



Characterization of the phytopathogenic fungal community in yellow pitahaya (*Selenicereus megalanthus*) crops from Boyacá, Colombia

Caracterización de la comunidad de hongos fitopatógenos en cultivos de pitahaya amarilla (*Selenicereus megalanthus*) de Boyacá, Colombia

Julián Esteban Másmela-Mendoza^{1,2} , Luz Marina Lizarazo-Forero¹ 

Abstract:

Background and Aims: Yellow pitahaya (*Selenicereus megalanthus*) is a high-value fruit crop in Colombia, but is highly susceptible to fungal phytopathogens affecting stems, flowers, and developing fruits. Improved knowledge of pathogen diversity and characterization is critical for accurate diagnosis and effective disease management. This study aimed to identify and characterize the mycobiota associated with diseases in yellow pitahaya crops in Boyacá, Colombia.

Methods: Phytopathogenic fungi were isolated both from symptomatic stem and fruit tissues of yellow pitahaya plants. Samples were surface-sterilized and cultured on potato dextrose agar (PDA). Fungal genomic DNA was extracted, and the ITS, LSU, *tef1*, and *BenA* gene regions were amplified and sequenced. Taxonomic identification was performed using BLAST and multilocus phylogenetic analysis. Pathogenicity tests were conducted on disinfected cladodes under controlled environmental conditions.

Key results: Key pathogens identified included *Fusarium foetens*, *F. guttiforme*, *F. sterilihyphosum*, and *Lasiodiplodia* species were linked to basal stem rot lesions. The bug *Leptoglossus zonatus* was identified as a vector transmitting *F. foetens*. *Diaporthe* species were associated with grayish-orange stem blight, while other fungi like *Neofusicoccum*, *Colletotrichum* sp., and *Alternaria* sp. were isolated from chlorotic lesions. Pathogenicity tests confirmed *F. foetens* as the cause of stem rot. Molecular analysis revealed that the *F. fujikuroi* species complex was primarily associated with rot symptoms.

Conclusions: Members of the *Fusarium fujikuroi* species complex were identified in pitahaya fruit as phytopathogenic mycobiota associated to crops in Boyacá, Colombia. This is the first report of *Fusarium foetens* causing stem rot in pitahaya crops.

Key words: basal stem rot, fungal pathogens, *Fusarium*, plant disease diagnosis.

Resumen:

Antecedentes y Objetivos: La pitahaya amarilla (*Selenicereus megalanthus*) es un cultivo frutal de alto valor en Colombia, pero es altamente susceptible a fitopatógenos fúngicos que afectan tallos, flores y frutos en desarrollo. Mejorar el conocimiento sobre la diversidad y caracterización de estos patógenos es fundamental para un diagnóstico preciso y un manejo eficaz de las enfermedades. Este estudio tuvo como objetivo identificar y caracterizar la micobiota asociada a cultivos enfermos de pitahaya amarilla en Boyacá, Colombia.

Métodos: Se aislaron hongos fitopatógenos a partir de tejidos sintomáticos de tallos y frutos de plantas de pitahaya amarilla. Las muestras fueron desinfectadas superficialmente y cultivadas en agar papa dextrosa (PDA). Se extrajo ADN genómico fúngico y se amplificaron y secuenciaron las regiones génicas ITS, LSU, *tef1* y *BenA*. La identificación taxonómica se realizó mediante búsquedas BLAST y análisis filogenético multilocus. Las pruebas de patogenicidad se realizaron en cladodios desinfectados bajo condiciones ambientales controladas.

Resultados clave: Los fitopatógenos claves identificados incluyeron *Fusarium foetens*, *F. guttiforme*, *F. sterilihyphosum* y las especies de *Lasiodiplodia*, las cuales fueron vinculadas a lesiones de podredumbre basal del tallo. El chinche *Leptoglossus zonatus* se identificó como un vector transmisor de *F. foetens*. Las especies de *Diaporthe* se asociaron con el tizón del tallo de color naranja grisáceo, mientras que otros hongos como *Neofusicoccum* sp., *Colletotrichum* sp. y *Alternaria* sp. se aislaron de lesiones cloróticas. Las pruebas de patogenicidad confirmaron que *F. foetens* era la causa de la podredumbre del tallo. El análisis molecular reveló que el complejo de especies de *F. fujikuroi* se asoció principalmente con los síntomas de podredumbre.

Conclusiones: Se identificaron miembros del complejo de especies *Fusarium fujikuroi* en cultivos de pitahaya como micobiota fitopatógena asociada a los cultivos de pitahaya en Boyacá, Colombia. Este es el primer reporte de *Fusarium foetens* causante de pudrición del tallo en cultivos de pitahaya.

Palabras clave: diagnóstico de enfermedades de las plantas, fitopatógenos fúngicos, *Fusarium*, pudrición basal del tallo.

¹Universidad Pedagógica y Tecnológica de Colombia, Facultad de Ciencias, Programa Biología, Grupo de investigación Biología Ambiental, Avenida Central del Norte 39-115, Tunja, Boyacá, Colombia.

²Autor para la correspondencia: julianmasmela@gmail.com

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Introduction

Dragon fruit, or pitahaya, is the fruit of several tropical climbing cacti belonging to the genera *Selenicereus* (A. Berger) Britton & Rose and *Hylocereus* (A. Berger) Britton & Rose (Cactaceae). Colombia has been a pioneer in the production and export of yellow pitahaya (*Selenicereus megalanthus* (K. Schum. ex Vaupel) Moran), ranking it among the country's top five export crops, with an annual production of approximately 470 tons. Within Latin America, Colombia is the second-largest producer after Ecuador, according to production data of 2022 (AGRONET, 2022; Morillo-Coronado et al., 2022a).

The predominant genotype cultivated in the production systems of Boyacá, Colombia, is yellow pitahaya, formerly known as *Hylocereus megalanthus* K. Schum. ex Vaupel) Ralf Bauer (Morillo-Coronado et al., 2022a). Although they were traditionally distinguished by external morphological characteristics—such as the presence of spines or scales on the pericarpel—, recent molecular phylogenetic studies have shown that there is no clear genetic evidence to maintain *Hylocereus* and *Selenicereus* as separate genera. Instead, *Hylocereus* has been proposed as a taxonomic synonym, now integrated within *Selenicereus* (Korotkova et al., 2017).

Pitahaya is susceptible to a range of biotic stressors, including bacteria, fungi, viruses, and certain insect pests. More than 17 genera and 25 species of pathogens have been reported in pitahaya crops, most of which are fungi affecting stems, fruits, and flowers (Balendres and Bengoa, 2019). Among the main constraints on pitahaya production in Boyacá, Colombia, are diseases caused by phytopathogenic fungi, which can significantly reduce yield and productivity (Mora, 2012; Morillo-Coronado et al., 2022b). These unidentified fungi can infect plants during both the establishment and growth stages, affecting stems, developing fruits, and even post-harvest fruit (Morillo-Coronado et al., 2016). In *Selenicereus*, *Fusarium oxysporum* Schltdl. f. sp. *cumini* Prasad & Patel, and *F. fujikuroi* Nirenberg are known to cause basal stem rot (Salazar-González et al., 2016; Huamán-Pilco et al., 2024).

Fusarium solani (Mart.) Sacc. and other fungal pathogens have also been associated with disease symptoms in

Hylocereus species. Recent reports have identified additional taxa such as *Lasiodiplodia* Ellis & Everh., *Diaporthe* Nitschke, and *Nigrospora sphaerica* (Sacc.) E.W. Masson (Rita et al., 2013; Briste et al., 2019; Karim et al., 2019; Liu et al., 2016; Ganesan et al., 2023).

Despite the economic relevance of pitahaya as an exotic fruit on Colombia's export market, research on fungal pathogens has largely focused on *Fusarium* Link ex Grey species. There is limited information on other fungal pathogens affecting *Hylocereus*, such as *Lasiodiplodia* and *Colletotrichum* Corda which are responsible for stem canker, stem rot, and reddish-brown spot, respectively (Liu et al., 2016; Briste et al., 2019; Karim et al., 2019).

In Colombia there are few reports of the identification of fungi isolated from signs and symptoms of soft rot. This study aimed to identify phytopathogenic fungi present in stems and fruits collected from commercial pitahaya plantations in the Miraflores region of Boyacá, Colombia. Morphological and molecular techniques were employed to characterize the fungal pathogens affecting pitahaya cultivation in this area.

Materials and Methods

Study area

The study was conducted in thirteen yellow pitahaya (*Selenicereus megalanthus*) production systems located in the municipality Miraflores, Boyacá, Colombia (5°14'12"N, 73°11'10"W) (Fig. 1). Local farmers cultivate pitahaya under two conditions: protected environments (greenhouses or "under cover" systems) and open-field systems. Vegetative propagation is practiced using stem cuttings, and crops are established in ground-level beds supported by cement, stone, or wooden structures. Fertilization practices include the application of products rich in macronutrients, while disease management relies on chemical, biological, and cultural control strategies.

Data on crop management and production systems were collected through structured surveys, direct observations, and on-site visits to each farm. Photographic documentation of disease symptoms and fungal signs was taken at each sampling site. Each sample was coded and documented for subsequent fungal isolation and analysis.



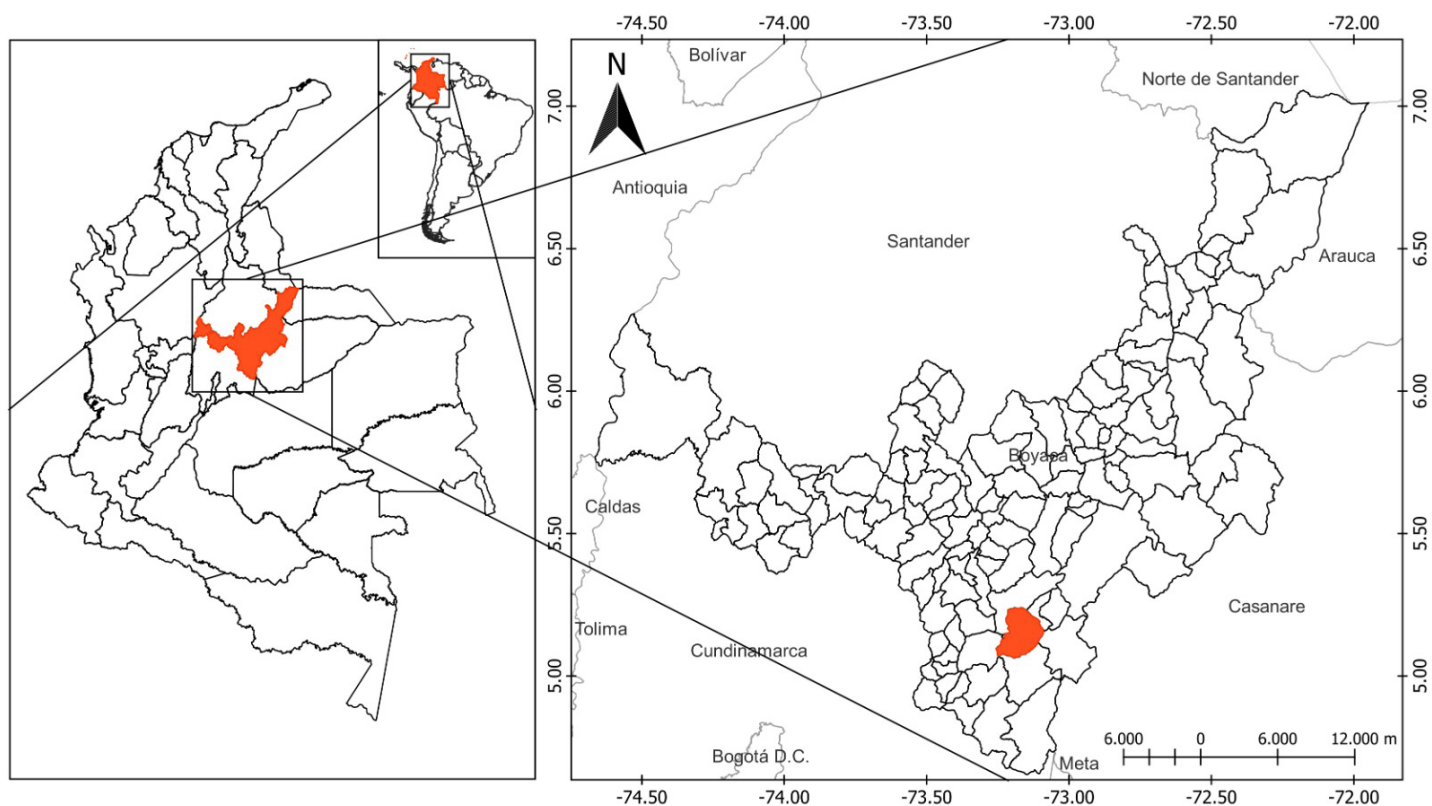


Figure 1: Location of the study area of pitahaya crops in the municipality Miraflores, Boyacá, Colombia.

