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NEW DISEASE REPORT

First report of secondary leaf fall in rubber trees caused by *Phyllosticta capitalensis* in the Eastern Plains of Colombia

A. Guevara[1] | **M. López**[1] | **F. Rivano**[2] | **O. Castro**[1]

[1]Corporación Colombiana de Investigación Agropecuaria (Agrosavia), C.I. La Libertad, Km 17 vía Puerto López, Villavicencio, Meta, Colombia

[2]Centre de Coopération Internationale en Recherche Agronomique pour le Développement (CIRAD), Convenio Agrosavia, C.I. La Libertad, Colombia

Correspondence
O. Castro, Corporación Colombiana de Investigación Agropecuaria (Agrosavia). C.I. La Libertad, Km 17 vía Puerto López, 500008, Villavicencio, Meta, Colombia.
Email: omcastro@agrosavia.co

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Hevea brasiliensis, leaf spot

Rubber trees (*Hevea brasiliensis*) are planted on 65,545 hectares in Colombia (Minagricultura, 2021). Since September 2020, unusual leaf spots were observed on mature leaves of rubber trees (clones RRIM 600 and FX 3864), in three plantations in the Eastern Plains region. The regular spots (usually 1–3 per leaf) were up to 8 mm in diameter, with a clearly defined brownish to blackish margin and a necrotic centre (Figure 1). The disease led to secondary leaf fall, causing up to 30% defoliation.

Diseased leaves were collected and transferred to the laboratory for diagnosis. After surface disinfection with ethanol (70%) and sodium hypochlorite (2%), 0.5 cm^2 sections of diseased tissue were transferred to potato dextrose agar (PDA) medium and incubated at 28°C for two weeks. The developing colonies had irregular borders and gradually turned dark greyish-green in colour, then black (Figure 2a). Sporulation was induced on a parboiled rice medium (Figure 2b) under controlled conditions (12 hr photoperiod, relative humidity 50%, 26 ±2°C). Pycnidia with conidia were observed after 44 days. Conidia (Figure 3a) were small, single-celled, hyaline, ovoid to elongate, measuring 8–11×5-6 μm. Pycnidia were dark, ostiolate and lenticular to globose (Figure 3b). These morphological characters were consistent with the descriptions of *Phyllosticta* species (Wikee et al., 2013).

FIGURE 1 Disease symptoms on mature rubber leaves (clone FX 3864) in the Eastern Plains region, Colombia

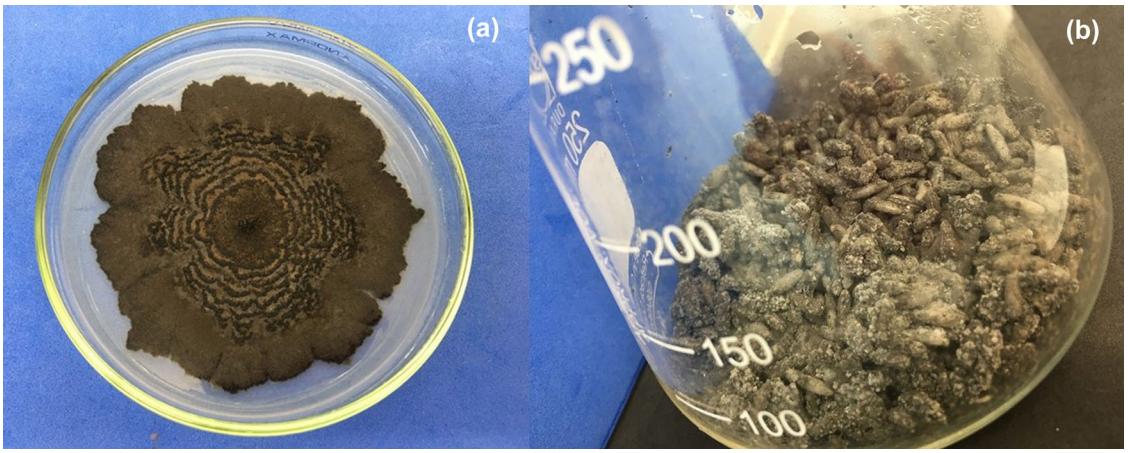

FIGURE 2 *Phyllosticta capitalensis* isolate grown in culture: (A) a 45-day-old colony on potato dextrose agar, and (B) sporulation on parboiled rice

