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ARTICLE

RESEARCH ARTICLE

Enhancing morphophysiological traits by inoculating a neotropical pine (*Pinus teocote*) with edible ectomycorrhizal mushrooms grown in environmentally friendly substrates

Mejora de los caracteres morfofisiológicos de un pino neotropical (*Pinus teocote*) mediante la inoculación con hongos ectomicorrízicos comestibles cultivados en sustratos ambientalmente sostenibles

Magdalena Martínez-Reyes
Colegio de Postgraduados, Campus Montecillo, Edafología. Texcoco 56230, Mexico
https://orcid.org/0000-0003-2352-917X

Karla I. Luciano-Dorado
Colegio de Postgraduados, Campus Montecillo, Edafología. Texcoco 56230, Mexico
https://orcid.org/0000-0003-3933-051X

José L. Barragán-Soriano
Colegio de Postgraduados, Campus Montecillo, Edafología. Texcoco 56230, Mexico and Consejo Mexiquense de Ciencia y Tecnología. 50120 Toluca de Lerdo, Mexico
https://orcid.org/0000-0003-2134-4788

Irma Díaz-Aguilar
Colegio de Postgraduados, Campus Montecillo, Edafología. Texcoco 56230, Mexico
https://orcid.org/0000-0001-9803-8231

Julián Delgadillo-Martínez
Colegio de Postgraduados, Campus Montecillo, Edafología. Texcoco 56230, Mexico
https://orcid.org/0000-0003-4777-0494

Samuel Araujo-Castillo
Colegio de Postgraduados, Campus Montecillo, Edafología. Texcoco 56230, Mexico
https://orcid.org/0009-0000-2735-9417

Jesús Pérez-Moreno
Colegio de Postgraduados, Campus Montecillo, Edafología. Texcoco 56230, Mexico
Corresponding author: jperezm@colpos.mx and jepemo@yahoo.com.mx, https://orcid.org/0000-0001-5216-8313

Abstract: *Aim of study*: To evaluate the effect of inoculating *Pinus teocote* Schiede ex Schltdl. & Cham. plants, a neotropical pine species highly resilient to stress conditions, with two edible ectomycorrhizal mushrooms, *Laccaria proxima* (Boud.) Pat. and *Suillus pungens* Thiers & A.H.Sm., on their growth and physiological quality. The plants were grown in different environmentally friendly substrate combinations. *Area of study*: Bioassays were conducted in the greenhouses of the Colegio de Postgraduados in Texcoco, Mexico. *Material and methods*: The influence of *L. proxima* and *S. pungens* on *P. teocote* was assessed through growth and physiological parameters using three distinct substrate formulations, two of which incorporated mixtures of sawdust and compost. The evaluated variables included plant biomass, photosynthesis, transpiration, chlorophyll a, b, and total chlorophyll content, as well as carotenoids and ectomycorrhizal colonization, measured 570 days after sowing. *Main results*: High ectomycorrhizal colonization, exceeding 84%, was recorded in the plants grown in sawdust and compost mixtures. Additionally, increases in biomass and physiological quality were observed in these plants compared to non-inoculated plants. Depending on the substrate mixtures evaluated, the inoculation with *S. pungens* produced higher transpiration and chlorophyll a, b, total chlorophyll, and carotenoid content than that with *L. proxima*. *Research highlights*: It was demonstrated that the production of ectomycorrhizal *Pinus teocote* plants in greenhouse, with high morphophysiological quality and elevated mycorrhization rates, is feasible when using environmentally friendly substrates.

Keywords: compost; *Laccaria proxima;* reforestation; resilience; sawdust; *Suillus pungens;* tree bark.

Resumen: *Objetivo del estudio:* Evaluar el efecto de la inoculación de plantas de *Pinus teocote* Schiede ex Schltdl. & Cham., una especie de pino neotropical altamente resiliente a condiciones de estrés, con dos hongos ectomicorrízicos comestibles, *Laccaria proxima* (Boud.) Pat. y *Suillus pungens* Thiers & A.H.Sm., sobre su crecimiento y calidad fisiológica. Las plantas fueron cultivadas en diferentes combinaciones de sustratos ambientalmente sostenibles. *Área del estudio:* Los bioensayos se realizaron en los invernaderos del Colegio de Postgraduados, en Texcoco, México. *Material y métodos:* La influencia de *L. proxima* y *S. pungens* sobre *P. teocote* se evaluó mediante parámetros de crecimiento y fisiológicos, utilizando tres formulaciones distintas de sustrato, dos de las cuales incorporaron mezclas de serrín y compost. Las variables evaluadas incluyeron biomasa vegetal, fotosíntesis, transpiración, contenido de clorofila a, b y total, así como carotenoides y colonización ectomicorrízica, medidos a los 570 días después de la siembra. *Resultados principales:* Se registró una alta colonización ectomicorrízica, superior al 84%, en las plantas cultivadas en mezclas de serrín y compost. Además, se observaron incrementos en la biomasa y la calidad fisiológica de estas plantas en comparación con las plantas no inoculadas. Dependiendo de las mezclas de sustrato evaluadas, la inoculación con *S. pungens* produjo mayores valores de transpiración y contenido de clorofila a, clorofila b, clorofila total y carotenoides en comparación con la inoculación con *L. proxima*. *Aspectos destacados de la investigación:* Se demostró que es factible producir plantas de *Pinus teocote* ectomicorrizadas en invernadero, con alta calidad morfofisiológica y elevadas tasas de micorrización, utilizando sustratos ambientalmente amigables.

Palabras clave: compost; *Laccaria proxima*; reforestación; resiliencia; serrín; *Suillus pungens*; corteza de árbol.

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Supplementary information ↓

1. INTRODUCTION

Forests play a crucial role in food security and human nutrition, not only for the communities living nearby but for humanity as a whole, by mitigating global climate change (FAO, 2025). Unfortunately, 75% of the planet’s land is degraded, directly affecting 3.2 billion people, and if this trend continues, we are projected to reach 90% by 2050 (Cherlet et al., 2018). An urgent strategy to halt or reverse this process is the promotion of successful plantations, reforestation, and afforestation, which presents a significant challenge from social, cultural, economic, technical, and geopolitical perspectives at the global level. A major limitation in the mass production of forest trees in Mexico is that historically, foreign-sourced substrates, such as peat, vermiculite, or agrolite, have been used in forest production. This has resulted in: *i*) a substantial increase in production costs (Martínez-Nevárez et al., 2023) and *ii*) significant environmental damage due to the extraction of raw materials or the artificial manufacturing of these materials (Atzoni et al., 2021). Therefore, there is a need for alternative use of environmentally friendly substrates, such as sawdust, which globally reached an annual production of approximately 38 billion tons in 2020 (Gang et al., 2023). In Mexico, 2.8 million m³ of sawdust, shavings, and bark, mainly from pine, were produced in 2017 (Fregoso-Madueño et al., 2017). Another promising alternative in forest production is the use of compost, which has the potential to completely replace peat, according to Calisti et al. (2023). Solid waste production worldwide is expected to increase from 2.1 billion tons in 2023 to 3.8 billion tons by 2050 (UNEP, 2024), with Mexico generating approximately 42 million tons of solid waste annually (SEMARNAT, 2025).

Furthermore, the vast majority of trees in temperate and boreal forests form obligatory mutualistic associations with ectomycorrhizal fungi (EMF). This relationship is crucial for the structure, function, and conservation of these forests (Pérez-Moreno et al., 2021). From an ecological perspective, ectomycorrhizal symbiosis provides various beneficial effects to the associated plants, including enhanced nutrient absorption, such as nitrogen and phosphorus (Smith & Read, 2008; Pickles & Simard, 2017), protection against pathogens, and tolerance to stress conditions such as transplant shock, drought, high temperatures, and potentially toxic elements (Smith & Read, 2008; Carrillo-Saucedo et al., 2022). Climate change mitigation may be achieved through the reforestation of degraded and deforested lands using native or endemic pine species inoculated with specific EMF. This strategy facilitates seedling establishment and survival in nutrient-deficient or drought-prone soils (Kothe et al., 2025). Ectomycorrhizal symbiosis serves as a significant driver of carbon sequestration within terrestrial ecosystems. This relationship functions not merely as a conduit for transferring plant-derived carbon into the soil but as a fundamental regulator of its long-term stability. Through the production of recalcitrant fungal necromass (primarily consisting of chitin and melanin), the promotion of soil aggregation, and, most critically, the suppression of decomposition rates via competitive interactions, EMF transition the carbon cycle from a rapid, plant-litter-based loop to a slower, soil-based storage system (Clemmensen et al., 2017; Dick et al., 2022; Yu et al., 2025). Consequently, understanding and managing these symbiotic relationships represents a critical frontier in the development of strategies for climate change mitigation. In Mexico, *Pinus* species are of primary importance in the country's timber production, both in terms of volume and economic value. Between 2002 and 2016, the value of pine timber production accounted for an average of 85.1% of the total forest species under management (Moctezuma-López & Flores, 2020). More recently, it has been recorded that the average pine timber production accounted for 72.5% (SEMARNAT, 2021). However, the country lacks a mass production program for ecologically important pines inoculated with ectomycorrhizal fungi, which has led to low field survival rates, ranging from 0% to 43% (Prieto & Goche, 2016), with the potential for drastic reductions in survival due to current climate change.