Collection of plant material and isolation of fungi
Stem and fruit samples exhibiting typical fungal lesions were collected in sterile plastic bags, labeled, refrigerated, and transported to the Biología Ambiental Laboratory at the Universidad Pedagógica y Tecnológica de Colombia (UPTC). Samples were washed with tap water to remove debris, and symptomatic tissues were excised and surface sterilized in 75% ethanol for 10 seconds, followed by a sterile distilled water rinse, immersion in 1.5% sodium hypochlorite for 10 seconds, and two additional rinses in sterile distilled water. The samples were then dried on sterile filter paper (Salazar-Gonzalez et al., 2016). Sterilized tissue segments were placed onto potato dextrose agar (PDA) plates and incubated at 24–26 °C for seven days (Pitt and Hocking, 2009; Rita et al., 2013).

To assess the potential role of insects as vectors of phytopathogenic bacteria, ten adult specimens of *Leptoglossus zonatus* (Dallas, 1852) (Hemiptera: Coreidae) were manually collected using entomological forceps. This species is known to cause damage to flower buds and clau-

dodes in yellow pitahaya (Medina and Kondo, 2012). Each insect was placed alive in a sterile glass container. Under aseptic conditions in a laminar flow hood, individuals were handled using sterile forceps, grasping them at the junction between the thorax and abdomen. Their legs were then gently pressed onto the surface of Potato-dextrose agar (PDA, Merck®) (24 °C, 7 d).

Molecular identification of fungal isolates

Serial cultures on PDA agar (24–26 °C, 7–14 days) were performed for the purification of the fungal isolates. Macroscopic and microscopic identification of the fungal strains was performed using the taxonomic keys of Barnett and Hunter (1972, 1998) and Watanabe (2010), which are widely used for the identification of phytopathogenic fungi, particularly those affecting tropical fruits. Fungal isolates were cryopreserved in 20% glycerol at -20 °C, by subculturing and refrigeration at 4 °C, and through standardized cryopreservation using microtubes with cryogenic beads (Cryobank®) in the microorganism collection of the Biología Ambiental

Laboratory at the Universidad Pedagógica y Tecnológica de Colombia (UPTC). The code of each fungal isolate, fungal specimen collector and isolator and the origin of the strains are described in the GenBank repository. These isolates also part of the collection of cryopreserved microorganisms of the Biología Ambiental Laboratory group with codes from A1 to A25, and 5.1B to 11B.

Pure fungal isolates were cultured in Sabouraud Dextrose Yeast (SDY) broth (Merck®) and incubated at 25 °C with shaking at 150 rpm for 7-10 days to promote mycelial biomass production (MaxQ™ 4450 benchtop orbital incubator/shaker, Thermo Fisher Scientific, Waltham, USA). Strains, vouchers, GenBank accession numbers, related diseases, signs, symptoms and a macroscopic description of the colonies were included in [Appendix 1](#) and [2](#). Mycelia were harvested by vacuum filtration, frozen at -80 °C for 24 hours, and lyophilized using a Thermo PowerDry PL9000 (Thermo Fisher Scientific, Allerød, Denmark) (under the following conditions: -45 °C core temperature, 1 hPa pressure, and a 24-hour drying program (5 h at -35 °C, 3 h at -15 °C, 3 h at 5 °C, 5 h at 20 °C, and 9 h at 28 °C) ([Borman et al., 2008](#)).

Lyophilized material was ground in liquid nitrogen and incubated in CTAB lysis buffer (2% CTAB, 1.4 M NaCl, 100 mM Tris-HCl pH 8.0, 20 mM EDTA, 0.2% β-mercaptoethanol, and proteinase K) at 65 °C for 1 hour. DNA was purified by sequential extraction with phenol:chloroform:isoamyl alcohol (25:24:1), followed by chloroform:isoamyl alcohol (24:1). DNA was precipitated with isopropanol and incubated at -30 °C for 12 h. Pellets were washed twice with 70% cold ethanol, dried at 35 °C in a SpeedVac (Thermo Fisher Scientific, Waltham, USA) for 30 min, and resuspended in nuclease-free water. RNA was removed using RNase treatment. DNA integrity was verified via 1% agarose gel electrophoresis at 100 V for 45 min ([Romanelli et al., 2014](#); [Chauhan et al., 2022](#)).

The ITS1-LSU region was amplified using primers ITS1 (5'-TCC GTA GGT GAA CCT GCG G-3') and LR5 (5'-TCC TGA GGG AAA CTT CG-3'). For *Fusarium* isolates, the elongation factor 1-alpha (*tef1*) gene was amplified with primers EF-1H and EF-2T. For isolates from the genera *Apiospora* Sacc. (*Apiosporaceae*), *Lasiodiplodia* Ellis & Everh. (*Botryosphaeriaceae*), *Diplodia* Fr., and *Diaporthe*, the β-tubulin (*BenA*)

gene was amplified using primers BT2A and BT2B. PCR reactions were prepared with 1 × GoTaq buffer, 3.5 mM MgCl₂, 0.2 mM dNTPs (Promega, USA), 1.75 U Taq polymerase, and primers at final concentrations of 0.0275 μM (forward) and 0.028 μM (reverse).

PCR conditions were specific according to the gene for the ITS Region (94 °C for 5 min; 35 cycles of 94 °C for 45 s, 54 °C for 45 s, 72 °C for 1 min; final extension at 72 °C for 10 min); *BenA* gene (94 °C for 5 min; 35 cycles of 94 °C for 45 s, 60 °C for 45 s, 72 °C for 1 min; final extension at 72 °C for 10 min); *tef1* gene (94 °C for 5 min; 35 cycles of 94 °C for 30 s, 52 °C for 30 s, 72 °C for 90 s; final extension at 72 °C for 7 min) ([Borman et al., 2008](#); [Romanelli et al., 2014](#)). PCR products were verified on 1% agarose gels (100 V, 45 min) and sequenced using the Sanger method on a 3500 Series Genetic Analyzer (Applied Biosystems, Thermo Fisher Scientific, Waltham, MA, USA) ([Borman et al., 2008](#)).

Sequence analysis and phylogenetics

Raw sequences were processed using Geneious Prime v. 2023.2 ([Geneious, 2023](#)). Quality filtering and consensus sequence generation were performed for ITS, *tef1*, and *BenA* regions. Taxonomic assignments were conducted using Basic local alignment search tool (BLAST) ([Sayers et al., 2022](#)) against the NCBI non-redundant (nr) database ([Altschul et al., 1990](#)), with filters restricted to type material ([Felsenstein, 2005](#)). The DNA sequences of the ITS1-LSU region gene are deposited under submission number SUB15326230 and GenBank accession numbers PV648697-PV648721 ([Appendix 1](#)). Phylogenetic relationships were inferred using the maximum likelihood (ML) method in MEGA v. 11.0 ([Tamura et al., 2021](#)). Phylogenetic trees were constructed with the Tamura-Nei genetic distance model and the nearest-neighbor interchange algorithm using global alignments ([Felsenstein, 2005](#)).

Establishment and maintenance of yellow pitahaya cactus cultures for pathogenesis assays Pathogenicity assays were conducted in the greenhouses and the Biología Ambiental Laboratory at the Universidad Pedagógica y Tecnológica de Colombia (UPTC) in Tunja, Boyacá, Colombia. Eight healthy yellow pitahaya cactus pads were collected from commercial farms, ensuring no



visible lesions caused by bacteria or fungi. The plant tissue was disinfected with Vitavax (9 g l^{-1}); (Adama, Colombia). To allow the wound cut to heal, the pads were placed on plastic for five days, after which they were planted in a sandy-loam substrate with high organic matter content, receiving weekly irrigation (Mohd-Hafifi et al., 2019). After 20 days, pads without root formation were submerged in a solution of indole-3-butyric acid (IBA) (4.5 g l^{-1}) for 3 min to stimulate root development. These pads were then replanted under the same conditions.

Pathogenesis assay

To assess the pathogenicity of the fungal isolates, healthy, vigorous pitahaya pads were used. *Fusarium* isolates were cultured in PDA medium ($26 \text{ }^\circ\text{C}$, 7 days), and a conidial suspension was prepared at concentrations of 1×10^4 conidia ml^{-1} and 1×10^8 conidia ml^{-1} (Choi et al., 2007; Singh et al., 2023). The pads were inoculated by injecting $40 \text{ }\mu\text{l}$ of the suspension into the tissue using a micropipette, creating a lesion of 3 mm in diameter and 5 mm in depth. The inoculated pads were then covered with polyethylene bags and incubated for 14 days (Rita et al., 2013; Mohd-Hafifi et al., 2019).

Maintenance of pitahaya tissue isolates

Twenty isolates from symptomatic pitahaya tissue with morphological features consistent with *Fusarium* were cultured on PDA. Since some isolates did not exhibit uniform growth, they were transferred to yeast extract-glucose-chloramphenicol (YGC) agar to obtain sufficient conidia for further pathogenicity assays (Pitt and Hocking, 2009; Rita et al., 2013).

Pathogenicity assays in stems

Pathogenicity tests were conducted using healthy, mature pitahaya leaves. Pathogenicity assays were performed exclusively with *F. foetens* isolates, as it is the most common and representative morphotype obtained in the sampling and more frequently isolated. A *Fusarium foetens* Schroers, O'Donnell, Baayen & Hooftman isolate cultured for 7 days on PDA was used to prepare conidial suspensions at concentrations of 1×10^4 and 1×10^8 conidia ml^{-1} (Singh et al., 2023). Each leaf was inoculated by injecting $40 \text{ }\mu\text{l}$ of the

suspension into the tissue using a micropipette, producing a lesion approximately 3 mm in diameter and 5 mm deep. Inoculated leaves were enclosed in polyethylene bags and incubated for 14 days under controlled conditions (Choi et al., 2007; Rita et al., 2013).

Results

Fungi associated with diseases in pitahaya crops in Boyacá, Colombia

A total of 53 fungal morphotypes were isolated from symptomatic tissues of pitahaya plants in Boyacá. These isolates correspond to one division, three classes, three orders, five genera, and twelve species, with sequence coverage values ranging from 99 to 100%, and identity percentages between 91.25 and 100%. Molecularly they are related to the genera *Fusarium* Link, *Nigrospora* Zimm., *Neofusicoccum* Crous, Slippers & A.J.L. Phillips., *Lasiodiplodia* Ellis & Everh., and *Diaporthe* Nitschke (Fig. 2). The DNA sequences of the ITS1-LSU region gene are deposited under submission number SUB15326230 and GenBank (Benson et al., 2023) accession numbers PV648697-PV648721. The accession numbers per isolate are described in Appendix 1.

Macroscopic and microscopic morphological analyses initially identified *Fusarium* species (Appendix 2) as *F. oxysporum* Schldl., *F. semitectum* Berk. & Ravenel, and *F. verticillioides* (Sacc.) Nirenberg. However, molecular identification using ITS and LSU genes sequences revealed isolates belonging to the *Fusarium fujikuroi* species complex (FFSC). Further classification using the more specific *tef1* and *BenA* genes allowed precise identification of isolates as *F. foetens*, *F. guttiforme* Nirenberg & O'Donnell, and *F. sterilihyphosum* Britz, Marasas & M.J. Wingf., all within the FFSC. These identifications showed sequence coverage between 99.9 and 100%, and identity values ranging from 97.97 to 100% (Appendix 1, Fig. 2).

Fusarium foetens isolates were obtained from lesions characteristic of basal stem rot. Initial symptoms included chlorosis or small yellowish spots at the base of the stem, which progressively expanded, turning brown and eventually leading to soft rot. The disease advanced through the xylem tissue, causing structural deterioration and necrosis (Fig. 3). Isolates were also recovered from advanced stages of infection. On fruits, symptoms included chlorosis or