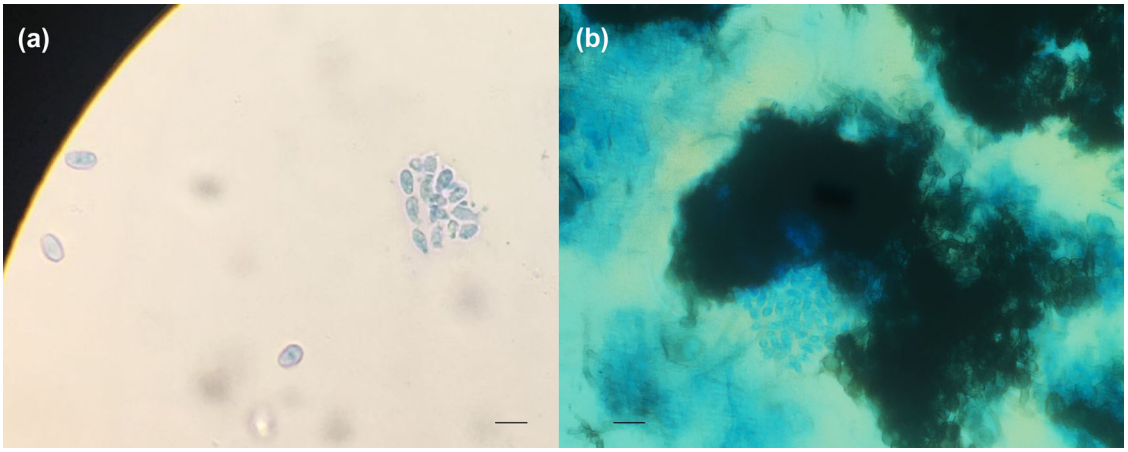

FIGURE 3 Morphological characters of *Phyllosticta capitalensis*: (A) conidia, and (B) pycnidium with conidia. Scale bars = 10 μm

Two isolates (14_SAT and 15_SAT) were deposited in the Agrosavia fungi collection and used for DNA-based identification. The ITS, TEF1 and ACT genes were amplified and sequenced using primers ITS5/ITS4 (White et al., 1990), EF1-728F/EF1-986R and ACT-512F/ACT-783R (Carbone & Kohn, 1999), respectively. BLASTn analysis showed that the ITS (GenBank Accession Nos. OL898551 and OL957043) had 100% identity with *P. capitalensis* strain CBS128856 (OL957169), ACT (OM782674 and OM782675) had 100% identity with *P. capitalensis* isolate CPC25327 (KY855640), and TEF1 (OM782672 and OM782673) had 100% identity with *Guignardia mangiferae* (syn. *P. capitalensis*) strain CBS 100175 (FJ538378). A multilocus phylogenetic analysis revealed isolates clustered together in a clade with reference strains of *P. capitalensis* (Figure 4).

For inoculation studies, *H. brasilisensis* seedlings of variety RRIM 600 were transferred to a growth chamber (24 hr dark initially, thereafter 12 hr light/dark; 26°C; relative humidity >90%). The abaxial surface of three healthy stage B leaves were sprayed with a conidial suspension of isolate 14_SAT (1×10^6 spores ml ^{1}), while three control leaves were sprayed with sterile water. The experiment was repeated after one month with the 15_SAT isolate. Chlorotic lesions were observed 48 hours after inoculation and necrotic spots appeared after five days. The pathogen was successfully re-isolated on PDA medium and morphologically confirmed as *P. capitalensis*. No symptoms were observed on the control leaves.

