

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

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Hevea brasiliensis is widely planted in tropical and subtropical regions and is the main source of natural rubber production. The growth of rubber trees is plagued by various leaf diseases, resulting in decreased rubber production. From January to March in 2020, a severe leaf spots disease on *Hevea brasiliensis* found in Agricultural Science Base in Haidian campus of Hainan University (20° 03' 31" N, 110° 19' 07" E), Haikou, Hainan province, China. Spots were only observed on the mature green rather than young and bronze-colored leaves. This symptom has never been reported on the leaves of *Hevea brasiliensis*. During the early stages of the disease, gray leaf spots were concentrated to the leaf margins, but later expanded forming irregular gray lesions with chlorotic edges (Figure 1A). Eventually, lesions became necrotic shot holed, and leaves curled, wilted, and dropped. Five small pieces were cut from the margin of spots from different infected leaves, and were surface disinfected with 75% alcohol three times for five seconds each time and 1% sodium hypochlorite solution (NaClO) for 60 s. After washing twice with sterile water, leaf pieces were placed in

the center of plates with Potato Dextrose Agar (PDA) medium and incubated for one week at 28 °C. After 7 days, mycelium developed and colonies were single-spore cultured for further study. One of the strains labeled HN01 developed a yellowish-brown to reddish-brown pigment on PDA, and the colonies were gray and cottony. The colony and pigment feature very consistent with *Stemphylium* sp. (Figure 2) (Li et al. 2017). Conidiophore were solitary, transparent to pale, mostly 102.1-228.8 $\mu\text{m} \times$ 4.0-5.8 μm , with 2-3 septa and apical vesicular swellings 6.5-7.9 μm . The dimensions of conidia were 28.3-45.1 \times 11.5-17.5 μm and one septum (Figure 3). Conidia of *S. lycopersici* were solitary, oblong with a conical end at the apex, with 1-2 septa, and 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'-GCACCGACCACAAAATC-3'/5'-GGGCCGTCAACGACCTTC-3'), calmodulin region (cmdA) was amplified with the primers CALDF1/CALDR2 (5'-AGCAAGTCTCCGAGTTCAAGG-3'/5'-CTTCTGCATCATCAYCTGGACG3') from genomic DNA of strain HN01 (Xie et al. 2018), and PCR products were sequenced. The ITS sequence of strain HN01 (GenBank Accession No. MZ496930) had 99.64% identity with isolates sl001, sl110, sl111, and sl112 of *Stemphylium lycopersici* (GenBank Accession No. KX858848.1, MF480547.1, MF480548.1, MF480549.1). Similarly GPD sequences (GenBank Accession No. MZ505106) had 100% identity with strain xiqing, HZ2114 and HZ2115 of *Stemphylium lycopersici* (GenBank Accession No. KR911809.1, KR911810.1, KT957742.1 and KT957743.1), and CMDA sequences (GenBank Accession No. MZ505105) had 99.85% identity with *Stemphylium lycopersici* strain LJ1609270201 (GenBank Accession No. MG742412.1). A phylogenetic analysis constructed by MEGA6.0 based on concatenated sequences of the HN01 and another 17 strains from GenBank by using the maximum-likelihood (ML) method showed that the HN01 was clustered and matched with *Stemphylium lycopersici* LJ1609270201 (Figure 4).

To satisfy Koch's postulates, we inoculated mature green leaves of *Hevea brasiliensis*

with mycelial plugs (diameter = 5 mm) of pure cultured strain HN01. All leaves of *Hevea brasiliensis* were wrapped in a freezer bag to maintain relative humidity >85%, and the temperature of greenhouse is 28°C. The disease developed on the inoculated leaves after 2-3 days, but not on control leaves (Figure 1B). We used the same method as before to re-isolate the pathogen, which had the same morphology and genotypes as the original isolate.

S. lycopersici has been reported to infect the leaves of a variety of plants, including pepper, tomato, eggplant, watermelon, *Physalis alkekengi*. (Yang et al. 2017; Ben et al. 2017; Yang et al. 2020). To our knowledge, this is the first record of *S. lycopersici* causing leaf spot of *Hevea brasiliensis* in China, and *Hevea brasiliensis* is the global new host of *S. lycopersici*. *Hevea brasiliensis* is the main source of natural rubber and is widely planted in southern China. Therefore, it is imperative to implement disease management measures to prevent potential threats.