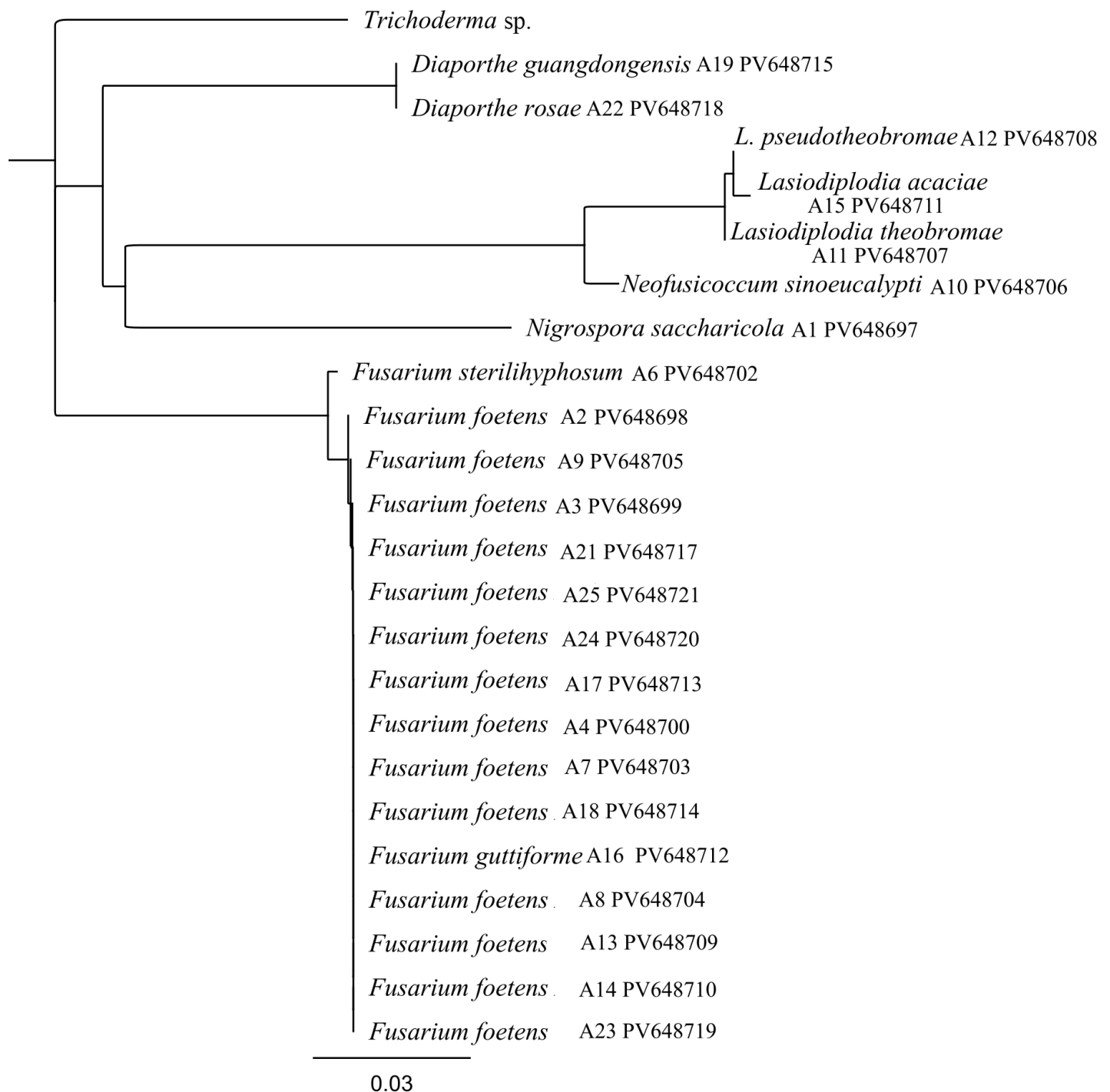


Figure 2: Phylogenetic tree of fungal isolates associated with disease symptoms in pitahaya crops from Boyacá, Colombia. The tree was constructed based on multiple sequence alignments of the ITS1 region using the Tamura-Nei genetic distance model and the neighbor-joining method. An ITS1 sequence of a *Trichoderma* sp. isolate was used as an outgroup.

yellowing at the base of the peduncle, soft rot on the fruit apex (nipples), and premature fruit ripening (Fig. 4). All the signs and symptoms described above have been reported in various studies of *Fusarium* in pitahaya (Hawa et al.,

2013, 2017; Rita et al., 2013; Balendres and Bengoa, 2019; Singh et al., 2023; Huaman-Pilco et al., 2024).

An entomofaunal survey conducted in collaboration with the Luis Gonzalo Andrade Natural History Museum

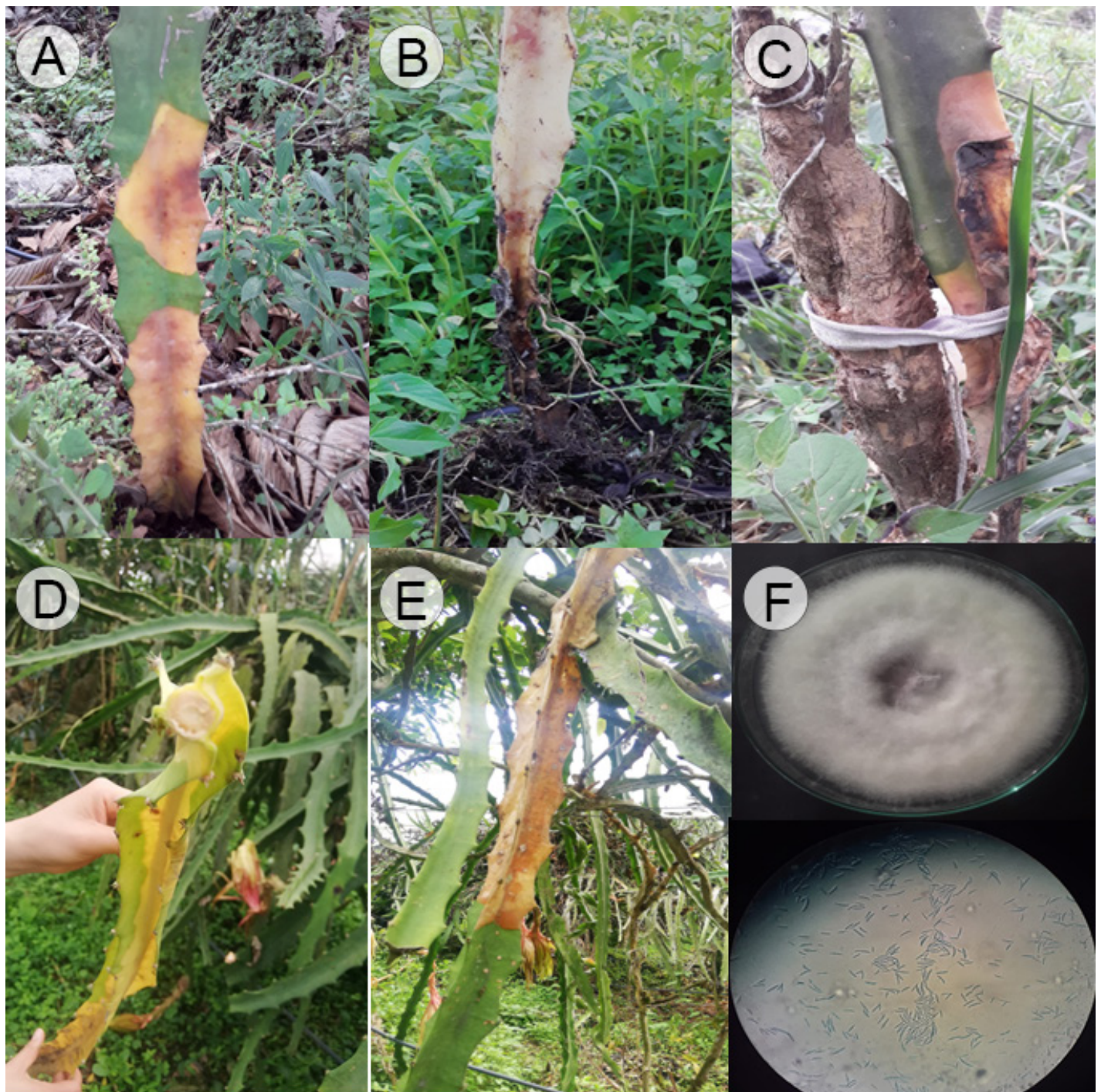


Figure 3: Basal stem rot and stem rot in pitahaya from tissues where morphotypes of *Fusarium foetens* Schroers, O'Donnell, Baayen & Hooftman, *F. sterilihyphosum* Britz, Marasas & M.J. Wingf., *F. guttiforme* Nirenberg & O'Donnell and *F. oxysporum* Schldl. were isolated. A-C. symptoms of basal stem rot; D, E. peduncle rot; F. *Fusarium* Link ex Grey colony and macroconidia (40×). Photos by J. Másmela-Mendoza.

(Programa de Biología Laboratories, Universidad Pedagógica y Tecnológica de Colombia) identified *Leptoglossus zonatus* (Coreidae) as a prevalent insect species in the crop environment. *Fusarium foetens* was isolated from three specimens of *L. zonatus*, suggesting that this insect could

potentially act as a vector in the transmission of the pathogen (Appendix 1).

Fungal isolates corresponding to *Lasiodiplodia theobromae* (Pat.) Griffon & Maubl., *L. pseudotheobromae* A.J.L. Phillips, A. Alves & Crous, and *L. acaciae* W. Zhang &



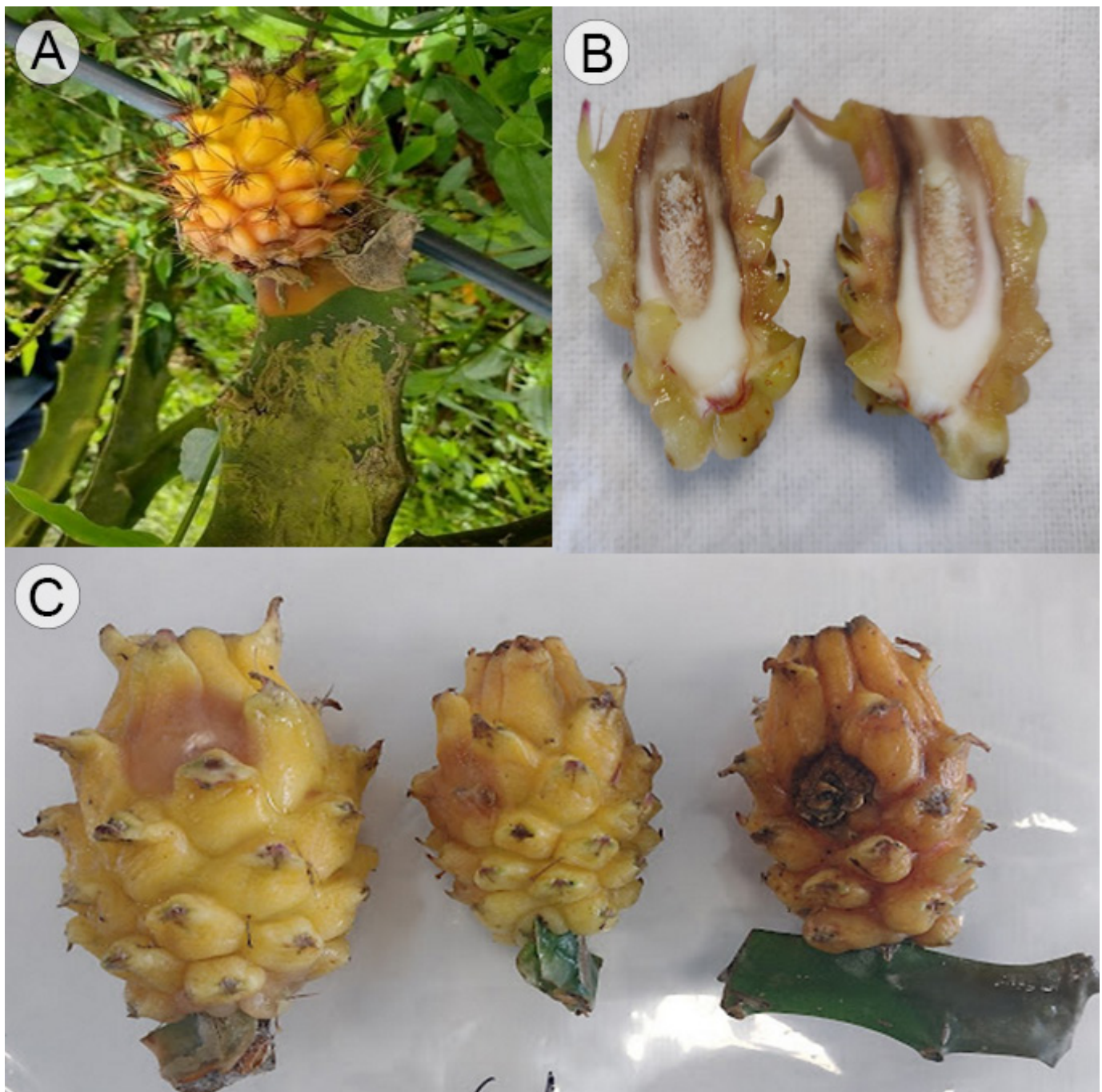


Figure 4: Symptoms observed in pitahaya fruits from which *Fusarium* Link ex Grey morphotypes corresponding to *F. oxysporum* Schldt., *F. foetens* Schroers, O'Donnell, Baayen & Hooftman, and *F. guttiforme* Nirenberg & O'Donnell were isolated. A. chlorosis at the base of the fruit peduncle; B. advanced internal soft rot affecting the central pulp of the fruit; C. various symptoms of rot and necrosis in the areoles (of the fruit, often associated with premature ripening). Photos by J. Másmela-Mendoza.

Crous were identified through sequencing of the β -tubulin gene (*BenA*), with both sequence coverage and identity percentages reaching 100% (Appendix 1, 2, Figs. 2). *Lasiodiplodia* spp. were isolated from stem rot lesions, which initially presented as chlorosis or yellowing, predom-

inantly at the apical regions of the stem. These symptoms progressed to more extensive yellow lesions, followed by tissue softening and eventual rot (Fig. 5).