Therefore, the biotechnological development of inoculating forest species with native ectomycorrhizal fungi represents an urgent need in forest production. Likewise, the search for locally produced substrates also represents a priority. Furthermore, one criterion for selecting EMF that has gained significant global attention is their edibility. Inoculating with edible ectomycorrhizal fungi provides dual benefits by not only enhancing tree growth and survival through improved nutrient and water uptake but also by generating an additional source of nutrition and income through the harvest of valuable fungal fruiting bodies. In contrast, while non-edible species may offer similar ecological benefits, they lack this supplementary socioeconomic advantage, thereby missing an opportunity to integrate food production and economic incentives into reforestation programs (Pérez-Moreno et al., 2021). In Mexico, *Laccaria proxima* (Boud.) Pat. (Lp) and *Suillus pungens* Thiers & A.H. Sm. (Sp) are two edible mushroom species of great social, economic, and environmental importance (Carrasco-Hernández et al., 2010; Garibay-Orijel et al., 2013; Pérez-Moreno et al., 2020, 2021). Species of the genera *Laccaria* and *Suillus* are considered cosmopolitan and pioneer fungi, colonizing plants in the early phenological stages and forming ectomycorrhizae with a wide range of hosts (Carrasco-Hernández et al., 2010; Pérez-Moreno et al., 2020). *Pinus teocote* Schiede ex Schlechtendal & Chamisso is an endemic species to Mexico and the gymnosperm with the widest distribution in the country, also having significant commercial timber value (Escobar-Alonso & Rodríguez-Trejo, 2021). It is characterized by its growth in dry, stony soils and its tolerance to long periods of drought, making it a key species in reforestation programs under current climate change conditions (CONAFOR, 2023). Against this backdrop, the aim of the present study was to evaluate the effect of inoculating *Pinus teocote* with Lp and Sp on the growth and physiological quality of the species, grown in environmentally friendly substrate combinations. As a comparative reference, a substrate named “Colpos,” previously used in research to evaluate the successful ectomycorrhizal colonization of native Mexican pines (Pérez-Moreno et al., 2021), was also employed.

2. MATERIALS AND METHODS

2.1. Plant material and inoculum

The experiment was conducted in the greenhouses Colegio de Postgraduados, Campus Montecillo, Texcoco, State of Mexico. The germplasm of *P. teocote* was collected from the Iztaccíhuatl-Popocatépetl National Park, State of Mexico. Prior to sowing, the seeds were submerged in water for 24 hours to eliminate sterile or empty seeds, preparing them for germination. Subsequently, they were sterilized with 30% H_2O_2 for 20 minutes and rinsed with sterile water. The basidiomes used for the preparation of ectomycorrhizal inoculum were collected from the same region than the *P. teocote* seeds. The inoculum was prepared according to the methodology established by Martínez-Reyes et al. (2012). The fruiting bodies were collected in June 2019. Subsequently, the pilei were excised from the stipes, and the hymenial tissues were separated for Lp and Sp, respectively. The pileal tissue of Lp and the hymenial tissue of Sp were desiccated at 35 °C in an industrial fruit-drying oven. Taxonomic identification of Lp and Sp was performed based on the morphological criteria described by Carrasco-Hernández et al. (2010) and Garibay-Origel et al. (2013). The dried pilei and hymenial tissues were weighed using an analytical balance (ESNOVA TH-II) and homogenized in a blender (Oster) with sterile distilled water at a 1:1 (w/v) ratio. The resulting inoculum was stored in amber glass containers at 5 °C until use three months after preparation. Spore concentration in the inoculant suspensions was quantified using a Neubauer hemocytometer chamber. A dosage of 10^6 to 10^8 spores was applied per plant.

2.2. Establishment of the experiment

Three substrates were used: S1) sand-bark-forest soil (40:40:20, v/v/v); S2) sawdust-bark-compost (50:30:20, v/v/v); and S3) sawdust-bark-compost (55:35:10, v/v/v). The bark and sawdust components were sourced from a local sawmill and comprised a mixture of native Mexican pine species, including *Pinus pseudostrobus* Lindl., *Pinus ayacahuite* Ehrenb. ex Schltdl., *P. teocote*, and *Pinus hartwegii* Lindl. All substrate components were sieved through a 1 cm mesh to achieve a uniform particle size prior to mixing. Substrate sterilization was performed using steam in a pressure cooker at 125 °C and 127 kPa (18.5 PSI) for a duration of four hours. Following sterilization, substrates were allowed to rest for 48 hours at ambient conditions before undergoing a second identical sterilization cycle. This protocol, established in prior studies, was implemented to ensure the complete elimination of pre-existing microbial inocula within the constituent materials.

For sowing, black plastic tubes of 130 cm^3, disinfected with 96% alcohol, were used. Three seeds were sown per tube at a depth of 1 cm. Two inoculations were performed: one during sowing and another 180 days after sowing, with the spores of the edible ectomycorrhizal mushrooms corresponding to each treatment. The plants were watered with sterile distilled water every three days, alternating with a fungicide solution (Captan) at a concentration of 2 $g \cdot L^{-1}$ as a preventive measure to avoid “damping off” during the initial growth stage until the plant stem became lignified. Although the experiment was conducted under greenhouse conditions, purpose-built experimental devices were utilized to control spore dispersal between treatments, based on prior experiences (see description and illustration in Carrasco-Hernández et al. 2010). These apparatuses consisted of seedling trays placed within wooden cubic frames. The frames were enclosed with transparent plastic sheeting on all sides, while the top was sealed with a mesh screen (< 1 mm) to permit gaseous exchange. A collection tray was placed at the base to capture irrigation leachate. One wall of the enclosure was designed to be removable to facilitate watering. This experimental setup effectively prevented cross-contamination between treatments.

2.3. Experimental design

A completely randomized 3x3 factorial design was used, with two factors: substrate (three levels: S1, S2, and S3) and fungal species [three levels: plants inoculated with Lp or Sp, and non-inoculated plants

(Nip)]. Each treatment had ten replications, resulting in a total of 90 experimental units. Five randomly selected plants were used to assess dry biomass, and the remaining five were divided into aerial and root parts. The aerial part of these later plants was used to evaluate chlorophyll and carotenoid content, while the root part was used for ectomycorrhizal colonization.

2.4. Evaluated variables

2.4.1. Dry biomass and plant growth

To determine plant growth and dry biomass (shoot, root, and total), five randomly selected plants per treatment were destructively sampled. The height and diameter of the plants were measured using a graduated ruler and a digital caliper at 120, 360, and 510 days after sowing. At 570 days after sowing, the shoot and root parts of the plants were separated, and the roots were washed with running water through a 0.18 mm mesh sieve to remove the substrate and prevent root loss. The shoot and root parts were then dehydrated in a drying oven (Santorius AC210S, Germany) at 70 °C for three days until a constant weight was achieved and subsequently weighed on an analytical balance.

2.4.2. Photosynthesis, transpiration, chlorophyll, and carotenoids content

Five plants per treatment were used for each variable. The photosynthetic rate was determined at 510, 540, and 570 days after sowing using a portable CO_2 meter (Model telaire7001, Spectrum Technologies). Transpiration was quantified gravimetrically 570 days after sowing, with measurements conducted at 12-hour intervals according to the following protocol:

i) Light irrigation was applied at 7:00 pm to each plant selected for evaluation; ii) At 07:00 am the following day, the substrate was watered to field capacity. Excess water was drained, and the base of each plant was sealed at the root collar using a transparent plastic bag. The initial weight (P_1) was then recorded using an analytical balance; iii) The plant weight (P_2) was recorded again at 7:00 pm; iv) A final weight measurement (P_3) was taken at 07:00 am the next day, after which the plastic bag was removed. Transpiration was calculated using the following formula:

Day and night transpiration = P1 - P2 - P3.

Chlorophyll a, b, total content, and carotenoids were determined 570 days after sowing using the acetone method (Zhang, 1986). Absorbance readings were made at wavelengths of 470, 646, and 663 nm according to the equations of Lichtenthaler (1987).

2.5. Ectomycorrhizal colonization and micro- and macromorphological characterization of morphotypes

Ectomycorrhizal colonization was evaluated by destructively sampling five plants per treatment 570 days after sowing. The root system was separated from the shoot part and washed with running water to remove the substrate, then passed through three sieves of different diameters (1.19, 0.180, and 0.085 mm) to collect as many roots as possible. The roots were quantified using a stereoscopic microscope (LEICA EZ4) to count live ectomycorrhizal short roots, non-mycorrhizal roots, and dead roots. The percentage of colonization was calculated by determining the proportion of live ectomycorrhizal short roots relative to the total number of short roots of each plant (Martínez-Reyes et al., 2012). The micro- and macromorphological characterization of the *L. proxima* and *S. pungens* morphotypes was performed through histological sections and semi-permanent preparations. The ectomycorrhizas selected were cleaned with water and a brush to remove substrate aggregates. Morphoanatomical characterization and measurement were carried out using the methodology proposed by DEEMY (2020) through stereoscopic microscopy to observe the color of various sections of the ectomycorrhizal tips, the branching pattern, and the presence or absence of rhizomorphs and external mycelium, and with light-field optical microscopy to record the arrangement of hyphae in the mantle, Hartig net, and external hyphae.

