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First Report of *Lasiodiplodia mahajangana* Causing Branch Dieback of Blueberry in South Africa

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In October 2021, several 2-year-old southern highbush blueberry plants in a commercial planting, located in Gauteng Province, South Africa, showed symptoms of dieback (Figure 1). Ten entire plants (with soil around the roots) showing symptoms were collected and transferred to the laboratory of the Agricultural Research Council-Plant Health Protection for further analysis. Pieces from diseased tissue were placed onto potato dextrose agar (PDA; Neogen, USA). The resulting isolates were identified as *Lasiodiplodia* based on morphological characteristics. Cultural characteristics included white, fluffy colonies with abundant aerial mycelium, which became pale olivaceous grey after 4 days,

TABLE 1 | Isolates of *Lasiodiplodia mahajangana* from *Vaccinium corymbosum* used in the phylogenetic analyses.

	GenBank Accession Numbers		
Collection number	**ITS**	**EF**	**Rpb2**
PPRI37791	OQ472517	PZ349717	PZ362547
PPRI29998	OQ472518	PZ349718	PZ362548
PPRI29999	OQ472519	PZ349719	PZ362549

FIGURE 1 | Branch dieback symptoms on southern highbush blueberries in the field in Gauteng Province, South Africa.

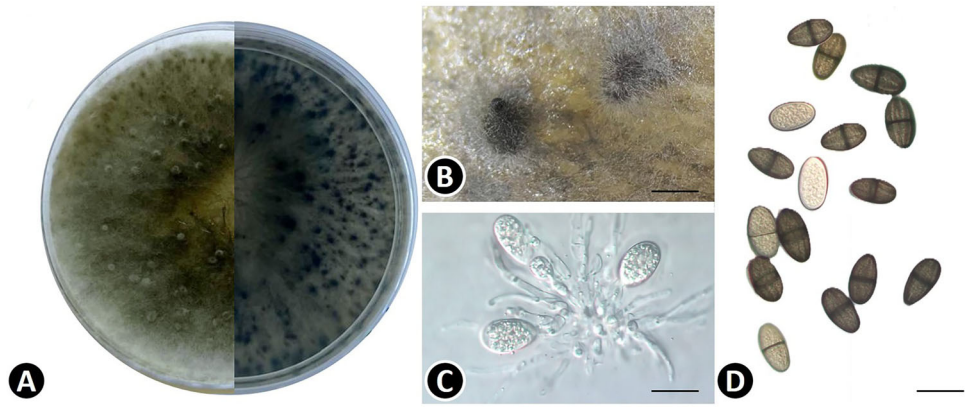

FIGURE 2 | Micrographs of *Lasiodiplodia mahajangana*: (A) culture morphology on potato dextrose agar; (B) pycnidium (Bar = 100 μm); (C) young conidia with attached conidiogenous cells; and (D) maturing conidia at various stages (Bar = 20 μm).

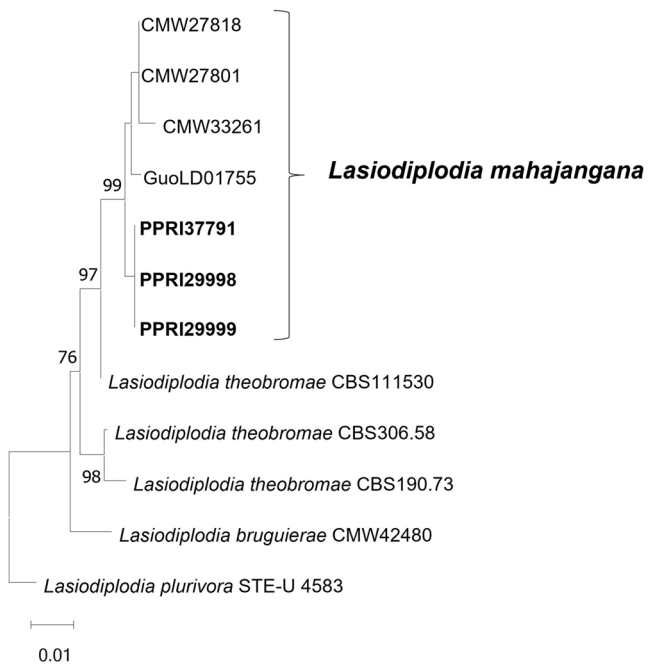

FIGURE 3 | Maximum likelihood (ML) tree of ITS, *tef1-α* and *rpb2* sequences of *Lasiodiplodia mahajangana* isolated from blueberry in Gauteng Province, South Africa. Bootstrap values ≥75% are given at the nodes. Isolates obtained in this study are indicated in bold.

at which point pycnidia began to form. The reverse side of the colonies was olivaceous grey. Conidia were initially aseptate, hyaline, ellipsoid to ovoid, thick-walled, and contained granular contents; after release, they became one-septate and pigmented, with vertical striations observed at maturity (Figure 2). All isolates obtained in this study have been maintained in the National Collection of Fungi at Roodeplaat, Biosystematics, South Africa. Identification of *Lasiodiplodia* species based on morphology alone is not possible, as the species within this genus are morphologically very similar; three representative isolates were used for further analysis.