For the genus *Diaporthe*, initial identification through sequencing of the ITS1 gene classified the isolates as *D.*

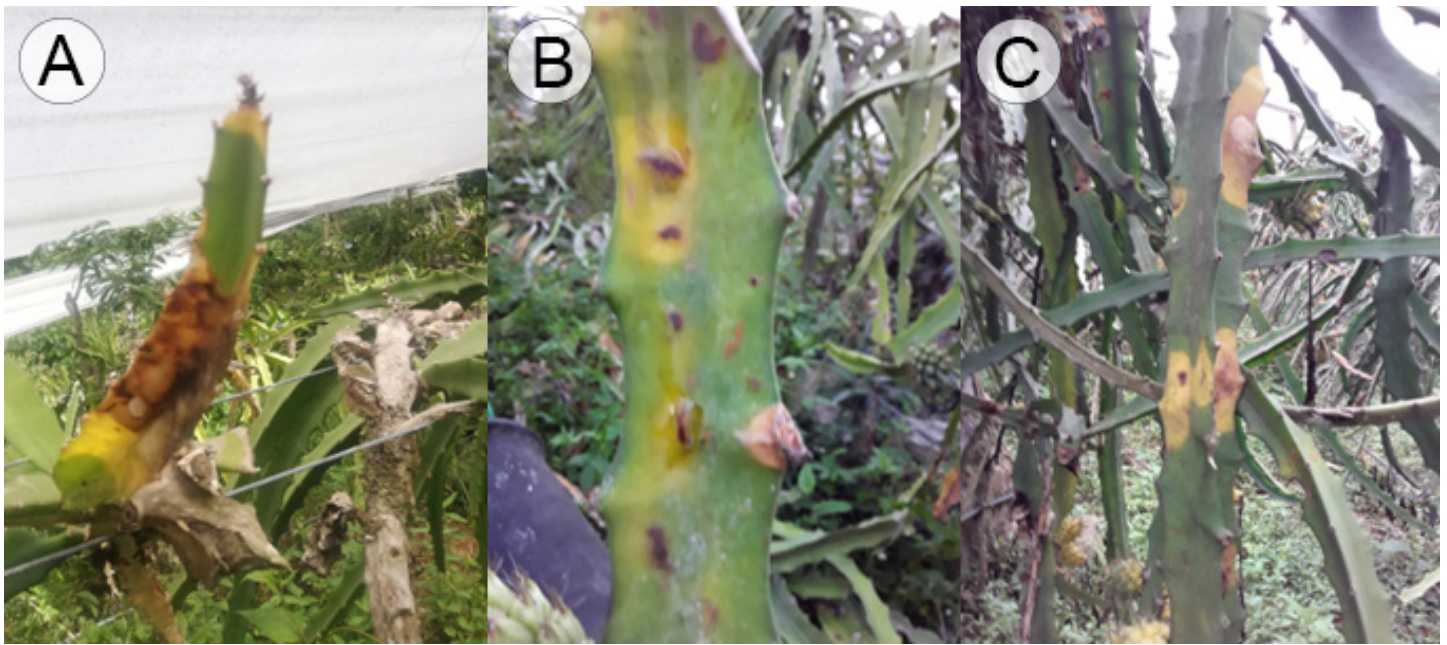


Figure 5: Symptoms observed on pitahaya stems from which *Lasiodiplodia* Ellis & Everh. isolates were obtained. A-C. yellowing at the margins and tips of the stems. As the disease progresses, the yellow discoloration expands, followed by stem softening, which manifests as brown to dark brown areas and ultimately leads to partial or complete stem rot. Lesions may contain masses of fungal mycelium at the center or margins. Photos by J. Másmela-Mendoza.

stewartii A.L. Harrison. However, further confirmation using the *BenA* β -tubulin gene revealed the presence of *D. guangdongensis* Z.Y. Dong, M. Luo, M.M. Xiang & K.D. Hyde and *D. rosae* M.C. Samar. & K.D. Hyde (Appendix 2, Fig. 2). These isolates were associated with small, reddish-orange or whitish-brown dot-like lesions on the stem. Among local farmers, the disease is commonly referred to as a type of gray-orange stem blight. Initial symptoms present as yellow lesions surrounded by pink to reddish hues, which gradually sink into the stem tissue and darken over time. As the disease progresses, some lesions dry out and form grayish concentric rings with visible black pycnidia at the center (Fig. 5). These necrotrophic lesions often show extensive tissue degradation, with black pycnidia and necrotized, hollowed-out stems clearly observable (Fig. 6).

The other genus identified in the symptoms was *Neofusicoccum*, in late signs and symptoms of the disease with production of black structures or pycnidia in necrotic tissues, with cankers at the base or tip of the stems and dark spots on the cladodes (Appendix 2; Fig. 7).

A total of 28 fungal isolates were identified at the genus level based on macroscopic and microscopic charac-

teristics (Appendix 2; Fig. 7). Several of these fungi have been reported as phytopathogens, while others appear to be saprophytic species colonizing necrotic tissue during the late stages of disease development. These include nine *Acromonium* Link morphotypes, one *Alternaria* Nees morphotype, five *Colletotrichum* isolates, two *Verticillium* Nees morphotypes, three *Geotrichum* Link isolates, and two *Trichoderma* Pers. isolates. Some *Colletotrichum* and *Alternaria* isolates were obtained from chlorotic lesions. *Colletotrichum* isolates were purified from stem lesions characterized by reddish-orange spots surrounded by concentric chlorotic halos, symptoms typically associated with anthracnose (Fig. 7). The reproductive structures observed in these *Colletotrichum* isolates included acervuli and unicellular, oval, hyaline conidia (Fig. 8).

Pathogenicity test

To evaluate the pathogenic potential of *Fusarium foetens* isolates, meristems induced from pitahaya cladodes were established under greenhouse conditions (Fig. 9A, B). Inoculum was prepared using isolate A10 at a concentration of 1×10^8 conidia ml^{-1} (Fig. 9C). Inoculation was performed

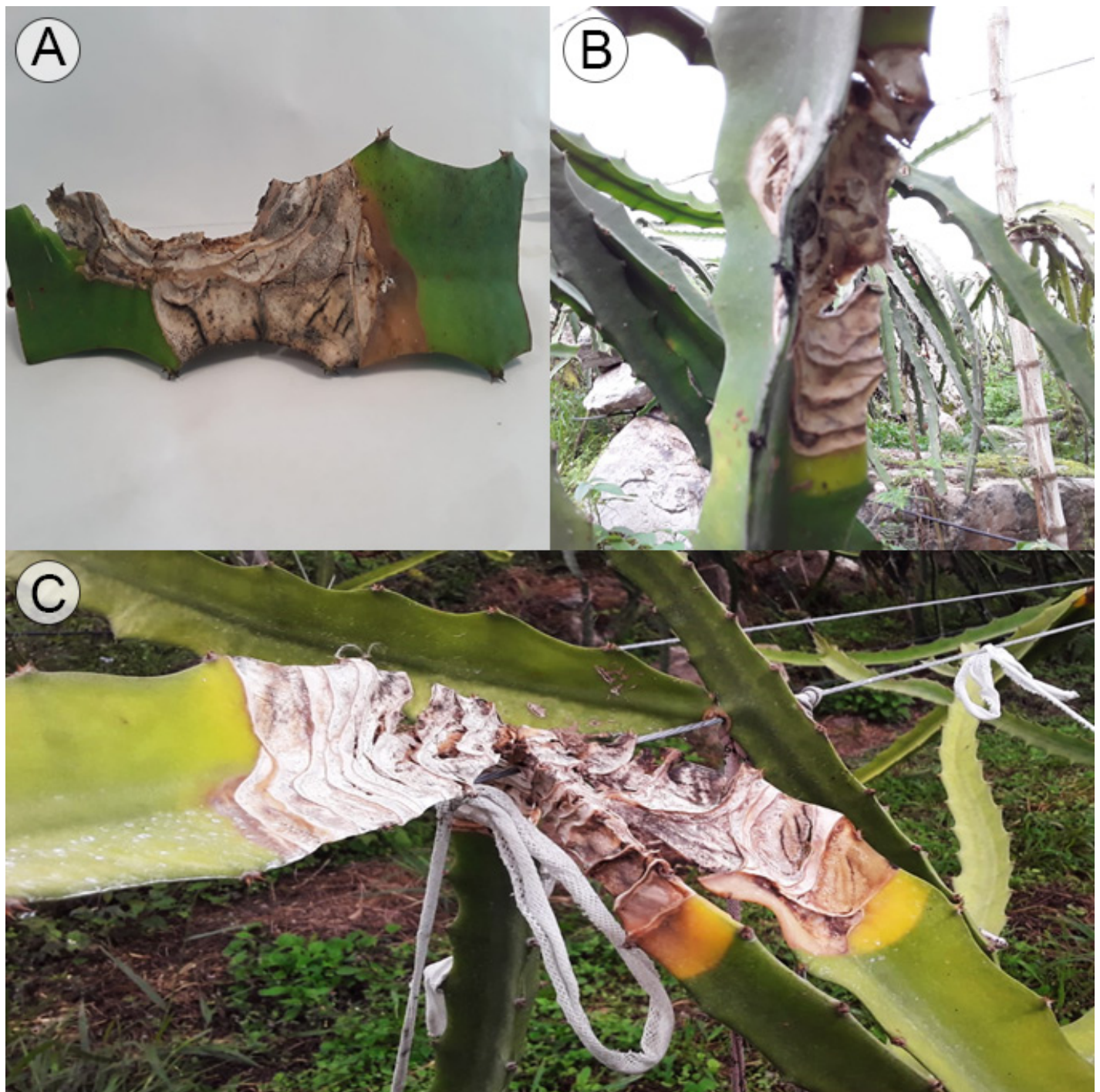


Figure 6: Symptoms of gray blight related to *Diaporthe guangdongensis* Z.Y. Dong, M. Luo, M.M. Xiang & K.D. Hyde and *Diaporthe rosae* M.C. Samar. & K.D. Hyde in pitahaya. A. black pycnidia and cancerous tissue identified in the final necrotrophic phase of the disease; B, C. as the disease progresses through healthy stems, symptoms of light green tones, chlorosis or yellow and brown symptoms are observed, finally forming grayish concentric rings with pycnidia on the edges. Photos by J. Másmela-Mendoza.

by creating standardized wounds (3 mm diameter, 5 mm deep) on the cladodes (Fig. 9D, E). Seven weeks after inoculation, isolates A2 and A4 induced visible symptoms characterized by brown to gray and pinkish discolorations,

along with extensive necrosis spreading from the inoculation site (Fig. 9G, H). Re-isolation of *F. foetens* from symptomatic tissue confirmed its pathogenicity and Koch postulates (Fig. 9I). Lesions developed on five pitahaya stems



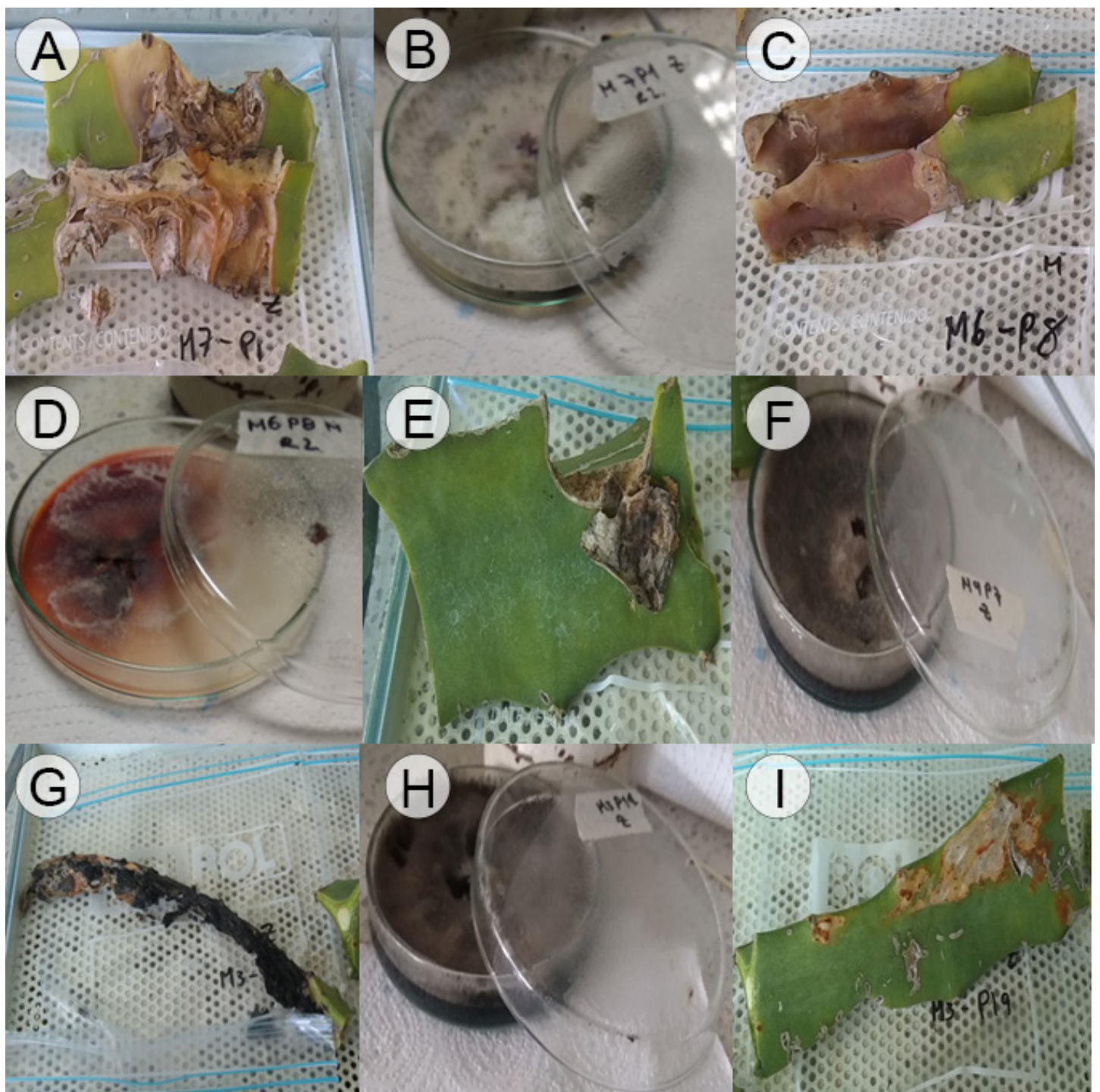


Figure 7: Symptoms and fungal isolations cultivated on PDA medium and obtained from diseased pitahaya leaves from crops in Miraflores, Boyacá, Colombia. The fungal isolates were identified by *tef1/BenA* gene sequencing. A, B. grayish blight caused by *Diaporthe rosae* M.C. Samar. & K.D. Hyde. The symptoms are lesions dry that out and form grayish concentric rings with visible black pycnidia at the center. Colonies contain pycnidia on the surface of the greyish mycelium; C, D. advanced stage of *Diaporthe guangdongensis* Z.Y. Dong, M. Luo, M.M. Xiang & K.D. Hyde symptoms. A concentric ring lesion with liquefied tissue. Colonies may have a cottony to floccose texture and a pinkish to pale grayish color; E, F. symptoms and isolation of *Lasiodiplodia theobromae* (Pat.) Griffon & Maubl. Final stage of the disease, with necrotic concentric tissue with pycnidia. Colony on PDA agar with pycnidia; G, H. black stem blight and fungic isolates of *Neofusicoccum sinoeucalypti* G.Q. Li & S.F. Chen. Production of black structures or pycnidia in necrotic tissues and pycnidia in colony grown in PDA medium (25 °C, 15 d); I. lesions related to *Nigrospora saccharicola* Raza & L. Cai. Small necrotic spots in initial lesions appear as small, circular to irregular spots on the surface of cladodes. Photos by L. Lizarazo-Forero.

