tests were performed on healthy leaves in the field, which were surface-sterilized with 70% alcohol and wounded with a sterilized needle. A 7-mm diameter mycelium plug from a 7-day-old PDA culture was aseptically placed on the middle of the wound and sealed to maintain moisture. Noncolonized PDA plugs served as the control. The mycelium plugs were removed 72 h after inoculation. All inoculated leaves reproduced similar symptoms 7 days after inoculation as observed initially in the field. For further characterization, pathogenicity tests were conducted by spraying a conidial suspension (1×10^5 conidia/ml) of *C. cassiicola* S18 onto young leaves of eight sweet viburnum plants. Three plants were sprayed with sterile distilled water as controls. All plants were incubated at high humidity for 6 days at 26°C. Mild symptoms appeared 4 days after inoculation and similar symptoms of those observed in the field from natural infections occurred for 6 days after inoculation. To fulfill Koch's postulates, the fungus was isolated on PDA from the margins of leaf spots on inoculated leaves and confirmed as *C. cassiicola* by morphological characters and ITS gene sequencing. Previously, *C. cassiicola* was reported as an endophyte on *Viburnum* spp. and *Viburnum odoratissimum* (Alfieri et al. 1994). More recently, *C. cassiicola* has been reported as a pathogen of many plant species in China, such as kiwifruit (Cui et al. 2015), American sweetgum (Mao et al. 2021), castor bean (Tang et al. 2020), and holly mangrove (Xie et al. 2021). To our knowledge, this is the first report of leaf spot disease on sweet viburnum caused by *C. cassiicola* in China and the precise identification of the causal agent will be useful for its management.

References:

- Alfieri, S. A., et al. 1994. Diseases and Disorders of Plants in Florida. Florida Department of Agriculture & Consumer Services, Division of Plant Industry, Bulletin 14.
- Carbone, I., and Kohn, L. M. 1999. Mycologia 91:553.
- Cui, Y. G., et al. 2015. Plant Dis. 99:725.
- Ellis, M. B. 1971. Page 372 in: Dematiaceous Hyphomycetes. Commonwealth Mycological Institute, Kew, U.K.
- Glass, N. L., and Donaldson, G. C. 1995. Appl. Environ. Microbiol. 61:1323.
- Mao, Y. F., et al. 2021. Plant Dis. 105:3743.
- Tang, J. R., et al. 2020. Plant Dis. 104:3056.
- White, T. J., et al. 1990. Page 315 in: PCR Protocols: A Guide to Methods and Applications. Academic Press, San Diego, CA.
- Xie, S. F., et al. 2021. Plant Dis. 105:509.

The author(s) declare no conflict of interest.

e-Xtra

Keywords: sweet viburnum, leaf spot, *Corynespora cassiicola*

[†]Indicates the corresponding authors.

F. Luan; FGLuan@jxau.edu.cn, and D. Li; jxld2008@163.com