Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Alternaria tenuissima* Causing Leaf Spot on Star Anise (*Illicium verum*) in China

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Funding: This work supported by the Scientific Research Foundation of Yulin Normal University, China (nos. 2018YJKY38 and G2019ZK49) and the Natural Science Foundation of Guangxi (2018GXNSFAA294110). Plant Dis. 105:229, 2021; published online as <https://doi.org/10.1094/PDIS-04-20-0758-PDN>. Accepted for publication 15 July 2020.

Star anise (*Illicium verum*), whose fruits are used as spice and traditional Chinese medicine, is an aromatic evergreen tree widely cultivated (about 404,000 ha) in Guangxi Zhuang Autonomous Region of China (Wang et al. 2011). However, disease is one of the major constraints limiting star anise production in this region. A survey conducted in Yulin city of Guangxi (22°35'N; 109°52'E) during 2018 to 2019 found that approximately 50% of star anise plants showed pathogen-induced symptoms. The symptoms initially appeared on leaves as small light brown spots and gradually expanded to circular or irregular water-soaked spots. Later on, spots became withered with grayish-white centers and surrounded by dark brown margins, and eventually leaves defoliated. The occurrence of the symptoms resulted in serious yield reduction and consequent economic loss. To explore the pathogen, leaves with typical symptoms, which were collected from different individual plants, were cut into small pieces (about 5 mm²), disinfected in 75% ethanol solution for 1 min, rinsed in sterile distilled water, and then transferred to Petri dishes (9 cm in diameter) containing potato dextrose agar (PDA). Cultures were incubated at 28°C in darkness for 8 days. Four fungal isolates were recovered by a single-spore isolation method. The resulting colonies were initially gray-white with loose aerial hyphae, then olive-green, and eventually turned brown with gray-white

margins. These colonies produced numerous conidia on simple conidiophores that occurred in sparsely branched chains. Conidia were oval or pyriform, brown, and ranged from 22.2 to 38.4 × 6.7 to 12.8 μm, with two to five transverse and zero to two longitudinal septa ($n = 50$). Conidial beaks, when present, were short (5 μm or less) and tapered. Based on the morphology, the fungus was identified as *Alternaria* sp. (Simmons 2007). Mycelial genomic DNA of the representative isolate GXBJ-1 was extracted as a template. Partial sequences of the internal transcribed spacer region (*ITS*) and histone 3 (*H3*) genes were amplified, according to the method of Zheng et al. (2015). The resulting fragments were sequenced and deposited in GenBank (accession nos. MN893910 for *ITS* and MN901870 for *H3* gene). BLAST analysis showed that the sequence of *ITS* was >99% identical to the corresponding sequences of *Alternaria tenuissima* in GenBank (MN593339, MN593338), and the sequence of *H3* gene shared >99% homology to the sequences of *A. tenuissima* (MK799631, MK799619). The fungus was identified as *A. tenuissima* based on phylogenetic analyses of *ITS-H3* gene sequences. Pathogenicity tests were performed by mechanical inoculation of mycelial plugs (4 mm in diameter) to healthy leaves of 1-year-old star anise plants in a greenhouse. Five plants were inoculated with each of two representative isolates. Plants inoculated with fresh PDA plugs were used as controls. All inoculated plants were maintained in a greenhouse (12 h/12 h light/dark, 25 ± 3°C, 90% relative humidity). Typical symptoms were observed on all leaves inoculated with mycelial plugs 7 days postinoculation, whereas no such symptoms were observed in the control. *A. tenuissima* was reisolated from infected leaves to fulfill Koch's postulates. The test was repeated three times with similar results. To date, *A. tenuissima* has been reported to cause leaf spot disease on many plants including lotus (*Nelumbo nucifera*) and kiwifruit (*Actinidia* spp.) in China (Li et al. 2019; Zhang et al. 2019). However, to our best knowledge, this is the first record of *A. tenuissima* causing leaf spot on star anise in China.

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The author(s) declare no conflict of interest.

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Keywords: *Alternaria tenuissima*, star anise, *Illicium verum*, China

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