First Report in Cuba: *Lasiodiplodia* pseudotheobromae as Causal Agent of Dieback in Murraya Paniculata (*L.*) *Jacq.* (Rutaceae)

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ABSTRACT

In Cuba and in other parts of the world, Murraya paniculata is an ornamental plant as well as agricultural crops of economic value that has presented chlorotic leaves, defoliation and regressive death, which suggests that it is a phytopathogenic fungus. The objective of this work was to demonstrate that the attack of this phytopathogenic agent is due to Lasiodiplodia pseudotheobromae, responsible for the regressive death in M. paniculata. In this sense, an investigation was carried out at the laboratory level of isolation and identification of the fungus based on the fact that DNA was extracted to ensure the correct identity of this genus and species of fungus that was cultivated with a commercial kit that supports a fungal attack of L. pseudotheobromae as a causal agent of regressive death in M. paniculata, with the help of the consulted literature. This is the first report found of this fungal disease caused by L. pseudotheobroame. This finding is important due to the risk of attacking other non-ornamental plants, as well as agricultural crops that affect agricultural production on the island of Cuba, to avoid a phytosanitary problem of greater impact if it is considered that the COVID-19 pandemic 19 caused other economic problems related to agriculture on the island.

Keywords: *Broad spectrum of plant attack, New phytopathogenic agent, Plant health, Ecomical crops, Phytosanitary, Ornamental.*

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Highlights of this paper

- In Cuba and in other parts of the world, Lasiodiplodia pseudotheobromae, which not only attacks ornamental plants, as Murraya paniculata is a phytopathogenic fungus that parasitizes commercially important agricultural crops, so knowing its life cycle mechanism of action and biological and/or chemical control is important to avoid a phytosanitary problem of economic importance.

1. INTRODUCTION

Lasiodiplodia is a fungal genus in the family *Botryosphaeriaceae (Botryosphaeria, Dothideomycetes*). This is a phytopathogen in tropical and subtropical regions, it is an endophytic, opportunistic phytopathogen [1, 2]. The main species of *Lasiodiplodia* are responsible for diseases in vegetables of commercial value subjected to biotic and abiotic stress that cause rotting of roots, stems and fruits, leaf spots, gummosis, with a descending death in avocado crops observed in leaves, cotton, coffee, sugar cane, etc. [3]. In these plants they can cause a transovarian infection, thus infecting seeds and other tissues, although they can be saprophytic in the soil [2, 4, 5].

On the island of Cuba it has not been reported in commercial agricultural crops and even less so in ornamental plants. *Murraya paniculata* is generally common in Cuba and elsewhere as an ornamental plant, especially for hedges around gardens and patios in urban areas. Lately in *M. paniculata* with chlorotic leaves, defoliation and dieback leading to eventual death prompted the following observations and laboratory studies aimed at determining a possible causal agent of these symptoms. In this sense, the objective of this research work is to demonstrate the presence of *L. pseudotheobromae* as a causal agent of regressive death in *M. paniculata* (L.) Jacq. (Rutaceae).

2. MATERIAL AND METHODS

To demonstrate that *L. pseudotheobromae* was attacking *M. paniculata*, plants from five symptomatic branches 15 cm long were collected from two locations in the Playa neighborhood of Havana, Cuba. The branch pieces, which presented areas of advanced necrosis, were placed in transparent polyethylene zip lock bags and transported to the Phytopathology Laboratory of the Tropical Fruit Research Institute in Havana for analysis.

The criteria of Koch's postulates were approached, first by isolating fungi in pure culture in "H" medium (dextrose 5g; sucrose 10g; mycological peptone 0.5g; yeast extract 5g, agar 15g and distilled water 1L + oxytetracycline 0.4g /L [6]. The resulting colonies were used to inoculate stems from five healthy *M. paniculata* plants from which the fungus was re-isolated.The test specimens were placed in the IIFT fungus collection. DNA was extracted from the cultured fungus using a commercial kit (Promega, Uniparts, S.A. México, City.) according to the manufacturer's instructions. The ITS1-5.8S-ITS2 region of r-DNA was amplified with the primer under the reaction conditions described by White, et al. [7]. The resulting amplicon was sequenced using the same primer from Macrogen Inc, (Seoul Korea). The BLAST-N program [8] was used to compare the sequence obtained with the stored databases. Phylogenetic analysis was performed using nearest neighbor reconstruction of evolution using the MEGA program version 4.0 [9]. The topology of the resultant tree was evaluated by resampling analysis over 1000 repetitions.

3. RESULTS AND DISCUSSION

Felted colonies grew rapidly on medium H at a daily rate of approximately 21.5 mm and were initially white, then turning grayish, then black. The conidia measured 24.8 ± 0.47 µm long and 13.6 ± 0.13 µm wide to give a length

to width ratio of 1.8 ± 0.03 µm. The ascal paraphyses measured 58.2 x 4.0 µm. These values are within the range indicated by Alves, et al. [10]; Abdollahzadeh, et al. [11]; Marques, et al. [12] for L. pseudotheobromae Alves, et al. [10] and Picos-Muñoz, et al. [13].

