

Morphological and molecular identification of foliar pathogenic fungi of *Hevea brasiliensis* in Costa Rica

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Abstract

Plantations of *Hevea brasiliensis* are vulnerable to a wide range of foliar fungal pathogens capable of causing substantial damage throughout the forest rotation cycle. This issue is particularly critical in Costa Rica, where the commercial cultivation of *H. brasiliensis* is a recent development, and no prior records exist of foliar pathogens affecting this species, thereby limiting timely detection and effective management. The study evaluated the pathogenicity of foliar fungi associated with *H. brasiliensis* through combined morphological and molecular characterization. Twenty plantation trees (2–4 years old) showing symptoms such as wilting, chlorosis, yellowing, partial to complete necrosis, punctate lesions, and anthracnose were sampled. Fungi were isolated from symptomatic leaves, cultured on potato dextrose agar supplemented with antibiotics, and identified based on colony and conidial morphology, complemented by sequencing of the internal transcribed spacer region (ITS4–ITS5). Pathogenicity was assessed by inoculating healthy detached leaves with mycelial discs from each isolate. A total of 30 isolates were obtained, representing the families Botryosphaeriaceae, Diaporthaceae, Nectriaceae, Apiosporaceae, and Glomerellaceae. Four species were confirmed as pathogenic: *Diaporthe tulliensis*, *Nigrospora sphaerica*, *Fusarium equiseti*, and *Lasiodiplodia theobromae*. All produced necrotic lesions, with *F. equiseti* and *N. sphaerica* generating the largest affected areas. Accurate identification of foliar pathogens is crucial for early disease diagnosis, which supports the development of targeted management strategies and safeguards the long-term health and productivity of *H. brasiliensis* plantations in Costa Rica.

Introduction

Hevea brasiliensis (rubber tree) is a deciduous tree species native to the lowland rainforests of the Amazon Basin in South America (Rincón Sepulveda, 1990, Nair, 2020). It naturally occurs in humid tropical environments such as low-altitude moist forests, wetlands, riparian zones, forest gaps, and disturbed areas (Oghenekome, 2004, Lim, 2011). This species serves as the primary source of natural rubber, a renewable material regarded for its remarkable elasticity, tensile strength, and resistance to abrasion and heat (Jacob et al., 1993, Candau & Maspoch, 2025). These qualities make natural rubber a valuable and more sustainable alternative to synthetic rubber, resulting in high demand across various industries, including pneumatic tires (Nair, 2020), footwear (Teoh et al., 2011), adhesives (Lim, 2011), and medical devices (Guerra et al., 2021). Due to its global demand, *H. brasiliensis* has become an economically significant crop cultivated across tropical and subtropical regions worldwide (Priyadarshan et al., 2005, Souza et al., 2013).

However, *H. brasiliensis* is highly susceptible to a broad range of fungal pathogens, particularly those that infect the foliage (Gasparotto et al., 1988). Foliar diseases substantially reduce photosynthetic capacity, thereby limiting overall growth and latex production (Sterling & Melgarejo, 2021). In juvenile trees, premature defoliation has been associated with increased mortality risk (Vineeth et al., 2024). Among the most commonly reported pathogens are *Colletotrichum acutatum* and *C. gloeosporioides*, which cause *Colletotrichum* leaf disease (Manju et al., 2002); *Corynespora cassiicola*, responsible for

leaf fall disease (Florence, 2018); *Phytophthora* spp., associated with abnormal leaf fall (Babu et al., 2024); and *Oidium heveae*, the causal agent of powdery mildew (Mohanta & Bae, 2015). Additional pathogenic fungi include *Fusarium* spp., *Lasiodiplodia theobromae*, *Nigrospora* spp., and *Diaporthe* spp., all of which induce symptoms such as necrotic lesions, anthracnose, chlorosis, wilting, and premature leaf drop (Gasparotto et al., 1988, Florence, 2018).

Misidentification or failure to detect the causal agents could result in inappropriate control measures, delayed responses, and increased economic losses (Ainusyifa et al., 2024, Lee, 2003, Mazlan et al., 2019b). Traditional identification methods based on morphological traits (e.g., colony appearance, spore structure, and reproductive features) provide an initial taxonomic framework but are often insufficient for species-level resolution due to overlapping phenotypic characteristics among genera (Jain et al., 2019, Thaochan et al., 2022). Consequently, molecular approaches, particularly sequencing of the internal transcribed spacer (ITS) region of ribosomal DNA, have become essential for the accurate identification of phytopathogenic fungi (Lieberei, 2007, Ainusyifa et al., 2024). Therefore, integrating morphological and molecular tools enhances diagnostic precision and enables the differentiation of closely related or cryptic species, thereby supporting the development of evidence-based phytosanitary practices in *H. brasiliensis* plantations (Thaochan et al., 2022, Hadi Ismail et al., 2024).

Understanding the identity and behavior of foliar pathogens is essential for developing effective disease management strategies in tropical regions, where *H. brasiliensis* cultivation has expanded notably in recent decades (Rincón Sepulveda, 1990, Lieberei, 2007). In Costa Rica, this renewed interest responds to the search for economically viable and environmentally sustainable crops within tropical agroforestry systems (Sell Biasetti, 2021, Seguin et al., 2003). However, the phytopathological knowledge regarding rubber cultivation in the country remains limited. Few formal studies have investigated the presence, distribution, or pathogenicity of fungi affecting *H. brasiliensis* foliage under local conditions (Robledo D'Angelo, 2016, Zainudin et al., 2023). This knowledge gap hinders early detection, timely intervention, and the implementation of integrated control measures by producers (Lee, 2003). As *H. brasiliensis* gains importance as a commercial species in the region, establishing a solid understanding of its foliar pathogens becomes critical to ensure plantation health, sustain productivity, and support long-term viability (Seguin et al., 2003, Rincón Sepulveda, 1990).

