Arbuscular mycorrhizal fungi affect early phenological stages of three secondary vegetation species in a temperate forest

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Abstract

The relationship between arbuscular mycorrhizal fungi (AMF) and secondary vegetation (SV) species at early phenological stages is critical for the successful establishment of these plants on disturbance sites in temperate forests. The main objective of this research is to evaluate the effect of AMF colonization on the early phenological stages (germination and early growth) of three shrub species present in the SV of a temperate forest in central Mexico. We collected soil from different sites in the *Abies religiosa* forest in central Mexico. We collected seeds of *Acaena elongata*, *Ageratina glabrata*, and *Solanum pubigerum*. We used a controlled experimental design with pasteurized soil (-AMF treatments) and unpasteurized soil (+ AMF treatments). We monitored germination percentage, growth (shoot and root weight and total biomass), AMF root colonization, and the mycorrhizal response index (MRI) for each plant species. All three species tested benefited by AMF, showing higher germination rates. Shoot and root weight and total biomass were significantly higher in the + AMF treatment. *Solanum pubigerum* showed greater stem length and *Ageratina glabrata* showed greater root development due to AMF. *Ageratina glabrata* and *Acaena elongata* were the most responsive to AMF as indicated by MRI. This research underscores the critical role of AMF in the early phenological stages of SV and highlights the potential ecological benefits of AMF in supporting plant germination and plant growth. This information suggests the integration of mycorrhizal inoculation into restoration practices to enhance ecological resilience in temperate forest ecosystems.

Introduction

Temperate forests are the second most widespread ecosystem in North America, Central America, Northeast Asia, Western Europe and Central Europe, accounting for 16% of the global forest area (665.8 million ha) (Gilliam 2016; FAO 2020). These forests face conservation challenges and have experienced significant degradation due to human activities, extreme climate events, fires, pests and diseases (Rawat et al. 2022; Lin et al. 2024). Consequently, there has been a notable increase in the expansion of secondary temperate forests worldwide (FAO 2020).

Disturbance alters the structure of plant communities, leading to the establishment of secondary vegetation (SV) species, which play a crucial role in increasing the resilience and functionality of temperate forests by mitigating the loss of ecological functions, interactions and processes (van Hall et al. 2017). Structurally, SV species contributes to the forest fragment connectivity (Sayer et al. 2004), and significantly influences soil formation and structure (Álvarez-Yépiz et al. 2008; van Hall et al. 2017). In addition, SV species actively participate in nutrient cycling and maintain critical biotic interactions through its root system (Post and Kwon 2000; Lesschen et al. 2008; van Hall et al. 2017).

A way to satisfy the low nutrient availability in temperate forest soils, plants are associated with soil microorganisms, such as arbuscular mycorrhizal fungi (AMF). These fungi are obligate symbionts forming arbuscular mycorrhizae with *ca*. 75% of plant families (Brundrett and Tedersoo 2018), and facilitate the efficient transfer of nutrients and water (Smith and Smith 2011). This symbiotic relationship enables host plants to withstand both biotic and abiotic stresses, while gaining advantages in germination (Kumar et al. 2023) and growth in their host plants (Smith and Smith 2012; Cavagnaro et al. 2015). On the other hand, SV species mitigate the loss of taxonomic and functional diversity of AMF by acting as temporary hosts for these fungi until mature forest species colonize the area (Guadarrama-Chávez et al. 2014).

Plant species of SV exhibit highly competitive ability, rapid germination and growth rates, and a tendency to rapidly colonize disturbed sites (Leck et al. 2008). These traits suggest a significant investment of resources by SV plants, including nutrients, water, and light. Thus, the association of SV species with AMF may serve as a buffer against the stressful environmental conditions they encounter at different life cycle stages in a changing environment due to anthropogenic disturbance.

The AMF are known to favor the germination and growth of their host plants (Sortibrán et al. 2018; Souza et al. 2023; Tian et al. 2023; Yang et al. 2024). The nutrient requirements of plants differ at early phenological stages due to the adaptive dynamics of their physiological and metabolic processes (Milla et al. 2009). This adaptation of nutrient requirements reflects the specific response of plants to changing demands during their germination and early growth phases.

The early phenological stages are critical for plant establishment as they are the periods during which seedlings overcome their vulnerability to abiotic and biotic stresses. This ensures the proper formation of tissues that will support critical processes such as photosynthesis and nutrient uptake (Ismail et al. 2012; Gioria et al. 2018). Recent studies have shown that AMF promote seed germination and seedling growth in the presence of abiotic stresses, such as drought and heavy metal toxicity (Van Der Heijden 2004; Soto-Cruz et al. 2022), and can even modulate plant-parasite interactions to the benefit of the host plant (Maighal et al. 2016).

The role of AMF in enhancing seed germination is of great importance (Kumar et al. 2023) and may have implications for the tolerance and establishment of plant species in disturbance sites. It has been proposed that AMF may contribute to the establishment of favorable microsites for seed germination through the formation of micro- and macroaggregates in the soil and the production of hyphal exudates that can scarify seeds (Koide 2000). However, the specific physical or chemical mechanisms underlying this enhanced germination remain largely unknown. It is also likely that the interacting plant and fungal species also regulate the effect of AMF on seed germination.

In contrast, during early growth, resource allocation shifts to biomass formation and accumulation, a critical process for tissue development and leaf growth (Lambers et al. 2019). This shift implies different requirements for essential nutrients such as nitrogen (N) and phosphorus (P) for optimal growth, both of which are critical for plant tissue formation. Ågren (2008) showed that nutrient concentrations in the tissues of *Lycopersicum esculentum*, *Betula pendula* and *Selenastrum minutum* vary with time. These variations are related to the plants relative growth rates and affect the stoichiometry of key elements such as carbon (C), N, and P (C:N:P). In particular, N concentrations increase more slowly than P concentrations. In this context, AMF can be an important option to meet the nutrient requirements of plants at each stage (Mosse and Hayman 1971; van der Heijden 2004). Thus, the shift in phenological phase and nutrient demand of the host plant also suggests a functional shift in the mycorrhizal association. During the early growth phase, the primary function of the AMF is nutrient and water transport, which is critical for both aboveground and belowground biomass plant production (Smith and Smith 2011; Chongtham and Pandey 2020; Zhou et al. 2022).