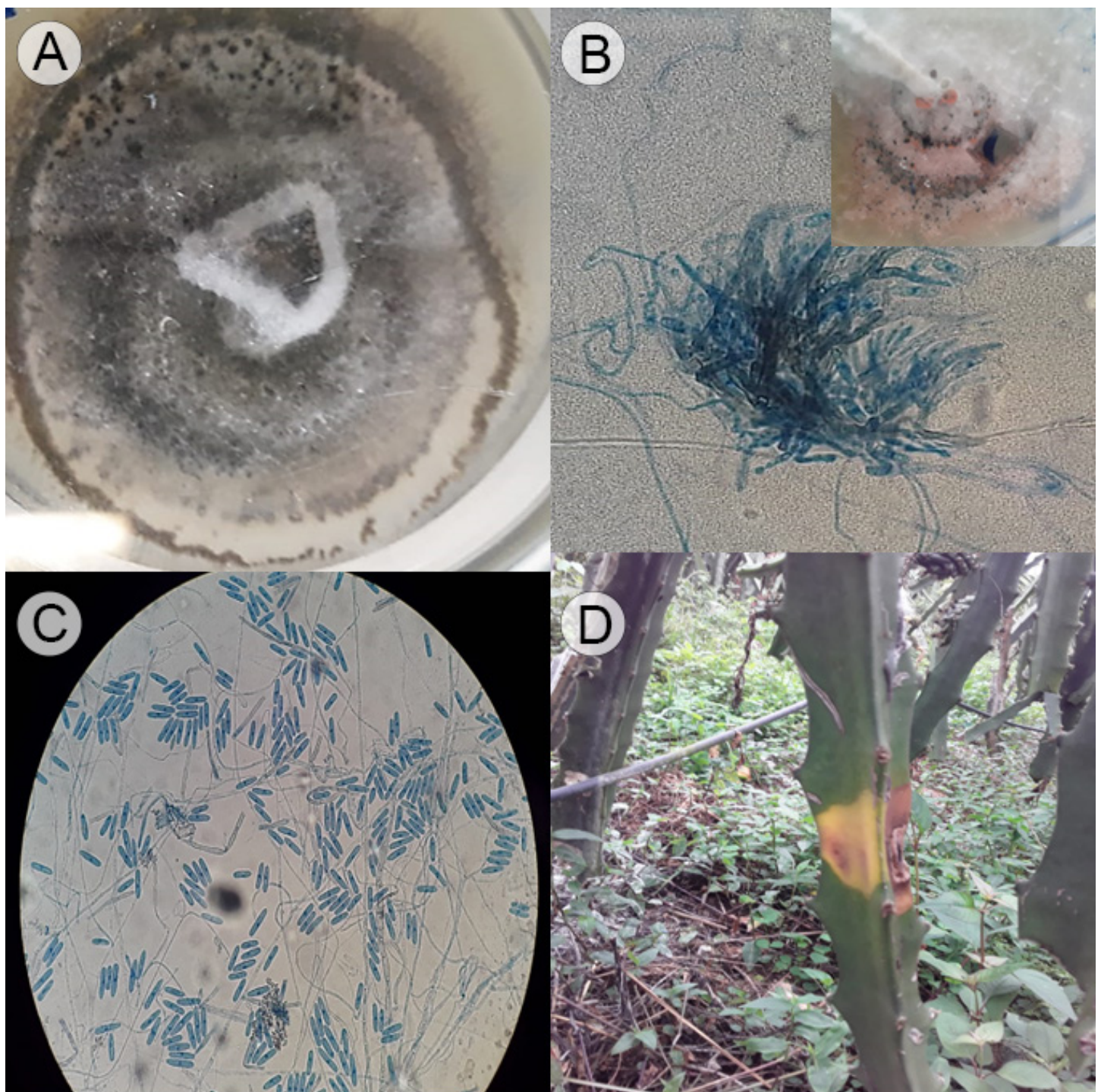


Figure 8: Macroscopic and microscopic morphology of *Colletotrichum* Corda isolated from pitahaya. A. *Colletotrichum* Corda morphotype 9.2B2 colony isolated from pitahaya anthracnose symptoms; B. acervulus (40×), structure where spores are formed and a sign of the disease; C. single-celled oval conidia seen under a 40× optical microscope; D. symptom of concentric yellow halos common in *Colletotrichum* anthracnose. Photos by J. Másmela-Mendoza.

seven weeks after inoculation with *F. foetens* isolates A2 and A4 (Fig. 9). Brown to gray discolorations appeared on the surface of the inoculated tissues, consistent with stem rot symptoms. The progression of symptoms under green-

house conditions differed from those typically observed in the field, particularly in the timing of disease onset and, the necrotic tissue was not observed. This variation may be related to the lower average temperature (~17 °C) in Tunja,

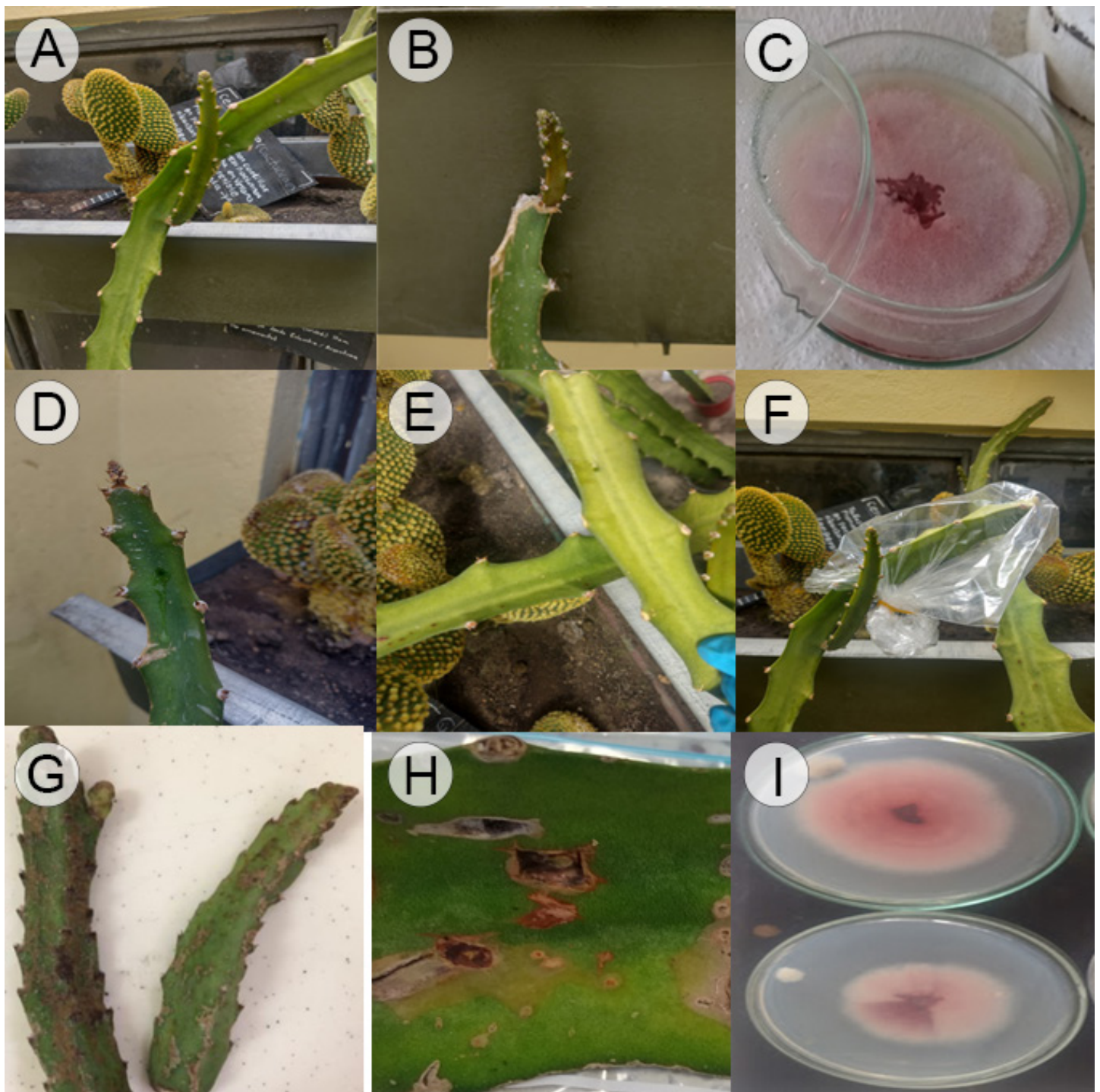


Figure 9: Pathogenicity test of *Fusarium foetens* Schroers, O'Donnell, Baayen & Hooftman isolates. A, B. meristems formed from pitahaya cladodes induced during plant establishment in the greenhouse; C. preparation of the *F. foetens* Schroers, O'Donnell, Baayen & Hooftman isolate A10 to obtain an inoculum of 100 μl at a concentration of 1×10^8 conidia ml^{-1} ; D, E. inoculation of the phytopathogen through the creation of a wound of 3 mm diameter; F. cladodes covered with polyethylene bags and incubated for 14 days; G–H. symptom development observed seven weeks after inoculation with isolates A2 and A4. Discoloration or lesions ranging from brown to gray and pinkish tones, with necrotic tissue spreading along the cladode; I. *Fusarium foetens* isolated from symptoms. Photos by L. Lizarazo-Forero and J. Másmela-Mendoza.

Colombia, where the pathogenicity assays were conducted, compared to the higher temperatures (25–28 °C) char-

acteristic of pitahaya-growing regions such as Miraflores, Boyacá. The pathogen was consistently re-isolated from

symptomatic tissues, confirming its association with the disease.

Discussion

Despite the implementation of various disease management practices in pitahaya production systems in Boyacá—ranging from chemical fungicides to organic and biological approaches—, a high incidence of fungal phytopathogens persists in the field. This study identified a broad diversity of pathogenic fungi, with 53 morphotypes belonging to 12 species across five genera, including *Fusarium*, *Lasiodiplodia*, *Diaporthe*, *Neofusicoccum*, and *Colletotrichum*. Key pathogens in the *Fusarium fujikuroi* species complex (FFSC), such as *Fusarium foetens*, *F. guttiforme*, and *F. sterilihyphosum* were linked to stem rot, while *Diaporthe stewartii*, *D. guangdongensis*, and *D. rosae* were associated with stem blight symptoms.

Disease control in yellow pitahaya production systems in Boyacá relies primarily on chemical management practices. Growers commonly apply a mixture of hydrogen peroxide and *Citrus × aurantifolia* (Christm.) Swingle (lime) extract (1.5% v/v). Other frequently used chemical products include copper oxychloride (0.4%), and Baladine®—a contact fungicide containing iodine and Propiconazol—applied either in liquid (0.5% v/v) or powder form (0.4% p/v). Additional fungicides employed in the region include Kasumin® (kasugamycin; 1.5–2 l ha⁻¹), the fungistatic product Azuco® (sulfur-based; 1.0–1.5 l ha⁻¹), and Timorex Gold® (1.0–1.5 l ha⁻¹), which contains bioactive compounds derived from *Melaleuca alternifolia* Cheel extract (Adama, 2022).

Some products used by farmers in Boyacá, Colombia, for chemical control of fungal phytopathogens in pitahaya do not contain active ingredients or modes of action specifically targeting *Fusarium* spp. Compounds such as hydrogen peroxide combined with lime (*Citrus × aurantifolia*) extract, copper oxychloride, and kasugamycin exhibit low to moderate efficacy against stem rot caused by *Fusarium*, primarily reducing superficial fungal load and functioning as preventive or supplementary disease management measures (Balendres and Bengoa, 2019). Among the products reported by growers, propiconazole was the only compound considered to have moderate to high effectiveness against *Fusarium* (Mondani et al., 2021).