For these reasons, this study aimed to isolate and identify phytopathogenic fungi associated with foliar symptoms in *H. brasiliensis*, integrating morphological characterization with molecular analysis of the internal transcribed spacer (ITS) region. In addition, pathogenicity tests were conducted to verify the causal role of the identified fungi in symptom development. By providing accurate identification of key foliar pathogens, the study aims to establish a baseline of knowledge that supports early diagnosis, informs effective disease management strategies, and promotes the long-term health and productivity of *H. brasiliensis* plantations in Costa Rica.

Materials and methods

Study area

The study site is located near El Cairo city, in the province of Limón, Costa Rica (10°10'06"N, 83°30'19"W), at an average elevation of 99 m. The regional climate is classified as tropical rainforest (Af) according to the Köppen-Geiger classification, characterized by high humidity, no defined dry season, and consistently warm temperatures throughout the year (Beck et al., 2018). The site has recorded a mean annual temperature of approximately 27°C, with an annual variation of less than 2°C. Annual precipitation ranges from 3,000 to 4,000 mm, with rainfall evenly distributed throughout the year due to the influence of Caribbean trade winds. Solar radiation ranges from 12.5 MJ m⁻² day⁻¹ during the cloudiest months (November-January) to 20.4 MJ m⁻² day⁻¹ during sunnier periods (March-April). Finally, vapor pressure deficit (VPD) varied from 0.3 to 1.2 kPa.

The soil is considered moderately undulating terrain (slopes ranging from 15–30%) and is classified as an Inceptisol (Bertsch et al., 2000). In terms of physical characteristics, the texture ranges from sandy loam to clay loam, providing favorable conditions for root development and water retention. Chemical properties assessed in the upper 60 cm of the soil profile revealed an average pH of 5.1, an organic matter content of at least 2.5%, and base saturation exceeding 50% (Alvarado et al., 2015). Within this depth, nutrient availability was considered adequate for the growth of *H. brasiliensis*.

Sample collection and fungal isolation

Sampling was conducted between February and March 2024 by collecting symptomatic leaves from *Hevea brasiliensis* trees aged 2 to 4 years (n = 20). The selected trees had an average diameter at breast height (DBH) of 10 cm and a total height (TH) of 8 m. The sampled individuals displayed foliar symptoms consistent with fungal infection, including varying degrees of wilting, chlorosis, yellowing, partial to complete necrosis, punctate lesions, and anthracnose. Under laboratory conditions, four segments measuring 1 cm² were extracted from the advancing margins of symptomatic tissue on each diseased leaf. Surface sterilization was carried out by immersing the samples in 70% (v/v) ethanol for 30 seconds, followed by 5% (v/v) sodium hypochlorite for 45 seconds, and then rinsing them in three consecutive washes with sterile distilled water. The disinfected segments were placed in Petri dishes containing Potato Dextrose Agar (PDA) supplemented with penicillin and streptomycin (0.2 g L⁻¹). Plates were incubated at 28°C for seven days under a 12-hour light/dark photoperiod.

After the incubation period, the most frequently occurring fungal colonies were recorded. Hyphal tips from distinct morphotypes were subcultured onto fresh PDA medium with antibiotics to obtain pure isolates. These isolates were incubated at 28°C for 15 days, and representative colonies were preserved on PDA slants at 5°C, following the protocols of the mycothèque at the Forest Pathology Laboratory, Tecnológico de Costa Rica (Cartago, Costa Rica).

Morphological identification

The shape, color, and organization of macroscopic fungal structures (such as stroma, pycnidia, and perithecia) were examined using a Nikon C-LEDS stereomicroscope, model SMZ25 (Nikon Corp., Japan) at a magnification range of 10× to 40×. Colony color and structural traits were quantitatively assessed using ImageJ software, version 1.54p (Schneider et al., 2012). For each plate, a random subsampling of ten points was performed to evaluate all measured variables, selecting representative areas from the surface and reverse sides, as well as the central and marginal zones of the colony.

Microscopic characterization was conducted on fungal structures derived from mycelium and reproductive bodies (perithecia or pycnidia), which were mounted on slides with lactic acid or lactophenol blue to enhance structural visibility. Photomicrographs were obtained using a Nikon Eclipse Ni optical microscope, model Ni-U (Nikon Corp., Japan), and morphometric measurements were performed with NIS-Elements imaging software. A total of 50 spores or conidia per isolate were measured for length and width.

Molecular identification

For molecular identification, representative fungal colonies growing uniformly on PDA were selected from the previously described cultures. Genomic DNA was extracted from actively growing mycelial tissue using the Wizard® Genomic DNA Purification Kit (Promega, USA), following the manufacturer's protocol (Tafur Salazar, 2017).

DNA concentration and purity were assessed using a Multiskan SkyHigh spectrophotometer (Thermo Scientific, USA), while DNA integrity was verified by electrophoresis on a 0.8% w/v agarose gel prepared with 1× TAE buffer, run at 70 V for 60 minutes. Samples showing a 260/280 absorbance ratio between 1.8 and 2.0, and a minimum concentration of 50 ng/μL (Hall, 2013), were selected for further processing. Qualified DNA extracts were submitted to the Centro Nacional de Innovaciones Biotecnológicas (CENIBiot, Costa Rica) for Sanger sequencing of the internal transcribed spacer (ITS) region.

Resulting chromatograms were quality-checked and edited using BioEdit software. Sequences with a base call accuracy $\geq 80\%$ were retained for identification. Taxonomic assignment was conducted using the BLASTn algorithm against the NCBI nucleotide database (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>), retrieving the closest matches with a similarity of 97% or higher.