Evolutionarily, some plant species are classified as 'obligate or facultative mycotrophic', while others fall into the 'non-mycotrophic' category (Li et al. 2016; Wang et al. 2021). AMF colonization shows variability among plant species, with four possible responses: a) no colonization and no growth response, b) no colonization and reduced growth, c) colonization with growth stimulation, and d) colonization with increased P concentration without changes in biomass (Helgason et al. 2002). Responses c and d do not fully capture changes in colonization

throughout plant phenology. Investigating these variations may provide a more complete understanding of the dynamics of plant-AMF associations and their effects on different plant phenological phases.

The outcome of this association depends on factors such as the identity of the host plant and fungus, interactions with other beneficial organisms, and the level of abiotic stress (Baslam et al. 2011; Copetta et al. 2011; Smith et al. 2011; Baslam and Goicoechea 2012; Carillo et al. 2020). For example, low P and high nitrogen (N) availability in the soil have been observed to promote AM association, whereas in soils with high availability of both nutrients, this association may become parasitic or even inhibited (Johnson 2010). In addition, under limited light conditions, AMF may have an antagonistic effect on the plant due to their ability to extract carbon from the plant. Therefore, changes in the cost-benefit balance of the mycorrhizal association occur in a continuous mutualism-antagonism (Johnson 2010).

Current knowledge on changes in intraradical colonization during early phenological stages in temperate forest species is still scare. Researchers often focus on the effect of AMF on later stages of plant growth. In turn, our study aims to contribute to the understanding of this interaction, particularly in temperate forest SV plant species. The hypothesis was that if the nutritional requirements of host plants vary according to their phenological stage, and this will affect arbuscular mycorrhizal colonization, then we could observe different degrees of colonization that vary interspecifically during each phenological stage of the host plant. The main objective of this research is to evaluate the effect of AMF colonization on the early phenological stages (germination and early growth) of three shrub species present in the SV of a temperate forest in central Mexico. The study of arbuscular mycorrhizal association in early phenological stages of SV species will allow a better understanding of the initial establishment and long-term effects of this symbiosis on plant development.

Material and methods

Study area

This research was carried out in the temperate forest dominated by *Abies religiosa* (H.B.K.) Schl. & Cham. (Fir) in the Magdalena river basin (MRB), located in Mexico City (Fig. 1). The climate is temperate subhumid, characterized by summer rains, with an average annual temperature of 18°C, an average annual rainfall of 1 250 mm, and a minimal thermal oscillation, typically below 5°C (García 1988). The forest has a strong seasonality, with a rainy season ranging from May to October and a dry season from November to April (Delgadillo-Duran 2011). The predominant soil group is classified as humic Andosol, according to the WRB (2022). This soil is characterized by its acidity, abundant presence of allophane, high organic matter content and remarkable ability to adsorb phosphates on the surface of amorphous mineral clays. This phosphate fixation, documented by Galicia et al. (2016) and INEGI (2011), may limit P availability to plants.

Study species

In this study, the effect of arbuscular mycorrhizal association on germination and growth of three SV species was addressed. The selected plant species are common shrubs in the SV of the study forest (Santibañez-Andrade et al. 2015): *Acaena elongata* L., *Ageratina glabrata* (Kunth) R.M. King & H. Rob., and *Solanum pubigerum* Dunal. These plants thrive in the understory and contribute significantly to natural regeneration through sources such as seed rain and the seed bank (Argüelles-Mayao 2018). Variations in flower and fruit types, seed size, and reproductive phenology patterns are observed among the three plant species.

Soil collection and processing

From July 2019 to January 2020, 56 kg of soil was collected randomly in the *A. religiosa* forest of the MRB. The soil was collected at 20 cm of depth, previously removing the litter. It was homogenized and divided into two equal parts: the first part was used as inoculum for the "+AMF" treatments, assuming that sufficient AMF propagules were present, such as spores (70 spores / 50 g soil), fragments of colonized roots and mycelium. The second part was subjected to a pasteurization process; this part of the soil was used for the "-AMF" treatments, where the AMF propagules were eliminated. To pasteurize the soil, we used a pressure cooker heated to 55 kPa at approximately 90°C (194°F). We maintained these conditions for 45 minutes before allowing the cooker to cool. This method was repeated on the same soil sample for three consecutive days at a constant temperature.

Inoculum description

Vázquez-Santos (2019) characterized the species richness and spore abundance of arbuscular mycorrhizal fungi (AMF) in 80 soil samples. The study reported 70 spores per 50 g of soil. These samples were collected from established plots within the *A. religiosa* forest in study. Analysis revealed a diverse array of 29 morphospecies representing nine families and ten genera. Notably, the genus *Acaulospora* dominated the AMF community, accounting for 77.7% of the total spores recorded. This was closely followed by *Ambispora* with 9.07%, *Claroideoglomus* with 4.96%, *Funneliformis* with 3.8%, while other genera such as *Archaeospora*, *Diversispora*, *Glomus*, *Rhizophagus*, *Sclerocystis* and *Scutellospora* accounted for less than 2.5% each.

Experiment set up: germination

From 50 individuals we randomly collected ripe fruits directly. For each study species, we had a collection of eight hundred seeds. The seeds were homogeneously mixed and a subsample of 160 mature seeds was extracted. General characterization (length, width, and weight) was performed. Subsequently, 80 seeds per species were disinfected with 10% of an initial concentration of 3.5% sodium hypochlorite for 10 min and washed with distilled water. The seeds were germinated in pots containing 250 g of soil, with pasteurized soil (-AMF) and unpasteurized soil (+ AMF). We set up a greenhouse (4 × 6 m) in March 2021, where we installed two HOBO DATA Loggers (easy LogUSB-ONSET, Massachusetts, USA) to record the variation in environmental temperature and moisture during the experimental period.

