



First report of *Fomitopsis meliae* causing *Damping off* in *Coffea arabica* seedlings in Mexico

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RESUMEN

Background/Objective. In the Frailesca region of Chiapas, Mexico, coffee nurseries (*Coffea arabica*) frequently experience high seedling mortality with symptoms attributed to the phytopathological complex known as *Damping off*. The objective of this study was to diagnose morphologically and molecularly new causal agents of *Damping off* in coffee seedlings

Experimental development. Coffee seeds and seedlings with symptoms of *Damping off* of the Typica, Marago, Bourbon, Marsellesa, Costa Rica, Sarchimor, Cerezo amarillo, and Pacamara varieties were collected from seedbeds of producers in the Frailesca region, Chiapas. From these, associated fungi were isolated and identified morphologically using dichotomous keys and molecularly using the ITS1 and ITS4 markers. To confirm their etiology, Koch's postulates were tested under field conditions using 20 seeds from each variety.

Results. Four fungal isolates associated with *Damping off* symptoms in coffee seedlings were identified: two species of *Fusarium* (*Fusarium oxysporum* and *Fusarium solani*), *Phoma costarricensis* and *Fomitopsis meliae*. The latter presented structures such as hyaline cystidioles and was 97.23% molecularly similar to *Fomitopsis meliae*. Pathogenicity tests confirmed that *Fomitopsis meliae* is a new causal agent of *Damping off*, causing symptoms such as root rot, basal stem rot, and seedling death.

Conclusion. This is the first report of *Fomitopsis meliae* as a new causal agent of *Damping off* in coffee nurseries of the Typica, Marago, Bourbon, Marsellesa, Costa Rica, Sarchimor, Cerezo amarillo, and Pacamara varieties in Mexico.

Keywords: Complex, Varieties, Coffee, Seedlings, Etiology, Drowning



INTRODUCTION

Coffee (*Coffea arabica*) is a highly important crop in Mexico, in both economic and social terms. It is a source of jobs for thousands of families, particularly in the states of the south and center of the country (Barrera Rodríguez *et al.*, 2021). Within this landscape, Chiapas, Mexico, stands out as the greatest producer, with 34.9% of its surface planted, followed by Veracruz and Oaxaca (SIAP, 2023). However, this crop faces several phytosanitary problems such as diseases and insect pests, which influence its yield and quality (Medina-Melendez *et al.*, 2016). Alongside this, Chiapas lacks scientific evidence in the diagnosis of phytopathogens associated to coffee seedlings, key information for the integrated management of diseases in this crop. In this context, the aim of this study was to morphologically and molecularly identify new causal agents associated to the *Damping off* of coffee seedlings.

Collection in seedbeds. In June, 2023, 20 coffee seeds and 10 coffee seedlings with symptoms of *Damping off* (Figure 1A–F) were collected from every one of the eight seedbeds belonging to the coffee farms in the municipal areas of Villa Corzo and Ángel Albino Corzo, Chiapas (Table 1).

Table 1. Coffee farms sampled with *Damping off* symptoms in the Frailesca region, Chiapas.

Municipality	Farm name	Coffea variety	Geographic coordinates
Villa Corzo	Santa Martha	Marago, Pacamara	15° 57' 51.7"N - 93° 22' 21.1" W
Villa Corzo	Gracias a Dios	Bourbón, Marsellesa	15° 58' 35.9 "N - 93° 21' 49.0" W
Ángel Albino Corzo	Solo Dios	Cerezo amarillo	15° 37' 55.0" N - 92° 33' 44.2" W
Villa Corzo	San Luis	Typica	16° 06' 14" N - 93° 35' 06" W
Villa Corzo	Ejido Sierra Morena	Costa Rica, Sarchimor	16° 09' 25.1" N - 93° 36' 08.0" W



Figure 1. Coffee seedlings of different varieties with symptoms of *Damping-off*; A) Marago; B) Typica; C) Costa Rica; D) Sarchimor; E) Marsellesa; F) Bourbon.

Fungal isolation. The seedlings and seeds with symptoms of *Damping off* were transported to the Biofertilizer and Bioinsecticide Laboratory of the Universidad de Ciencias y Artes de Chiapas (UNICACH), campus Villa Corzo, Chiapas, Mexico, for their diagnose. The seedlings were washed with tap water, segmented into root, stem and leaf with a scalpel, obtaining 5 mm explants of diseased tissue, and the same was carried out with the seeds. Subsequently, all explants (seeds and plant tissue) were disinfected with sodium 10% hypochlorite, then washed twice with sterile distilled water. Five explants were placed in each Petri dish with potato dextrose agar (PDA) medium and a nutrient culture (V8) in triplicate and incubated in a plant growth chamber with a photoperiod of 12 h light, 12 h darkness at 28 °C (Ríos-Velasco *et al.*, 2016). Mycelial growth was monitored every 24 hours for seven days; afterwards, the isolated fungi were purified using hypha tip.