For phylogenetic inference, the three most similar reference sequences for each isolate were selected from the GenBank database. A Neighbor-Joining phylogenetic tree was constructed in MEGA version 10.1 (Kimura, 1980), using Kimura's two-parameter model and 1,000 bootstrap replicates (Tamura et al., 2021) to assess branch support. The analysis included 11 nucleotide sequences, with ambiguous positions removed using the pairwise deletion method (Robledo D'Angelo, 2016).

Pathogenicity testing

Pathogenicity assays were conducted using healthy *H. brasiliensis* leaves collected in September 2024 from the same location where the fungus was isolated. Healthy leaves were randomly selected from five different trees showing no visible disease symptoms. A completely randomized experimental design was employed, with eight replicate leaves per fungal isolate and a negative control treated with sterile PDA (n = 40 leaves). Prior to inoculation, leaves were disinfected by washing with commercial bleach (sodium hypochlorite) and antibacterial soap, rinsed thoroughly with distilled water, and dried with sterile paper towels.

Each leaf was superficially wounded with a sterilized needle, and a 5 mm-diameter mycelial disc from an actively growing culture was placed over the wound. Control leaves received only sterile PDA discs. Inoculated leaves were then placed in Petri dishes containing 2%w/v. water-agar (AA) and sealed with adhesive film. The dishes were incubated under darkness for 24 hours to promote infection (Zainudin et al., 2023), followed by 12-hour light/dark photoperiod at 24–26°C for eight days.

After the incubation period, each leaf was photographed alongside a millimetric ruler. The leaf area and lesion area were quantified using the software ImageJ, version 1.54p (Schneider et al., 2012) to calculate the percentage of affected tissue. To confirm the pathogenic role of each isolate, reisolation procedures were carried out from the advancing lesion margins. Small fragments were surface-sterilized and plated on PDA, incubated at 28°C for eight days, and the identity of reisolates was confirmed morphologically, thereby fulfilling Koch's postulates (Rahman, 1988).

Statistical analysis

The percentage of leaf area affected by each treatment was calculated from lesion measurements obtained in the pathogenicity assay. To evaluate differences in pathogenicity among fungal isolates, a linear mixed-effects model (LMM) was fitted, with fungal treatment as a fixed effect and tree identity as a random effect, to account for potential non-independence among leaves collected from the same individual (Eq. 1).

$$Y_{ij} = \mu + \alpha_i + \beta_j + \epsilon_{ij}$$

1

Where: Y_{ij} is the percentage of lesion area for the i th treatment on the j th tree; μ is the overall mean; α_i is the fixed effect of the i th fungal treatment ($i = 1, \dots, 5$); β_j is the random effect of the j th tree ($j = 1, \dots, 5$), assumed to follow $\beta_j \sim N(0, \sigma^2_{\beta})$; and, ϵ_{ij} is the residual error, assumed to follow $\epsilon_{ij} \sim N(0, \sigma^2_{\epsilon})$.

Model assumptions of normality of residuals and homoscedasticity were evaluated using the Shapiro–Wilk test and Levene's test, respectively. Once assumptions were confirmed, Tukey's Honestly Significant Difference (HSD) test was applied for multiple comparisons at a 95% confidence level. All statistical analyses were performed using R software (version 4.4.3) (R Core Team, 2024).

Results

A total of 30 fungal isolates belonging to the families Botryosphaeriaceae, Diaporthaceae, Nectriaceae, Apiosporaceae, and Glomerellaceae were obtained from diseased leaves of *Hevea brasiliensis*. ITS gene sequencing using primers ITS4–ITS5 identified four species: *Diaporthe tulliensis*, *Nigrospora sphaerica*, *Fusarium equiseti*, and *Lasiodiplodia theobromae*. These identifications were consistent with genus-level determinations based on colony and conidial morphology.

Diaporthe tulliensis

The colony morphology on potato dextrose agar (PDA) showed a slightly cottony mycelium with concentric growth and a distinct black stromatic layer of firm consistency. Initially white (RGB: 211, 205, 245), colonies darkened after 15 days, particularly at the margins with medium gray (RGB: 205, 197, 183) (Figure 1a); The reverse of the colonies showed brownish pigmentation (RGB: 216, 158, 116). On *Pinus caribaea* var. *hondurensis* needles, globose black pycnidia (RGB: 195, 151, 128) covered by a thin layer of white mycelium (RGB: 211, 205, 190) were produced (Figure 1b). Microscopic examination revealed hyaline, ellipsoid α -conidia measuring $4.3\text{--}5.1 \times 1.3\text{--}2.2 \mu\text{m}$ (L $\times$ W; n = 50) (Figure 1c).

PCR amplification of the ITS region yielded a 503 bp fragment (GenBank accession no. PV865276). BLAST analysis showed 99% sequence identity with *D. tulliensis* (GenBank accession no. OL353699.1). In the phylogenetic analysis, which incorporated *D. tulliensis* sequences from multiple geographic origins, our isolate clustered with reference strains with high bootstrap support (Figure 1d).

Figure 1 is here.

Nigrospora sphaerica

Colony morphology on PDA showed white, cottony mycelium with concentric growth rings (RGB: 220, 218, 210) (Figure 2a). On synthetic nutrient-poor agar (SNA), sporulation was induced, producing a thin mycelial layer (RGB: 203, 202, 198) and abundant spores with black to gray pigmentation (RGB: 207, 184, 142 to 220, 214, 204) (Figure 2b). Conidia were smooth-walled, black (RGB: 203, 202, 198), globose to ellipsoidal, and measured $14.1\text{--}16.2 \times 14.7\text{--}16.7 \mu\text{m}$ (L $\times$ W; n = 50) (Figure 2c).

ITS region amplification produced a 476 bp fragment (GenBank accession no. PV865279). BLAST analysis indicated 100% identity with *N. sphaerica* (GenBank accession no. MZ724895.1). Phylogenetic analysis, incorporating sequences from diverse geographic origins, placed our isolate within the *N. sphaerica* clade with strong bootstrap support (Figure 2d), corroborating the morphological and DNA identification.