The pots were watered daily at 10:00 a.m. to field capacity while seed germination was monitored by checking for signs of radicle emergence, and once the germination process had begun, radicle or root fragments were made, with a minimum of 5 individuals and a maximum of 8 individuals per extraction. The radicles and/or roots were processed according to Koske and Gemma´s method (1989), in order to obtain the mycorrhizal colonization percentage and the fungal structures characteristics of AM according to the method of McGonigle et al. (1990). Seedling collection was completed when cotyledon emergence was observed. Therefore, the remaining seedlings are destined for the next phase.

Experiment set up: growth

After the previous experiment, another 90 seeds of each species were set to germinate, in pots with a capacity of 250 g, in pasteurized soil (-AMF) and unpasteurized soil (+ AMF). The total number of pots was 360 (12 individuals × 3 species × 2 mycorrhization treatments × 5 crops). The pots were distributed in species × treatment blocks design.

Measurements started in August 2021. We worked with a minimum of 30 individuals/species/treatment, with a total of 180 seedlings (6 individuals × 3 species × 2 treatments × 5 harvests). Five harvests were made each month, with an extraction of six individuals per species and treatment, to determine plant height, root length, stem length, fresh root weight and fresh shoot weight. The seedlings were then dried in a Riossa ® oven at 72 ºC for 24 hours (until constant weight) and the dry weight of the root and aerial part was determined using an OHAUS ® analytical balance. The fresh weight and the dry weight of the root and stem were combined to give the total dry weight (TDW). These dry tissue samples were used for chemical analysis of the phosphorus and nitrogen content of the plant tissue for each harvest.

Arbuscular mycorrhizal fungi roots colonization

Simultaneously with the seed extraction for growth measurements, we carried out five root extractions from six individuals/species/treatment (6 individuals × 3 species × 2 treatments × 5 harvests), during the same months, to determine the AMF colonization percentage. We extracted the finest roots to process them according to Koske and Gemma (1989) and to obtain the mycorrhizal colonization percentage and per AM structures according to McGonigle et al. (1990).

The effect of mycorrhizal fungi on plants TDW was estimated using the mycorrhization response index (MRI). Treatments were compared where the only difference was the presence of mycorrhizae. This index ranges from –∞ to 100% (Plenchette et al. 1983), and expresses the difference in dry weight between mycorrhizal and non-mycorrhizal plants as a percentage of the dry mass of the mycorrhizal plant.

$$\mathrm{MRI} = \left(\frac{\mathrm{TDWm(g)} - \mathrm{TDWnm(g)}}{\mathrm{TDWm}}\right) * 100$$ [Equation 1]

Where: TDWm = total dry weight of the mycorrhizal plant and TDWnm = total dry weight of the non-mycorrhizal plant.

Data analysis

We used a simple random design with a 3 × 2 factorial arrangement (three species, two mycorrhization treatments), with five replicates. The accumulated germination percentages for each treatment were calculated using the following equation:

$$\mathrm{Germination}\,(\%) = \left(\frac{\mathrm{Germinatedseeds}}{\mathrm{Seedstotal}}\right)(100)$$

[Equation 2]

Two-way analyzes of variance (Two-way ANOVA) was used to determine the effects of species (*Acaena elongata, Ageratina glabrata* and *Solanum pubigerum*), mycorrhization (+ AMF and -AMF) and the interaction between these factors on germination, growth, phosphorus and nitrogen contents of the plants tissue and arbuscular mycorrhizal colonization.

To meet the assumptions of normality and homogeneity of variances, the cumulative germination percentages were transformed using Eq. 3 (Zar 1974). Similarly, the first day of germination, the maximum day of germination, the average germination time and seed vigor were transformed using the natural logarithm (ln).

$$\left(\text{arc}\sqrt{\frac{\text{Germination}\backslash\%}{100}} \right)$$

[Equation 3]

Growth parameters such as root and shoot length, root and shoot dry weight, total dry weight, and root/shoot ratio were transformed with the natural logarithm to meet the assumptions of normality and homogeneity of variances. On the other hand, total colonization percentages by hyphae, arbuscules and spores were transformed with the arcsine function (Eq. 2). For all the parameters evaluated, a multiple comparison of means was performed using Tukey tests, with a significance level of $p < 0.05$. All statistical analyzes were performed with the RStudio software.

Results

Germination

The two-way ANOVA showed significant differences between treatments with mycorrhiza (+AMF) and without mycorrhiza (-AMF) for the three species studied (Figure 2). The +AMF treatments showed a higher value of accumulated germination percentages compared to the -AMF treatments.

Mycorrhiza had a positive effect on the accumulated germination values of the species over time. We observed that this value was significantly higher in all species with the (+AMF) treatment compared to the treatment without mycorrhiza (-AMF) (Figure 3).

Our results show that the first day of germination, maximum day of germination, and the average germination time differed significantly between the mycorrhization treatments (+AMF, -AMF) (Table I). On the other hand, differences in seed vigor were observed between species. No differences were observed between treatments (+AMF, -AMF).

We observed that *Acaena elongata* and *Solanum pubigerum* seeds with mycorrhiza (+AMF) germinated earlier than seeds without it (-AMF). However, *Ageratina glabrata* seeds showed no significant differences in the first day of germination between the mycorrhiza treatments. The day of maximum germination was significantly earlier for *Ageratina glabrata* in the mycorrhiza treatment. Similarly, the mean germination time occurred earlier in the +AMF treatment compared to the -AMF treatment, for *Ageratina glabrata* and *Solanum pubigerum* (Table II).

Growth

According to two-way ANOVA, significant differences were found for shoot length, shoot dry weight, root dry weight, total dry weight and root/shoot ratio between plant species, mycorrhization and the interaction of these factors (Table III).