2.6. Statistical analysis

Data analysis for all variables was conducted using analysis of variance and Tukey's mean comparison test ($\alpha \leq 0.05$) using the SAS® statistical package, version 9.0. When the assumptions of normality were not met, the data were transformed to their logarithmic values before performing the statistical tests. For those data of colonization rates expressed as percentages, they were always log-transformed.

3. RESULTS

3.1. Dry biomass and plant growth

For plants grown in S1 and S2, differences were observed between inoculated and non-inoculated plants in terms of dry weight of the shoot, root, and total compartments, regardless of the inoculated fungus. Among plants inoculated with S1, those inoculated with Lp exhibited higher dry weight in the root and total compartments compared to those inoculated with Sp. A contrasting trend was observed in plants grown in S3, where differences were only observed in the dry weight of the root between inoculated and non-inoculated plants (Table 1). The highest total dry biomass production was recorded in plants inoculated with Lp and grown in S1. The effect was 11.6, 8.7, and 8.7 times greater compared to non-inoculated plants across the various substrates (Table 1). Overall, the height of the inoculated plants was greater than the non-inoculated plants, irrespective of the inoculated fungus and substrate, particularly at 360 and 510 days after sowing. Differences in height between inoculated plants were observed 510 days after sowing. Plants inoculated with Sp exhibited greater height than those inoculated with Lp grown in S1, while the opposite trend was seen in plants grown in S3, 510 days after sowing (Figure 1). At 510 days after sowing, greater stem diameters were recorded in inoculated plants compared to non-inoculated plants, particularly in plants grown in S1 and S2. A different trend was observed in S3, where plants inoculated with Sp showed stem diameters equal to those of non-inoculated plants (Figure 2). No differences were observed in stem diameters between plants inoculated with Sp or Lp at any of the three evaluation times.

Table 1. Dry weight of the shoot, root and total of non-inoculated *Pinus teocote* plants or inoculated with *Laccaria proxima* and *Suillus pungens*, grown in three substrates, 570 days after sowing

Treatment ¶	**Dry weight (g)**		
	Shoot	**Root**	**Total**
S1			
Ip with Lp	1.52 ± 0.05 a	1.28 ± 0.05 a	2.80 ± 0.07 a
Ip with Sp	1.10 ± 0.04 ab	0.76 ± 0.07 b	1.86 ± 0.07 b
Nip	0.14 ± 0.02 e	0.10 ± 0.00 c	0.24 ± 0.02 d
S2			
Ip with Lp	0.74 ± 0.12 bc	0.70 ± 0.13 b	1.44 ± 0.22 b
Ip with Sp	0.86 ± 0.21 bc	0.82 ± 0.05 b	1.68 ± 0.25 b
Nip	0.14 ± 0.02 e	0.18 ± 0.02 c	0.32 ± 0.02 cd
S3			
Ip with Lp	0.54 ± 0.02 ced	0.58 ± 0.04 b	1.12 ± 0.05 bc
Ip with Sp	0.68 ± 0.21 bcd	0.72 ± 0.16 b	1.40 ± 0.37 b
Nip	0.16 ± 0.02 de	0.16 ± 0.02 c	0.32 ± 0.03 cd

The data are means ± Standard deviation, n = 5. Values in the same column with the same letter are not different according to the Tukey test at ($p \leq 0.05$). Ip = Inoculated plants; Nip = Non-inoculated plants; Lp = *Laccaria proxima*; Sp = *Suillus pungens*; Substrates: S1 = sand, pine bark, and forest soil (2:2:1); S2 = sawdust, bark, and compost (50:30:20); S3 = sawdust, bark, and compost (55:35:10).

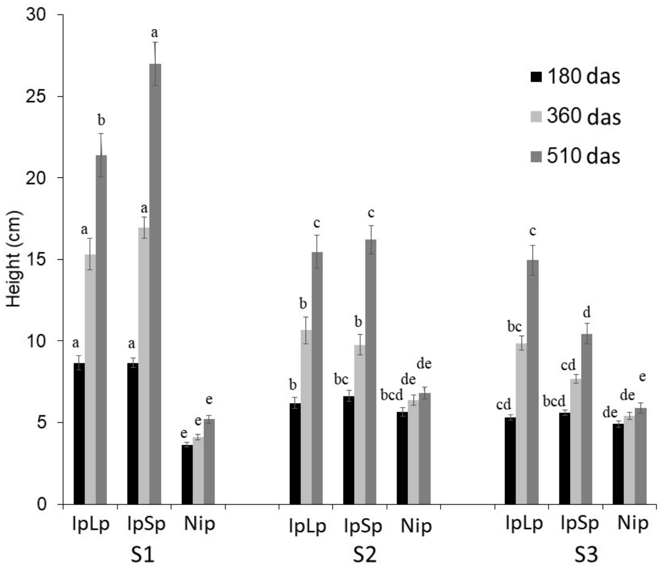

Figure 1. Height of *Pinus teocote* plants at 120, 360, and 510 days after sowing (das), inoculated or non-inoculated with two ectomycorrhizal fungi, grown in three substrates. Values represent the mean ± standard error of the mean (n = 5). Bars sharing the same letter within each date are statistically equivalent, according to Tukey's test (p ≤ 0.05). Ip = Inoculated plants; Nip = Non-inoculated plants; Lp = *Laccaria proxima*; Sp = *Suillus pungens*; Substrates: S1 = sand, pine bark, and forest soil (2:2:1); S2 = sawdust, bark, and compost (50:30:20); S3 = sawdust, bark, and compost (55:35:10).

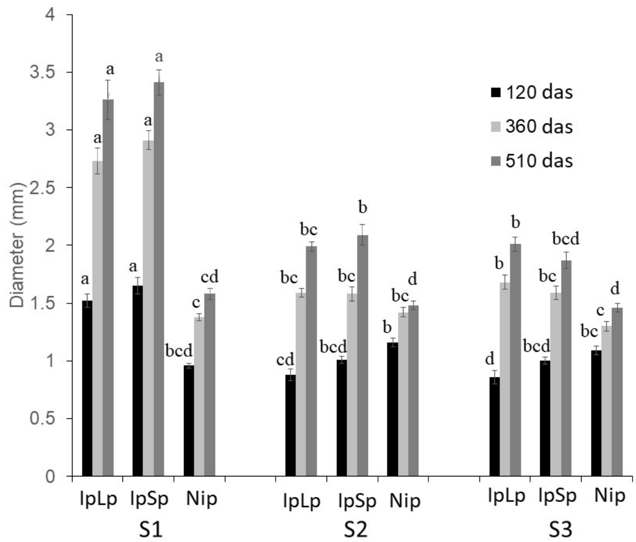

Figure 2. Diameter of *Pinus teocote* plants at 120, 360, and 510 days after sowing (das), inoculated or non-inoculated with two ectomycorrhizal fungi, grown in three substrates. Values represent the mean ± standard error of the mean (n = 5). Bars sharing the same letter within each date are statistically equivalent, according to Tukey's test (p ≤ 0.05). Ip = Inoculated plants; Nip = Non-inoculated plants; Lp = *Laccaria proxima*; Sp = *Suillus pungens*; Substrates: S1 = sand, pine bark, and forest soil (2:2:1); S2 = sawdust, bark, and compost (50:30:20); S3 = sawdust, bark, and compost (55:35:10).

3.2. Photosynthetic rate and transpiration

In general, the photosynthetic rate was higher in inoculated plants compared to non-inoculated plants, regardless of the substrate and inoculated fungus. The greatest increase in the photosynthetic rate was recorded at 570 days after sowing in plants inoculated with Lp and grown in S3, which was 4.9 times greater than in non-inoculated plants. A similar trend was observed in transpiration. In plants grown in S1, higher transpiration was recorded in those inoculated with Lp compared to those inoculated with Sp, which did not occur in plants grown in S2 and S3 (Table 2).

