Diseases Caused by Fungi and Fungus-Like Organisms

Leaf Spot Caused by *Colletotrichum fruticola* on Star Anise (*Illicium verum*) in China

Jiang Zhao,¹ Zhihe Yu,^{1,†} Qili Li,^{2,†} Lihua Tang,² Tangxun Guo,² Suiping Huang,² Jianyou Mo,² and Tom Hsiang³

¹ College of Life Sciences, Yangtze University, Jingzhou, Hubei, 434025, China

² Institute of Plant Protection, Guangxi Academy of Agricultural Sciences and Guangxi Key Laboratory of Biology for Crop Diseases and Insect Pests, Nanning, Guangxi, 530007, China

³ School of Environmental Sciences, University of Guelph, Guelph, Ontario, Canada

Funding: The research was supported by Guangxi Natural Science Foundation (grant no. 2018GXNSFBA281077) and Technology Development Fund of Guangxi Academy of Agricultural Science (grant nos. 2015JZ43 and 2015JZ56). Plant Dis. 106:1060, 2022; published online as <https://doi.org/10.1094/PDIS-04-21-0685-PDN>. Accepted for publication 12 September 2021.

Star anise (*Illicium verum*) has been cultivated for centuries in southern China. Its fruit can be used as a spice and as medicine (Wang et al. 2011). Anthracnose seriously affects yield and quality by infecting twigs, pedicels, fruit stalks, and fruits (Liao et al. 2017). Leaf spots first appear as round, water-stained, small, dark brown spots, which expand into round separated spots, then become yellowish brown with small black acervuli arranged in a circular pattern. In August 2019, four leaf spot samples of star anise were collected, two each from Shanglin and Jinxiu counties of Guangxi Province. This 8 ha area had more than 80% leaf spot incidence. Small tissue pieces (5 × 5 mm) were taken from the zone between symptomatic and healthy plant tissues, surface-disinfected in 75% ethanol for 10 s and 1% NaClO (sodium hypochlorite) for 1 min, and washed three times in sterilized distilled water. The tissues were placed on PDA and incubated at 28°C in darkness for a week. Hyphae growing from tissue pieces were subcultured onto fresh PDA. Three of the four leaves yielded cultures resembling *Colletotrichum* spp. Four fungal isolates were obtained by single-spore isolation. Isolates JX1-2 and JX1-5 were collected from Jinxiu while SL1-2 and SL2-1 were collected from Shanglin. Genomic DNA was extracted followed by PCR amplification and sequencing of the rDNA internal transcribed spacer (ITS), actin (ACT), Apn2-Mat1-2 intergenic spacer, partial mating type (Mat1-2) (ApMat), calmodulin (CAL), chitin synthase (CHS-1), and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (Weir et al. 2012). The sequences were deposited in GenBank (ITS: MW301215–18; ACT: MW348965–68; ApMat: MW348973–76; CAL: MW348957–60; CHS-1: MW348969–72; GAPDH: MW348961–64). For phylogenetic analysis, MEGAX (Kumar et al. 2018) was used to produce a maximum likelihood (ML) tree with 1,000

bootstrap replicates, based on a concatenation of the sequenced genomic regions for each of the four isolates from this study as well as sequences of other *Colletotrichum* spp. obtained from GenBank. The results revealed that isolates JX1-2, JX1-5, and SL1-2 were *C. horii*, and SL2-1 was *C. fruticola* (Weir et al. 2012). The resulting colonies were initially white with abundant aerial hyphae, and white-gray after 3 days at 28°C on PDA. Isolate SL2-1 eventually turned greenish-gray after 14 days, while the center of *C. horii* isolates turned iron-gray with white-gray margins. Both *Colletotrichum* spp. had hyaline conidia that were terete, smooth, apex obtuse, base truncate, and no significant differences ($P > 0.05$) in size between *C. horii* (10.5 to 33.6 × 3.6 to 9.3 μm) ($n = 300$) and *C. fruticola* (13.1 to 16.2 × 4.7 to 7.1 μm) ($n = 100$). Pathogenicity tests were conducted in the greenhouse on 1-year-old star anise seedlings and performed with a conidial suspension (10 μl of 10⁶ conidia/ml) containing 0.1% Tween 20 placed onto lightly wounded sites on healthy leaves. Sterilized toothpicks gently scratched the surface without piercing the leaf. Each isolate was inoculated onto three seedlings, with at least eight leaves per seedling inoculated in two spots after light wounding. Controls were inoculated with water containing 0.1% Tween 20. All inoculated seedlings were maintained in the greenhouse (12/12 h light/dark, 25 ± 2°C) and covered with plastic bags to maintain humidity. Wound sites inoculated with *C. horii* darkened to greenish-brown after 24 h, and *C. fruticola* gave similar symptoms after 36 h. Then the wounds turned to light brown round spots, and after 5 days, the spots expanded to water-stained spots with dots of acervuli arranged in a circular pattern. No symptoms were observed on controls. Each fungal isolate was consistently reisolated from inoculated leaves, fulfilling Koch's postulates. There were significant differences ($P < 0.05$) in aggressiveness between the two species, with *C. horii* showing larger diameter lesions (10.2 mm) than *C. fruticola* (8.4 mm). Anthracnose of star anise caused by *C. horii* (Liao et al. 2017) and *C. coccodes* (Wu et al. 2003) has been previously reported in China; however, to our knowledge, this is the first report of *C. fruticola* infecting star anise in China. This study may provide reference for further epidemiological study and prevention of anthracnose on star anise.

References:

- Kumar, S., et al. 2018. Mol. Biol. Evol. 35:1547.
- Liao, W. J., et al. 2017. Southwest China J. Agric. Sci. 30:2242.
- Wang, G. W., et al. 2011. J. Ethnopharmacol. 136:10.
- Weir, B. S., et al. 2012. Stud. Mycol. 73:115.
- Wu, Y. J., et al. 2003. Guangxi For. Sci. 3:118.

The author(s) declare no conflict of interest.

e-Xtra

Keywords: *Illicium verum*, anthracnose, pathogenicity

[†]Indicates the corresponding authors.

Z. H. Yu; zhiheyu@hotmail.com, and Q. L. Li; 65615384@qq.com