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Investigation note

Manila mango dieback disease: histological characterization and occurrence in the central area of Veracruz

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Abstract

Dieback is one of the diseases that generates the greatest impact on mango, it affects the entire structure of the tree, leading it to cause losses in production. This research studied the impact of the disease in five localities in the central area of the state of Veracruz from 2016 to 2017 and carried out a histological characterization of the damage caused by *Lasiodiplodia pseudotheobromae* in Manila mango. The results revealed an incidence ranging from 38% to 80%, a severity ranging from 1.5 to 2.59 degrees, and a high disease index of 0.58 to 2. Histologically, the fungus affected various plant tissues, mainly the vascular cambium, so the inner and outer bark was affected, causing the destruction of the xylem and phloem vessels as well as the production of gum. This pathogenic activity could result in damage to the phloem, preventing the transport of nutrients and mineral salts, and damage to the xylem, preventing the passage of water, which would eventually trigger the death of the plant. Knowing how the pathogenic fungus acts places us in the opportunity for early intervention for the management of the disease.

Keywords:

Mangifera indica, *Lasiodiplodia pseudotheobromae*, decline, rot.

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Mango (*Mangifera indica* L.) is one of the most produced and traded tropical fruits in the world. Mexico grows mango varieties with participation in the international market (Ataulfo, Kent and Keitt) and the national market (Manila and Criollo). Within the country, the Manila variety is the second most important, where Veracruz is the large producing state with a planting area of 224 646.5 ha (SIAP, 2023).

Currently, the aim is to improve the quality of the plant through productive and fruit genetic improvement by applying various controls or products that delay ripening (Alonso-Barrera *et al*., 2023). Fungal diseases are a latent problem in mango crops; one of the most important is dieback disease, which affects the structure of the tree, causing death, which eventually results in a reduction in production (Rodríguez-Gálvez *et al*., 2017).

The species responsible for this disease can appear individually or form fungal complexes; in some cases, they rot the peduncle and damage the fruit in the postharvest stage (de Oliveira Costa *et al*., 2010; Zhan *et al*., 2023). In Mexico, some genera that cause the disease have been reported, such as *Lasiodiplodia*, *Neofusicoccum*, *Diplodia* and *Pestalotiopsis*, among others, in states such as Veracruz, Colima, Jalisco, Nayarit, Guerrero, and Michoacán (Sandoval-Sánchez *et al*., 2013; Saucedo-Picazo *et al*., 2022).

At the histological level, the infection of *L. theobromae* has been characterize in sapote, grapevine, and peach, affecting the plant's vascular system in tissues such as xylem, parenchyma and epidermal cells, among others (Al-Saadoon *et al*., 2012; Tovar-Pedraza *et al*., 2013; Li *et al*., 2014). In Veracruz, the species *L. pseudotheobromae* was reported as the causal agent of dieback disease in Manila mango; however, host damage has not been characterized at the histological level during the infective process of the fungus.

Despite its global relevance, the incidence and severity of this disease in the field have not been documented in Mexico, which highlights the need to investigate its regional status. Therefore, this work aimed to histologically characterize the infection of *Lasiodiplodia pseudotheobromae* and to know the impact on commercial orchards in Manila mango in the central area of the state of Veracruz.

In 2016 and 2017, Manila mango plots were visited in Veracruz, Mexico, where symptoms of dieback disease were observed (Figure 1). Five commercial orchards in Actopan were selected to analyze the incidence and severity, recording the coordinates of each site: Actopan (19° 30.161" north latitude, 96° 35.460" west longitude), Ídolos (19° 25.828" north latitude, 96° 31.615" west longitude), La Esperanza (19° 27' 33.9" north latitude, 96° 34' 20.1" west longitude), Palmas de Abajo (19° 33.377" north latitude, 96° 24.292" west longitude) and San Nicolás (19° 33.32" north latitude, 96° 46.136" west longitude).

The incidence, severity, and index of the disease were evaluated according to the methodology described by Cardoso *et al*. (2004). To carry out the corresponding analyses, the data of a total of 500 trees, 100 trees per plot in each study site, were documented.

