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First report of dieback in jackfruit (*Artocarpus heterophyllus*) caused by *Lasiodiplodia theobromae* in Nayarit, Mexico

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ABSTRACT

Background/Objective. Jackfruit (*Artocarpus heterophyllus*) is an economically important fruit for Nayarit, accounting for over 90% of the national crop. Branch dieback has been detected in jackfruit trees in recent years, so the objective of this study was to identify the causative agent of branch dieback in *A. heterophyllus* in the state of Nayarit, Mexico.

Materials and Methods. From a collection of *Lasiodiplodia theobromae* strains from commercial orchards of jackfruit, mango (*Mangifera indica*), soursop (*Annona muricata*), avocado (*Persea americana*), and Persian lime (*Citrus latifolia*) in Nayarit, the pathogenicity of *L. theobromae* was evaluated on one-year-old jackfruit trees. The isolates were molecularly identified by sequencing the internal transcribed spacer (ITS5 and ITS4 primers) and translation elongation factor 1-alpha (TEF1; primers EF-728/EF-986) regions.

Results. All isolates were pathogenic to jackfruit. LY1 and LG4, from jackfruit and soursop, respectively, showed high aggressiveness on branches. Dieback symptoms were evident two months post-inoculation, with shoot rot, internal necrotic lesions, and bark fissures on branches and stems, as well as tree death. The ITS and EF1-α sequences of isolates recovered from pathogenicity testing showed 99–100% similarity to previously published sequences for *L. theobromae* in GenBank.

Conclusion. This is the first report of *L. theobromae* as a causal agent of dieback of jackfruit *A. heterophyllus* in Nayarit, Mexico.

Keywords: Botryosphaeriaceae, ITS, EF1-α, fungus, necrosis

INTRODUCTION

Jackfruit (*A. heterophyllus*) is of high economic importance for the producing regions of Nayarit. The fruit, which is sold fresh or industrialized, is rich in vitamins, carbohydrates and minerals. The state of Nayarit concentrates more than 90% of the surface planted in the country (1,632.95 ha), with a yield of 26 t ha^{-1}, and a production worth 441 million pesos.

Among the phytosanitary problems of economic importance in this fruit, fungal diseases in inflorescences, fruits and branches stand out. Fungi belonging to the Botryosphaeriaceae family affect a large diversity of botanical species and induce symptoms such as rots, cancers, branch deaths, the overall decline of the tree, and others. Species of the *Lasiodiplodia* genus attack more than 500 plant species, mainly commercially important tropical and subtropical fruit trees (Luoye *et al*., 2020; Abdollahzadeh *et al*., 2010). The progressive symptoms of descending death (MD) induced by *Lasiodiplodia* are wilting in the apex of peripheral or annual branches, the leaves turning brown and dying then falling, and the branch having a dry appearance. It is common to observe adjacent branches that are dry, as well as the decline or death of the tree.

L. theobromae is considered a species able to induce a severity of up to 100%. The presence of a dark, gummy exudate on the bark and necrotic vascular tissue in soursop (*A. muricata*) are the main symptoms reported for *L. theobromae*. A similar behavior has been observed in other fruit trees. In macadamia (*Macadamia integrifolia*), it causes the necrosis of the apex, rotting of the peduncle and MD. In mango (*M. indica*), mamey (*Pouteria sapota*), grapevines (*Vitis vinifera*) and Persian lime (*C. latifolia*) peduncle rot has been found, along with stem cancers and MD caused by *L. citricola* and *L. pseudotheobromae* (Valle de la Paz *et al*., 2019; Polanco *et al*., 2019; Dukare *et al*., 2019; Abdollahzadeh *et al*., 2010). In Nayarit, *L. theobromae* was reported as the causal agent for the soft rot in jackfruit (A Betancourt and G. Luna-Esquivel. Not published). However, its etiological role in the MD of this fruit species has not been reported. Therefore, the aim of this research was to identify the causal agent of MD in *A. heterophyllus* in commercial orchards in the state of Nayarit, Mexico.

The methodology for this investigation was developed from five *Lasiodiplodia* strains from the fungal collection of the Agricultural Parasitology Laboratory of the UAN. These strains were originally isolated from jackfruits and branches with MD from mango, Persian lime, soursop and avocado trees located in the production areas of Nayarit (Table 1). These strains were previously identified based on their micro- and macroscopic morphological characteristics in chosen colonies cultured on PDA medium at 7 days of age at 28±2 °C, using the keys by Abdollahzadeh *et al.* (2010).