Table 2. Photosynthetic rate and transpiration in *Pinus teocote* plants at 510, 540, and 570 days after sowing, inoculated or non-inoculated with *Laccaria proxima* and *Suillus pungens*, grown in three substrates

Treatment¶	Photosynthetic rate (g CO2·m^{-2}·h^{-1})			Transpiration (g)
	510 days	540 days	570 days	
S1				
Ip with Lp	1.15 ± 0.131 ab	1.19 ± 0.116 bc	1.24 ± 0.112 ab	0.05 ± 0.010 b
Ip with Sp	1.17 ± 0.101 ab	1.34 ± 0.212 b	1.67 ± 0.277 a	0.08 ± 0.005 a
Nip	0.20 ± 0.014 d	0.38 ± 0.054 d	0.48 ± 0.042 b	0.01 ± 0.003 c
S2				
Ip with Lp	1.17 ± 0.174 ab	1.65 ± 0.213 ab	2.02 ± 0.201 a	0.05 ± 0.005 b
Ip with Sp	1.43 ± 0.248 ab	1.34 ± 0.174 b	1.92 ± 0.253 a	0.04 ± 0.005 b
Nip	0.36 ± 0.112 cd	0.52 ± 0.041 cd	0.45 ± 0.060 b	0.01 ± 0.003 c
S3				
Ip with Lp	1.65 ± 0.117 a	2.16 ± 0.130 a	1.88 ± 0.177 a	0.05 ± 0.002 b
Ip with Sp	0.96 ± 0.087 bc	1.69 ± 0.202 ab	2.02 ±0.249 a	0.03 ± 0.005 bc
Nip	0.41 ± 0.063 cd	0.44 ± 0.030 d	0.44 ± 0.033 b	0.01 ± 0.007 c

The data are means ± Standard deviation, n = 5 for photosynthetic rate and transpiration, Values in the same column with the same letter are not different according to the Tukey test at ($p \leq 0.05$). Ip=Inoculated plants; Nip = Non-inoculated plants; Lp = *Laccaria proxima*; Sp = *Suillus pungens*; Substrates: S1 = sand, pine bark, and forest soil (2:2:1); S2 = sawdust, bark, and compost (50:30:20); S3 = sawdust, bark, and compost (55:35:10).

Table 3. Chlorophyll a, b, total and carotene content of non-inoculated *Pinus teocote* plants or inoculated with *Laccaria proxima* and *Suillus pungens*, grown in three substrates, 570 days after sowing

Treatments	Chlorophyll (mg·g^{-1})			Carotene (mg·g^{-1})
	a	b	Total	
Substrate 1				
Ip with Lp	7.32 ± 0.4 ab	2.91 ± 0.21 a	10.24 ± 0.61 ab	2.14 ± 0.11 ab
Ip with Sp	6.22 ± 0.69 abc	2.31 ± 0.25 ab	8.53 ± 0.95 abc	1.75 ± 0.16 abc
Nip	5.90 ± 0.65 abcd	2.10 ± 0.23 ab	8.01 ± 0.89 abc	1.83 ±0.18 abc
Substrate 2				
Ip with Lp	4.65 ± 0.70 cd	2.07 ± 0.21 ab	6.72 ± 0.73 c	1.18 ± 0.35 c
Ip with Sp	8.12 ± 0.39 a	2.86 ± 0.26 a	10.99 ± 0.43 a	2.24 ± 0.21 a
Nip	3.64 ± 0.32 d	1.67 ± 0.22 b	5.32 ± 0.32 c	0.99 ± 0.17 c
Substrate 3				
Ip with Lp	5.32 ± 0.38 bcd	2.00 ± 0.13 ab	7.32 ± 0.51 bc	1.50 ± 0.09 abc
Ip with Sp	7.73 ± 0.59 ab	2.80 ± 0.24 a	10.53 ± 0.82 ab	2.09 ± 0.12 ab
Nip	3.99 ± 0.51 cd	1.45 ± 0.17 b	5.45 ± 0.69 c	1.32 ± 0.13 bc

The data are means ± Standard deviation, n = 5. Values in the same column with the same letter are not different according to the Tukey test at ($p \leq 0.05$). Ip = Inoculated plants; Nip = Non-inoculated plants; Lp = *Laccaria proxima*; Sp = *Suillus pungens*; Substrates: S1 = sand, pine bark, and forest soil (2:2:1); S2 = sawdust, bark, and compost (50:30:20); S3 = sawdust, bark, and compost (55:35:10).

3.3. Chlorophyll a, b, total chlorophyll, and carotenoid content

Substantial differences were observed in the concentrations of chlorophylls and carotenoids depending on the substrates in which the plants grew. In plants grown in S1, no differences were found between inoculated and non-inoculated plants regarding chlorophyll and carotenoid content, regardless of the inoculated fungus. In contrast, in plants grown in S2, those inoculated with Sp exhibited higher concentrations of chlorophyll a, b, total chlorophyll, and carotenoids compared to plants inoculated with Lp or non-inoculated plants. Similarly, in plants grown in S3, those inoculated with Sp showed higher concentrations of chlorophyll b and total chlorophyll compared to non-inoculated plants (Table 3).

3.4. Mycorrhizal colonization and morphoanatomical characterization of morphotypes

The percentages of colonization in inoculated plants were high regardless of the inoculated fungi and the substrates in which the plants grew, ranging from 84.7% to 98.8%. Although no differences were found between the two inoculated fungi, the highest recorded value was observed in plants inoculated with Sp in S2, which was higher than the values recorded for plants inoculated with Sp in S1 and those inoculated with Lp in S3 (Table 4).

Table 4. Mycorrhizal colonization of inoculated *Pinus teocote* plants with *Laccaria proxima* and *Suillus pungens*; or not inoculated, grown in three substrates, 570 days after sowing

Treatments¶	Living short-roots		Dead short-roots		% Colonization
	Mycorrhizal	Non- mycorrhizal	Mycorrhizal	Non- mycorrhizal	
Substrate 1					
Ip with Lp	546.0±163.1b	15.3±6.6b	217.6±107.5a	22.3±6.7c	97.5ab
Ip with Sp	275.3±95.1b	51.6±19.9ab	131.3±19.5ab	24.6±4.1c	84.7b
Nip	0.0±0.0b	69.6±6.3a	0.0±0.0b	324.6±42.9a	0.0c
Substrate 2					
Ip with Lp	638.0±224.9ab	38.0±16.6ab	233.0±36.7a	10.0±5.2c	91.2ab
Ip with Sp	1361.3±201.5a	15.6±0.8b	84.6±5.0ab	8.0±4.3c	98.8a
Nip	0.0±0.0b	56.0±8.1ab	0.0±0.0b	214.3±27.0b	0.0c
Substrate 3					
Ip with Lp	328.6±81.4b	54.0±2.0ab	153.0±20.5ab	25.6±12.9c	84.8b
Ip with Sp	594.0±250.3b	56.0±2.5ab	76.6±22.9ab	34.6±2.1c	88.6ab
Nip	0.0±0.0b	81.3±8.2a	0.0±0.0b	226.6±34.4ab	0.0c

The data are means ± Standard deviation, n = 3. Values in the same column with the same letter not different according to the Tukey test at ($p \leq 0.05$). Ip = Inoculated plants; Nip = Non-inoculated plants; Lp = *Laccaria proxima*; Sp = *Suillus pungens*; Substrates: S1 = sand, pine bark, and forest soil (2:2:1); S2 = sawdust, bark, and compost (50:30:20); S3 = sawdust, bark, and compost (55:35:10).

The morphoanatomical characteristics of the ectomycorrhizas of Lp and Sp in *P. teocote* were as follows: a) Lp: The hyphal branching was simple or dichotomous, with lengths ranging from 0.9 to 2.5 mm. The shape of the non-branching apex was sinuous. In the juvenile stage, the surface was light brown with a brown apex (Figure 3a), and in the adult stage, it turned brown with yellow to golden brown apices and a cylindrical end (Figure 3c). Occasionally, transparent to white hyphae were observed at the apex. The external mantle had a smooth, slightly shiny, and gelatinous surface. No rhizomorphs or sclerotia were observed. The internal and external mantle organization was plectenchymatous, with the Hartig net extending inside the root, penetrating two to three layers of cortical cells (Figure 3b). Additionally, a senescent primordium of Lp was found at the base of the tubes 570 days after sowing, evidencing the establishment of the ectomycorrhizal symbiosis with the inoculated ECM species (Figure 3c); and b) Sp: The hyphal branching was dichotomous and coraloid, with lengths ranging from 2 to 4.5 mm (Figure 3d). The shape of the non-branching apex was straight in the juvenile stage, with a light ochre surface, and yellow ochre when mature, with a cylindrical end. In both stages, the fungal mantle was clearly visible, with a cottony white surface, often covered by substrate mineral particles. A plentiful presence of rhizomorphs with emanating white hyphae was observed, with long-distance exploration (Figure 3e). The presence of oxalate crystals was observed, and no sclerotia were found. The internal and external mantle organization was plectenchymatous, with the emanating hyphae intertwined, irregularly shaped, with slightly thickened walls and simple septa. The Hartig net extended inside the root, penetrating two to three layers of cortical cells (Figure 3f).

