Research Note

FIRST REPORT OF *LASIODIPLODIA BRASILIENSIS* CAUSING LEAF BLIGHT ON RAMBUTAN[1]

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Rambutan (*Nephelium lappaceum* L.) is a large sized, evergreen tropical fruit tree belonging to the family *Sapindaceae*. Originally from Southeast Asia it has been cultivated since 1998 in commercial orchards at Mayagüez, Puerto Rico. During a disease survey from 2014 to 2016, leaf blight on Rambutan was observed at greenhouses, and commercial and experimental orchards throughout the island. Diseased leaves were disinfected with 70% ethanol, rinsed with sterile-distilled-water, and plated in potato dextrose agar (PDA) and oatmeal agar (OA). Two isolates of *Lasiodiplodia brasiliensis* A1 and A2 were obtained, purified, and identified based on cultural characteristics and conidial morphology (Netto et al., 2014) (Figure 1).

In PDA and OA, *L. brasiliensis* initially produced grey mycelia that turned dark brown with age. Pycnidia were dark brown to black. Immature one-celled conidia were hyaline, thick-walled, sub-ovoid to ellipsoid, apex rounded and truncate at the base. Mature two-celled conidia were dark brown with irregular longitudinal striations. Conidia (n = 50) in isolate A1 and A2 averaged 26.1 µm long by 13.7 µm wide. Polymerase chain reaction (PCR) products of three genetic regions were sequenced to support morphological identification. Sequences of β-tubulin (βtub), internal transcribed spacer (ITS), and translation elongation factor 1-alpha (EF1-α) were compared with other homologous sequences using the NCBI GenBank Basic Local Alignment Search Tool (BLAST). The best Maximum-Likelihood (RAxML) phylogenetic tree of the *Lasiodiplodia* spp. (Figure 2) based on the concatenated sequences of βtub, ITS, and EF1-α, clustered both isolates of *L. brasiliensis* in one clade corresponding to the *L. brasiliensis* species [bootstrap support (BS)=100]. All three genetic regions were able to distinguish *L. brasiliensis* from other *Lasiodiplodia* spp. Accession numbers of gene sequences of *L. brasiliensis* from Puerto Rico submitted to GenBank were: MT784900 and MT784901 for ITS region; MT796209 and MT796210 for EF1-α; and MT796213 and MT796214 for β-tubulin.

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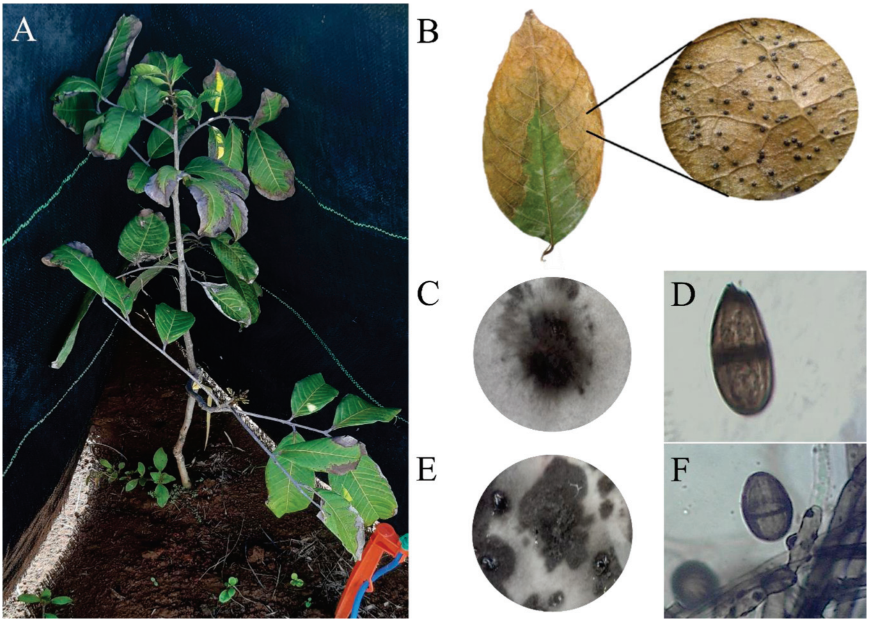

FIGURE 1. Symptoms of (A) leaf blight caused by *Lasiodiplodia brasiliensis* on one-year-old rambutan seedlings in the field; (B) leaf blight and pycnidia observed on leaves under greenhouse conditions; (C and E) eight-day-old colonies on PDA; and (D and F) conidia.

Pathogenicity tests were conducted on six one-year-old rambutan seedlings using six healthy non-detached leaves per isolate (Figure 3). Two trees per isolate were inoculated using 5 mm mycelial disks from *L. brasiliensis* pure cultures grown on PDA. Leaves were kept in a humid chamber made of plastic bags for up to eight days under greenhouse conditions. Two of the six trees were untreated, inoculated with PDA disks only, and were used as controls. Experiments were repeated twice. Isolates of *L. brasiliensis* caused leaf blotch and leaf blights eight and 14 days after inoculation (DAI). Diseased leaves turned from light brown to dark brown starting from the apex and spreading through the lamina. The necrotic tissues ranged from 10 mm to 40 mm in length. Untreated controls did not develop symptoms, and no fungi were re-isolated from tissue.