The pathogenicity of each isolate was evaluated in January 2023, in 18 jackfruit trees aged one year (three per treatment) under greenhouse with shade mesh at 50%, in Xalisco, Nayarit (21° 25' 45.3" N y -104° 36' 46" W; a 930 masl). Parts of the epidermis of experimental trees were removed using a sterile scalpel in one single place (area of 0.4 x 1 cm) on the main stem or branch, depending on the characteristic of the tree, to expose the woody tissue (approximately 65 cm above the base of the stem). On the exposed xylem tissue of each experimental plant, a 5-10 mm PDA disk (explants) with 7-day-old fungal growth was inoculated. Trees inoculated with PDA disks without fungal growth were used as controls. The inoculated area of the experimental trees was covered with wet sterile cotton and plastic wrap to maintain humidity and prevent external contamination.

Table 1. Location and botanical source of *Lasiodiplodia theobromae* isolates used in pathogenicity tests to determine the etiological implication in branch dieback of jackfruit (*Artocarpus heterophyllus*).

Isolated	Municipality	Locality	Crop[x]	Coordinates	Altitude (m)
LY1	San Blas	La Palma	Jackfruit (fruto)	21° 30’ 19.9” N - 105° 04’ 41.4” O	16
LM2	Tepic	Atonalisco	Mango	21° 38’ 55.9” N - 104° 49’ 24.5” O	362
LL3	Tepic	La Fortuna	Persian Lime	21° 33' 36.8” N - 104° 56' 29.6” O	752
LG4	Compostela	Tonino	Soursop	21° 07’ 33.9” N - 105° 12’ 09.3” O	39
LA5	San Blas	Mecatan	Avocadpo	21° 32' 43.1” N - 105° 08' 14.6" O	365

[X] Isolates were obtained from branches with symptoms of descending death, except in jackfruit, which came from fruits.

From the jackfruit trees that developed branch necrosis or progressive dieback, the inoculated isolates were re-isolated and verified morphologically and molecularly. For molecular identification, the isolates were cultured on PDA at 25±2 °C for 7 days. The mycelial growth was collected and genomic DNA (gDNA) was extracted using the CTAB II methos according to Allers and Lichten (2000).

The amplification conditions for the PCR with the ITS4/ITS5 primers were an initial denaturation at 94 °C for 5 min, 38 denaturation cycles at 94 °C for 1 min, annealing at 60 °C for 1 min, extension at 72 °C for 1 min, and a final extension stage at 72 °C for 4 min. For the EF-728/EF-986 primers, the conditions were denaturation at 95 °C for 4 min, 35 denaturation cycles at 95 °C for 1 min, annealing at 55 °C for 1 min, extension at 72 °C for 2 min, and a final extension at 72 °C for 10 min. The PCR products were visualized by electrophoresis on 1.5% agar gels in 1X TBE 1X buffer at 80 V for 1 h, and then sequenced by Macrogen Inc (Seoul, Korea).

The results of the application of Koch's postulates showed that *L. theobromae* was pathogenic in jackfruit. Symptoms began in the 15 days after inoculation with apex necrosis and the subsequent wilting of leaves and the descending progress of the necrotic tissue. Symptoms at 60 days included wilting in branch apices, tearing and necrosis of bark, and plant MD. These symptoms were similar to those reported for soursop, macadamia, mango, grapevine, mamey and Persian lime (Valle de la Paz *et al*., 2019; Polanco *et al*., 2019; Dukare *et al*., 2019; Abdollahzadeh *et al*., 2010). In some cases, the damage descended down to the base of secondary branches or stem, which coincide with the symptoms caused by *L. pseudotheobromae* in *Ormosia pinnata* (Luoye Li *et al.,* 2020).

All *Lasiodiplodia* isolates necrotized the wood of the inoculated area and induced MD in the inoculated trees. Isolate LG4, from soursop, was the most aggressive, since its damage extended down to the base of the stem and caused the death of the tree. Isolate LY1, from jackfruit, caused the bark to tear, near the inoculation zone. Isolates LM2, LA5 and LL3 from mango, avocado and Persian lime, respectively, only induced the drying of apex branches with a downward trend.

After re-isolating the fungal isolations from the symptomatic tissues of young jackfruit trees, their identity was confirmed as *L. theobromae* form its microscopic morphological characteristics (Figure 1).

