Diseases Caused by Fungi and Fungus-Like Organisms

First Report of Leaf Spot Disease Caused by *Stemphylium eturmiunum* on American Sweetgum (*Liquidambar styraciflua*) in China

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American sweetgum (*Liquidambar styraciflua* L.) is an important tree for landscaping and wood processing. In recent years, leaf spots on American sweetgum with disease incidence of about 53% were observed in about 1,200 full-grown plants in a field (about 8 ha) located in Pizhou, Jiangsu Province, China. Initially, dense reddish-brown spots appeared on both old and new leaves. Later, the spots expanded into dark brown lesions with yellow halos. Symptomatic leaf samples from different trees were collected and processed in the laboratory. For pathogen isolation, leaf sections (4 × 4 mm) removed from the lesion margin were surface sterilized with 75% ethanol for 20 s and then sterilized in 2% NaOCl for 30 s, rinsed three times in sterile distilled water, and incubated on potato dextrose agar (PDA) at 25°C in the darkness. After 5 days of cultivation, the pure culture was obtained by single-spore separation. Six isolate samples from different leaves named FXA1 to FXA6 shared nearly identical morphological features. The isolate FXA1 (codes CFCC 54675) was deposited in the China Center for Type Culture Collection. On the PDA, the colonies were light yellow with dense mycelium, rough margin, and reverse brownish yellow. Conidiophores (23 to 35 × 6 to 10 μm) (*n* = 60) were solitary, straight to flexuous. Conidia (19 to 34 × 10 to 21 μm) (*n* = 60) were single, muriform, oblong, mid- to deep brown, with one to six transverse septa. These morphological characteristics resemble *Stemphylium eturmiunum* (Simmons 2001). Genomic DNA was extracted from mycelium following the CTAB method. The ITS region, *gapdh*, and *cmdA* genes were amplified and sequenced with the primers ITS5/ITS4

(Woudenberg et al. 2017), *gpd1/gpd2* (Berbee et al. 1999), and CALDF1/CALDR2 (Lawrence et al. 2013), respectively. A maximum likelihood phylogenetic analysis based on ITS, *gapdh*, and *cmdA* (accession nos. MT898502 to MT898507, MT902342 to MT902347, and MT902336 to MT902341) sequences using MEGA 7.0 revealed that the isolates were placed in the same clade as *S. eturmiunum* with 98% bootstrap support. All seedlings for pathogenicity tests 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. For pathogenicity testing, the conidial suspension (10⁵ spores/ml) of each isolate was sprayed respectively onto healthy leaves of *L. styraciflua* potted seedlings (2 years old, three replicate plants per isolate). As a control, three seedlings were sprayed with sterile distilled water. After 7 days, dense reddish-brown spots were observed on all inoculated leaves. In another set of tests, healthy plants (three leaves per plant, three replicate plants per isolate) were wound inoculated with mycelial plugs (4 × 4 mm) and inoculated with sterile PDA plugs as a control. After 7 days, brown lesions with a light yellow halo were observed on all inoculation sites with the mycelial plugs. Controls remained asymptomatic in the entire experiment. The pathogen was reisolated from symptomatic tissues and identified as *S. eturmiunum* but was not recovered from the control. The experiment was repeated twice with the similar results, fulfilling Koch's postulates. *S. eturmiunum* had been reported on tomato (Andersen et al. 2004), wheat (Poursafar et al. 2016), and garlic (Fu et al. 2019) but not on woody plant leaves. To our knowledge, this is the first report of *S. eturmiunum* causing leaf spot on *L. styraciflua* in the world. This disease poses a potential threat to American sweetgum and wheat in Pizhou.

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