Diseases Caused by Fungi and Fungus-Like Organisms

First Report of Leaf Spot on American Sweetgum (*Liquidambar styraciflua*) Caused by *Corynespora cassiicola* in China

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American sweetgum (*Liquidambar styraciflua* L.) is a forest plant native to North America, which has been introduced into other countries due to its ornamental and medicinal values. In June 2019, symptoms of leaf spot on sweetgum were observed in a field (5 ha) located in Xuzhou, Jiangsu Province, China. In this field, approximately 45% of 1,000 trees showed the same symptoms. Symptoms were observed showing irregular or circular dark brown necrotic lesions approximately 5 to 15 mm in diameter with a yellowish margin on the leaves. To isolate the pathogen, diseased leaf sections (4 × 4 mm) were excised from the margin of the lesion, surface-sterilized with 0.1% NaOCl for 90 s, rinsed four times in sterile distilled water, air dried, and transferred to potato dextrose agar (PDA) medium at 25°C in the dark. Pure cultures were obtained by monospore isolation after subculture. Ten purified isolates, named FXI to FXR, were transferred to fresh PDA and incubated as above to allow for morphological and molecular identification. After 7 days, aerial mycelium was abundant, fluffy, and exhibited white to grayish-green coloration. The conidia were dark brown or olive, solitary or produced in chains, obclavate, with one to 15 pseudosepta, and measured 45 to 200 μm × 10 to 18 μm. Based on morphological features, these 10 isolates were identified as *Corynespora cassiicola* (Ellis 1971). Genomic DNA of each isolate was extracted from mycelia using the cetyltrimethylammonium bromide (CTAB) method. The EF-1α gene and ITS region were amplified and sequenced with the primer pairs rDNA ITS primers (ITS4/

ITS5) (White et al. 1990) and EF1-728F/EF-986R (Carbone and Kohn 1999), respectively. The sequences were deposited in GenBank. BLAST analysis revealed that the ITS sequence had 99.66% similarity to *C. cassiicola* MH255527 and that the EF-1α sequence had 100% similarity to *C. cassiicola* KX429668A. Maximum likelihood phylogenetic analysis based on EF-1α and ITS sequences using MEGA 7 revealed that 10 isolates were placed in the same clade as *C. cassiicola* (isolate: XQ3-1; accession numbers: MH572687 and MH569606, respectively) at 98% bootstrap support. Based on the morphological characteristics and phylogenetic analyses, all isolates were identified as *C. cassiicola*. For the pathogenicity test, a 10 μl conidial suspension (1 × 10⁵ spores/ml) of each isolate was dripped onto healthy leaves of 2-year-old sweetgum potted seedlings. Leaves inoculated with sterile water served as controls. Three plants (three leaves per plant) were used for each treatment. The experiment was repeated twice. All seedlings were enclosed in plastic transparent incubators to maintain high relative humidity (90 to 100%) and incubated in a greenhouse at 25°C with a 12-h photoperiod. After 10 days, leaves inoculated with conidial suspension of each isolate showed symptoms of leaf spots, similar to those observed in the field. Control plants remained healthy. In order to reisolate the pathogen, surface-sterilized and monospore isolation was conducted as described above. The same fungus was reisolated from the lesions of symptomatic leaves, and its identity was confirmed by molecular and morphological approaches, thus fulfilling Koch's postulates. Chlorothalonil and boscalid can be used to effectively control *Corynespora* leaf spot (Chairin et al. 2017). To our knowledge, this is the first report of leaf spot caused by *C. cassiicola* on *L. styraciflua* in China.

References:

- Carbone, I., and Kohn, L. M. 1999. Mycologia 91:553.
Chairin, T., et al. 2017. Australas. Plant Dis. Notes 12:16.
Ellis, M. B. 1971. Dematiaceous Hyphomycetes. Commonwealth Mycological Institute, Kew, U.K.
White, T. J., et al. 1990. Page 315 in: PCR Protocols: A Guide to Methods and Applications. Academic Press, San Diego, CA.

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