Figure 2 is here.

Lasiodiplodia theobromae

Colony showed abundant aerial mycelium that was light gray on the surface (RGB: 190, 185, 176) and dark gray on the reverse (RGB: 137, 126, 109) (Figure 3a). On *P. caribaea* var. *hondurensis* needles, black cone-shaped pycnidia (RGB: 137, 137, 131) covered by gray mycelium (RGB: 121, 121, 113) were produced (Figure 3b). Conidia were hyaline, cylindrical to subovoid, and measured $18.4\text{--}21.1 \times 9.5\text{--}10.3 \mu\text{m}$ (L $\times$ W; n = 50) (Figure 3c).

PCR amplification yielded a 360 bp ITS fragment (GenBank accession no. PV865278). BLAST analysis showed 93% identity with *L. theobromae* (GenBank accession no. MK734053.1). In the phylogenetic analysis, our isolate clustered with *L. theobromae* sequences from other regions (Figure 3d), reinforcing its identification and supporting the morphological observations.

Figure 3 is here.

Fusarium equiseti

Colony morphology was irregular, with abundant white cottony mycelium (RGB: 208, 196, 174) interspersed with brown aerial patches (RGB: 221, 143, 78), and a caramel-colored reverse (RGB: 157, 93, 70) (Figure 4a). Conidia were elongated, curved, sharp-tipped, septate, hyaline, and measured $20.9\text{--}26.1 \times 3.5\text{--}4.2 \mu\text{m}$ (L $\times$ W; n = 50) (Figure 4b, 4c).

PCR amplification of the ITS region produced a 486 bp fragment (GenBank accession no. PV865277). BLAST analysis revealed 100% sequence identity with *F. equiseti* (GenBank accession no. MT907512.1). Phylogenetic analysis grouped our isolate with *F. equiseti* sequences from other geographic regions (Figure 4d), confirming its identity and complementing the morphological diagnosis.

Figure 4 is here.

Pathogenicity tests

Analysis of lesion development revealed that *Fusarium equiseti*, *Nigrospora sphaerica*, *Diaporthe tulliensis*, and *Lasiodiplodia theobromae* produced necrotic areas on *H. brasiliensis* leaves significantly larger than those in the control treatment ($p < 0.05$; Figure 5). Lesion size differed significantly among species: *F. equiseti* and *N. sphaerica* caused the largest mean necrotic areas (exceeding 2.8 mm^2), followed by *D. tulliensis* and *L. theobromae* (from 1.9 to 2.7 mm^2). The variability within treatments was minimal, as reflected by the narrow standard error bars. In all inoculation trials, the same fungal species were successfully reisolated from symptomatic leaves, thereby fulfilling Koch's postulates. Control leaves remained symptom-free throughout the assay, confirming the pathogenic role of the tested species.

Figure 5 is here.

Regarding morphometric comparisons, the conidial dimensions recorded in this study, as summarized in Table 1, generally aligned with those reported in the literature, thereby supporting the species identifications. Minor deviations were noted, including slightly shorter conidia in *F. equiseti* and a narrower size range in *L. theobromae* compared with certain published values. Such differences are consistent with intraspecific variability, which can be influenced by factors such as host origin, and incubation conditions. This morphometric evidence complements the molecular analyses, reinforcing the taxonomic assignments of the fungi associated with *H. brasiliensis* leaf spot in tropical environments.

Table 1. Conidial dimensions of fungal species isolated from *H. brasiliensis* leaves compared with measurements reported in the literature.

Species	Conidia dimension (µm)		Reference
	Our study	Literature	
<i>D. tulliensis</i>	4.3–5.1 × 1.3–2.2	2.6–5.9 × 1.4–3.9; 3.7–6.5 × 1.0–2.6	(Alves et al., 2008, Pan <i>et al.</i> , 2024)
<i>N. sphaerica</i>	14.1–16.2 × 14.7–16.7	11.5–15.7 × 13.3–19.6; 13.3–19.7 × 10.8–17.8	(Villanueva et al., 2023, Bansal et al., 2024)
<i>F. equiseti</i>	20.9–26.1 × 3.5–4.2	24.0–40.0 × 4.0–6.0	(Manghwar <i>et al.</i> , 2021, Pérez-Corral et al., 2015)
<i>L. theobromae</i>	18.4–21.1 × 9.5–10.3	(19–) 21–31 (–32.5) × (12–) 13–15.5 (–18.5); 20–21.8 × 9.1–10.9	(He et al., 2025, Munirah <i>et al.</i> , 2017)

Note: Conidial dimensions are presented as length × width (µm). Ranges in parentheses indicate extreme minimum or maximum values reported in the literature. Values from this study are based on 50 conidia per isolate (n = 50).

Discussion

Accurate identification of foliar pathogens in *Hevea brasiliensis* is essential to assess their potential impact on tree health and long-term plantation productivity. In the present study, morphological and molecular analyses confirmed the occurrence of *Diaporthe tulliensis*, *Nigrospora sphaerica*, *Fusarium equiseti*, and *Lasiodiplodia theobromae* associated with symptomatic leaves in Costa Rica. The genus *Diaporthe* (Ascomycota: Diaporthaceae) is well known to include important plant pathogens, endophytes, and saprobes on a wide range of hosts, including economically valuable agricultural and forestry species (Diogo et al., 2010). Consistent with our findings, *Diaporthe* spp. and *Colletotrichum* spp. have previously been reported as causal agents of foliar diseases in *H. brasiliensis* plantations in Asia, particularly in Malaysia and Bangladesh (Hao et al., 2020, Kee et al., 2019). Foliar infections by *Diaporthe* spp. could lead to premature leaf senescence, reduction of canopy photosynthetic capacity,

and, over time, significant declines in growth and natural latex production (Ogunsiji et al., 2020, Oghenekome, 2004).