Morphological and molecular identification. The isolated fungi were morphologically identified using the staining technique described by Sinclair and Dhingra, (2017) and taxonomic keys (Barnett and Hunter, 1998; Watanabe, 2002), observed in an optical microscope (Axiolab 5, Carl Zeiss). For molecular identification, genomic DNA was extracted using the protocol described by Raeder and Broda (1985) and the PCR amplification and the sequencing of the internal transcribed spacer (ITS) of the rDNA were carried out using the ITS4/ITS1 primers (White *et al.*, 1990). The PCR products were sequenced in the Genomic Services Laboratory of the CINVESTAV-Irapuato and compared with the National Center for Biotechnology Information (NCBI) database using BLAST (Altschul *et al.*, 1990) and the sequences were deposited in GenBank.

Pathogenicity test. Koch's postulates were carried out under field conditions, in a semi-controlled environment with a 75% shade mesh, simulating the agroforestry system of the coffee plantation in which excess sunlight is regulated. Twenty seeds of every coffee variety (Typica, Marago, Bourbón, Marsellesa, Costa Rica, Sarchimor, Cerezo amarillo, and Pacamara) were disinfected with 10% sodium hypochlorite and planted in 14 fl oz Styrofoam cups, using sterile sand as a substrate. The substrate was inoculated with 1000 µL of a *Fomitopsis meliae* conidial suspension (4×10^4 spores mL⁻¹). The inoculations were repeated every 15 days until the seedlings emerged (59 days), with a total of five inoculations. The control seeds were watered with sterile distilled water. The seedlings were monitored until symptoms were observed.

Based on the morphological and molecular characteristics, four fungi associated with *Damping off* in coffee seedbeds were identified; two belonged to the genus *Fusarium*: *F. oxysporum* (PX216556) and *F. solani* (PV242014), with a frequency of 100%, presenting macroconidia in the shape of crescent moons, phytopathogenic species related to phytopathogens associated to root diseases in coffee plants (López-Lima *et al.*, 2020; Gamboa-Becerra *et al.*, 2021). Another fungus associated to the disease was *Phoma costarricensis* (PX244525), with a frequency of 33% and the presence of hyalin pycnidia and solitary chlamidospores. Likewise, *Fomitopsis meliae* (PV242016) is reported for the first time as a causal agent of *Damping off* in coffee, with a frequency of 100%. This species displayed hyaline cystidioles with thin walls ending in a point with dimensions of 3.912 x 1.377 µm (length x width) (n=30) (Figure 2) (Han and Cui, 2014; Salazar-Vidal, 2016; Li, *et al.*, 2013; Han and Cui, 2014; Alcantara-Varga *et al.*, 2020). Molecularly, the isolate displayed a similarity of 97.23% with *Fomitopsis meliae*.

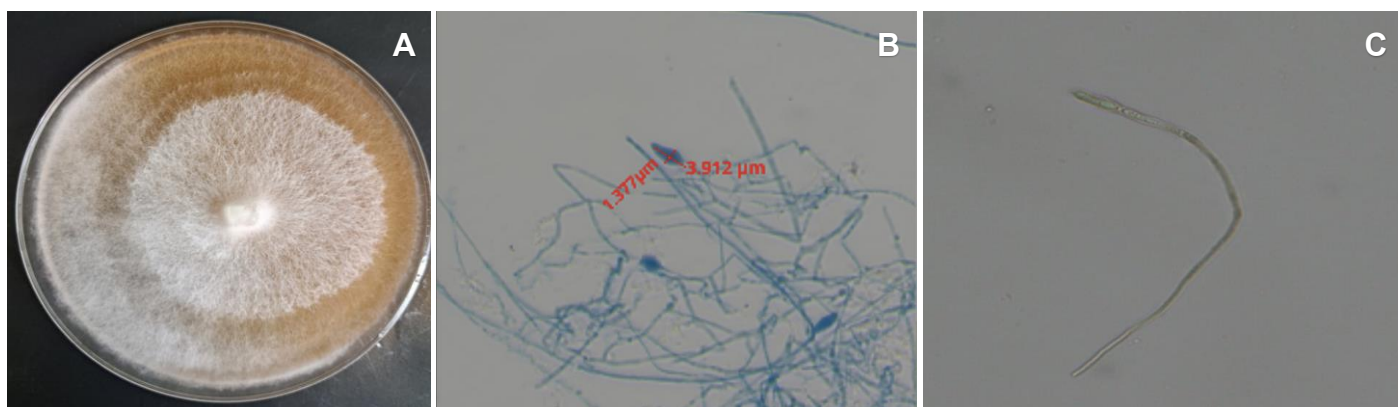


Figure 2. Morphological characteristics of *Fomitopsis meliae*; **A)** Colony grown on PDA medium; **B)** Length and width of the cystidioles; **C)** Cystidioles in water observed under the microscope at 40X.