The severity (S) was estimated according to the equation $S= \sum(x_i n_i)/n$. Where: x represents the degree of the disease [0) no symptoms; 1) small and few cankers, small cracks with no exudation from the branches; 2) cracked cankers on trunks and branches that reach up to ⅓ in diameter, with little or no exudation and 3) cracked cankers more than ⅓ in diameter with abundant exudation or 4) cracked cankers completely surrounding the trunk or branches, foliage yellowing, dieback and exudation of the gums], x_i represents the number of plants per degree and n refers to the total number of diseased plants evaluated.

The incidence (I) was analyzed with formula $I= \sum x/N$. Where: x represents the number of diseased plants divided by N, which is the total number of plants evaluated. The disease index (DI) was estimated through the formula $\sum(x_i n_i)/N$, result of the incidence and severity (Groth *et al*., 1999).

To characterize the infection of *L. pseudotheobromae* histologically, a wound was made in one-year-old Manila mango seedlings. The isolate, from the BioMolFito laboratory (INBIOTECA), was cultured in PDA medium for seven days at 28 °C. A 1 mm wound was made on the trunk of the seedlings, where a disk of mycelium of the fungus was placed, whereas a PDA disk was used in the

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control. After 15 days, the infected tissues were fixed in FAA, dehydrated in increasing alcohols, rinsed with xylol, and included in paraffin. The cuts were made with a microtome, stained with safranin and green, and observed and photographed with an Eclipse E600 microscope.

This study revealed a considerable impact of the disease, where there was a predomination of the characteristic symptoms of dieback disease in the early stages of the infestation, such as cracking in the bark and gummosis (Gao *et al*., 2016, Rodríguez-Gálvez *et al*., 2017). In trees that showed greater severity, with degrees equal to or greater than 2, there was an excessive proliferation of shoots at points other than the middle part of the branches, suggesting a possible association with another symptom of the disease.

In terms of incidence, the Ídolos site stood out with the highest incidence compared to the other locations, whereas Palmas de Abajo and La Esperanza showed a similar incidence of 77%. Actopan had a 49% incidence, with San Nicolás being the place with the least impact (Table 1). In terms of severity, Ídolos had the highest degree of damage, with a value of 2.59, followed by La Esperanza with 2.26, Actopan with 1.98, and San Nicolás with 1.5, and the lowest severity was observed in Palmas de Abajo, with a value of 1.35.

Table 1. Incidence, severity, and index of the disease in commercial orchards.

Sites	Incidence	Severity (0-4)	Disease index (0-2)	Altitude (m)
San Nicolás	38.7	1.5	0.58	580
Actopan	49	1.98	0.97	220
Palmas de Abajo	77	1.95	1.04	20
La Esperanza	77	2.26	1.81	100
Ídolos	80	2.59	2	100

The impact of the disease could be correlated with the climatic factors of the environment where the plots are located, favoring the development of fungi or increasing the susceptibility of the tree to dieback disease. In the sites with lower altitudes (Table 1), located between 20 and 220 masl, higher incidences, severities, and indices of the disease were observed. In pine plantations in Tunisia, a correlation was established between dendrometric parameters, ecological factors, and disease incidence (Hlaiem *et al*., 2023).

Histological sections revealed the presence of the infection caused by *L. pseudotheobromae* in the mango bark. At 30 days after infection (DAI), the pathogen caused significant damage to the tissue of the inoculated plants. The mycelium, characterized by its septate and branched structure, invaded both the outer and inner bark, resulting in the destruction of the xylem and phloem vessels (Figure 1).

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Figure 1. Manila mango trees with symptoms of dieback disease caused by *Lasiodiplodia pseudotheobromae* in Veracruz: a) decay of the tree in the terminal phase of death; b) cankers and cracks in the trunk; c) cankers and cracks of branches and d) secretion of reddish-amber substances commonly known as gummosis.

A notable accumulation of gum was identified, mainly located in the vessels of the inner bark and phloem. This production is closely linked to the defensive response of glycometabolism during infection caused by the genus *Lasiodiplodia* (Gao *et al*., 2016).