Phyllosticta capitalensis has been identified previously as a rubber foliar pathogen in Sri Lanka (Herath et al., 2019). Narayanan & Reju

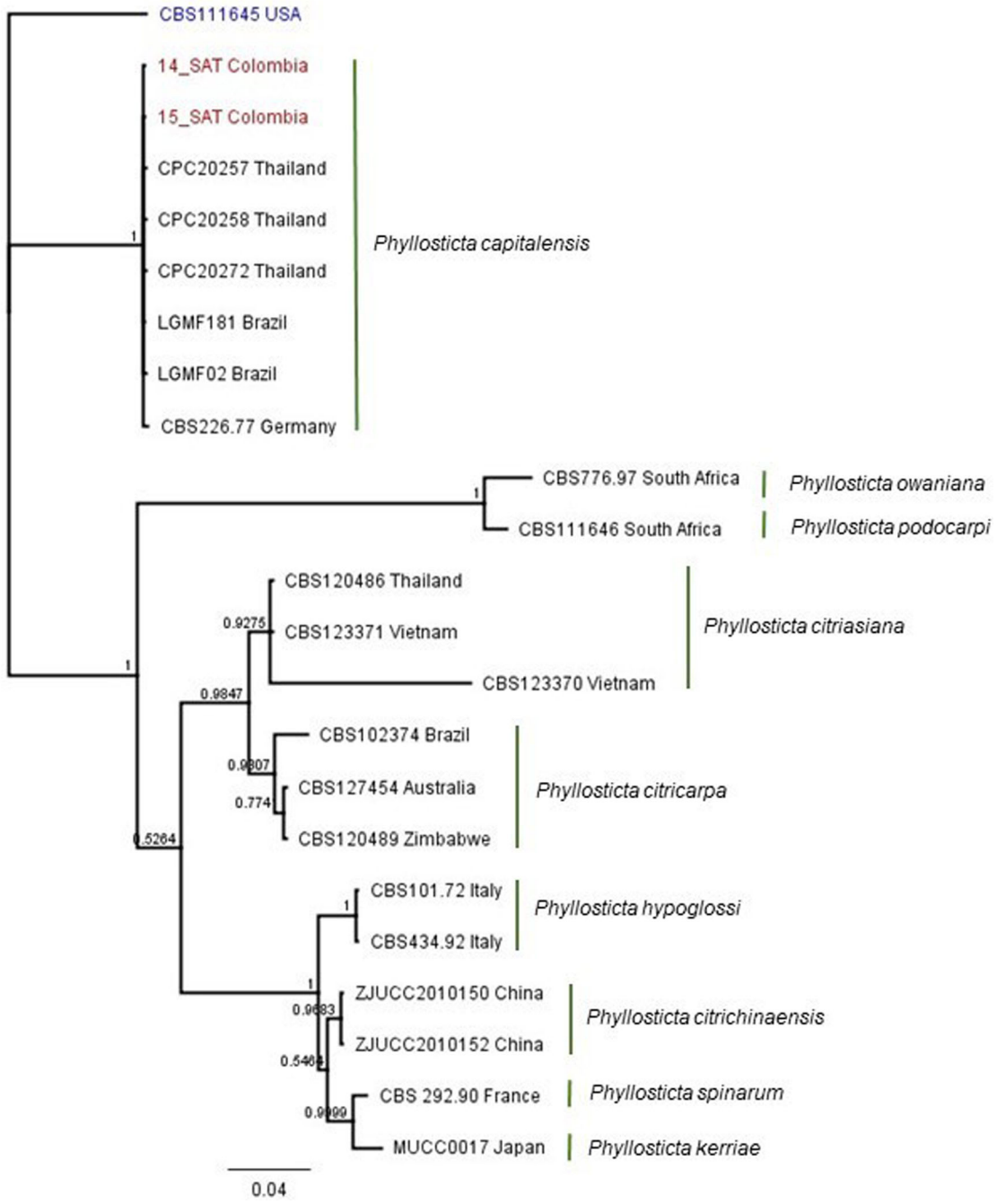

FIGURE 4 Phylogenetic analysis of *Phyllosticta* species based on a combined dataset of ITS, ACT and TEF1 gene regions using MEGA X. The analysis was conducted using the maximum likelihood method bootstrapped at 1,000 replicates. Isolates from this study are shown in red. *Guignardia bidwellii* CBS111645 was used as an outgroup

(2020) isolated the fungus from rubber leaves in India showing the same symptoms as reported here. This is the first report of *P. capitalensis* causing secondary leaf fall in rubber plantations in Colombia. Further monitoring of this new disease is required to determine its impact.

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