References:

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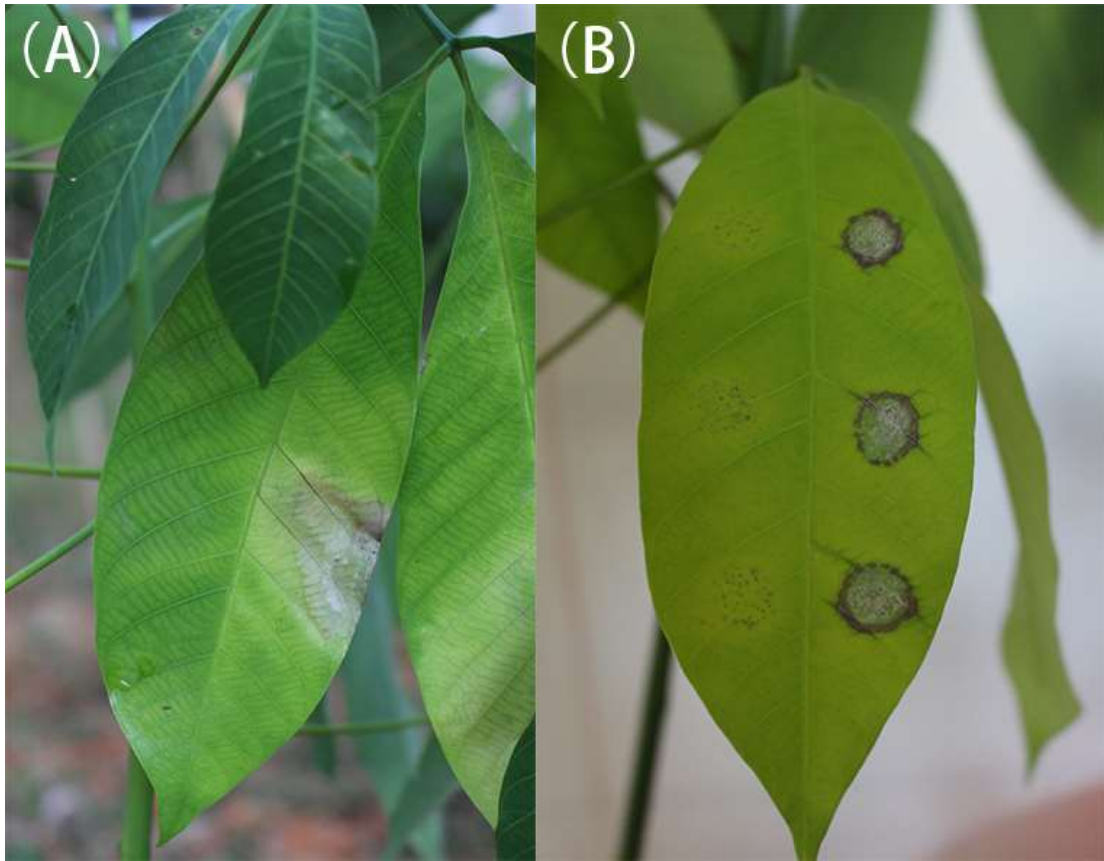


Figure 1. Symptoms of leaf spot on *Hevea brasiliensis* leaves. (A) Natural infection. (B) Inoculated on *Hevea brasiliensis* leaf (right of the leaf) and blank control (left of the leaf).