Solanum pubigerum showed a higher stem length in the +AMF treatment, but not *Ageratina glabrata* and *Acaena elongata*. Root length was greater in *Ageratina glabrata* in the +AMF treatment, but not in the other species. Shoot, root and total biomass were significantly higher in the +AMF treatments for *Acaena elongata* and *Ageratina glabrata*, but not for *Solanum pubigerum*. The root-shoot ratio was significantly higher in the -AMF treatments only for *Ageratina glabrata* and *Solanum pubigerum* (Figure 4).

Growth-Nutrient content

Concentrations of nitrogen and phosphorus in plant tissues varied significantly among species, between aerial parts and roots, and among mycorrhization treatments. However, no significant differences were observed for the three-way interaction among species, plant part, and mycorrhization treatment, as reported in Table IV for the species:AMF interaction.

Arbuscular mycorrhizal fungi (AMF) generally increase nutrient levels in plant tissues, although the extent of this increase varies among species. In *Acaena elongata*, the presence of AMF increased nitrogen and phosphorus levels in both shoot and root tissues. In *Ageratina glabrata*, AMF treatments also increased nitrogen and phosphorus concentrations, with the most notable difference occurring in the roots. In the case of *Solanum pubigerum*, the AMF effect was more muted, but there was a noticeable increase in nitrogen and phosphorus levels in the aerial parts when AMF were present (Figure 5).

Significant differences were observed in the MRI of the three species (X^2=28.24, P<0.001) (Figure 6). *Ageratina glabrata* showed the highest growth response to AMF colonization ($\bar{x}$ = 77 ±S.D.= 13), followed by *Acaena elongata* ($\bar{x}$ =59±27), and *Solanum pubigerum* ($\bar{x}$ =-14±41) showed the lowest. However, the species with significant differences in MRI was *Solanum pubigerum*.

Arbuscular mycorrhizal fungi roots colonization

Significant differences between species and between mycorrhization treatments were observed for the total and hyphal colonization percentages (Table V).

Acaena elongata and *Solanum pubigerum* showed the highest percentages of AMF colonization. *Ageratina glabrata* showed significantly lower percentages of total and hyphal colonization (Figure 7). Colonization by arbuscules and spores showed no differences between species.

Discussion

This study provides evidence of the positive effect of AMF on the germination and early growth of three characteristic SV plant species, which are considered as important in natural regeneration sources of this temperate forest: *Acaena elongata*, *Ageratina glabrata*, and *Solanum pubigerum*. Germination and growth are key stages in a plant's life cycle that are strongly influenced by the symbiosis with AMF (Smith et al. 2011), as observed in this study. Knowledge of the role of AMF in the early phenological stages of SV plant species is crucial for understanding their ecological dynamics. These fungi are key players in enhancing plant establishment in disturbed ecosystems, contributing to ecosystem resilience and natural regeneration. This has been demonstrated in studies of different plant species (Ramos-Zapata et al. 2012; Ramos-Zapata et al. 2013).

The influence of AMF on germination was found to vary between plant species. The greatest benefit was shown by *Ageratina glabrata*, followed by *Solanum pubigerum* and the least for *Acaena elongata* (Fig. 2). This variability can be attributed to differences in biological traits between plant species, such as fruit type, seed dormancy, seed size, and reserve content (Closa and Goicoechea 2011; Orozco-Segovia and Sanchez-Coronado 2013; Zamora-Crescencio et al. 2018). For instance, *Ageratina glabrata* produces the smallest seed size (0.0001 g), while *Solanum pubigerum* has the intermediate seed size (0.002 g). In contrast, *Acaena elongata* produces the largest seed size (0.02 g) (Calderón and Rzedowski 2005). It is possible to observe a direct correlation between seed size and seed reserve content (Soriano et al. 2011), since smaller seeds (such as those of *Ageratina glabrata*), which have fewer stored reserves, may need to establish arbuscular mycorrhizal associations more quickly to obtain

nutritional benefits and be able to allocate them to plant tissue production. In contrast, larger seeds, such as those of *Acaena elongata*, take longer to establish such symbiotic relationships and may benefit less due to their higher reserve content. In this context, Jin et al. (2009) found a strong negative correlation between seed size and AMF colonization. This suggests that species with smaller seeds may be more dependent on AMF for nutrient acquisition, especially during early growth stages, possibly due to their limited seed reserves.

A positive effect of AMF on accumulative germination was observed in the three species studied. Germination rates were significantly higher in treatments with AMF (+ AMF) than without (-AMF). This indicates that these fungi can induce seed germination (Tian et al. 2023). In this context, Varga (2015) reported a negative effect of AMF spores on seed germination, while Rueda-Puente et al. (2010) suggested that AM fungi could induce seed germination, although the underlying mechanisms remain unclear. AMF may contribute to the establishment of favorable microsites for seed germination through the formation of micro and macro aggregates in the soil and the production of hyphal exudates (Koide 2000). The presence of micro and macroaggregates can increase nutrient availability and maintain a more stable moisture regime, AMF create a nurturing environment that can enhance germination rates and subsequent growth of plant seeds. Therefore, AMF may play a role in creating moisture microenvironment conditions conducive to seed germination of tree species in temperate forests (Olivera-Morales et al. 2011).

It is also possible that seed exudates may stimulate germination of AMF spores, and as a result, rapid establishment of AM association has been observed. These exudates contain a variety of organic compounds such as sugars, amino acids, and other signaling molecules that may act as chemical cues to induce AMF spores to germinate (Besserer et al. 2008). However, the specific physical or chemical processes of AMF were not examined in this study.

Acaena elongata and *Solanum pubigerum* showed an earlier germination in the + AMF treatment. This early response could give mycorrhizal plants an initial competitive advantage, facilitating early establishment (Van Der Heijden 2004; Varga 2005; Jansa et al 2008). These results highlight the importance of arbuscular mycorrhiza in the early stages of the life cycle of SV plant species in temperate forests. *Ageratina glabrata*, however, did not show a significant difference in the first day of germination in any treatment. It is very important to note that other mechanisms, such as the presence of beneficial fungi and bacteria, may also contribute to seed germination in + AMF treatments. These microorganisms could provide a "priming" effect similar to AMF, increasing nutrient availability and possibly even providing protection against soil pathogens.