Lasiodiplodia brasiliensis was re-isolated and sequenced from diseased leaves, fulfilling Koch's postulates. DNA sequences from re-isolated fungi were submitted to GenBank with the following accession numbers: MT784902 for ITS region; MT796211 and MT796212 for EF1-α; and MT796215 for β-tubulin. *Lasiodiplodia brasiliensis* has been reported causing dieback and corky bark on rambutan (Serrato-Diaz et al., 2019). To our knowledge, this is the first report of *L. brasiliensis* causing leaf blight on rambutan.

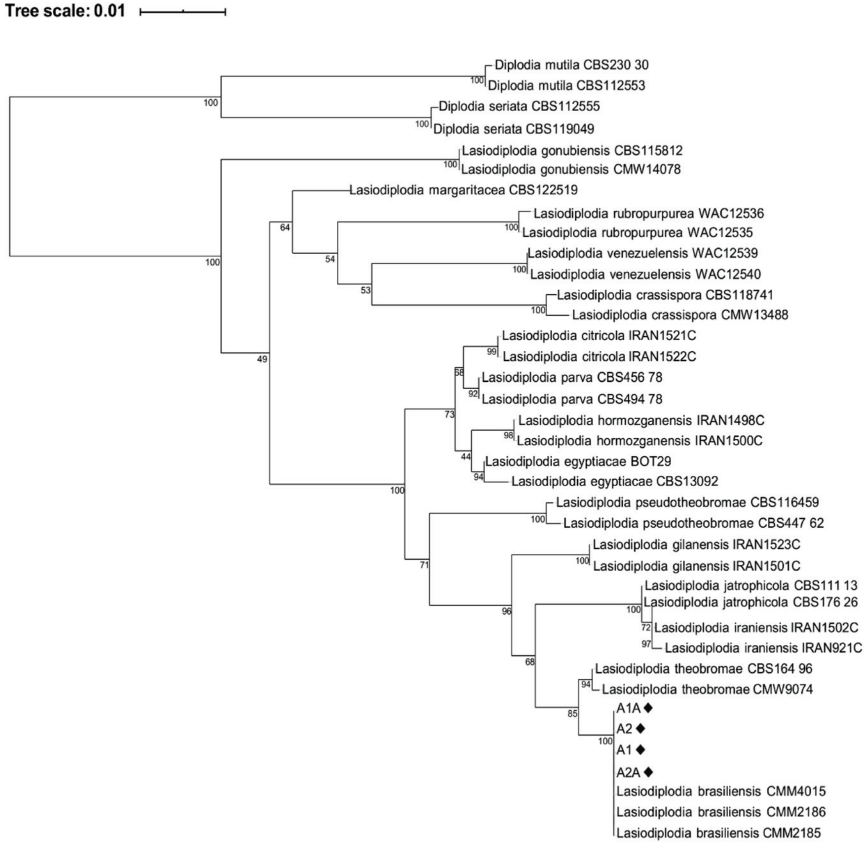

FIGURE 2. Best Maximum-likelihood tree of *Lasiodiplodia* spp. based on the concatenated sequences of β-tubulin (βtub), internal transcribed spacer (ITS) and translation elongation factor 1 alpha (EF1-α). Bootstrap support values shown in the nodes and isolates of *L. brasiliensis* are marked (♦), where A1 and A2 are the original isolates, and A1A and A2A are isolates recovered from Koch's postulates. The tree was rooted to the outgroups *Diplodia seriata* (CBS 119049 and CBS 112555) and *D. mutila* (CBS 230.30 and CBS 112553).

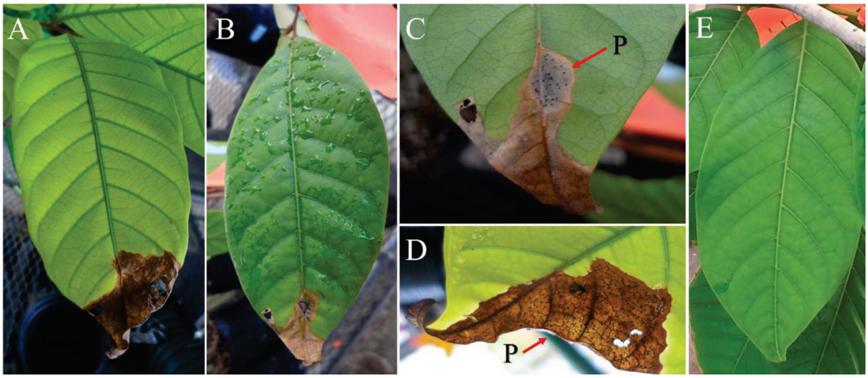

FIGURE 3. Symptoms observed during pathogenicity tests on non-detached rambutan leaves, 14 days after inoculation (A-D), showing leaf blight and pycnidia (P) production on lesions; (E) healthy rambutan leaf (control).

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