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NEW DISEASE REPORT

First report of *Lasiodiplodia theobromae* causing *Paulownia elongata* dieback in Costa Rica

M. Rodríguez-Solís[1] | **M. Guevara-Bonilla**[1] | **D. Méndez-Álvarez**[1] | **E. Esquivel-Segura**[2]

[1]School of Forest Engineering, Instituto Tecnológico de Costa Rica, Cartago, Costa Rica

[2]School of Agricultural Engineering, Instituto Tecnológico de Costa Rica, San Carlos, Costa Rica

Correspondence
M. Rodríguez-Solís, School of Forest Engineering, Instituto Tecnológico de Costa Rica, Cartago, Costa Rica. Email: maria.rodriguez@itcr.ac.cr

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Paulownia species (Paulowniaceae) are known for their great economic potential due to their rapid growth and short rotations for timber production. In Costa Rica, *P. elongata* has been planted extensively since 2016 to produce wood for sawmilling and as a shade tree for coffee crops.

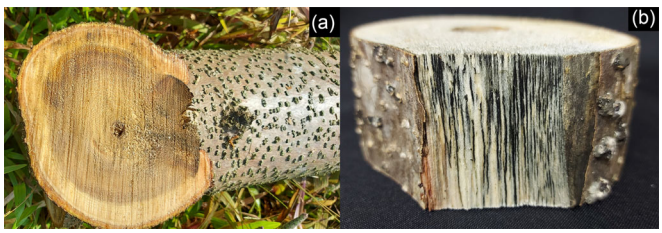


FIGURE 1 Symptoms of *Lasiodiplodia theobromae* on *Paulownia elongata*: a) radial cut with a stain on the wood; b) tangential cut of the stained wood

In the rainy season of 2023, three two-year-old *P. elongata* trees, 12 cm in diameter, showing symptoms of die-back were observed in San Carlos, Costa Rica (10° 26 37.91 N and 84° 16 16.52 W). When the trees were cut down, the wood had a black stain (Figure 1). Segments of 1.5 cm were taken from the border zone between healthy and discoloured wood. The segments were surface sterilised and placed on potato dextrose agar (PDA) medium and incubated at 25°C for seven days with a 12-hour photoperiod. After incubation, hyphal tips were transferred to new PDA medium to obtain individual colonies. Colonies growing on PDA had abundant aerial, cottony mycelium. The

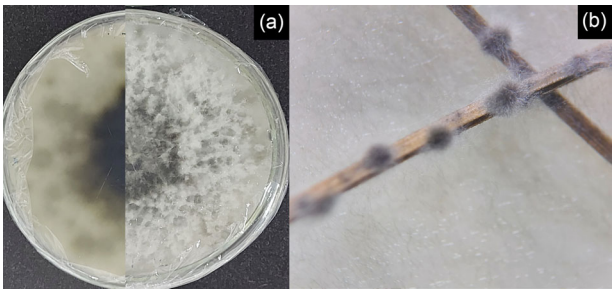


FIGURE 2 Colony and pycnidia of *Lasiodiplodia theobromae* on *Paulownia elongata*: a) colony on potato dextrose agar pictured from the bottom (left) and top (right) of the plate; b) conidiomata (pycnidia) on pine needles

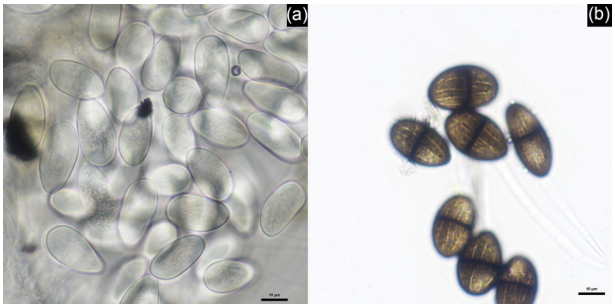


FIGURE 3 Morphological characteristics of conidia of *Lasiodiplodia theobromae* on *Paulownia elongata*: a) immature conidia; b) mature conidia. Bar = 10 μm