Pathogenicity tests. The entirety (100%) of the plants inoculated with *Fomitopsis meliae* began displaying symptoms 45 days after emergence. The yellowing of leaves, change in color of the base of the stem from green to brown, and later the lesions became largely extended until they caused the rotting of the root, stem and death of the seedlings, similar to those observed in the seedlings of the seedbeds sampled. The control displayed no symptomatology. The seedlings with symptoms were used to reisolate the fungus and *F. meliae* was verified to be a causal agent of the *Damping off* complex in coffee seedlings, confirming its pathogenicity in all the coffee varieties evaluated (Typica, Marago, Bourbon, Marsellesa, Costa Rica, Sarchimor, Cerezo amarillo and Pacamara) (Figure 3 A-D).

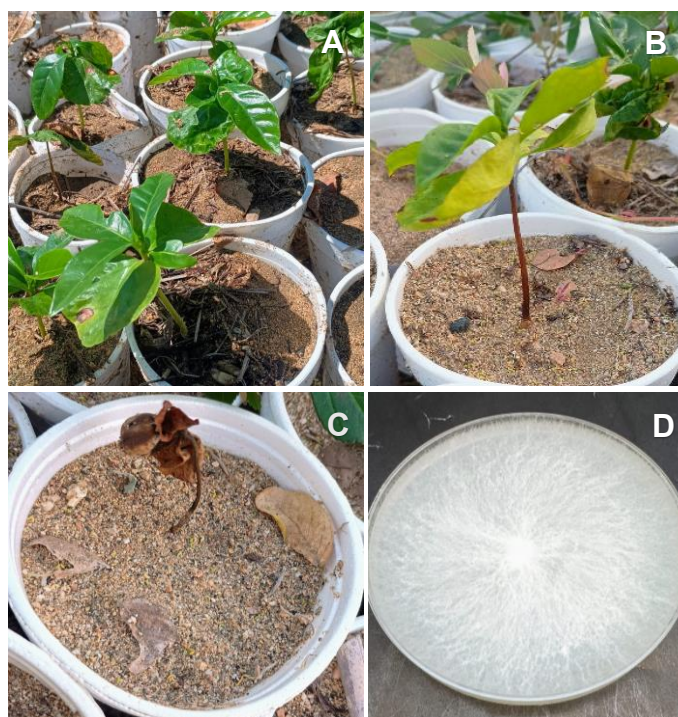


Figure 3. Symptoms produced on coffee seedlings inoculated with *Fomitopsis meliae*; **A)** Control seedling; **B)** Diseased; **C)** dead plant; **D)** *F. meliae* reisolated in PDA medium.

In Mexico, there is documented evidence that *F. meliae* is the causal agent of the regressive death of citrus plants (Polanco-Florián *et al.*, 2020) and the brown rot of the wood of lemon trees in Arizona and California (Hu and Wright, 2021). Likewise, this pathogen has been isolated from Arabic coffee fruits and leaves with symptoms of anthracnose (Sosa-Rubio, 2023), where this disease is caused by the *Colletotrichum gloeosporioides* complex, and there is no scientific evidence of the pathogenicity of *Fomitopsis meliae* causing diseases in coffee plants.

The results obtained in this study coincide with descriptions by López-Lima *et al.* (2020) and Gamboa-Becerra *et al.* (2021), who reported the presence of diverse species of *Fusarium* (*F. oxysporum* and *F. solani*) associated to diseases in the roots of coffee plants. On the other hand, the presence of *Phoma costarricensis* was also identified, according to SENASICA (2014), causing the “coffee Phoma blight”, affecting mainly young organs such as leaves, stems, flowers and fruits, which coincides with the observations made in this study.

Although pathogenicity tests were only performed on *Fomitopsis meliae*, the importance of experimentally evaluating the pathogenic potential of the rest of the isolated fungi has been acknowledged. Therefore, research will continue in order to carry out pathogenicity tests and biological control strategies with an integrated management of the coffee plant.

CONCLUSIONS

Four fungal species associated to *Damping off* were identified: *F. oxysporum*, *F. solani*, *Phoma costarricensis* and the first report of *Fomitopsis meliae* causing symptoms characteristic of *Damping off* in coffee seedbeds, both in local and introduced varieties, resistant to coffee rust (*Hemileia vastatrix*) in Chiapas, Mexico. As a result, the number of causal agents of this disease increases the early development stages of coffee seedlings in the seedbeds.

Limitations

No limitations in the methodological parts.

Conflict of interest

All authors declare having no conflict of interest.

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Contributions of the authors

Vidal Hernández-García, experimental design. Orlando Cruz-Sarmiento, taking data and follow-up of the experiment. Miguel Ángel Salas-Marina, writing and manuscript

review. Claudio Ríos-Velasco, extraction and sequencing of phytopathogenic fungi. Luis Alfredo Rodríguez-Larramendi, writing and manuscript review.

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