Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Stemphylium lycopersici* Causing Leaf Spot on *Hevea brasiliensis* in China

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Hevea brasiliensis is widely grown in the tropics and subtropics and is the main source of natural rubber. It is plagued by various leaf diseases that decrease rubber production. In January to March 2020, severe leaf spot disease on *H. brasiliensis* was widespread in the Agricultural Science Base of Haidian Campus of Hainan University (20°03'31" N, 110°19'07" E), Haikou, Hainan Province, China. Spots were seen on mature green leaves rather than young, bronze leaves. This symptom has never been seen on *H. brasiliensis* leaves before. Initially, gray leaf spots were concentrated on leaf edges. Then, spots expanded, forming irregular gray lesions with chlorotic edges. Eventually, lesions of some leaves turned black with a perforated center, and leaves curled, wilted, and dropped. Five small pieces were cut from the margin of spots on different leaves and surface disinfected with 75% alcohol three times for 5 s and 1% NaOCl for 60 s. After washing twice with sterile water, pieces were put in the center of plates of potato dextrose agar (PDA) medium and incubated for 1 week at 28°C. After 7 days, mycelium appeared in plates and was purified by single-spore culture for further study. One strain, HN01, showed yellowish-brown to reddish-brown pigment on PDA, and colonies were gray and cottony. These features were very consistent with *Stemphylium* sp. (Li et al. 2017). Conidiophores were solitary, transparent to pale, mostly 102.1 to 228.8 × 4.0 to 5.8 µm, and had two to three septa and apical vesicular swellings (6.5 to 7.9 µm). Conidia were 28.3 to 45.1 × 11.5 to 17.5 µm with one septum. Conidia of *Stemphylium lycopersici* were solitary, oblong with a conical end at the apex, had one to two septa, and were constricted at

the transverse septum. The internal transcribed spacer region of rDNA was amplified with primers ITS1/ITS4 (5'-TCCGTAGGTGAACCTGCGG-3'/5'-TCCTCCGCTTATTGATATGC-3'), glyceraldehyde-3-phosphate dehydrogenase (gpd) was amplified with primers GPD-F/R (5'-GCACCGACCA CAAAAATC-3'/5'-GGGCCGTCAACGACCTTC-3'), and the calmodulin region (cmdA) was amplified with primers CALDF1/CALDR2 (5'-AGCAA GTCTCCGAGTTCAGG-3'/5'-CTTCTGCATCATCAYCTGGACG-3') from genomic DNA of HN01 (Xie et al. 2019), and PCR products were sequenced. The ITS sequence of HN01 (GenBank no. MZ496930) showed 99.64% identity to isolates sl001, sl110, sl111, and sl112 of *S. lycopersici* (GenBank nos. KX858848.1, MF480547.1, MF480548.1, and MF480549.1). The gpd sequence (GenBank no. MZ505106) showed 100% identity to strain xiqing, HZ2114, and HZ2115 of *S. lycopersici* (GenBank no. KR911809.1, KR911810.1, KT957742.1, and KT957743.1). The cmdA sequence (GenBank no. MZ505105) showed 99.85% identity to *S. lycopersici* strain LJ1609270201 (GenBank no. MG742412.1). A phylogenetic analysis constructed by MEGA6.0 based on concatenated sequences of HN01 and 17 strains from GenBank using the maximum-likelihood method showed that HN01 was clustered and matched with *S. lycopersici* LJ1609270201. To satisfy Koch's postulates, we inoculated hypha cakes (diameter = 5 mm) of HN01 on mature green leaves of *H. brasiliensis*. All leaves were wrapped in a freezer bag to maintain >85% relative humidity in a 28°C greenhouse. The hypha cakes were removed after 24 h. The disease appeared in the inoculated group after 2 to 3 days but not in the control group. We used the same method as before to reisolate the pathogen, which had the same morphology and pigmentation on PDA as the original isolate. *S. lycopersici* has been reported to infect leaves of plants including pepper, tomato, eggplant, watermelon, and *Physalis alkekengi* (Ben et al. 2021; Yang et al. 2017, 2020). To our knowledge, this is the first record of *S. lycopersici* causing leaf spot of *H. brasiliensis* in China, and *H. brasiliensis* is a new global host of *S. lycopersici*. *H. brasiliensis*, the main source of natural rubber, is widely grown in southern China. Thus, it is warranted to implement disease management measures to prevent potential threats.

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