



NEW DISEASE REPORT

Branch die-back of *Cojoba arborea* caused by *Lasiodiplodia brasiliensis*

L. da Mata¹ | J. Garlet¹ | L. Eburneo¹ | V. A. Morais¹ | C. Gonzatto Maciel² ¹Faculdade de Ciências Biológicas e Agrárias da Universidade do Estado de Mato Grosso – Campus de Alta Floresta, Mato Grosso, Mato Grosso, Brazil²Departamento de Horticultura e Silvicultura, Faculdade de Agronomia Universidade Federal do Rio Grande do Sul, Porto Alegre, Rio Grande do Sul, Brazil

Correspondence: C. Gonzatto Maciel, Departamento de Horticultura e Silvicultura, Faculdade de Agronomia Universidade Federal do Rio Grande do Sul, Porto Alegre, Rio Grande do Sul, Brazil. Email: caciaraconzatto@gmail.com

KEYWORDS

Botryosphaeriaceae, *Lasiodiplodia* sp., urban afforestation

In June 2023, *Cojoba arborea* trees in urban areas of Alta Floresta county, Mato Grosso State, Brazil showed symptoms of branch dieback, including leaf discolouration and dark-coloured staining of the branches (Figs. 1–2). These symptoms were observed in c. 50 % of the 158 trees surveyed.

Branches were surface sterilised with 70% alcohol and 1.5% sodium hypochlorite, rinsed with sterile water, and small sections of wood taken from the leading edge of lesions and plated onto potato dextrose agar (PDA). The resulting fungi were transferred to new PDA plates and incubated at 25 ± 2°C for 21 days. Isolates had colonies and conidia consistent with members of the *Lasiodiplodia* genus. Immature conidia were single-celled, ellipsoidal and hyaline while mature conidia were two-celled, had a central septum, and were dark brown (Fig. 3). The size of mature conidia (n = 45) were 9.91–21.02 × 17.41–28.15 µm (mean = 12.2 × 24.4 µm).

Primer pairs ITS1/ ITS4 and EF1-T/ EF1-567R were used to amplify three regions, ITS1.8S, ITS2 and elongation factor-1α (TEF 1-α) of one isolate (ISO1). BLASTn analysis showed 98% and 96 % identity for ITS and TEF 1-α, respectively, with reference sequences of various isolates of *Lasiodiplodia brasiliensis*. (ITS: GenBank with Accession Nos MW274145.1 and MF436021.1; TEF 1-α: OR271542.1 and OR271539.1). Based on the morphological and molecular analysis, the isolate was identified as *L. brasiliensis* and sequences were deposited in GenBank with Accession Nos. PQ778067 (ITS) and PV405281

(TEF 1-α). A phylogenetic tree based on neighbour-joining analysis of sequences derived from region TEF 1-α was produced (Fig. 4), based on 1,000 replicates of bootstrap by *Lasiodiplodia* from *Cojoba arborea* (LBCA1*) and sequences of the same genus obtained from Rathnayaka et al. (2023).

Twelve four-month-old plants (c. 35 cm in height) of *C. arborea* were inoculated with mycelial plugs from the margins of 10-day-old cultures of three *L. brasiliensis* isolates. Stems were drilled with a sterile 5-mm borer and a mycelial plug was placed into the wound and sealed with plastic film. Sterile PDA was inserted as a negative control in 12 replicates. After inoculation, plants were kept at 25°C in a growth room with a 12/12 hour light/dark cycle and 56% relative humidity. Ninety days post-inoculation, branch dieback and discolouration symptoms were recorded (mean lesion length = 9.90 mm). No symptoms were observed in the controls (mean lesion length = 4.0 mm). The pathogen was reisolated from the lesions of inoculated plants to confirm its pathogenicity according to Koch's postulates.

In Brazil *L. brasiliensis* has been reported in apple (Martins et al., 2018) and *Annona* species (Machado et al., 2019). This is the first report of the pathogen causing die-back on *C. arborea*. However, this identification should be interpreted with caution, as the taxonomic status of *L. brasiliensis* remains uncertain with some researchers suggesting that *L. brasiliensis* may reflect intraspecific variation within *L. theobromae* rather than a distinct species (Ko et al., 2023).

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FIGURE 1 Diseased *Cojoba arborea* in urban areas of Alta Floresta county, Mato Grosso State, Brazil.