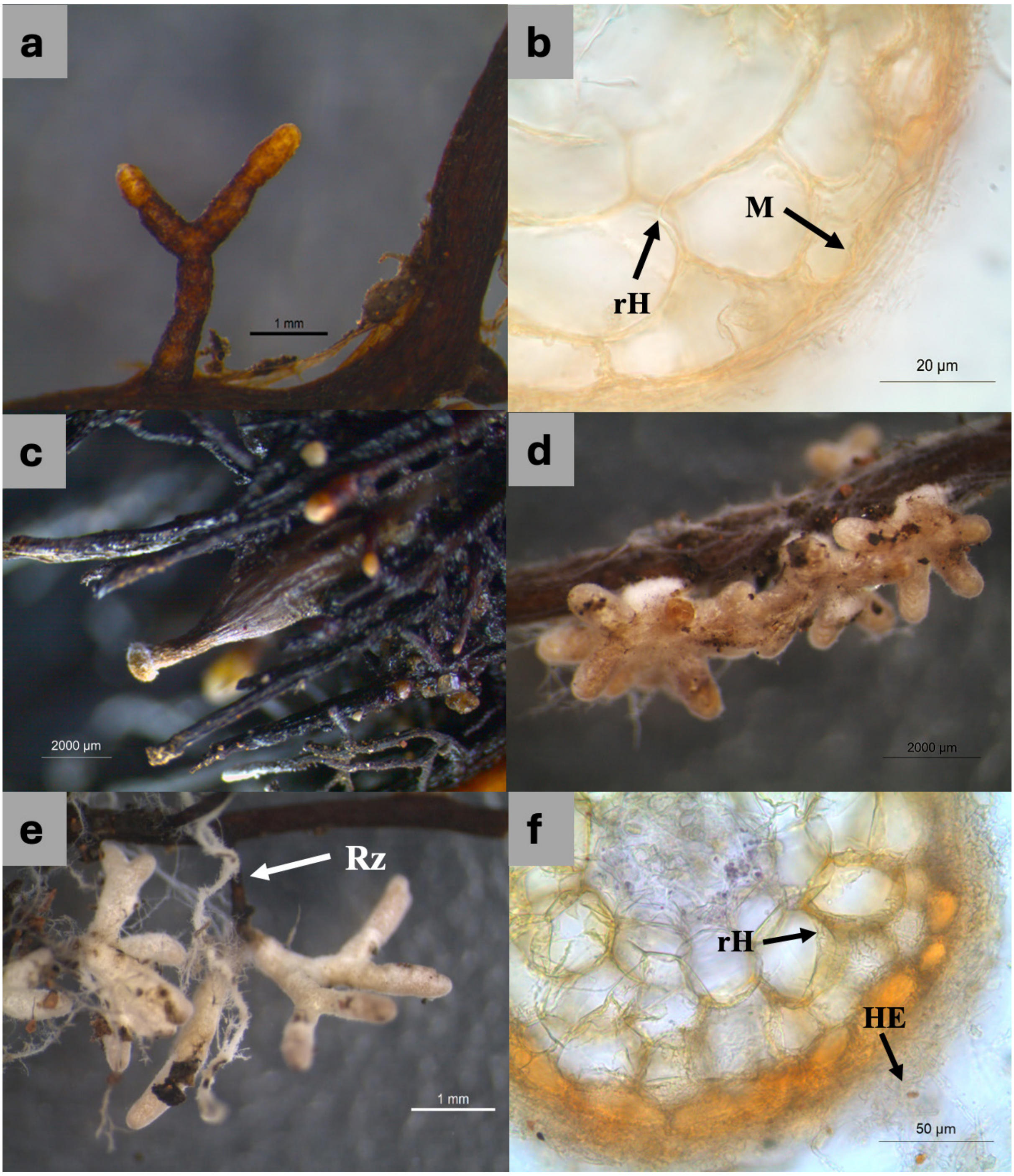

Figure 3. Ectomycorrhizae between *Pinus teocote* and *Laccaria proxima* (Lp); and *Suillus pungens* (Sp). a) Dichotomous ectomycorrhiza of Lp; b) Cross-section view of the Lp ectomycorrhiza showing the Hartig net (rH) and fungal mantle (M); c) Senescent primordium of L. proxima growing between the roots of P. teocote; d) Coraloid ectomycorrhiza of P. teocote with Sp; e) Abundant rhizomorphs (Rz) associated with the ectomycorrhiza of Sp; f) Cross-section view of the Sp ectomycorrhiza showing the Hartig net (rH) and emanating hyphae (HE).

4. DISCUSSION

This is the first study to evaluate the effect of ectomycorrhizal inoculation of Lp and Sp on morphophysiological traits of *P. teocote* grown in different substrates, including environmentally friendly combinations without chemical fertilization. Due to the low survival rates in pine forest plantations, the production of high-quality seedlings becomes increasingly important (Vicente-Arbona et al., 2019). Traditionally, the standards for seedling production in nurseries have been based solely on the morphological and physiological characteristics of the plants (Sánchez et al., 2014; Vicente-Arbona et al., 2019), without considering the importance of ectomycorrhizal inoculation. However, one of the most important criteria for the adaptation of forest species in the field is the appropriate selection of mycobionts (Pérez-Moreno & Martínez-Reyes, 2014) for ectomycorrhiza formation. The promotion of plant growth and biomass production in conifers has been reported as a consequence of ectomycorrhizal inoculation. Among the ectomycorrhizal fungi still used as inoculum sources, species from the *Laccaria* and *Suillus* genera have been previously included (Pera & Parlade, 2005; Erlandson et al., 2022; Salazar-Alemán et al., 2023).

In the present study, an increase in biomass was recorded, regardless of the ectomycorrhizal inoculum source. Plants grown in substrate S1 (sand-bark and forest soil) showed higher dry biomass and plant growth compared to those grown in different ratios of sawdust-bark-compost (S2 and S3). Previous studies have found the efficiency of substrate S1, with favorable results in the growth of various forest species inoculated with a wide range of ectomycorrhizal fungi. For example, Carrasco-Hernández et al. (2011) obtained greater dry weight of the aerial, root, and total parts of *Pinus patula* Schl. et Cham and *P. pseudostrobus* plants when inoculated with *Laccaria bicolor* (Maire) P.D. Orton., *L laccata* (Scop.) Cooke, and Lp. Similarly, López-Gutiérrez et al. (2018) used the same substrate in *Pinus pringleii* Shaw plants inoculated with Lp and recorded greater height, stem diameter, and dry weight compared to non-inoculated plants. However, two of the substrates used in these studies, sand and forest soil, are non-renewable forest resources since they take thousands of years to naturally regenerate. In contrast to the results found in this study, Rincón et al. (2005) stated that the substrate does not influence the development of *Pinus pinea* L. plants inoculated with *Pisolithus tinctorius* (Pers.) Coker & Couch, *Rhizopogon luteolus* Fr., *R. roseolus* (Corda) Th. M. Fr., and *Scleroderma verrucosum* (Bull.) Pers.; rather, the effect on plant growth is mainly attributed to the mycobiont.

The highest transpiration was recorded in plants inoculated with Sp in S1, being 8.0 times higher compared to non-inoculated plants. This beneficial effect could be linked to the presence of rhizomorphs in the roots of Sp, which are structures with high water mobilization capacity. Therefore, these structures are considered an extension of the plant's root system to tolerate water-stress environments (Pérez-Moreno & Read, 2004). Other studies have demonstrated that colonization by ectomycorrhizal fungi enhances photosynthetic rate and water-use efficiency. Xu et al. (2015), when inoculating *L. bicolor* in *Picea glauca* (Moench) Voss, showed that fungal hyphae promote higher transpiration and an increase in photosynthetic rate. Li et al. (2021) mentioned that under drought stress, *Pinus massoniana* Lamb. plants inoculated with *Suillus placidus* (Bonord.) Singer showed greater growth and photosynthetic performance compared to non-inoculated plants.

Increases in the concentrations of chlorophylls a, b, total chlorophyll, and carotenoids in plants inoculated with some species of ectomycorrhizal fungi have been reported. In this study, plants inoculated with Sp in S2 showed significant differences in chlorophyll and carotenoid content. Likewise, plants grown in S1 and S3, inoculated with Lp and Sp, respectively, showed significant differences in chlorophyll a content compared to non-inoculated plants. Some authors have mentioned that at the beginning of symbiosis, the pigment content in non-inoculated plants may be higher than in inoculated plants (Vodnik & Gogala, 1994; Kraj & Grad, 2013). For instance, *Picea abies* (L.) Karsten plants inoculated with *Laccaria laccata*, *P. tinctorius*, and *Lactarius piperatus* (L.) Pers. showed a notably lower pigment content compared to non-inoculated plants in spring. However, this effect changed during autumn (Vodnik & Gogala, 1994). Among the possible causes attributed to this effect, hormonal modifications during plant growth have been considered, as mycorrhizal plants contain more cytokinins, and the increasing concentrations of these hormones may influence chlorophyll synthesis (Kuraishi et al., 1992). Several studies have been conducted on the influence of inoculation with various species of *Suillus* and *Laccaria* in *Pinus* species, and in all cases, a significant increase in chlorophyll a, b, and carotenoid concentrations was observed compared to the control group (Yin et al., 2016; Chu et al., 2019; Barragán-Soriano et al., 2022).