In the case of *Nigrospora sphaerica*, this species induced necrotic lesions comparable to those previously reported on *Chrysanthemum morifolium* (Luo et al., 2022) and *Vigna unguiculata* (Deepika et al., 2021). Widely distributed across tropical and subtropical regions, *N. sphaerica* is recognized for its opportunistic infection strategy, which facilitates rapid colonization of wounded or physiologically stressed tissues (Bansal et al., 2024). In the context of *H. brasiliensis* plantations, such infection dynamics indicate that factors such as mechanical injury, foliar stress, or inadequate cultural practices may act as predisposing conditions for disease onset, potentially compromising canopy integrity and long-term plantation productivity (Villanueva et al., 2023, Seephueak et al., 2010).

Regarding lesion development, *Fusarium equiseti* exhibited the most extensive necrotic area formation in the pathogenicity assays, corroborating previous reports of its aggressive behavior in a wide range of hosts, including *Triticum aestivum* in China (Han et al., 2022) and *Malus domestica* in Mexico (Pérez-Corral et al., 2015). This species is recognized for its ability to cause both foliar and root diseases, reflecting a high degree of ecological plasticity and adaptability to varied environmental conditions (Raja et al., 2017, Jia et al., 2020). In *H. brasiliensis*, this pathogenic versatility raises significant concerns regarding its capacity to impair canopy function and root system health, potentially reducing tree vigor and latex productivity (Roesler et al., 2022). The lesion development patterns observed in the present study are consistent with prior descriptions of disease progression, in which small necrotic spots rapidly enlarge and coalesce, culminating in extensive foliar necrosis (Rehman et al., 2023, Hami et al., 2021).

Lasiodiplodia theobromae is a cosmopolitan pathogen in tropical and subtropical regions, frequently existing as a latent endophyte until host stress conditions trigger pathogenic development (Wang et al., 2023). In the present study, this species caused extensive necrotic lesions on *H. brasiliensis* leaves, comparable to those documented in plantations in Thailand (He et al., 2025) and China (Wang et al., 2023). Its wide host range, combined with a marked capacity for adaptation to diverse environmental conditions, underscores the importance of implementing preventive management strategies in both young and mature *H. brasiliensis* stands to mitigate potential canopy damage and productivity losses (Alves et al., 2008, Pornsuriya et al., 2023).

The precise identification of foliar pathogens in *H. brasiliensis* underscores the importance of integrating morphological and molecular approaches to ensure reliable diagnostic practices in tropical forestry systems (Rincón Sepulveda, 1990, Sell Biasseti, 2021). This study demonstrates strong concordance between colony and conidial morphological traits and internal transcribed spacer (ITS) region sequencing, reinforcing the utility of this molecular marker as a DNA barcode for fungal identification (Raja et al., 2017, Pornsuriya et al., 2023). Taxonomic placement of the isolates was supported by identity values exceeding 95%, while morphometric measurements (Table 1) closely matched published ranges, accounting for intraspecific variation attributable to host origin and environmental conditions (Vineeth et al., 2024, Alves et al., 2008). This integrative approach is particularly valuable in tropical

environments, where morphological plasticity frequently obscures species boundaries and hinders timely pathogen detection (Havanapan et al., 2016, Mazlan et al., 2019a). Establishing precise pathogen baselines is critical for enabling early diagnosis, informing targeted management strategies, and safeguarding the long-term health and productivity of *H. brasiliensis* plantations (Zainudin et al., 2023, Sagaff et al., 2022).

Anchored in this diagnostic framework, the identification of these pathogens provides a solid foundation for formulating integrated disease management strategies aimed at reducing productivity losses and lowering tree mortality (Mohanta & Bae, 2015, Ogunsiji et al., 2020). Effective management requires the deployment of resistant or tolerant clones from well-defined breeding programs, the enforcement of stringent sanitation protocols, and systematic surveillance to track pathogen prevalence and spread (Syed Sagaff et al., 2022, Fajarningsih, 2016). In the context of climate change adaptive management and the incorporation of resilient genotypes into breeding efforts are increasingly critical (Rubilar et al., 2024). The continued expansion of *H. brasiliensis* cultivation in Costa Rica, proactive exclusion and containment measures are essential to prevent large-scale outbreaks that could compromise canopy integrity and latex yield (Seguin et al., 2003, Sell Biasseti, 2021). Future research should investigate the epidemiology and environmental thresholds for infection by these species, along with their interactions with other foliar pathogens in co-infections, thereby enhancing the resilience and long-term sustainability of tropical *H. brasiliensis* plantations (Teoh et al., 2011, Sterling & Melgarejo, 2021, Mazlan et al., 2019a).

Conclusions

This study integrated morphological and molecular approaches to accurately identify foliar fungi associated with *Hevea brasiliensis* in Costa Rica. Thirty isolates were obtained from symptomatic leaves belonging to the families Botryosphaeriaceae, Diaporthaceae, Nectriaceae, Apiosporaceae and Glomerellaceae. Sequencing of the internal transcribed spacer region enabled species-level identification, which was consistent with genus-level determinations based on colony and conidial morphology.

Pathogenicity assays demonstrated that *Fusarium equiseti*, *Nigrospora sphaerica*, *Diaporthe tulliensis*, and *Lasiodiplodia theobromae* are capable of inducing necrotic foliar lesions in *H. brasiliensis*. Among these, *F. equiseti* and *L. theobromae* produced the largest areas of lesion, highlighting their potential as major threats to plantation health. Accurate identification of these pathogens represents the first report of their association with *H. brasiliensis* in Costa Rica and establishes a baseline reference for disease surveillance. These findings provide critical information for the development of early detection protocols and integrated management strategies, ultimately contributing to the long-term health and productivity of tropical *H. brasiliensis* plantations.