Significant differences were found in the time to 50% germination (T_{50}) between treatments, with *Ageratina glabrata* and *Solanum pubigerum* taking longer to germinate 50% of their seeds in the + AMF treatment. This suggests that AMF symbiosis affects not only the germination rate and total percentage, but also the synchrony of germination, as seen in the T_{50} (Table II). Such synchronization may be advantageous for establishment as it allows seeds to remain viable in the soil for a longer period of time. Seed priming can be facilitated by treating seeds with specific osmotic agents, soaking times, cold or heat to achieve more synchronized germination in a seed population. Marimuthu et al. (2018) propose that AMF may have a priming effect on seeds, and seedlings inoculated with AMF at the seed stage may show increased resistance to water stress. However, the ecophysiological mechanisms underlying these observations remain poorly understood.

For *Acaena elongata*, no significant differences were observed between treatments, suggesting that mycorrhizae do not significantly affect germination synchrony in this species. *Acaena elongata* is typically found in disturbed

areas of temperate forests and is the most common species near agricultural fields and open canopy forest sites among the three species studied. High seed viability rates (95% as determined by the tetrazolium test) have been previously documented for this species. However, the presence of high seed heteromorphism within woody Rosaceae species and a non-deep physiological dormancy, as in the case of *Acaena elongata* (Martínez-Camacho et al. 2018) could contribute to a lack of synchrony in germination, even in the presence of AMF. In relation to *A. elongata*'s response to AMF, it has been previously shown that there are negative and significant correlations between colonization and some phenological phases, and this varies seasonally (Vázquez-Santos et al. 2019).

For the growth stage, Closa and Goicoechea (2011) demonstrated interspecific variation in the percentage of colonization by AMF in *Oxalis acetosella* and *Viola riviniana*. In this case, root structure and root hair characteristics are considered to be the fundamental factors regulating the mycorrhizal association (Roumet et al. 2006). *Solanum pubigerum* has a taproot system (Bui et al. 2015), which could correlate with its ability to store nutrients and therefore show a low colonization response during early phenological stages. We suggest that root systems should be considered in future analyses of AMF dynamics.

A recently emerged seedling loses the resistance provided by the seed coat, making this phenological stage particularly vulnerable as it lacks the physical robustness of an adult plant (Kitajima and Fenner 2000). During this critical period, the plant must prioritize rapid growth and the development of a deep and branched root system to ensure adequate water and nutrient supply. At the same time, the allocation of resources for maintaining the AMF association increases (Vázquez-Santos et al. 2021). In our study, colonization percentages were significantly higher in *Acaena elongata* and *Solanum pubigerum* compared to *Ageratina glabrata*. Despite the high colonization rates observed in the first two species, they showed a low growth response.

It has been shown that AMF inoculation significantly enhances seedling growth, biomass, nutrient uptake and improves biomass partitioning (Souza et al. 2023; Tian 2023; Yang et al. 2024). In this study, the greatest stem length observed in *Solanum pubigerum* treated with AMF, together with the most notable response in total dry weight in *Acaena elongata* and *Ageratina glabrata*, indicates the role of AMF in resource allocation to vegetative structures. This was corroborated by the positive influence of AMF on shoot N y P concentration for *Acaena elongata* and *Ageratina glabrata*, suggesting that association with AMF may be critical for the efficiency of soil nutrient uptake and translocation to shoot. Notably, *Solanum pubigerum* showed the least response to mycorrhizal colonization, with no significant differences in root length, stem dry weight, root dry weight or total dry weight. In addition, this species showed a less pronounced effect of AMF, since only an increase in P accumulation in the aerial part was observed in the presence of AMF, but not of N. This is consistent with its significantly lower rate of mycorrhizal response compared to *Acaena elongata* and *Ageratina glabrata*, suggesting that *Solanum pubigerum* is least dependent on AMF association during early growth.

It is important to clarify that plants can be colonized by different species of arbuscular mycorrhizal fungi (AMF) and that AMF species do not colonize two different plant species with the same intensity (Helgason et al. 2002). This AMF diversity may lead to different responses in germination and growth in different plant species. The specificity of the relationship between plants and their fungal symbionts could reflect adaptations to particular environmental conditions, leading to variation in colonization efficiency and consequently in the benefits derived from mycorrhizal symbiosis, such as improved nutrient uptake and resistance to soil pathogens. Thus, it is important for future research to recognize that intraradical colonization varies between plant and fungal species. This consideration may lead to a more accurate understanding of AMF functions at different phenological stages and the processes involved in their association.

We conclude that the early phenological stages of a plant have a significant influence on the rate of AMF colonization, as nutrient requirements vary at different stages of growth and development. During early germination, rapid root development and robust vegetative growth are critical, requiring a substantial nutrient supply in which AMF can play a key role. As the plant grows to later stages, the need for resource allocation for AMF maintenance may change, reflecting a dynamic association that adapts to the developmental needs of the plant. Furthermore, the variability in AMF colonization between host species suggests that specific plant-fungal interactions are finely tuned to the plant's life cycle and environmental conditions, including soil properties.

Declarations

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Competing interest

The authors declare that they have no competing financial interests or personal relationships that could have appeared to influence the research reported in this paper.

Availability of data and materials

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Author Contributions

Author Contributions**:** [Yasmin Vázquez-Santos], [Silvia Castillo-Argüero], [Noé Montaño] and [Francisco J. Espinosa-García]: Conceptualization, reviewing and editing; [Yasmin Vázquez-Santos] [Yuriana Martínez-Orea] and [Silvia Castillo-Argüero]: Methodology and Fieldwork; [Yasmin Vázquez-Santos], and [César Flores-Ortiz]: Data Collection and Data Analysis [César Flores-Ortiz]. [Yasmin Vázquez-Santos]: Writing - Original Draft; All authors Writing- Reviewing and Editing.