In different regions of central and southern Mexico, Lp and Sp are of great importance as edible species. Particularly, Lp is associated with a wide range of hosts such as *Abies*, *Eucalyptus*, *Larix*, *Fagaceae*, *Picea*, *Pinus*, *Quercus*, and *Tsuga* (Pérez-Moreno et al., 2008). Meanwhile, Sp is associated with species of the *Pinus*, *Larix*, and *Tsuga* genera. It has been demonstrated that inoculation with species from these ectomycorrhizal genera increases growth and nutritional content, particularly in *Pinus* species (Barroetaveña et al., 2012; Galindo-Flores et al., 2015; López-Gutiérrez et al., 2018). However, these effects had not been reported for *P. teocote* until now. Regarding ectomycorrhizal colonization, it ranged from 84.7% to 98.8% in inoculated plants and across different substrates, with the highest value observed in plants inoculated with Sp in S2. The percentages obtained in this study are considered high, as they exceed the 25% threshold, which is the minimum acceptable for determining the vigor and quality of ectomycorrhizal plants (Wang & Hall, 2004; Smith & Read, 2008). These results are consistent with several inoculation studies with the mycobionts and phytobionts described here, obtaining mycorrhization percentages ranging from 90% to 98.5% with *Laccaria* species (Zong et al., 2015; López-Gutiérrez et al., 2018; Rodríguez-Gutiérrez et al., 2019). However, for the *Suillus* genus, the colonization percentages reported in other studies have been lower than those observed in this study, ranging from 35% to 71% at most (Barroetaveña et al., 2012; Castrillón et al., 2015; Yin et al., 2016). Regarding the relationship between the increase in mycorrhization percentage and physiological variables such as photosynthesis, authors like Choi et al. (2005) suggest that inoculated seedlings have higher nutrient and water absorption, which leads to better nutritional status and results in a more vigorous physiological response, particularly in photosynthetic activity.

The diagnostic characters of ectomycorrhizae (Table 5) align with those previously described for both mycobiont genera by various authors (Carrasco-Hernández et al., 2010; Barroetaveña et al., 2012; Garibay-Orijel et al., 2013; López-Gutiérrez et al., 2018; Rodríguez-Gutiérrez et al., 2019). To date, few studies have reported the production of sporocarps or fruiting bodies of Lp in nurseries or greenhouses as a result of mycorrhizal inoculation. However, this study found sporocarps at the bottom of the tubes in plants inoculated with this mycobiont. Previous reports in conifers have documented sporocarp formation in greenhouses, such as *Hebeloma leucosarx* P.D. Orton with *Pinus montezumae* Lamb. (Villegas-Olivera et al., 2017), *Hebeloma mesophaeum* (Pers.) Quél. with *Pinus greggii* Engelm. ex Parl. (Martínez-Reyes et al., 2012), and *L. laccata* with *Pinus sylvestris* L. and *Pinus resinosa* Aiton (Massicotte et al., 2005).

The results of this study demonstrate that substrates composed solely of compost and sawdust can not only effectively support the growth of *Pinus* seedlings but are also exceptionally conducive to achieving high rates of ectomycorrhizal colonization, consistently exceeding 80%. This finding is of significant relevance for sustainable forest nursery practices, as it provides a robust, environmentally friendly alternative to conventional fertilized substrates. Previous research has established that substrates based on pine sawdust and bark can produce quality plants of *P. pseudostrobus* and *P. montezumae* (Aguilera-Rodríguez et al., 2016a, b), and that *P. greggii* can be cultivated in fertilized sawdust-based substrates (Vicente-Arbona et al., 2019). It is well-documented that chemical fertilization often inhibits the mycorrhization process (Smaill & Walbert, 2013). However, the present study is, to the best of our knowledge, the first successful report of controlled ectomycorrhization with native fungi in a greenhouse setting using unfertilized sawdust and compost substrates, and its subsequent positive impact on both the morphological and physiological traits of the plants. This contrasts sharply with most other studies reporting positive effects from inoculation with *Laccaria* or *Suillus* genera, which typically rely on mixtures of commercial substrates like peat moss, vermiculite, or agrolite, supplemented with chemical fertilization (Chu et al., 2019; Rodríguez-Gutiérrez et al., 2019). The avoidance of such fertilizers in our protocol is a critical factor, as it prevents the nutrient saturation that suppresses host-fungus signaling and symbiotic establishment, thereby creating the low-nutrient conditions that select for and promote a robust mycorrhizal partnership.

The consistently high colonization rates (> 80%) achieved are interpreted as a result of the synergistic interplay between: i) the aggressivity of the compatible ectomycorrhizal fungal symbionts, ii) an extended developmental period of 570 days that facilitated extensive colonization, and iii) the optimized methodological conditions employed in this study. The compost and sawdust substrate provide a physical structure and a complex organic nutrient base that favors fungal development over rapid, fertilizer-driven plant biomass accumulation. This partnership, once established, becomes a powerful biological substitute for chemical inputs. The significant enhancement in photosynthetic rate, observed between 510 and 570 days after sowing and representing increases of 4.4 and 4.5 times in plants inoculated with Lp and Sp, respectively, can be attributed to the establishment of an effective symbiotic relationship. These findings are consistent with those reported by Barragán-Soriano et al. (2018), who documented a 4.0-fold increase in photosynthetic rate in *P. montezumae* seedlings co-inoculated with *Hebeloma mesophaeum* and *Azospirillum brasilense* Tarrand, Krieg y Döbereiner. The comparative magnitude of the physiological response observed in the present study corroborates the beneficial impact of ectomycorrhizal symbiosis on host plant photosynthesis. This physiological enhancement is likely mediated by the ectomycorrhizal fungi's extensive extraradical mycelium, which dramatically expands the functional root area. This network potentially may act as a highly efficient auxiliary absorption system, not only for water (Smith & Read, 2008), mitigating plant drought stress and facilitating higher stomatal conductance, but also for mobilizing essential nutrients like nitrogen and phosphorus from organic matter in the compost, directly fueling photosynthetic processes. The importance of this friendly-environmental technology in forestry cannot be overstated. Firstly, it valorizes local organic waste streams (sawdust, bark and compost), promoting a circular economy and reducing the nursery's dependency on external, expensive, and carbon-intensive inputs like peat (whose harvest raises ecological concerns) and synthetic fertilizers. Secondly, it eliminates the environmental risks associated with fertilizer runoff, such as soil and water eutrophication. Most importantly, it produces seedlings that are not merely larger but are ecologically primed for success in reforestation sites. A seedling entering the field with a pre-established, high-rate mycorrhizal symbiont is already adapted to nutrient-limited conditions and possesses a sophisticated network for resource acquisition. This translates to superior resilience to transplantation shock, water stress, and soil pathogens, ultimately leading to higher survival rates and more successful, cost-effective forest restoration programs. Therefore, the use of unfertilized compost and sawdust substrates is a cornerstone of a sustainable forestry paradigm, leveraging biological synergy to produce hardy, ectomycorrhized trees while minimizing the environmental footprint of nursery operations.

5. CONCLUSIONS

To the best of our knowledge, this study is the first to demonstrate the feasibility of producing *Pinus* plants with high ectomycorrhizal colonization, exceeding 84%, and good morphological and physiological quality, using environmentally friendly substrate mixtures. Additionally, for the first time, ectomycorrhizal colonization of Lp and Sp with *P. teocote*, a neotropical forest species highly tolerant to environmental stress and, therefore, of significant importance for reforestation efforts in the current context of climate change, is reported. In general terms, the inoculation of Lp and Sp improved plant growth, particularly when plants were grown in substrate S1. Moreover, Sp demonstrated greater efficiency with respect to transpiration in S1 and chlorophyll a, b, total chlorophyll content, and carotenoids in S2. These results highlight the considerable potential of producing ectomycorrhizal *P. teocote* plants grown with organic material combinations and inoculated with ectomycorrhizal mushrooms, Lp and Sp. These edible mushrooms are of great sociocultural and economic importance in Mexico and hold substantial potential for reforestation or afforestation projects within the current context of climate change.

Supplementary information ↑

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Supplementary material

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Data availability

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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Authorship contribution statement

Magdalena Martínez-Reyes: Conceptualization, Data curation, Formal analysis, Supervision, Writing – original draft, Writing – review & editing.

Karla Iveth Luciano-Dorado: Conceptualization, Data curation, Formal analysis, Validation, Writing – original draft.

José Luis Barragán-Soriano: Conceptualization, Validation, Writing – review & editing.

Irma Díaz-Aguilar: Methodology, Validation, Writing – review & editing.

Julián Delgadillo-Martínez: Methodology, Validation, Writing – review & editing.

Samuel Araujo-Castillo: Methodology, Validation, Writing – review & editing.

Jesús Pérez-Moreno: Conceptualization, Methodology, Supervision, Validation, Writing – original draft, Writing – review & editing.

Competing interests

The authors have declared that no competing interests exist.

Statement on the use of Artificial Intelligence

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REFERENCES

Agerer R, Rambold G, 2009. DEEMY. An information system for characterization and determination of ectomycorrhizae. München, Germany.

Aguilera-Rodríguez M, Aldrete A, Martínez-Trinidad T, Ordaz-Chaparro VM, 2016a. Producción de *Pinus pseudostrobus* Lindl. con sustratos de serrín y fertilizantes de liberación controlada. Rev Mex Cien For 7, 7-20.