Declarations

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Author Contributions: M.S.E: morphological and molecular analysis, inoculation, pathogenicity tests. M.R.S: sample collection, isolation, morphological description of the fungus. D.M.A.: de-scription and analysis of molecular identification and pathogenicity tests. D.A.A: project management, statistical analysis. J.C.V.: writing - original draft preparation. All authors reviewed and editing the manuscript.

Conflict of interest: The authors declare no conflict of interest.

Data Availability: Data will be made available on reasonable request.

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Figures

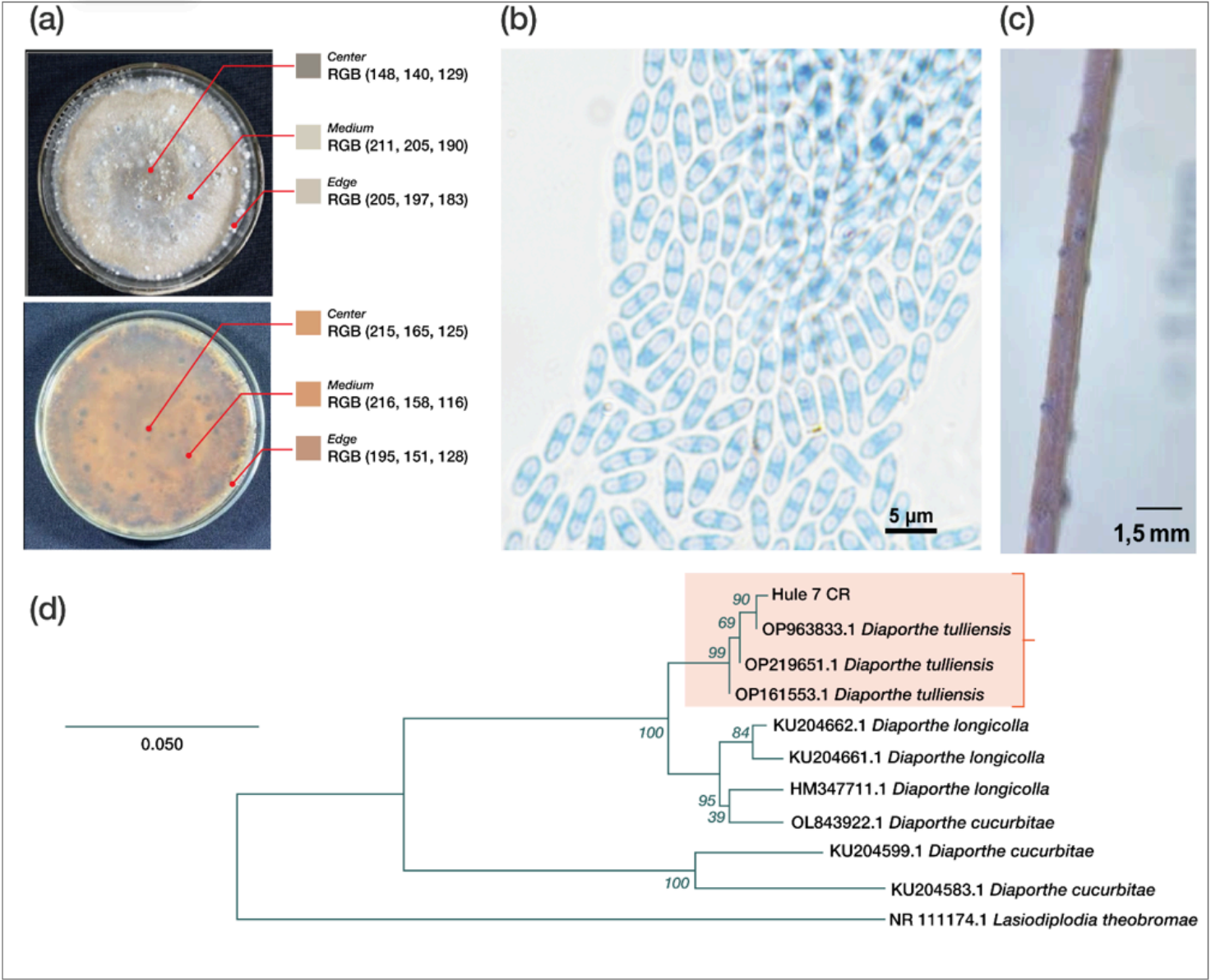


Figure 1

Morphological and molecular characterization of *Diaporthe tulliensis*. (a) Colony color on surface and reverse of PDA plates. (b) Pycnidia on *Pinus caribaea* var. *hondurensis* needles. (c) α -conidia. (d) Neighbor-Joining phylogenetic tree of ITS sequences, with *L. theobromae* as the outgroup (The red bracket marks the accessions grouping with the sequence obtained in this study).