From the observations made, it is inferred that the presence of hyphae (Hi) in the xylem vessels (Figure 2a) could cause obstruction of the flow of water and mineral salts, causing the drying out of the mango branches, as has been found in similar cases in grapevines and sapote (Morales *et al*., 2008, Al-Saadoon *et al*., 2012). Likewise, the production of gums is a natural defense mechanism typical of several species of the family Anacardiaceae, such as mango; these gums appear on tree branches (Figure 1d) as they have intercellular resin canals in the bark (Figure 2b), which allows resinous secretions once they present diseases (Verbeken *et al*., 2003).

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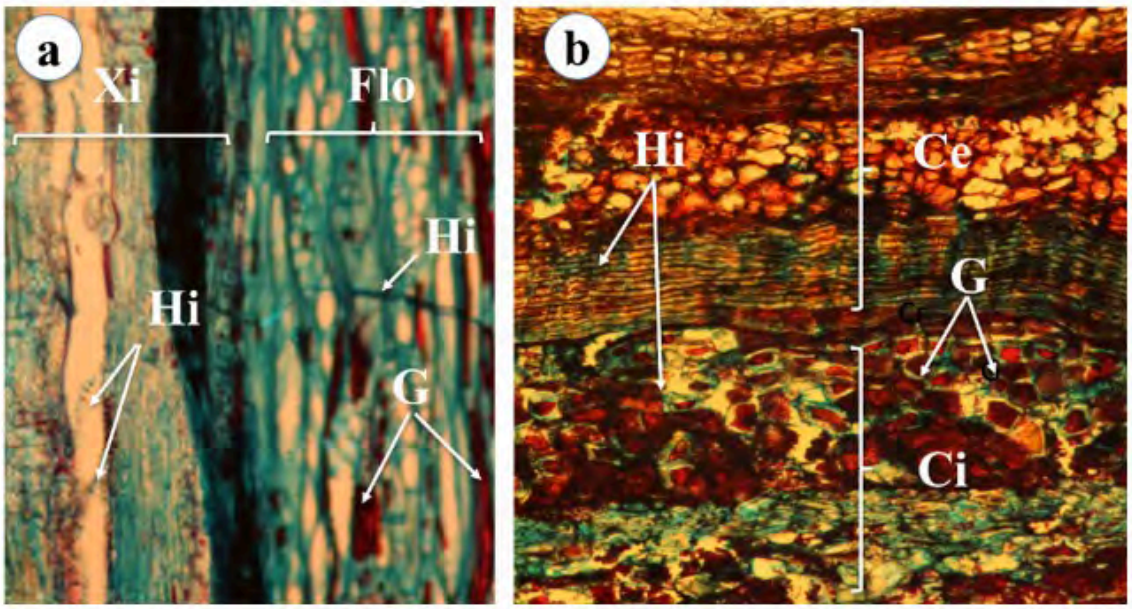
Figure 2. Structure of tissues of the stem bark of *Mangifera indica* seedlings infected with *Lasiodiplodia pseudotheobromae*: a) longitudinal section of the bark with (20X), invasion of the hyphae in Xylem (Xi) and Phloem (Flo) and b) tangential section of the bark (10X), showing the layers of cells of the outer bark (Ce) and inner bark (Ci), as well as accumulation of gum (G) and the presence of hyphae of *Lasiodiplodia pseudotheobromae* (Hi).



On the other hand, the lack of other fungal structures in the micrographs, unlike the findings reported by Tovar-Pedraza *et al*. (2012) in sapote, could be attributable to the variability in the duration of the experiment, the particular characteristics of the host used, and the differences in the virulence of the strain used in our study.

Conclusions

Dieback disease was detected in all the sites studied, evidencing its impact on Manila mangoes in the central region of Veracruz and its negative effect on the productive life of the trees. Histologically, *L. pseudotheobromae* causes degeneration of the bark, cambium and vascular bundles, facilitating host invasion. This is the first study to quantify the incidence, severity, and index of this disease in Veracruz, providing key information for its understanding and management.

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