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Tables

Table 1. Statistical summary of the two-way ANOVA for germination percentage = germination, day of first germination = First germination, day of maximum germination = Max germination, average germination time = T50 and seed vigor, for *Acaena elongata, Ageratina glabrata* and *Solanum pubigerum*, for treatments with mycorrhiza (+AMF) and without mycorrhiza (-AMF).

Sources of variation		Germination		First germination		Max germination		T50		Vigor	
	df	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Species	2	4.972	**0.001**	4.093	**0.029**	1.648	0.21	0.207	0.81	39.32	**0.001**
AMF	1	59.93	**0.001**	14.24	**0.001**	9.905	**0.004**	32.28	**0.001**	0.063	0.93
Species×AMF	2	0.741	0.487	2.47	0.105	2.13	0.14	4.6	**0.0203**	0.277	0.75

*Significant differences in bold

Table 2. Means values ($\bar{x}$ ± S.D.) of germination traits for species and treatments with mycorrhiza (+AMF) and without mycorrhiza (-AMF).

	Acaena elongata		*Ageratina glabrata*		*Solanum pubigerum*	
	+AMF	-AMF	+AMF	-AMF	+AMF	-AMF
Firts germination day	8.4 ± 04^{b}	13.7 ± 0.44^{a}	8 ± 0.86^{b}	8.5 ± 0.44^{a}	8.25 ± 0.22^{b}	14 ± 3.07^{a}
Maximum germination day	35.4 ± 4.2^{b}	37.5 ± 2.04^{a}	26.5 ± 4.9^{b}	40 ± 1.9^{a}	31.25 ± 3.6^{b}	40.75 ± 1.1^{a}
T_{50}	20.3 ± 2.6^{b}	26 ± 4.6^{a}	12.6 ± 1.2^{b}	39.5 ± 5^{a}	14.6 ± 1.2^{b}	32.37 ± 4.4^{a}
Vigor (mm)	14.28 ± 3.9^{a}	15.3 ± 5.0^{a}	5.46 ± 2.7^{c}	5.31 ± 1.6^{c}	11.92 ± 8.4^{b}	12.69 ± 9.2^{b}

* Significant differences are indicated by superscript letters

Table 3. Statistical summary of the two-way ANOVA for stem length, root length, SDW= stem dry weight, RDW= root dry weight, TDW= total dry weight and root/stem ratio for *Acaena elongata*, *Ageratina glabrata* and *Solanum pubigerum*. Var.exp= percentage variance explained.

Source of variation	Stem length			Root length			SDW		
	df	*F*	*P*	Var. exp	*F*	*P*	Var. exp	*F*	*P*	Var. exp
Species	2	10.03	**0.001**	11.29	14.64	**0.001**	17.25	19.25	**0.001**	13.02
AMF	1	15.73	**0.001**	8.85	1.81	0.18	1.06	87.13	**0.001**	29.46
Species:AMF	2	4.44	**0.013**	4.99	2.83	0.062	3.33	18.51	**0.001**	12.52

Source of variation	RDW			TDW			Root/stem		
	df	*F*	*P*	Var. exp	*F*	*P*	Var. exp	*F*	*P*	Var. exp
Species	2	24.813	**0.001**	19.7	12.66	**0.001**	7.58	74.36	**0.001**	46.79
AMF	1	47.56	**0.001**	18.89	58.28	**0.001**	17.69	20.04	**0.001**	6.3
Species×AMF	2	10.8	**0.001**	8.58	12.36	**0.001**	7.51	8.03	**0.001**	5.05

*Significant differences are shown in bolds

Table 4. Statistical summary of the two-way ANOVA for nitrogen and phosphorus content in plant tissue for *Acaena elongata*, *Ageratina glabrata* and *Solanum pubigerum* in +AMF and -AMF treatments.

Source of variation		Nitrogen		Phosphorus	
	df	*F*	*P*	*F*	*P*
Species	2	3.89	**<0.01**	10.359	**<0.001**
Plant tissue	2	47.37	**<0.0001**	0.156	0.855
AMF	1	9.63	**<0.001**	10.483	**<0.01**
Species:Plant tissue	2	1.99	0.153	6.057	**<0.01**
Species:AMF	2	1.28	0.291	0.463	0.633
Plant tissue:AMF	1	5.12	**<0.05**	16.315	**<0.001**
Species:Plant tissue:AMF	2	3.26	0.052	0.044	0.957

*Significant differences are shown in bolds

Table 5. Statistical summary of the two-way ANOVA for the colonization by AMF parameters for *Acaena elongata*, *Ageratina glabrata* and *Solanum pubigerum* in treatments with mycorrhiza (+AMF) and without mycorrhiza (-AMF).

Source of variation		AMF Colonization		Hyphae colonization		Arbuscules colonization		Spores colonization	
	df	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Species	2	5.701	**0.004**	5.97	**0.003**	1.26	0.28	2.31	0.102
AMF	1	330.107	**0.001**	334.7	**0.001**	26.13	**0.001**	6.42	**0.01**
Species:AMF	2	1.29	0.29	1.28	0.27	1.25	0.28	1.71	0.18