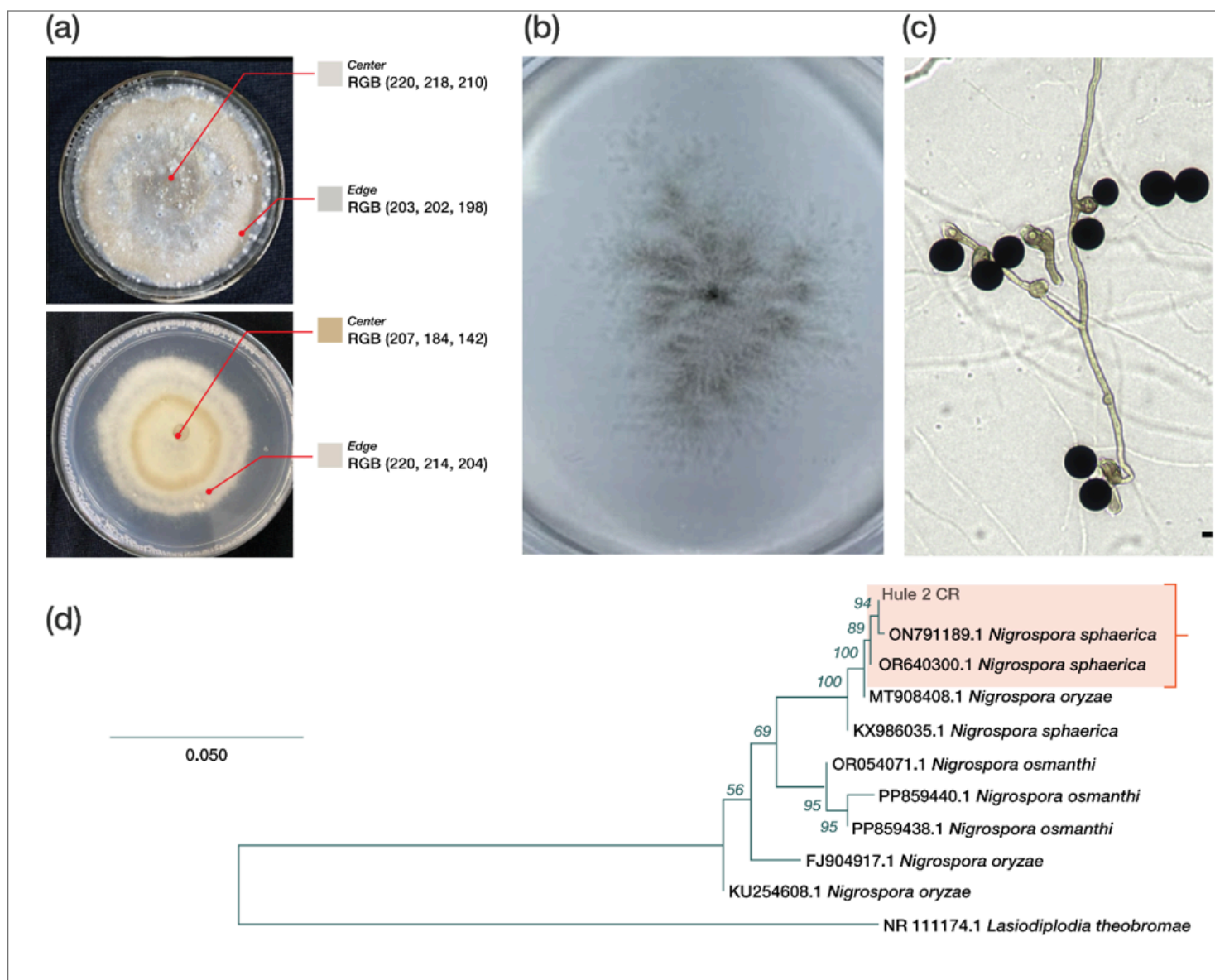


Figure 2

Morphological and molecular characterization of *Nigrospora sphaerica*. (a) Colony color on surface and reverse of PDA plates. (b) Mycelium with spores on SNA medium. (c) Conidia and hyaline hyphae. (d) Neighbor-Joining phylogenetic tree of ITS sequences, with *L. theobromae* as the outgroup (The red bracket marks the accessions grouping with the sequence obtained in this study).