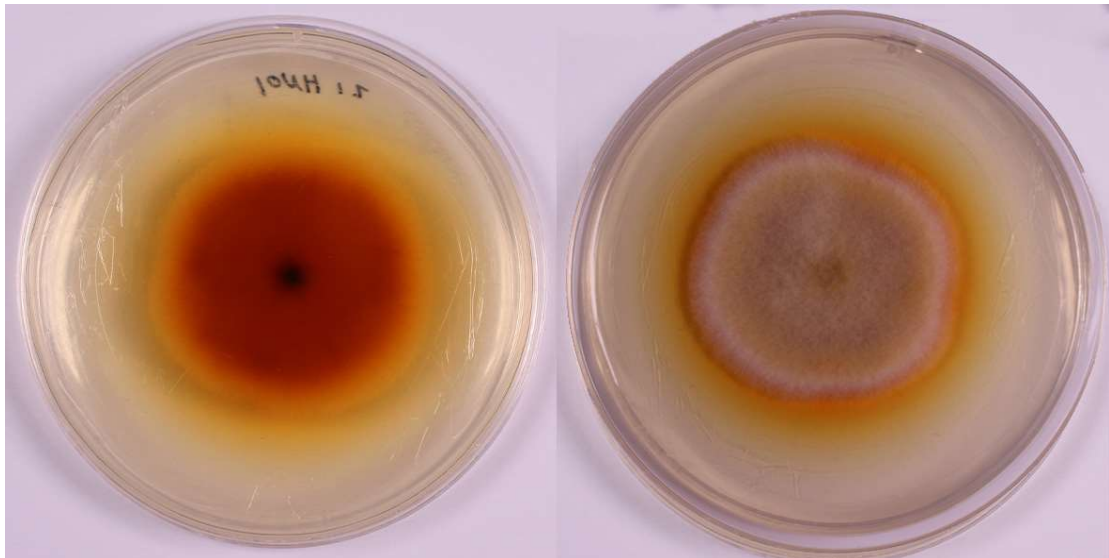


Figure 2. Colony morphology and pigmentation on PDA.



Figure 3. Microscopic observation of *Stemphylium lycopersici*. (A) Microscopic structures of conidiophores. (B) Single conidia. Bar = 50 μm

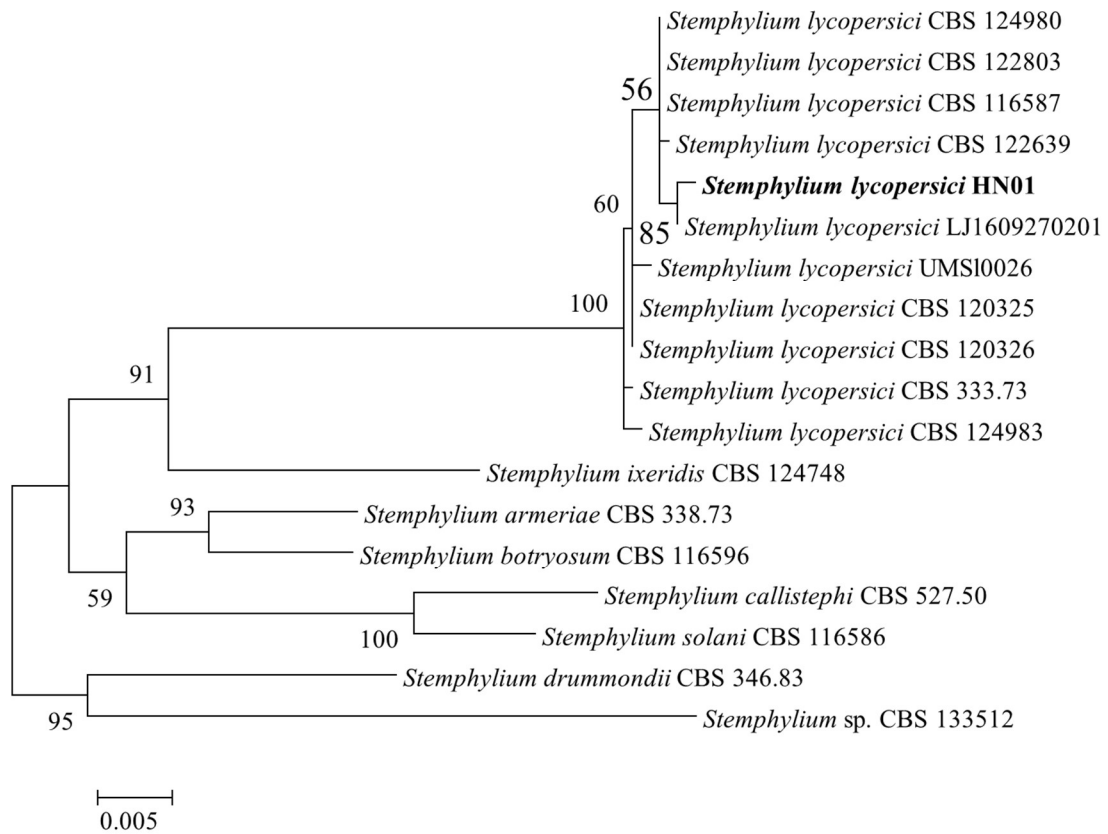


Figure 4. Polygene tandem phylogenetic tree derived from the concatenated sequences of the ITS, GPD, CAL using MEGA6.0.