*Significant differences are observed in bolds

Figures

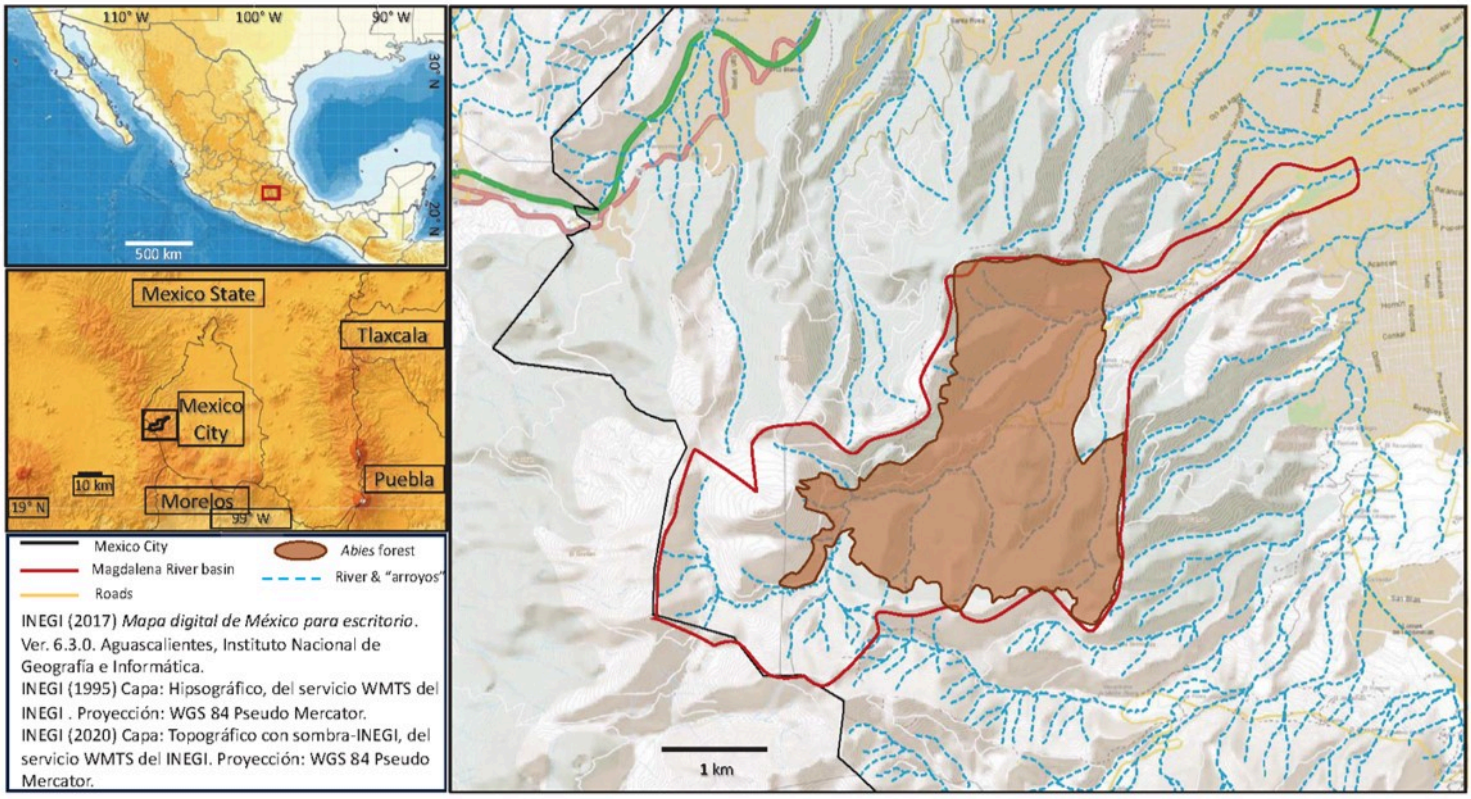

Figure 1

Figure 1

Geographical location of the *Abies religiosa* forest within the Magdalena River Basin, Mexico City, Mexico showcasing A) Mexico, B) Mexico City, C) the Magdalena River Basin, with the Oyamel Forest area highlighted in brown. Prepared by Romero-Romero M.A. (2024).