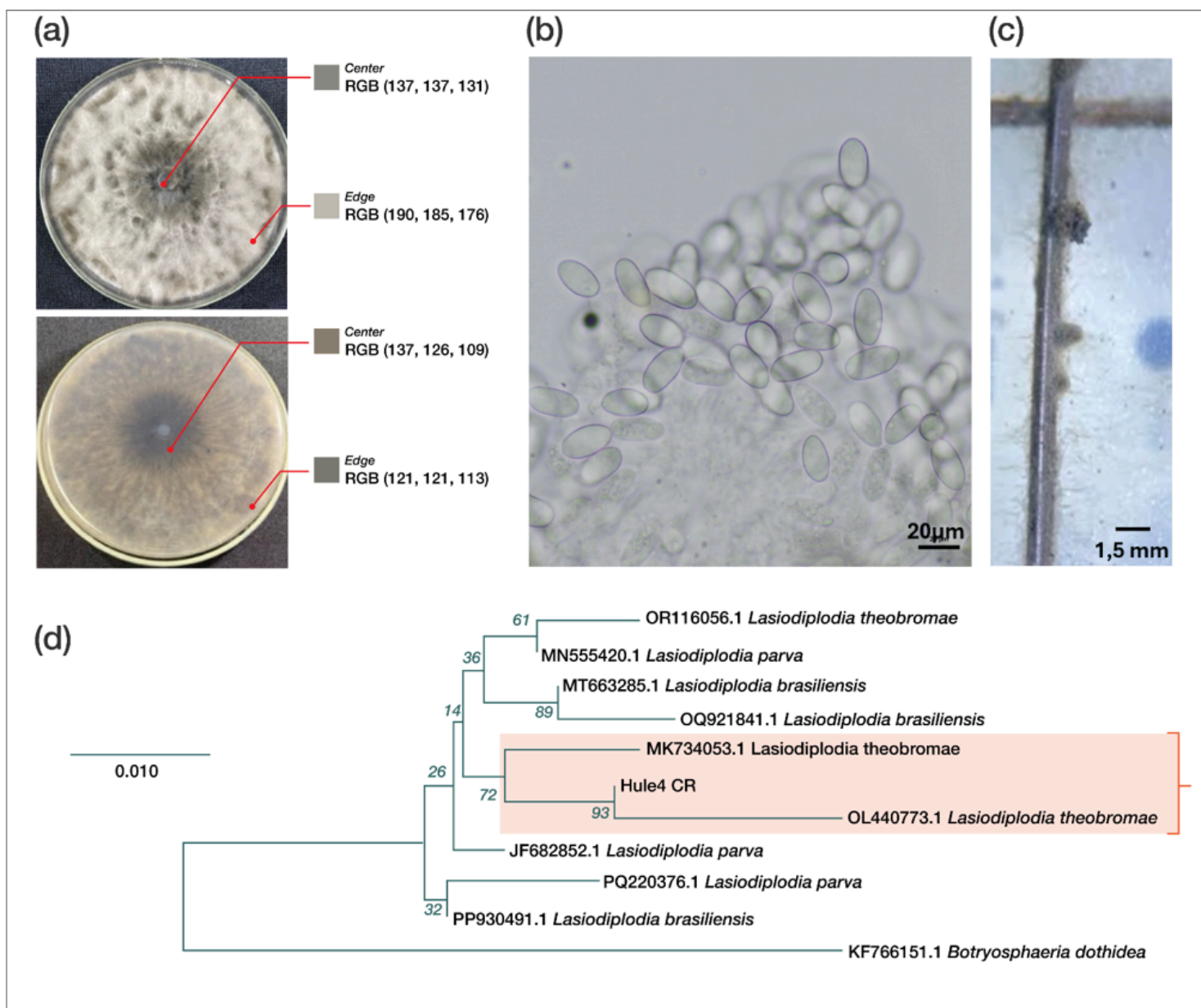


Figure 3

Morphological and molecular characterization of *Lasiodiplodia theobromae*. (a) Colony color on surface and reverse of PDA plates. (b) Pycnidia on *Pinus caribaeavar. hondurensis* needles. (c) α-conidia. (d) Neighbor-Joining phylogenetic tree of ITS sequences, with *Botryosphaeria dothidea* as the outgroup (The red bracket marks the accessions grouping with the sequence obtained in this study).

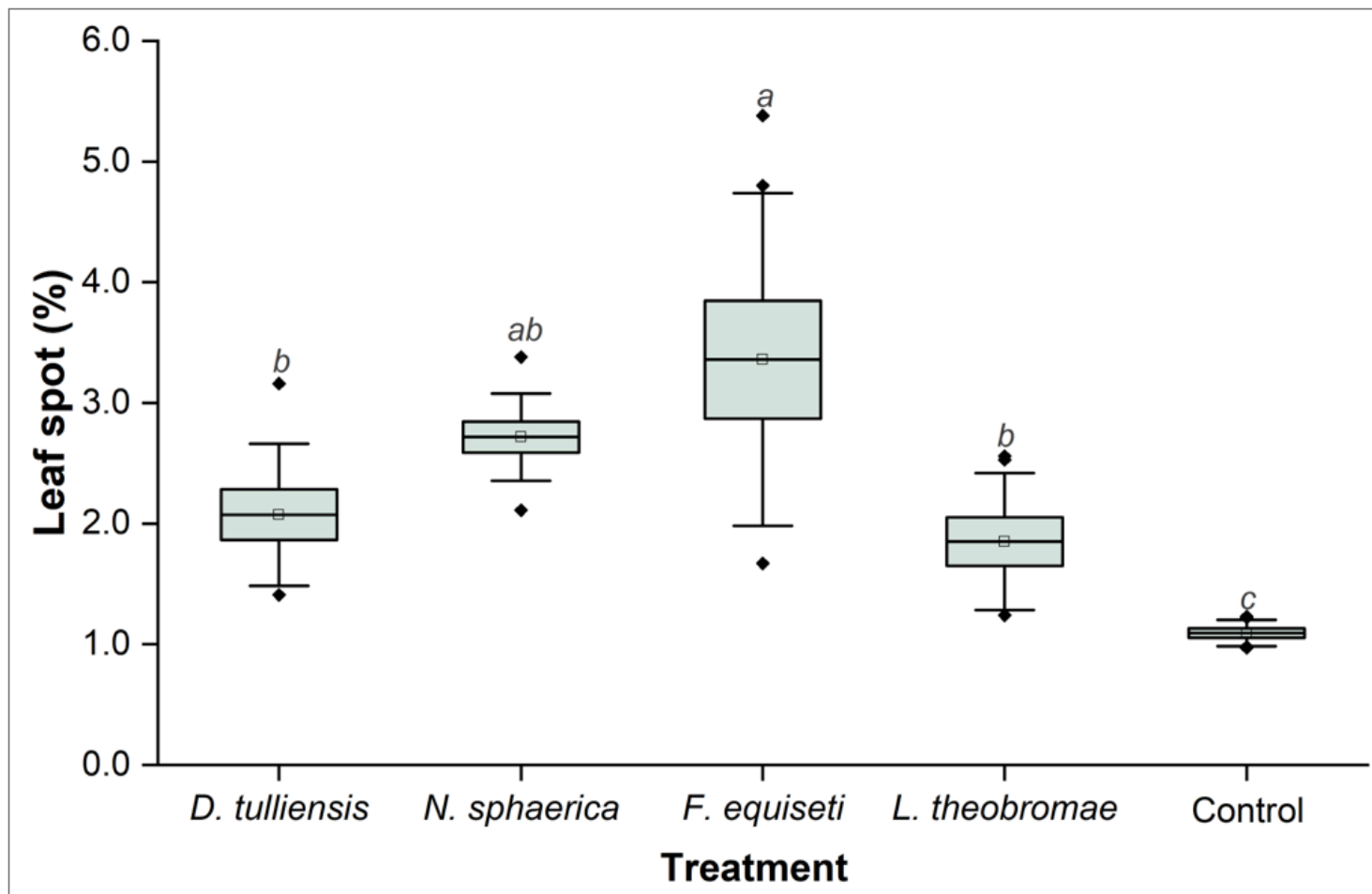


Figure 5

Boxplot of the percentage of leaf spot severity on *H. brasiliensis* leaves inoculated with different fungal species during laboratory pathogenicity assays (The horizontal line within each box represents the mean, the box boundaries correspond to the standard error, and the whiskers denote the standard deviation. Different letters indicate statistically significant differences among treatments ($p < 0.05$)).