Aguilera-Rodríguez M, Aldrete A, Martínez-Trinidad T, Ordaz-Chaparro VM, 2016b. Producción de *Pinus montezumae* Lamb. con diferentes sustratos y fertilizantes de liberación controlada. Agrociencia 50, 107-118.

Atzori G, Pane C, Zaccardelli M, Cacini S, Massa D, 2021. The Role of Peat-Free Organic Substrates in the Sustainable Management of Soilless Cultivations. Agronomy 11, 1236. https://doi.org/10.3390/agronomy11061236

Barragán-Soriano JL, Perez-Moreno J, Almaraz-Suárez JJ, Carcaño-Montiel MC, Delgadillo-Martínez J, Cetina-Alcalá M, Mata G, 2022. Coinoculación de *Pinus montezumae* (Pinaceae) con un hongo comestible ectomicorrízico y bacterias promotoras de crecimiento vegetal. Acta Bot Mex 129, 1-17. https://doi.org/10.21829/abm129.2022.2024

Barragán-Soriano JL, Pérez-Moreno J, Almaraz-Suárez JJ, Carcaño-Montiel MG, Medrano-Ortiz KI, 2018. Inoculation with an edible ectomycorrhizal fungus and bacteria increases growth and improves the physiological quality of *Pinus montezumae* Lamb. Rev Chapingo Ser Hortic 24. https://doi.org/10.5154/r.rchscfa.2017.01.010

Barroetaveña C, Bassani VN, Rajchenberg M, 2012. Inoculación micorrízica de *Pinus ponderosa* en la Patagonia Argentina: colonización de las raíces, descripción de morfotipos y crecimiento de las plántulas en vivero. Bosque 33, 163-169. http://dx.doi.org/10.4067/S0717-92002012000200006

Calisti R, Regni L, Pezzolla D, Cucina M, Gigliotti G, Proietti P, 2023. Evaluating Compost from Digestate as a Peat Substitute in Nursery for Olive and Hazelnut Trees. Sustainability*,* 15, 282. https://doi.org/10.3390/su15010282

Carrasco-Hernández V, Pérez-Moreno J, Espinosa-Hernández V, Almaraz-Suárez JJ, Quintero-Lizaola R, Torres-Aquino M, 2010. Caracterización de micorrizas establecidas entre dos hongos comestibles silvestres y pinos nativos de México. Rev Mex Cienc Agríc 567-577.

Carrasco-Hernández V, Pérez-Moreno J, Espinosa-Hernández V, Almaraz-Suárez JJ, Quintero-Lizaola R, Torres-Aquino M, 2011. Contenido de nutrientes e inoculación con hongos ectomicorrízicos comestibles en dos pinos neotropicales. Rev Chil Hist Nat 84, 83-96. http://dx.doi.org/10.4067/S0716-078X2011000100006

Carrillo-Saucedo SM, Puente-Rivera J, Montes-Recinas S, Cruz-Ortega R, 2022. Las micorrizas como una herramienta para la restauración ecológica. Acta Bot Mex 129. https://doi.org/10.21829/abm129.2022.1932

Castrillón M, de León J, Carvajal D, Osorio NW, 2015. Effectiveness of single and combined ectomycorrhizal inocula on three species of *Pinus* at Nursery. Commun Soil Sci Plant Anal 46, 169-179. https://doi.org/10.1080/00103624.2014.967856

Cherlet M, Hutchinson C, Reynolds J, Hill J, Sommer S, von Maltitz G, 2018. (Eds.) World Atlas of Desertification, Publication Office of the European Union, Luxembourg.

Choi DS, Quoreshi AM, Maruyama Y, Jin HO, Koike T, 2005. Effect of ectomycorrhizal infection on growth and photosynthetic characteristics of *Pinus densiflora* seedlings grown under elevated CO2 concentrations. Photosynthetica 43, 223-229.

Chu H, Wang C, Li Z, Wang H, Xiao Y, Chen J, Tang M, 2019. The dark septate endophytes and ectomycorrhizal fungi effect on *Pinus tabulaeformis* carr. seedling growth and their potential effects to pine wilt disease resistance. Forests 10, 140. https://doi.org/10.3390/f10020140

Clemmensen KE, Bahr A, Ovaskainen O, Dahlberg A, Ekblad A, Wallander H, Stenlid J, Finaly RD, Wardle DA, Lindahl BD, 2013. Roots and associated fungi drive long-term carbon sequestration in boreal forest. Science 339 (6127): 1615-1618. https://doi.org/10.1126/science.1231923

CONAFOR, Comisión Nacional Forestal, 2023. *Pinus teocote* Schiede ex Schltdl. Secretaría del Medio Ambiente y Recursos Naturales. Mexico City: Comisión Nacional Forestal. http://www.conafor.gob.mx:8080/documentos/docs/13/983Pinus%20teocote.pdf [01 December 2023].

DEEMY, 2020. An information system for characterization and determination of ectomycorrhizae. http://www.deemy.de/ [19 December 2020].

Dick LA, Gardner TG, Frene JP, Heitman JL, Sucre EB, Leggett ZH, 2022. Forest floor manipulation effects on the relationship between aggregate stability and ectomycorrhizal fungi. For Ecol Manage 505: 119873. https://doi.org/10.1016/j.foreco.2021.119873

Erlandson SR, Margis R, Ramirez A, Nguyen N, Lofgren LA, Liao HL, Vilgalys R, Kennedy PG, Peay KG, 2022. Transcriptional acclimation and spatial differentiation characterize drought response by the ectomycorrhizal fungus *Suillus pungens*. New Phytol, 234: 1910-1913. https://doi.org/10.1111/nph.17816

Escobar-Alonso S, Rodríguez-Trejo DA, 2021. *Pinus teocote* Schiede ex Schlechtendal & Chamisso (Pinaceae). In: Semillas de especies forestales; Rodríguez-Trejo DA (ed.). pp: 271-274.

FAO. Food and Agriculture Organization of the Unites Nations, 2025. International Day of Forest 21 March.

Fregoso-Madueño JN, Goche-Télles JR, Rutiaga-Quiñones JG, González- Laredo RF, Bocanegra-Salazar M, Chávez-Simental JA, 2017. Alternative uses of sawmill industry waste. Rev Chapingo Ser Cienc For Ambient 23, 243-260. https://doi.org/10.5154/r.rchscfa.2016.06.040

Galindo-Flores G, Castillo-Guevara C, Campos-López A, Lara C, 2015. Caracterización de las ectomicorrizas formadas por *Laccaria trichodermophora* y *Suillus tomentosus* en *Pinus montezumae*. Bot Sci 93, 855-863. https://doi.org/10.17129/botsci.200

Gang Z, Li S, Meng Q, Niu Ch, Zhang X, Wa Q, 2023. A new type of highly efficient fir sawdust-based super adsorbent: Remove cationic dyes from wastewater. Surf Interfaces 36. https://doi.org/10.1016/j.surfin.2023.102637

Garibay-Orijel R, Morales-Marañon E, Domínguez-Gutiérrez M, Flores-García A, 2013. Caracterización morfológica y genética de las ectomicorrizas formadas entre *Pinus montezumae* y los hongos presentes en los bancos de esporas en la Faja Volcánica Transmexicana. Rev Mex Biodivers 84, 153-169. https://doi.org/10.7550/rmb.29839

Kothe E, Bogdanova O, Abdulsalam OA, Ezediokpu MN, Krause K, 2025. Ectomycorrhizae for the rescue: ectomycorrhizosphere signaling in agroforestry and afforestation. In: The Mycota 16, Agricultural and Industrial Applications; Grüttner S, Kollath-Leiß K, Kempken F (eds.). Springer, Cham. https://doi.org/10.1007/978-3-031-81904-9_5

Kraj W, Grad B, 2013. Seasonal dynamics of photosynthetic pigment, protein and carbohydrate contents in *Pinus sylvestris* L. seedlings inoculated with *Hebeloma crustuliniforme* and *Laccaria bicolor*. J Plant Nutr 36, 633-650. https://doi.org/10.1080/01904167.2012.754035

Kuraishi S, Sakurai N, Tazaki K, Sadatoku K, 1992. A posible relationship between chlorophyll synthesis and endogenous cytokinin levels in whole seedlings and excised cotyledons of squash. In: Physiology and biochemistry of cytokinins in plants; Kaminek M, Mok DWS, Zažímalová E (ed.). pp: 283-288. SPB Academic Publishing, The Hague.