In the Figure 1, comparative analysis using BLAST-N confirmed that the isolated species (IIFT E64) belonged to the genus *Lasiodiplodia* with 99-97% identity with isolates of *L. theobromae* and *L. pseudotheobromae*. The clade grouping the *L. pseudotheobromae* strains was very robust according to resampling values 84 and included the gb EF622077.1 sequence corresponding to the type material for this species.

These phenotypic and genotypic results combined with the pathogenicity tests that complete Koch's postulates support the conclusion that *L. pseudotheobromae* is the causal agent of the dieback and final death observed in *M. paniculata*. *L. pseudotheobromae* has been reported in Africa, Asia, Europe, and Latin America on forests and fruit trees, including citrus [14]. However, to our knowledge, this is the first report of *L. pseudotheobromae* as a pathogen of *M. paniculata* in some Caribbean countries, especially Cuba. This represents a phytosanitary risk for agricultural crops on the island of Cuba due to the damage that this fungus is capable of causing.

At the Figure 2 is showed rapid necrosis was observed in the stem cuttings of *M. paniculata* in the regions adjacent to each inoculation point, followed by desiccation, defoliation and subsequent death of the five inoculated plants. These results were similar to those described by Cabrera, et al. [6] for the response of *Citrus macrophylla* Wester to *L. theobromae*.

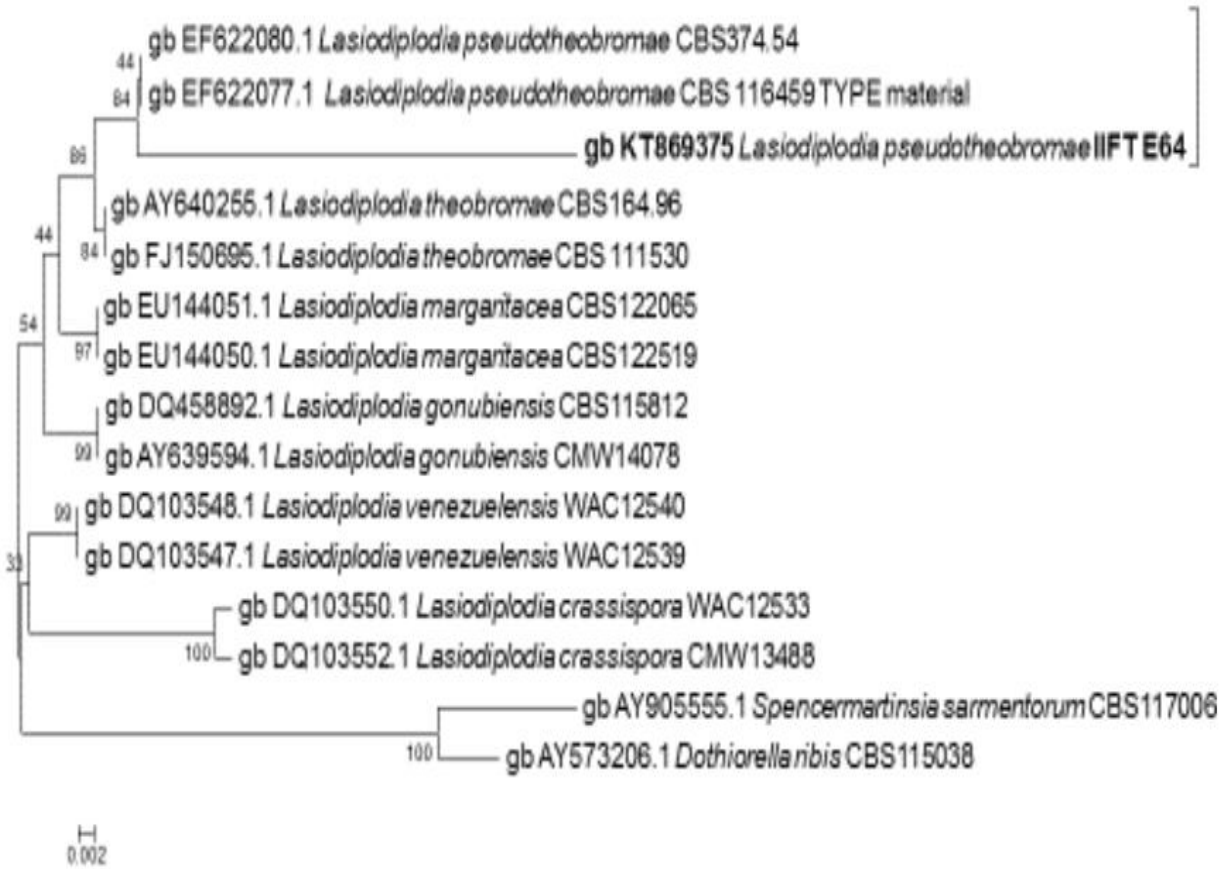

Figure 1. Phylogenetic analysis by the nearest neighbor method using nucleotide sequences of the ITS region of rDNA strains of the genus *Lasiodiplodia*. Sampling analysis was performed using 1000 replicates. STI *Dothiorella ribis* (CBS115038) and *Spencermartinsia sarmentorum* (CBS117006) sequences were used to make the phylogenetic tree of the new *Lasiodiplodia* isolate.

Figure 2. *Murraya paniculata* plants with symptoms of *Lasiodiplodia pseudotheobromae.* Left, blighted branches, right advanced wound.

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