Fungicides such as trifloxystrobin, prochloraz, and tebuconazole have shown high efficacy against *Fusarium oxysporum* and *F. concentricum* Nirenberg & O'Donnell in red-fleshed pitahaya (*Hylocereus polyrhizus* (F.A.C. Weber) Britton & Rose) in China, with EC₅₀ values ranging from 0.13 to 0.14 µg ml⁻¹ (Li et al., 2022; Zhao and Huang, 2023). Molecular identification of phytopathogenic fungi is essential to guide rational use of fungicides, allowing for the rotation of active ingredients and integration with ecological practices to prevent fungicide resistance (Balendres and Bengoa, 2019).

As part of integrated disease management, pitahaya producers in Boyacá, Colombia, implement various biological, organic and cultural practices aimed at reducing disease incidence and improving crop health. Organic disease management strategies include mineral preparations such as agricultural lime and Bordeaux mixture (2%), the latter comprising copper sulfate and agricultural lime. Other products applied include eucalyptus leaf extracts at 10% concentration, and botanical formulations such as CapsiAlil EC®, garlic, and chili extracts (0.05 %) (Mora, 2012; Choudhury et al., 2018).

Biological control practices involve soil applications (5%) of bacterial consortia produced through anaerobic and aerobic fermentation systems. These formulations contain fungal biocontrol agents such as *Beauveria bassiana* (Bals.-Criv.) Vuill. and *Trichoderma* spp. (Mora, 2012; Lizarazo-Forero and Másmela-Mendoza, 2024). These organisms are cultured in liquid media composed of molasses, wheat bran, yeast, and wheat flour (Medina et al., 2020). Among local growers, this formulation is colloquially known as “mountain microorganisms” (Medina et al., 2020; Lizarazo-Forero and Másmela-Mendoza, 2024). In our study, *Trichoderma* isolates were recovered from pitahaya cladodes, highlighting the importance of this genus as a biological control agent (Mora, 2012). *Trichoderma* is widely recognized for its multiple mechanisms of action against plant pathogens, including mycoparasitism, antibiosis through the production of secondary metabolites (e.g., peptaibols, gliotoxins, and viridin), competition for nutrients and space, and the induction of systemic resistance in host plants (Harman et al., 2004). These diverse strategies make *Trichoderma* a key component in integrated disease



management programs. Likewise, these formulations may contain fungal biocontrol agents and lactic acid bacteria such as *Lactobacillus plantarum* Orla-Jensen (1919) Bergey 1923 and *Weissella cibaria* (Björkroth, Schillinger, Geisen, Weiss, De Vos & Holzapfel (2002), which have demonstrated antifungal activity against *Fusarium oxysporum* and *F. fujikuroi*, causal agents of basal rot in yellow pitahaya fruit (Valencia-Hernández et al., 2021; Lozada et al., 2022).

In this context, Colombian farmers also adopt organic and cultural alternatives as part of integrated disease management (IDM) (Mora, 2012; Morillo-Coronado et al., 2022b; Lizarazo-Forero and Másmela-Mendoza, 2024). Cultural disease management includes preventive and sanitary pruning, removal of infected plant tissues, the disposal of pruning residues in isolated areas away from production zone and residues are either buried with the addition of agricultural lime or incinerated (Balendres and Bengoa, 2019). However, it was observed that many farmers rely exclusively on chemical control measures, with limited adoption of integrated disease management practices (Mora, 2012). Cultural practices such as sanitation and pruning are essential for reducing pathogen inoculum and limiting disease spread in pitahaya crops (Balendres and Bengoa, 2019). Their integration with chemical and biological strategies is critical, as the continued presence of fungal pathogens despite fungicide use indicates that chemical control alone is insufficient. Strengthening the adoption of integrated disease management (IDM) could substantially lower disease pressure in these production systems (Mora, 2012; Balendres and Bengoa, 2019).

Species from the *Fusarium fujikuroi* complex (FFSC) were isolated from diseased tissues of pitahaya stems exhibiting stem rot. Fungal identification was conducted through gene sequencing. Using specific molecular markers, such as the *tef1* gene and ITS gene, the species *F. foetens*, *F. guttiforme*, and *F. sterilihyphosum*—all members of the FFSC— (Yilmaz et al., 2021) were identified in pitahaya crops in Boyacá, Colombia.

In Colombia, species such as *Fusarium oxysporum*, *F. oxysporum* f. sp. *cumini*, and *F. fujikuroi* have been reported by ITS and TEF 1 α region sequencing as causal agents of basal stem rot in yellow pitahaya crops in Valle del Cauca, Risaralda, Boyacá, and Cundinamarca (Salazar-González et

al., 2016). These species were isolated from characteristic symptoms and identified by sequencing the ITS1-ITS4 and TEF-1 α regions (Salazar-González et al., 2016). Similarly, in Peru, *F. oxysporum* has been reported associated with basal rot in yellow pitahaya crops (Huaman-Pilco et al., 2024).

Fusarium oxysporum, *Fusarium proliferatum* and *Fusarium fujikuroi* have also been isolated as the causal agent of stem blight and stem rot in red-fleshed dragon fruit (*Hylocereus polyrhizus*) in Malaysia (Hawa et al., 2013, 2017; Mohd-Hafifi et al., 2019; Mohd et al., 2023). In pitahaya (*Hylocereus undatus* (Haw.) Britton & Rose) crops in southern Philippines, *F. oxysporum* and *F. incarnatum* were reported from soft and watery yellow symptoms associated to stem rot (Emejas et al., 2023). Likewise, *Fusarium* species like *F. equiseti* and *F. fujikuroi* have been identified and associated to causal agent stem rot in red-fleshed dragon fruit (*Hylocereus polyrhizus*) in India (Hawa et al., 2017; Singh et al., 2023).

To date, *F. foetens*, *F. guttiforme*, and *F. sterilihyphosum* had not been reported in pitahaya. *Fusarium foetens* is relatively host-specific to *Begonia* spp. (Judet et al., 2019). However, since 2017, it has been reported as a pathogen of rooibos seedlings and Solanaceae crops such as tomatoes, bell peppers, and cayenne peppers (Lamprecht and Tewoldemedhin, 2017). *Fusarium guttiforme* is considered one of the greatest threats to pineapple cultivation, as it can infect the entire plant, particularly the fruit, and cause production losses of up to 80%, depending on the harvest season, production region, and inoculum load (Matos et al., 2009). *Fusarium sterilihyphosum* has been identified as a cause of malformations in mango in Brazil (Lima et al., 2009).

Lasiodiplodia theobromae, *L. pseudotheobromae*, and *L. acaciae* were isolated from typical basal stem rot lesions in yellow pitahaya diseases in Boyacá, Colombia. Ganesan et al. (2023) found an association between *Lasiodiplodia* species and basal stem rot of pitahaya (*Hylocereus* spp.) in the coastal belt of Odisha, eastern India. Additionally, stem canker in dragon fruit caused by *L. theobromae* has been reported (Briste et al., 2019).

In this study, fungal pathogens including *Fusarium oxysporum*, *F. foetens*, and *Lasiodiplodia* spp. were isolated from diseased tissues exhibiting basal stem rot, stem rot,



and fruit soft rot symptoms, which were frequently found in conjunction with the bacterial species previously reported. Likewise, in previous reports, we isolated phytopathogenic bacteria from the symptoms of soft rot of stems identified as *Enterobacter cloacae* (Jordan 1890) Hormaeche & Edwards 1960, *Pectobacterium carotovorum* (Jones 1901) Waldee 1945, *Paenibacillus glucanolyticus* (Alexander & Priest 1989) Shida et al. 1997, *Pantoea cyripedii* (Hori 1911) Brenner et al. 1973, *Kluyvera intermedia* (Izard et al. 1980) Pavan et al. 2005 and *Klebsiella oxytoca* (Flügge 1886) Lautrop 1956, whose DNA sequences are deposited in Genbank with submission numbers SUB14634657 and Genbank accession number PV661852-PV661867 (Lizarazo-Forero and Másmela-Mendoza, 2024). These findings suggest that the disease is the result of a complex of polymicrobial infection pathosystem and diverse mycobiota, where multiple phytopathogens—both fungal and bacterial—may be interacting synergistically, antagonistically, or sequentially in the infection process (Lamichhane et al., 2014; Martins et al., 2018). Such interactions are referred to as polymicrobial infections or pathobiome and they often involve facilitative, antagonistic, or commensal relationships that influence disease expression and development (Lamichhane et al., 2014; Vayssier-Taussat et al., 2014; Trivedi et al., 2020). The observed co-occurrence may also reflect processes of microbial succession, in which initial colonization by one pathogen alters host tissue and creates conditions favorable for subsequent invasion by others (Martins et al., 2018). This conceptual shift from “one pathogen-one disease” to the pathobiome framework emphasizes the ecological complexity of plant disease and highlights the importance of community-level approaches to diagnosis and management (Vayssier-Taussat et al., 2014).

The genus *Diaporthe* was associated with small reddish-orange or whitish-brown punctate lesions, commonly known among farmers as a type of grayish-orange stem blight. HudaShakirah et al. (2021) characterized a novel fungal stem disease of red-fleshed dragon fruit (*Hylocereus polyrhizus*) in Malaysia, known as “gray blight.” Based on morphological characteristics, DNA sequences, and phylogeny (ITS, TEF1- α , and β -tubulin), the fungal isolates were identified as *Diaporthe arecae* (H.C. Srivast., Zakia

& Govindar.) R.R. Gomes, Glienke & Crous, *D. eugeniae* R.R. Gomes, C. Glienke & Crous, *D. hongkongensis* R.R. Gomes, Glienke & Crous, *D. phaseolorum* Kulik, and *D. tectonendophytica* M. Doilom, A.J. Dissanayake & K.D. Hyde.

Molecular taxonomic identification of phytopathogens is an important laboratory tool for guiding integrated disease management practices. Likewise, the use of several molecular markers may be more effective for the correct identification of plant pathogens down to genus and species, since markers of conserved regions such as ITS1 may be less precise than other genes such as LSU, *tef1* and *BenA* (O'Donnell et al., 2021). The ITS1 region, while widely used for fungal identification, lacks sufficient resolution to distinguish closely related *Diaporthe* species due to low interspecific variability. In contrast, the β -tubulin gene (*BenA*), with higher sequence variability, allows more accurate species delimitation. Studies have shown that only protein-coding genes like *BenA* and TEF1- α can reliably resolve species within the *Diaporthe* complex (Udayanga et al., 2014). For instance, the *tef1* and *BenA* genes were effective in identifying *Neofusicoccum* at the genus level and in distinguishing it from *Diaporthe*, as the ITS region did not provide sufficient resolution to clearly separate these two genera in isolate A10. Similarly, the *Fusarium* isolates were initially identified only at the species complex level (*Fusarium fujikuroi* species complex, FFSC) based on ITS sequencing alone. However, the use of multilocus approaches, including *tef1* and *BenA* gene sequences, enabled species-level resolution, allowing the identification of *F. foetens*, *F. guttiforme*, and *F. sterilihyphosum* (O'Donnell et al., 2021).

Conclusions

This study identified a diverse group of phytopathogenic fungi associated with pitahaya crops in Boyacá, Colombia, including *Fusarium*, *Lasiodiplodia*, *Diaporthe*, and *Colletotrichum* species, linked to symptoms such as basal stem rot, stem blight, and fruit soft rot. *Fusarium foetens*, *F. guttiforme*, and *F. sterilihyphosum*, members of the *F. fujikuroi* species complex, were identified, with *F. foetens* confirmed as a causal agent of stem rot and transmitted by the insect vector *Leptoglossus zonatus* isolated from three specimens. *Lasiodiplodia* spp. were associat-



ed with basal stem rot, *Diaporthe* spp. with gray-orange stem blight, and *Colletotrichum* sp. with anthracnose-like symptoms. This study contributes to the understanding of fungal pathogens affecting pitahaya cultivation in Colombia, providing a basis for the development of targeted management strategies. The identification of these fungi, including species not previously reported in pitahaya, underscores the importance of tailored control measures to mitigate the impact of these pathogens on crop health. Future research should focus on evaluating the pathogenicity of the isolates under controlled conditions and on designing integrated management approaches to reduce disease incidence and support sustainable pitahaya production.

Author contributions

Project administration: LMLF; Formal analysis: JEMM; Conceptualization: LMLF, JEMM; Data curation: JEMM; Investigation: JEMM; Methodology: JEMM; Funding acquisition: LMLF; Resources: LMLF; Writing - original draft: LMLF, JEMM; Writing - review and editing: JEMM, LMLF.

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Data Availability Statement

The dataset that supports the results of this study was published in SciELO Data and can be accessed at DOI: <https://doi.org/10.48331/SCIELODATA.NO6YIC>

Literature cited

Adama, 2022. Timorex Gold® 23.8 EC – Product leaflet [Ficha técnica]. Adama Colombia. https://www.adama.com/colombia/sites/adama_colombia/files/product-

<documents/2022-04/Timorex%20Gold.pdf> (Consulted September, 2025).