For molecular identification, DNA sequences were generated for the internal transcribed spacer (ITS) region using primers ITS-1 (Gardes and Bruns 1993) and ITS-4 (White et al. 1990), the translation elongation factor 1- α (*tef1-α*) gene using primers EF1-728F and EF1-986R (Carbone and Kohn 1999) and the partial RNA polymerase II second largest subunit (*rpb2*) using primers RPB2-5F and RPB2-7cR (Liu et al. 2012). Sequences of the isolates were edited using CLCBio Workbench V9.0 (Qiagen, Germany). Phylogenetic analyses for all datasets were performed using maximum likelihood (ML) using PhyML 3.0 online (http://www.atgc-montpellier.fr/phyml). The isolates in this study were identified as *Lasiodiplodia mahajangana* (Figure 3). All sequences generated from this study were deposited in GenBank (Table 1).

Pathogenicity was confirmed by inoculating ten healthy 2-year-old plants in a greenhouse at 25°C, under normal daylight conditions. For inoculation, a section of bark was removed from the main stems of the plants with a 6 mm sterilised corkborer to expose the cambium. A 6 mm plug of agar covered with mycelium of the fungus was placed, mycelial surface facing inwards, onto the wound, while clean agar discs were used in control inoculations. The inoculated wounds were sealed with plastic film to minimise contamination and to prevent desiccation of the inoculum. Lesion lengths were measured 6 weeks after inoculation. *Lasiodiplodia mahajangana* produced significant lesions on the stems, followed by branch dieback while no lesions developed on the control plants (Figure 4). The pathogen was re-isolated from the inoculated plants and identified based on morphology.

This is the first report of *Lasiodiplodia mahajangana* causing branch dieback of blueberry in South Africa. The fungus has been reported causing disease in other hosts including *Adansonia digitata*, *Euphorbia ingens*, *Mangifera indica* and *Sclerocarya birrea* subsp. *caffra* in South Africa (Jami et al. 2017). The occurrence of the disease in blueberry plantings in South Africa is currently not causing significant losses, and farmers, through the removal and disposal of diseased branches and the implementation of sanitary protocols, have the disease under control.

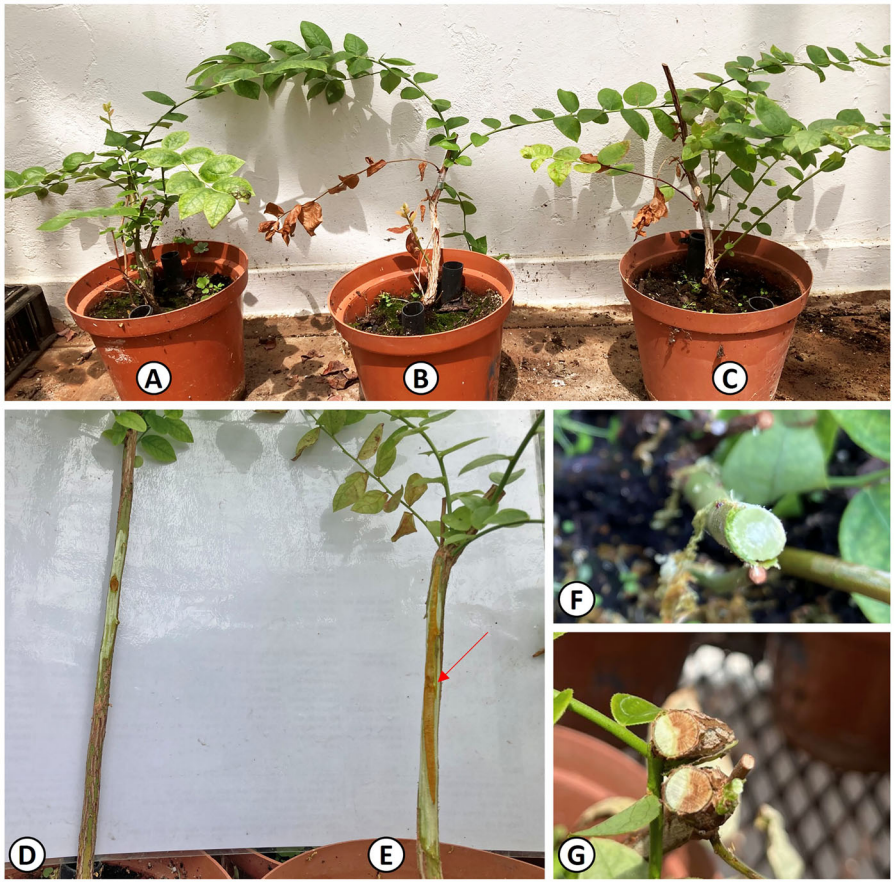

FIGURE 4 | Pathogenicity trials with *Lasiodiplodia mahajangana* on southern highbush blueberry: (A) control; (B and C) branch dieback above inoculation; (D) length of lesion on control; (E) length of lesion after inoculation; (F) healthy main stem in control; and (G) main stem discolouration after inoculation.

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