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ORCID

J. Garlet <https://orcid.org/0000-0002-0791-7060>

C. Gonzatto Maciel <https://orcid.org/0000-0003-3719-1026>

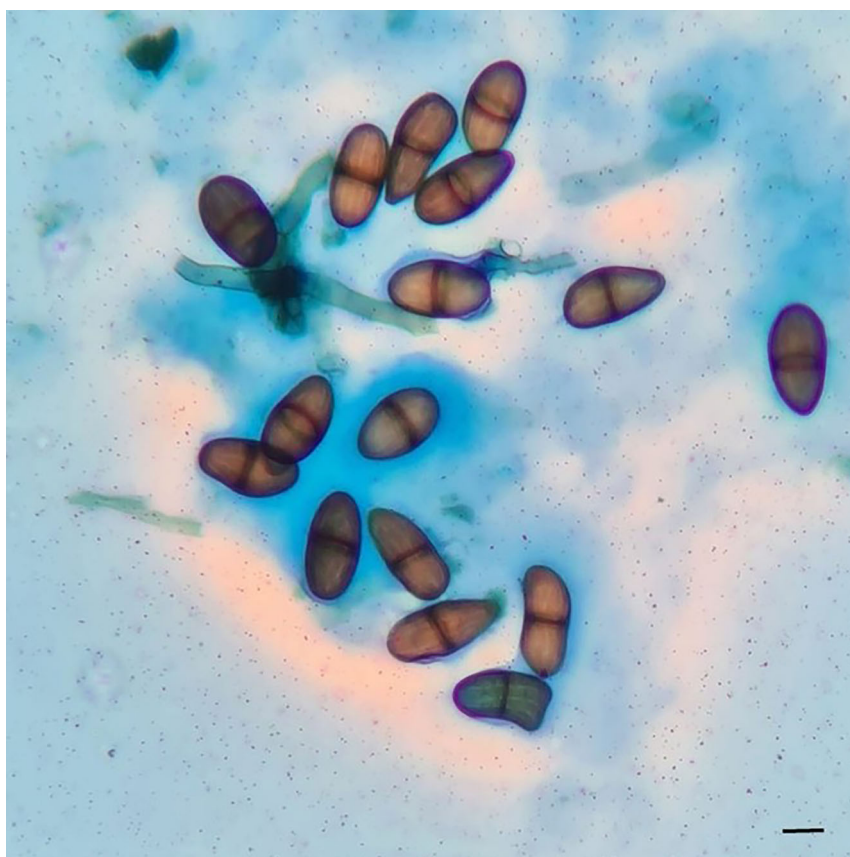
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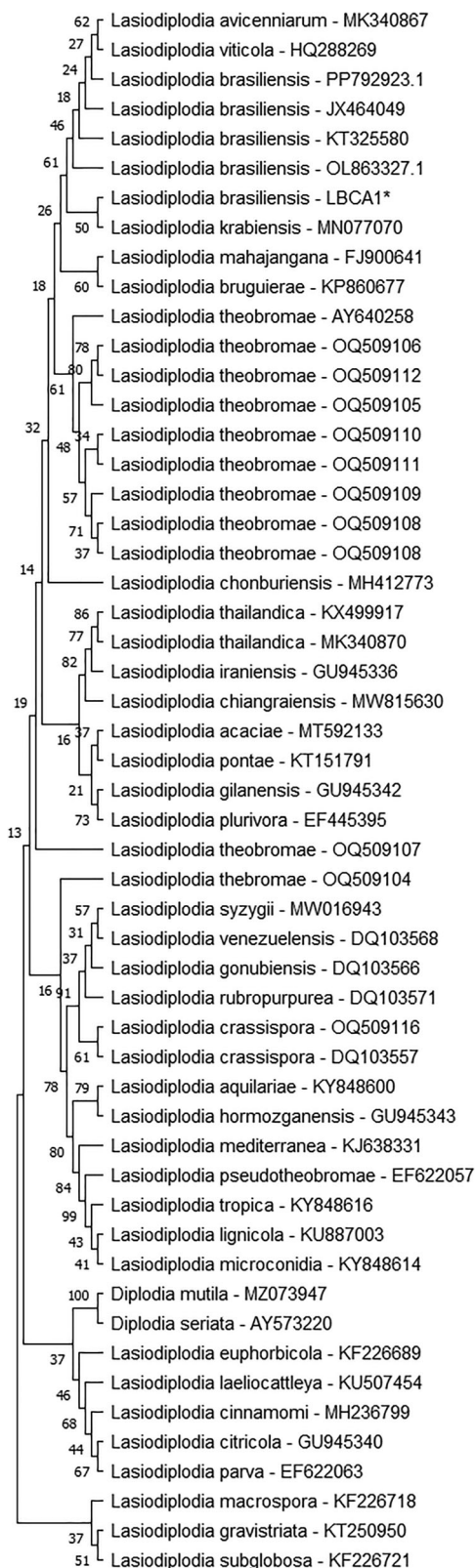
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FIGURE 2 Branches of diseased *Cojoba arborea* tissue showing dark-coloured colonisation.



FIGURE 3 Conidia of *Lasiodiplodia brasiliensis*. Bar = 10 μ m.





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FIGURE 4 Phylogenetic tree based on neighbour-joining analysis of sequences derived from region TEF 1-α. Based on 1,000 replicates of bootstrap by *Lasiodiplodia* from *Cojoba arborea* (LBCA1*) and sequences of the same genus (Rathnayaka et al., 2023).