AGRONET. 2022. Red de información y comunicación del sector Agropecuario Colombiano. <https://www.agronet.gov.co/estadistica/Paginas/home.aspx?cod=1> (consulted April, 2023).

Altschul, S. F., W. Gish, W. Miller, E. W. Myers and D. J. Lipman. 1990. Basic local alignment search tool. *Journal of Molecular Biology* 215(3): 403-410. DOI: [https://doi.org/10.1016/S0022-2836\(05\)80360-2](https://doi.org/10.1016/S0022-2836(05)80360-2)

Balendres, M. A. and J. C. Bengoa. 2019. Diseases of dragon fruit (*Hylocereus* species): etiology and current management options. *Crop Protection* 126: 104920. DOI: <https://doi.org/10.1016/j.cropro.2019.104920>

Barnett, H. L. and B. Hunter. 1972. Illustrated genera of imperfect fungi. 4a ed. McMillan Publishing Company. New York, USA. 241 pp.

Barnett, H. L. and B. B. Hunter. 1998. Illustrated genera of imperfect fungi. 4a ed. APS Press, St. Paul, MN, USA. 218 pp.

Benson, D. A., M. Cavanaugh, K. Clark, C. Karsch-Mizrachi, D. J. Lipman, J. Ostell and E. W. Sayers. 2023. GenBank. *Nucleic Acids Research* 51(D1): D141-D147. <https://www.ncbi.nlm.nih.gov/genbank/> (consulted October, 2025).

Borman, A. M., C. J. Linton, S. J. Miles and E. M. Johnson. 2008. Molecular identification of pathogenic fungi. *Journal of Antimicrobial Chemotherapy* 61(supl. 1): i7-i12. DOI: <https://doi.org/10.1093/jac/dkm425>

Briste, P. S., M. M. Rahman, M. M. Hossain, M. J. Alam and M. T. Hossain. 2019. First report of dragon fruit stem canker caused by *Lasiodiplodia theobromae* in Bangladesh. *Plant Disease* 103(10): 2686. DOI: <https://doi.org/10.1094/pdis-03-19-0619-pdn>

Chauhan, B., K. Kunjadiya and S. Parekh. 2022. Comparison of extraction techniques for isolation of fungal DNA. *International Journal for Research in Applied Science and Engineering Technology* 10(IV): 41542. DOI: <https://doi.org/10.22214/ijraset.2022.41542>

Choi, H.-W., I.-M. Chung, M.-H. Sin, Y. S. Kim, J. B. Sim, J. W. Kim, K. D. Kim and S. C. Chun. 2007. The effect of spent mushroom sawdust compost mixes, calcium cyanamide and solarization on basal stem rot of the cactus *Hylocereus trigonus* caused



- by *Fusarium oxysporum*. Crop Protection 26(2): 162-168. DOI: <https://doi.org/10.1016/j.cropro.2006.04.017>
- Choudhury, D. C., P. Dobhal, S. Srivastava, S. Saha and S. Kundu. 2018. Role of botanical plant extracts to control plant pathogens. Indian Journal of Agricultural Research 52(4): 341-346. DOI: <https://doi.org/10.18805/IJARE.A-5005>
- Emejias, D. P. Jr., C. M. Jumao-As and F. R. P. Salvaña. 2023. Identification of common fungal pathogens of dragon fruit (*Hylocereus undatus*) in Sarangani Province, Philippines. Environmental and Experimental Biology 21(1): 93-99. DOI: <https://doi.org/10.22364/eeb.21.11>
- Felsenstein, J. 2005. Evolutionary trees from DNA sequences: A maximum likelihood approach. Journal of Molecular Evolution 17: 368-376. DOI: <https://doi.org/10.1007/BF01734359>
- Ganesan, S., N. Kumari, S. Sahu, M. Pattanaik and K. Kishore. 2023. Identification of *Lasiodiplodia* species inciting stem rot of dragon fruit in India through polyphasic approach. 3 Biotech 13(7): 333. DOI: <https://doi.org/10.1007/s13205-023-03754-1>
- Geneious. 2023. Geneious version 2023.2. Created by Biomatters. <https://www.geneious.com> (consulted November, 2024).
- Harman, G. E., C. R. Howell, A. Viterbo, I. Chet and M. Lorito. 2004. *Trichoderma* species—opportunistic, avirulent plant symbionts. Nature Reviews Microbiology 2(1): 43-56. DOI: <https://doi.org/10.1038/nrmicro797>
- Hawa, M. M., B. Salleh and L. Zakaria. 2013. Characterization and pathogenicity of *Fusarium proliferatum* causing stem rot of *Hylocereus polyrhizus* in Malaysia. Annals of Applied Biology 163(2): 269-280. DOI: <https://doi.org/10.1111/aab.12057>
- Hawa, M., N. Faziha, M. Izham and Z. Latiffah. 2017. *Fusarium fujikuroi* associated with stem rot of red-fleshed dragon fruit (*Hylocereus polyrhizus*) in Malaysia. Annals of Applied Biology 170(3): 434-446. DOI: <https://doi.org/10.1111/aab.12348>
- Huaman-Pilco, A. F., M. Arce-Inga, J. Huaman-Pilco, V. Aguilar-Rafael, S. M. Oliva-Cruz, E. Hernández-Díaz, Y. Fernández-Rodríguez, T. J. Torres-Cruz and J. R. Díaz-Valderrama. 2024. First report of basal rot of yellow dragon fruit (*Selenicereus megalanthus*) caused by *Fusarium oxysporum* in Peru. Plant Disease 108(10): 3189. DOI: <http://doi.org/10.1094/PDIS-05-24-1060-PDN>
- Huda-Shakirah, A. R., Y. J. Kee, K. L. Wong, L. Zakaria and M. Hawa Mohd. 2021. *Diaporthe* species causing stem gray blight of red-fleshed dragon fruit (*Hylocereus polyrhizus*) in Malaysia. Scientific Reports 11: 3907. DOI: <https://doi.org/10.1038/s41598-021-83551-z>
- Judet, P., C. Fourrier, C. Saurat, R. loos and C. Casset. 2019. First report of *Begonia* Elatior wilt disease caused by *Fusarium foetens* in France. Plant Disease 97(1): 144. DOI: <https://doi.org/10.1094/PDIS-07-12-0659-PDN>
- Karim, M. M., M. M. E. Rahman, M. N. Islam, M. S. Akhter, F. Khatun, M. L. Rahman and B. K. Goswami. 2019. Occurrence of stem rot disease of *Hylocereus undatus* in Bangladesh. Indian Phytopathology 72(4): 545-549. DOI: <http://doi.org/10.1007/s42360-019-00166-1>
- Korotkova, N., T. Borsch and S. Arias. 2017. A phylogenetic framework for the Hylocereeae (Cactaceae) and implications for the circumscription of the genera. Phytotaxa 327(1): 1-46. DOI: <https://doi.org/10.11646/phytotaxa.327.1.1>
- Lamichhane, J. R., A. Fabi and L. Varvaro. 2014. Synergisms and antagonisms among plant pathogens and microbial communities: current knowledge and potential implications. Critical Reviews in Plant Sciences 33(4): 293-309.
- Lamprecht, S. C. and Y. T. Tewoldemedhin. 2017. *Fusarium* species associated with damping-off of rooibos seedlings and the potential of compost as soil amendment for disease suppression. South African Journal of Botany 110: 110-117. DOI: <https://doi.org/10.1016/j.sajb.2016.07.009>
- Li, Y., H. Chen, L. Ma, Y. An, H. Wang and W. Wu. 2022. Laboratory screening of control agents against isolated fungal pathogens causing postharvest diseases of pitaya in Guizhou, China. Frontiers in Chemistry 10: 942185. DOI: <http://doi.org/10.3389/fchem.2022.942185>
- Lima, C. S., L. H. Pfenning, S. S. Costa, M. A. Campos and J. F. Leslie. 2009. A new *Fusarium* lineage within the *Gibberella fujikuroi* species complex is the main causal agent of mango malformation disease in Brazil. Plant Pathology 58(1): 33-42. DOI: <http://doi.org/10.1111/j.1365-3059.2008.01946.x>
- Liu, F., J. B. Wu, R. L. Zhan and X. C. Ou. 2016. First report of reddish-brown spot disease on pitaya caused by *Nigrospora sphaerica* in China. Plant Disease 100(9): 1792. DOI: <https://doi.org/10.1094/PDIS-01-16-0063-PDN>
- Lizarazo-Forero, L. M. and J. E. Másmela-Mendoza. 2024. First



- report of bacteria associated with soft rot in yellow pitahaya (*Selenicereus megalanthus* Haw.) in Colombia crops. *Revista Facultad Nacional de Agronomía Medellín* 77(3): 10797-10809. DOI: <https://doi.org/10.15446/rfnam.v77n3.111234>
- Lozada, L., C. Aguilar, C. Vargas, C. Bedoya and L. Serna-Cock. 2022. Biological control for basal rot in yellow pitahaya fruits (*Selenicereus megalanthus*): Ex vivo trials. *Journal of King Saud University – Science* 34(5): 102042. DOI: <https://doi.org/10.1016/j.jksus.2022.102042>
- Martins, L. M., R. R. Silva, R. B. Souza, L. R. Almeida and P. S. Costa. 2018. Microbial succession in the rhizosphere of *Hylocereus undatus* during the development of stem rot disease. *Journal of Phytopathology* 166(9): 585-593.
- Matos, A. P., N. F. Sanches, F. A. Teixeira and J. J. Elias. 2009. Integrated management of fusariosis in pineapple fields under integrated production system. *Acta Horticulturae* 822: 199-204. DOI: <http://doi.org/10.17660/ActaHortic.2009.822.24>
- Medina, J. and T. Kondo. 2012. Listado taxonómico de organismos que afectan la pitaya amarilla, *Selenicereus megalanthus* (K. Schum. ex Vaupel) Moran (Cactaceae) en Colombia. *Ciencia y Tecnología Agropecuaria* 13: 41-46.
- Medina, S., C. Lobo-Sanahuja, A. Moya-Quirós and M. Flores-Mora. 2020. Abono orgánico, microorganismos de montaña (MM) y Fertilbiol para el control biológico de la hernia de las crucíferas (*Plasmiodiophora brassicae*) en mostaza china (*Brassica rapa* sp. *pekinensis* var. *Taranko* F1). *Agronomía Costarricense* 44(2): 31-49. Retrieved from: <https://archivo.revistas.ucr.ac.cr/index.php/agrocost/article/view/43088/43352> (consulted September, 2025).
- Mohd, M. H., N. M. I. M. Nor, N. F. Azuddin and L. Zakaria. 2023. Mycotoxin production by *Fusarium proliferatum* and *Fusarium fujikuroi* causing stem rot of *Hylocereus polyrhizus* in Malaysia. *Malaysian Applied Biology* 52(3): 13-22. DOI: <https://doi.org/10.55230/mabjournal.v52i3.2644>
- Mohd-Hafifi, A. B., Y. J. Kee and M. H. Mohd. 2019. First report of *Fusarium oxysporum* as a causal agent of stem blight of red-fleshed dragon fruit (*Hylocereus polyrhizus*) in Malaysia. *Plant Disease* 103(5): 1040. DOI: <https://doi.org/10.1094/pdis-07-18-1249-pdn>
- Mondani, L., G. Chiusa and P. Battilani. 2021. Chemical and biological control of *Fusarium* species involved in garlic dry rot at early crop stages. *European Journal of Plant Pathology* 160(3): 575-587. DOI: <https://doi.org/10.1007/s10658-021-02265-0>
- Mora, D. P. 2012. Manejo fitosanitario del cultivo de la pitahaya *Hylocereus megalanthus* (K. Schum. ex Vaupel) Ralf Bauer.: medidas para la temporada invernal. Instituto Colombiano agropecuario, Bogotá, Colombia. 20 pp. <http://hdl.handle.net/20.500.12324/2283> (consulted November, 2025).
- Morillo-Coronado, A. C., Y. P. Tovar and Y. Morillo. 2016. Caracterización morfológica de *Selenicereus megalanthus* (K. Schum. ex Vaupel) Moran en la provincia de Lengupá. *Ciencia en Desarrollo* 7: 23-33.
- Morillo-Coronado, A. C., E. H. Manjarres-Hernández, O. J. Saenz-Quintero and Y. Morillo-Coronado. 2022a. Morphoagronomic evaluation of yellow pitahaya (*Selenicereus megalanthus* Haw.) in Miraflores, Colombia. *Agronomy* 12(7): 1582. DOI: <https://doi.org/10.3390/agronomy12071582>
- Morillo Coronado, A., E. Manjarres Hernández, L. Lizarazo Forero, I. Morales Castaño, R. Pérez Martínez, I. Ruíz Rosas y J. Velásquez Arias. 2022b. Fortalecimiento de las condiciones de producción, comercialización y gestión de la cadena productiva de la pitahaya amarilla en el municipio de Miraflores (Boyacá). Universidad Pedagógica y Tecnológica de Colombia. <http://repositorio.uptc.edu.co/handle/001/9054> (consulted January, 2025).
- O'Donnell, K., B. Whitaker, I. Laraba, R. Proctor, D. Brown, K. Broders, H. S. Kim, S. McCormick, M. Busman, T. Aoki, T. Torres-Cruz and D. Geiser. 2021. DNA sequence-based identification of *Fusarium*: a work in progress. *Plant Disease* 106(6): 1597-1609. DOI: <https://doi.org/10.1094/PDIS-09-21-2035-SR>
- Pitt, J. I., y A. D. Hocking. 2009. *Fungi and Food Spoilage*. 3ª ed. CSIRO Food and Nutritional Sciences, Springer. New York, USA. 537 pp.
- Rita, W. S., D. N. Suprpta, I. M. Sudana and I. M. D. Swantara. 2013. First report on *Fusarium solani*, a pathogenic fungus causing stem rot disease on dragon fruits (*Hylocereus* sp.) in Bali. *Journal of Biology, Agriculture and Healthcare* 3: 93-99.
- Romanelli, A. M., J. Fu, M. L. Herrera and B. L. Wickes. 2014. A universal DNA extraction and PCR amplification method for fungal rDNA sequence-based identification. *Mycoses* 57(10): 612-622. DOI: <https://doi.org/10.1111/myc.12208>
- Sayers, E. W., J. Beck, E. E. Bolton, D. Bourexis, J. R. Brister, K. Canese, D. C. Comeau, K. Funk, S. Kim, W. Klimke, N. O'Leary,