Li M, Wang H, Zhao X, Lu Z, Sun X, Ding G, 2021. Role of *Suillus placidus* in improving the drought tolerance of Masson pine (*Pinus massoniana* Lamb.) seedlings. Forests 12, 332. https://doi.org/10.3390/f12030332

Lichtenthaler HK, 1987. Chlorophyll and carotenoids: pigments of photosynthetic biomembranes. Methods Enzymol 148, 350-382. https://doi.org/10.1016/0076-6879(87)48036-1

López-Gutiérrez A, Pérez-Moreno J, Hernández-Santiago F, Uscanga-Mortera E, García-Esteva A, Cetina-Alcalá VM, Xoconostle-Cázares B, 2018. Nutrient mobilization, growth and field survival of *Pinus pringlei* inoculated with three ectomycorrhizal. Bot sci 96 (2) https://doi.org/10.17129/botsci.1239

Martínez-Nevárez LE, Prieto-Ruíz JÁ, Sigala-Rodríguez JÁ, García-Rodríguez JL, Martínez-Reyes M, Carrillo-Parra A, Dominguez-Calleros PA, 2023. Growth and Efficiency in the Use of Nutrients of *Pinus cooperi* C. E. Blanco Seedlings Produced in Nurseries with a Controlled Release Fertilizer. Terra Latinoam 41, 1-12. https://doi.org/10.28940/terra.v41i0.1707

Martínez-Reyes M, Pérez-Moreno J, Villarreal-Ruiz L, Ferrera-Cerrato R, Xoconostle-Cázares B, Vargas-Hernández JJ, Honrubia-García M, 2012. Crecimiento y contenido nutrimental de *Pinus greggii* Engelm. inoculado con el hongo comestible ectomicorrízico *Hebeloma mesophaeum* (Pers.) Quél. Rev Chapingo Ser Cienc For Ambient 18, 183-192. https://doi.org/10.5154/r.rchscfa.2010.11.112

Massicotte HB, Melville LH, Peterson RL, 2005. Building a basidiocarp: a case study of *Laccaria* spp. fruitbodies in the extraradical mycelium of *Pinus* ectomycorrhizas. Mycologist 19, 141-149. https://doi.org/10.1017/S0269-915X(05)00402-7

Moctezuma LG, Flores A, 2020. Importancia económica del Pino (*Pinus* spp.) como recurso natural en México. Rev Mex Cienc Forestales 11(60). https://doi.org/10.29298/rmcf.v11i60.720

Pera J, Parladé J, 2005. Inoculación controlada con hongos ectomicorrícicos en la producción de planta destinada a repoblaciones forestales: estado actual en España. Investig Agrar Sist Recur For 14, 419-433.

Pérez-Moreno J, Read JD, 2004. Los hongos ectomicorrízicos, lazos vivientes que conectan y nutren a los árboles en la naturaleza. Interciencia 29, 239-247.

Pérez-Moreno J, Martínez-Reyes M, Yesca-Pérez A, Delgado-Alvarado A, Xoconostle-Cázares B, 2008. Wild mushroom markets in central México and a case study at Ozumba. Econ Bot 3, 425-436. https://doi.org/10.1007/s12231-008-9043-6

Pérez-Moreno J, Martínez-Reyes M, 2014. Edible ectomycorrhizal mushrooms: biofactories for sustainable development. In: Biosystems engineering: Biofactories for food production in the century XXI. Guevara-Gonzalez R, Torres-Pacheco I (ed.). pp: 151-233. Springer International Publishing. https://doi.org/10.1007/978-3-319-03880-3_6

Pérez-Moreno J, Martínez-Reyes M, Hernández-Santiago F, Ortiz-Lopez I, 2020. Climate Change, Biotechnology, and Mexican Neotropical Edible Ectomycorrhizal Mushrooms In: Mushrooms, Humans and Nature in a Changing World Perspectives from Ecological, Agricultural and Social Sciences; Pérez-Moreno J, Guerin-Laguette A, Flores-Arzú R, Fu-Qiang Y (eds). pp: 61-69. Springer NatureSwitzerland. Suiza. https://doi.org/10.1007/978-3-030-37378-8_3

Pérez-Moreno J, Guerin-Laguette A, Rinaldi AC, Yu F, Verbeken A, Hernández-Santiago F, Martínez-Reyes M, 2021. Edible mycorrhizal fungi of the world: What is their role in forest sustainability, food security, biocultural conservation and climate change? Plants People Planet 3, 471–490. https://doi.org/10.1002/ppp3.10199

Pickles BJ, Simard SW, 2017. Mycorrhizal networks and forest resilience to drought. In: Mycorrhizal mediation of soil: Fertility, structure, and carbon storage; Johnson NC, Gehring C, Jansa J. (ed.). pp: 319-339. Elsevier, Amsterdam.

Prieto RJÁ, Goche TJR, 2016. Las reforestaciones en México: Problemática y alternativas de solución. Editorial de la Universidad Juárez del Estado de Durango. Dgo., México. 79 pp.

Rincon A, Parlade J, Perea, J, 2005. Effects of ectomycorrhizal inoculation and the type of substrate on mycorrhization, growth and nutrition of containerised *Pinus pinea* L. seedlings produced in a commercial nursery. Ann For Sci 62, 817-822.

Rodríguez-Gutiérrez I, Ramírez-Martínez D, Garibay-Orijel R, Jacob-Cervantes V, Pérez-Moreno J, Ortega-Larrocea MDP, Arellano-Torres E, 2019. Sympatric species develop more efficient ectomycorrhizae in the *Pinus-Laccaria* symbiosis. Rev Mex Biodivers 90, 1-11. https://doi.org/10.22201/ib.20078706e.2019.90.2868

Salazar-Alemán T, García-Rivas JL, Garcia GB, Martínez-Reyes M, Pérez-Moreno J, Cortés-Sarabia J, 2023. Lyophilized biopolymeric beads of chitosan-xanthan with edible fungus *Laccaria laccata* (Scop.) Cooke as forest ectomycorrhizal biofertilizers. Agro Productividad 16, 79-85. https://doi.org/10.32854/agrop.v15i4.2411

Sánchez MJJ, Capulín-Grande J, Santana AMR, Islas SA, Moreno MB, 2014. Crecimiento de *Acacia retinodes* Schltdl. en sustratos a base de serrín de pino y envases tratados con cobre. Rev Cub Cienc For 2, 1-19.

SEMARNAT, 2025. Secretaría de Medio Ambiente y Recursos Naturales. Residuos sólidos urbanos: la otra cara de la basura. www.gob.mx 01/05/2025

SEMARNAT, 2021. Anuario Estadístico de la Producción Forestal. Secretaría del Medio Ambiente y Recursos Naturales. Mexico City.

Smaill SJ, Walbert K, 2013. Fertilizer and fungicide use increases the abundance of less beneficial ectomycorrhizal species in a seedling nursery. Appl Soil Ecol 65, 60-64. https://doi.org/10.1016/j.apsoil.2013.01.007

Smith SE. Read DJ, 2008. Mycorrhizal symbiosis. Third edition. Academic Press, UK. https://doi.org/10.1016/B978-0-12-370526-6.X5001-6

UNEP, 2024. Global Waste Management Outlook 2024. https://www.unep.org [01 May 2025] https://doi.org/10.59117/20.500.11822/44939

Vicente-Arbona JC, Carrasco-Hernández V, Rodríguez-Trejo DA, Villanueva-Morales A, 2019. Calidad de planta de *Pinus greggii* producida en sustratos a base de serrín. Madera Bosques 2, 1-14. https://doi.org/myb.2019.2521784

Villegas-Olivera JA, Pérez-Moreno J, Mata G, Almaraz-Suárez JJ, Ojeda-Trejo E, Espinosa-Hernández V, 2017. Type of light and formation of basidiomata of two species of edible ectomycorrhizal mushrooms associated with neo-tropical pines and the description of basidiomata development. Rev Fitotec Mex 40, 405-413. https://doi.org/10.35196/rfm.2017.4.405-413

Vodnik D, Gogala N, 1994. Seasonal fluctuations of photosynthesis and its pigments in 1-year mycorrhized spruce seedlings. Mycorrhiza 4, 277-281. https://doi.org/10.1007/BF00206777

Wang Y, Hall IR, 2004. Edible ectomycorrhizal mushrooms: challenges and achievements. Canad J Bot 82, 1063-1073. https://doi.org/10.1139/b04-051

Xu H, Kemppainen M, El Kayal W, Lee SH, Pardo AG, Cooke JE, Zwiazek JJ, 2015. Overexpression of *Laccaria bicolor* aquaporin JQ585595 alters root water transport properties in ectomycorrhizal white spruce (*Picea glauca*) seedlings. New Phytol 205, 757-770. https://doi.org/10.1111/nph.13098

Yin D, Deng X, Song R, 2016. Synergistic effects between *Suilllus luteus* and *Trichoderma virens* on growth of Korean spruce seedlings and drought resistance of scotch pine seedlings. J For Res 27, 193-201. https://doi.org/10.1007/s11676-015-0131-z

Yu SJ, Shen R, Lin DM, 2025. Research advances in the impacts of ectomycorrhizal fungi on the formation and decomposition of soil organic matter in forests. Ying Yong Sheng Tai Xue Bao 18; 36(3): 943-949. https://doi:10.13287/j.1001-9332.202503.034

Zhang ZX, 1986. Determination of chlorophyll content of plants - acetone and ethanol mixture method. Liaoning Agric Sci 3, 26-28

Zong K, Huang J, Nara K, Chen Y, Shen Z, Lian C, 2015. Inoculation of ectomycorrhizal fungi contributes to the survival of tree seedlings in a copper mine tailing. J For Res 20, 493-500. https://doi.org/10.1007/s10310-015-0506-1