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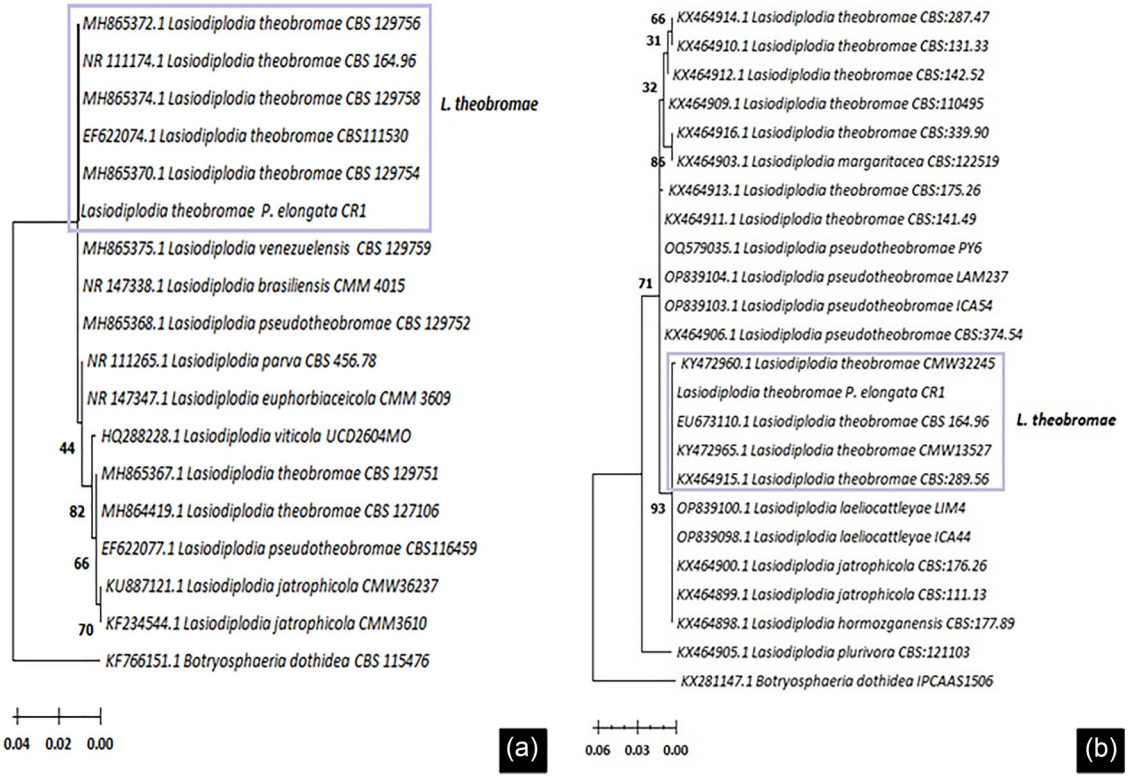


FIGURE 4 Maximum likelihood trees based on a) ITS and b) beta-tubulin DNA sequences of representative isolates of *L. theobromae* and other species of Botryosphaeriales. Inference of phylogeny was conducted in MEGA 11 (Tamura et al., 2021) using the Kimura 2-parameter model. The isolate from this study (CR1) is highlighted in each tree. *Botryosphaeria dothidea* was used as an outgroup. Bootstrap values (1,000 replications) are indicated on the nodes Scale bar indicates number of substitutions per site

colonies were initially white, darkening from the centre to the edge after the fourth day (Figure 2a). Black conidiomata (pycnidia) were globose and covered with grey mycelium (Figure 2b) when formed on *Pinus* sp. needles. Immature, unicellular, hyaline, ellipsoidal to oblong conidia with thick walls were observed. Conidia measured 23–28 × 13–16 µm (mean 26 ± 1.3 × 14 ± 0.7 µm) (Figure 3a). Mature conidia were bicellular, dark brown with transverse lines (Figure 3b). The size range and shape of these conidia is consistent within those reported by Alves et al. (2008) and Huda-Shakirah et al. (2022) for *Lasiodiplodia theobromae*.

Molecular identification was performed using DNA extracted directly from the mycelium, followed by PCR with primers for the internal transcribed spacer (ITS) and beta-tubulin gene (tub2). The ITS (Genbank Accession No. OR490957) and beta-tubulin (PQ682537) sequences had 99% and 100% identity with *Lasidioplodia theobromae* (MH793582 and LC468783, respectively). Based on maximum-likelihood phylogenetic inference of the ITS and beta-tubulin DNA sequences, the *Lasidioplodia theobromae* isolate from this study (CR1) clustered with other *L. theobromae* isolates for both genes (Figure 4).

A pathogenicity test was done on five three-month-old *P. elongata* plants. The collar area was disinfected with 70% ethanol and a wound was made with a 6-mm diameter punch, 10 cm from the base. A mycelium disc was placed on the cambium exposed by the wound and sealed with plastic film. Five plants were inoculated with sterile PDA as controls. All plants were kept in a greenhouse with daily watering. Lesions measuring 50.4 and 4.5 mm (mean) were observed on the plants inoculated with *L. theobromae* and on the controls, respectively, 22 days after inoculation. These results are similar to those reported by Biju et al. (2021) on nutmeg saplings in India. Subsequently, to comply with Koch's postulates, the fungus was re-isolated from the inoculated plants and identified based on morphology.

Lasiodiplodia theobromae is a widespread species found in tropical and subtropical countries on about 500 hosts, mainly woody species. The fungus can produce various symptoms associated with dieback including general decay, spots on branches and trunks of trees, branch dieback, necrotic lesions and necrosis of vascular tissues (Mohali et al., 2005; Biju et al., 2021). In Costa Rica, *L. theobromae* is reported to have damaged teak plantations (Méndez-Álvarez et al., 2019) and other agricultural crops. To our knowledge, this is the first report of *L. theobromae* causing dieback symptoms and wood staining on *P. elongata* in Costa Rica and globally.

ORCID

M. Rodríguez-Solís https://orcid.org/0000-0002-4605-6152

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