- L. Phan, K. D. Pruitt, V. A. Schneider y S. T. Sherry. 2022. Database resources of the National Center for Biotechnology Information. *Nucleic Acids Research* 50(D1): D20–D26. DOI: <https://doi.org/10.1093/nar/gkab1112>
- Salazar-González, C., L. Serna-Cock and E. Gómez-Lopez. 2016. Caracterización molecular de *Fusarium* asociado a pudrición basal del fruto en pitahaya (*Selenicereus megalanthus*). *Agronomía Mesoamericana* 27(2): 277-285. DOI: <https://doi.org/10.15517/am.v27i2.21269>
- Singh, A., S. Sharma, V. Dayal, S. Dutta, S. Singh, S. Phurailatpam, T. Boopathi, L. Singson, S. Saha, P. Irungbam, S. Thokchom and E. Nongtdu. 2023. *Fusarium equiseti* as the pathogen causing stem rot disease of red-fleshed dragon fruit (*Hylocereus polyrhizus*) in India. *Crop Protection* 173: 106380. DOI: <https://doi.org/10.1016/j.cropro.2023.106380>
- Tamura, K., G. Stecher and S. Kumar. 2021. MEGA11: Molecular Evolutionary Genetics Analysis version 11. *Molecular Biology and Evolution* 38(7): 3022-3027. DOI: <https://doi.org/10.1093/molbev/msab120>
- Trivedi, P., J. E. Leach, S. G. Tringe, T. Sa and B. K. Singh. 2020. Plant–microbiome interactions: from community assembly to plant health. *Nature Reviews Microbiology* 18: 607-621. DOI: <https://doi.org/10.1038/s41579-020-0412-1>
- Udayanga, D., L. A. Castlebury, A. Y. Rossman and K. D. Hyde. 2014. Species limits in *Diaporthe*: molecular re-assessment of *Diaporthe* species in *Diaporthe eres* complex. *Fungal Diversity* 67: 203-229.
- Valencia-Hernández, L., K. López-López, E. Gómez-López, L. Serna-Cock and C. Aguilar. 2021. In-vitro assessment for the control of *Fusarium* species using a lactic acid bacterium isolated from yellow pitahaya (*Selenicereus megalanthus* (K. Schum. Ex Vaupel Moran)). *Journal of Integrative Agriculture* 20(1): 159-167. DOI: [https://doi.org/10.1016/s2095-3119\(20\)63284-1](https://doi.org/10.1016/s2095-3119(20)63284-1)
- Vayssier-Taussat, M., A. Albina, C. Citti, J. F. Cosson, M. A. Jacques, M.-H. Lebrun, Y. Le Loir, M. Ogliastro, M.-A. Petit, P. Roumagnac and T. Candresse. 2014. Shifting the paradigm from pathogens to pathobiome: new concepts in the light of meta-omics. *Frontiers in Cellular and Infection Microbiology* 4: 29. DOI: <https://doi.org/10.3389/fcimb.2014.00029>
- Watanabe, T. 2010. Pictorial atlas of soil and seed fungi: Morphologies of cultured fungi and key to species. 3rd ed. CRC Press. Boca Raton, Florida, USA. 426 pp.
- Yilmaz, N., M. Sandoval-Denis, L. Lombard, C. M. Visagie, B. D. Wingfield, P. W. Crous. 2021. Redefining species limits in the *Fusarium fujikuroi* species complex. *Persoonia* 46: 129-162. DOI: <https://doi.org/10.3767/persoonia.2021.46.05>
- Zhao, J. and M. Huang. 2023. Characterization and in vitro fungicide sensitivity of two *Fusarium* spp. associated with stem rot of dragon fruit in Guizhou, China. *Journal of Fungi* 9(12): 1178. DOI: <https://doi.org/10.3390/jof9121178>



Appendix 1: Results of molecular identification using ITS and *tef1/BenA* sequences of twenty-four fungal isolates obtained from pitahaya, symptoms and associated disease, from Boyacá, Colombia.

GenBank accession number	Voucher or strain number	Identification with ITS gene	Identification with <i>tef1/BenA</i> gene	Coverage (%)	Identity (%)	Related disease	Crop system
PV648698	A2	<i>Fusarium fujikuroi</i> Nirenberg (FFSC)	<i>Fusarium foetens</i> Schroers, O'Donnell,	99.9	97.97	Soft rot of peduncles	Under cover
PV648700	A4	species complex	Baayen & Hooftman	99.9	97.97		
PV648703	A7			99.9	97.97	Basal stem rot	Open field
PV648704	A8			100	97.97		
PV648705	A9			100	96.79	Root rot	
PV648709	A13			99.9	97.97		
PV648710	A14			99.9	97.97	Fruit rots during ripening and post-harvest	Under cover
PV648713	A17			99.9	97.97		
PV648714	A18			99.9	97.97		
PV648716	A20			98	98.79		
PV648702	A6		<i>Fusarium sterilihyphosum</i> Britz, Marasas & M.J. Wingf.	99	99.65		
PV648699	A3	<i>Fusarium vanleeuwenii</i> Crous & Sand.-Den	<i>Fusarium foetens</i> Schroers, O'Donnell, Baayen & Hooftman	100	97.85		
PV648717	A21	<i>Fusarium napiforme</i> Marasas, P.E. Nelson & Rabie	<i>Fusarium foetens</i> Schroers, O'Donnell, Baayen & Hooftman	100	97.97		Open field
PV648712	A16	<i>Fusarium napiforme</i> Marasas, P.E. Nelson & Rabie	<i>Fusarium guttiforme</i> Nirenberg & O'Donnell	100	100		
PV648697	A1	<i>Apiospora</i> Sacc.	<i>Nigrospora saccharicola</i> Raza & L. Cai	100	91.26	Reddish-brown spot disease	Under cover
PV648706	A10	<i>Lasiodiplodia</i> Ellis & Everh.	<i>Neofusicoccum sinoeucalypti</i> G.Q. Li & S.F. Chen	100	99.54	Black stem blight	
PV648707	A11	<i>Lasiodiplodia</i> Ellis & Everh.	<i>Lasiodiplodia theobromae</i> (Pat.) Griffon & Maubl.	100	100	Stem rot	
PV648708	A12	<i>Lasiodiplodia</i> Ellis & Everh.	<i>Lasiodiplodia pseudotheobromae</i> A.J.L. Phillips, A. Alves & Crous	100	100		
PV648711	A15	<i>Diaporthe stewartii</i> A.L. Harrison	<i>Lasiodiplodia acaciae</i> W. Zhang & Crous.	100	100		
PV648715	A19	<i>Diaporthe stewartii</i> A.L. Harrison	<i>Diaporthe guangdongensis</i> Z.Y. Dong, M. Luo, M.M. Xiang & K.D. Hyde	100	96.5	Grayish blight	
PV648718	A22	<i>Diaporthe</i> Nitschke	<i>Diaporthe rosae</i> M.C. Samar. & K.D. Hyde	100	94.78		



GenBank accession number	Voucher or strain number	Identification with ITS gene	Identification with <i>tef1/BenA</i> gene	Coverage (%)	Identity (%)	Related disease	Crop system
PV648719	A23	<i>Fusarium fujikoro</i> Nirenberg (FFSC) species complex	<i>Fusarium foetens</i> Schroers, O'Donnell, Baayen & Hooftman	99.9	97.97	<i>Leptoglossus zonatus</i> (Dallas, 1852) Crop pest; leaf-footed bug; vector of the phytopathogenic fungus	Under cover
PV648720	A24		<i>Fusarium foetens</i> Schroers, O'Donnell, Baayen & Hooftman		97.97		
PV648721	A25		<i>Fusarium foetens</i> Schroers, O'Donnell, Baayen & Hooftman		97.97		



Appendix 2: Fungal isolates identified in pitahaya disease symptoms by macroscopic and microscopic morphology.

Isolate code	Taxon	Description	Related disease
10.1B3 13.1B1 13.1B2 5.1B2 10.1B3 5.1B3 10.1B5 10.1B6 10.1B8	<i>Acremonium</i> Link	Colonies are yellowish-white in color, powdery, cottony and moist in appearance. Septate, hyaline mycelium. Elongated conidiophores perpendicular to the hyphae. Conidia grouped in the shape of heads; conidia are unicellular and oval.	Isolates in the necrotrophic phase of soft stem rot
6.1B2	<i>Alternaria</i> Nees	Colonies have a hairy, grayish appearance and after incubation time they acquire olive-black tones in the center. Conidiophores simple, septate, elongated. Brown conidia, with septa that cross the conidia transversely and vertically.	Black rot or accompanying microbiota in anthracnose symptoms
4.1C7	<i>Colletotrichum gloeosporoides</i> (Penz.) Penz. & Sacc.	Colonies with abundant aerial mycelium, white, orange center, with brown tones. The hyphae are hyaline or stained with lactophenol blue, unicellular.	Anthracnose
9.2B2.1 9.2B3 4.1C2.1 4.1C1	<i>Colletotrichum</i> Corda	Chlamydospores are irregular in size. Conidia hyaline or unicellular, oval, with a single nucleus, formation of acervules.	
10.14B4 10.2C1 8.1C2 6.1C9 4.1C2 10.2C7	<i>Fusarium oxysporum</i> Schltdl. <i>Fusarium oxysporum</i> Schltdl. <i>Fusarium</i> Link	White colony, purple, pink reverse, structures called phialides thin, bottle-shaped, hyaline macroconids, crescent-shaped, with septa. Chlamydospores with thick, smooth double walls.	Soft fruit rot, basal stem rot
10.2C3 11B3 8.1C1	<i>Geotrichum</i> sp. 1 <i>Geotrichum</i> sp. 2 <i>Geotrichum</i> sp. 3	Rapidly growing white colonies, thick hyphae that partition or divide forming numerous arthroconidia, hyaline, rectangular to square.	Accompanying microbiota in rot symptoms



Isolate code	Taxon	Description	Related disease
A10	<i>Neofusicoccum</i> Crous, Slippers & A.J.L. Phillips	Mycelium is white and cottony, gradually turning dark gray to black over time. The texture is dense, woolly, or floccose, and the underside of the colony acquires a brownish to blackish color. Under prolonged conditions, dark pycnidia may form on the surface of the mycelium. Pycnidia globose to subglobose, dark. Conidia: hyaline, unicellular, ellipsoidal or ovoid, with rounded ends and thin, smooth walls.	Black stem blight
5.1C2	<i>Trichoderma</i> sp. 1	Colonies with a cottony appearance, dark green, white, yellow, greenish, areas of the mycelium with conidia are observed as concentric rings. Conidiophores hyaline, branched, straight, solitary or in groups. Bottle-shaped phialides, single-celled globose or ovoid, hyaline or green conidia in the form of a mass at the apices of the phialides.	Epiphytic fungus isolated from samples with biological disease control practices
6.1C1	<i>Trichoderma</i> sp. 2		
A15	<i>Diaporthe</i> Nitschke	Colony surface is initially white to off-white with a cottony or aerial mycelium that becomes grayish or olivaceous after 7 d to 15 d at 25 °C.	Grayish blight
A19		Concentric ring patterns may develop as the colony matures. Pycnidia may form on the surface after prolonged incubation, appearing as dark, globose structures embedded in the mycelium. Pycnidia are dark brown to black, globose to subglobose, and often ostiolate structures, either immersed or superficial in the agar. Inside the pycnidia, conidiophores are hyaline, simple or branched. Conidia: hyaline, aseptate, fusiform to ellipsoidal.	
A22			
A10	<i>Lasiodiplodia</i> Ellis & Everh.	Mycelium is initially white and cottony, but turns dark gray to black with age as reproductive structures develop. The underside of the colony is usually dark brown to black. Concentric zones and diffuse dark pigmentation are often observed, which may extend into the medium. Under favorable conditions, black pycnidia form on the surface of the mycelium starting after 10-15 days of incubation. Pycnidia globose or ovoid, dark, thick-walled structures, immersed or superficial.	Stem rot
A11		Conidiophores: hyaline, short, and cylindrical, lined with phialidic conidiogenic cells. Immature conidia hyaline, unicellular, ellipsoidal or ovoid, with thin walls.	
A12			