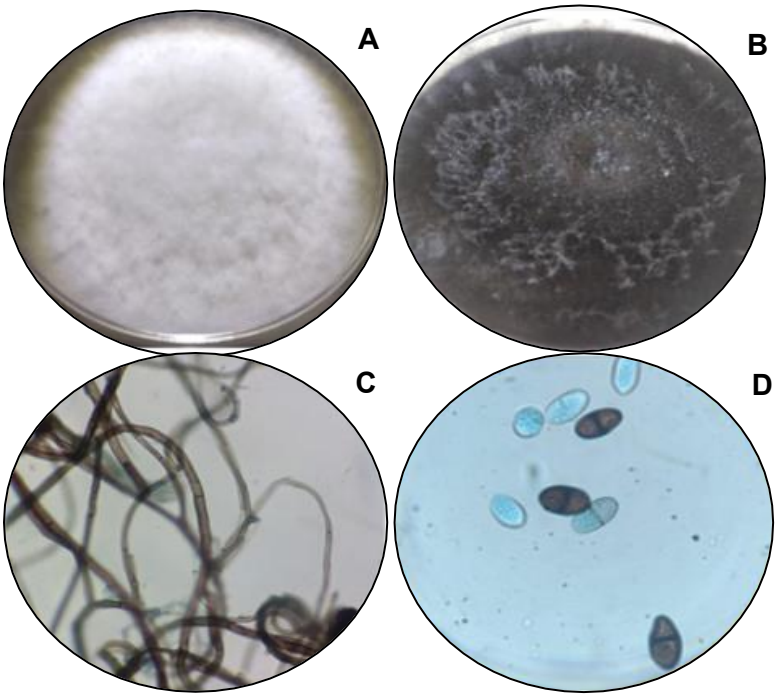

Figure 1. A. *Lasiodiplodia* culture on day 3 in PDA. **B.** *Lasiodiplodia* culture after 7 days in PDA. **C**. Septated maroon-brown hyphae viewed under a compound microscope at 40X. **D**. Conidia, characteristically two-celled and dark, at maturity under a compound microscope at 40X.

From the PCR products, 600 and 350 pb amplicons were obtained for the regions ITS5 (GGAAGTAAAAGTCGTAACAAGG)/ITS4 (TCCTCCGCTTATTGATATGC) and EF-728 (GGAAGTAAAAGTCGTAACAAGG)/ EF-986 (TCCTCCGCTTATTGATATGC), respectively. The consensus sequence of the PCR products was 530 and 280 pb. The five sequences of the fungal isolates share a nucleotide similarity of 99 to 100% with previously published sequences of the ITS regions in the GenBank (OP725658.1).

Our results suggest that virulence (the ability to cause diseases, *sensu* Vanderplank) of *L. theobromae*, is not host-specific, as the fungus is capable of inducing MD in various tropical or subtropical fruit trees, different to those from which it was isolated. However, the intensity of the observed symptoms suggests pathogenic variability (i.e., aggressiveness) of the fungus depending on the host. The control trees displayed no symptoms. Dukare *et al.* (2019) documented the pathogenicity of an *L. theobromae* isolate from guava (*Psidium guajava*) on mango fruits inoculated post-harvest.

Lime plants displayed chlorosis on leaves, branch MD, the formation of cancers and gummy exudates 22 days after inoculation with *L. citricola*, *L. pseudotheobromae* and *L. theobromae* (Valle de la Paz *et al.*, 2019). In sweet orange (*C. sinensis*), Polanco y collaborators (2019) reported wilting, necrosis and branch MD, as well as external necrotic lesions caused by *L. theobromae* 32 days after inoculation. That same fungus in mamey caused necrotic lesions after 30 days. Two years later, the MD of vegetative shoots was found (Tovar-Pedraza *et al*., 2012).

In conclusion, five *L. theobromae* isolates from jackfruit and MD from mango, avocado, Persian lime and soursop branches led to symptoms of MD from jackfruit branches (*Artocarpus heterophyllus*), confirming Koch's postulates. The symptoms began with the rotting of apices, followed by internal necrotic lesions on branches and stems, and finally, the death of the tree. *L. theobromae* is reported for the first time as the causal agent of MD

in *A. heterophyllus* trees in Nayarit, Mexico. Isolate LG4, from soursop, was highly aggressive, since it led to the death of the tree 60 days after being inoculated. The jackfruit isolate was the second most aggressive, with the tearing of branches.

Limitations

No severity scale was implemented, nor were there events of pathogenesis determined. No statistical analysis was performed.

Conflict of interest

The authors declare no conflict of interest related to this article.

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Author contributions

Betancourt-Aranguré participated in data acquisition, analysis and interpretation, and wrote the manuscript. G. Luna-Esquivel, Graciela Guadalupe López Guzmán, C. Rios-Velasco, O.J. Cambero-Campos and E. Cruz-Crespo carried out the conceptualization, design and revision of the document. E. Andrea Martínez-Mera participated in writing and revising the manuscript.

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