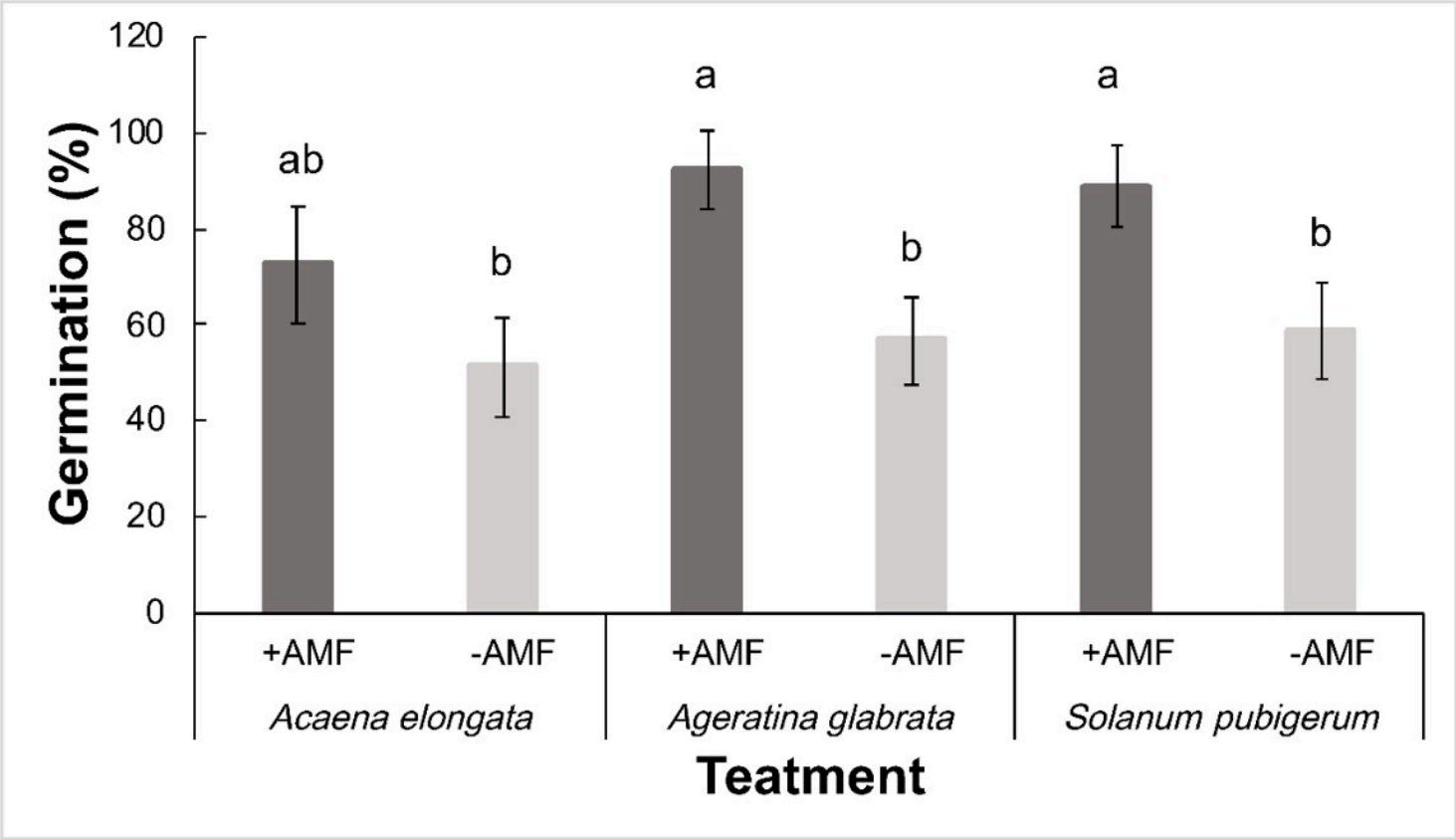

Figure 2

Figure 2

Mean value (±S.D.) of germination percentages for *Acaena elongata*, *Ageratina glabrata* and *Solanum pubigerum* seeds treated with mycorrhiza (+AMF) and without mycorrhiza (-AMF). Significant differences are indicated by letters (p<0.001).

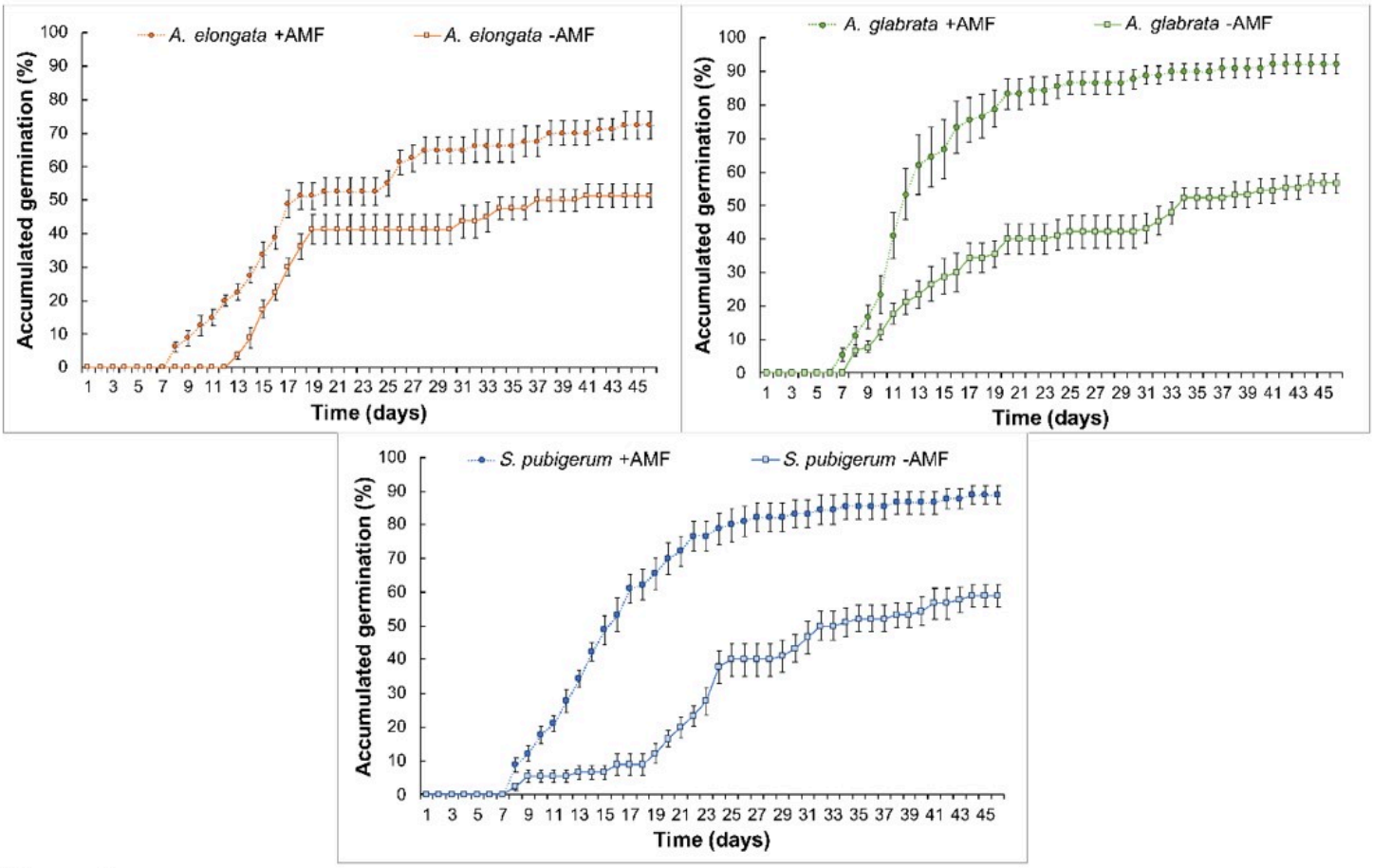

Figure 3

Figure 3

Mean values of accumulated germination percentages ($\bar{x}$ ±S.D.) for *Acaena elongata*, *Ageratina glabrata* and *Solanum pubigerum* in two treatments: +AMF (with mycorrhiza) and -AMF (without mycorrhiza), over time in days.

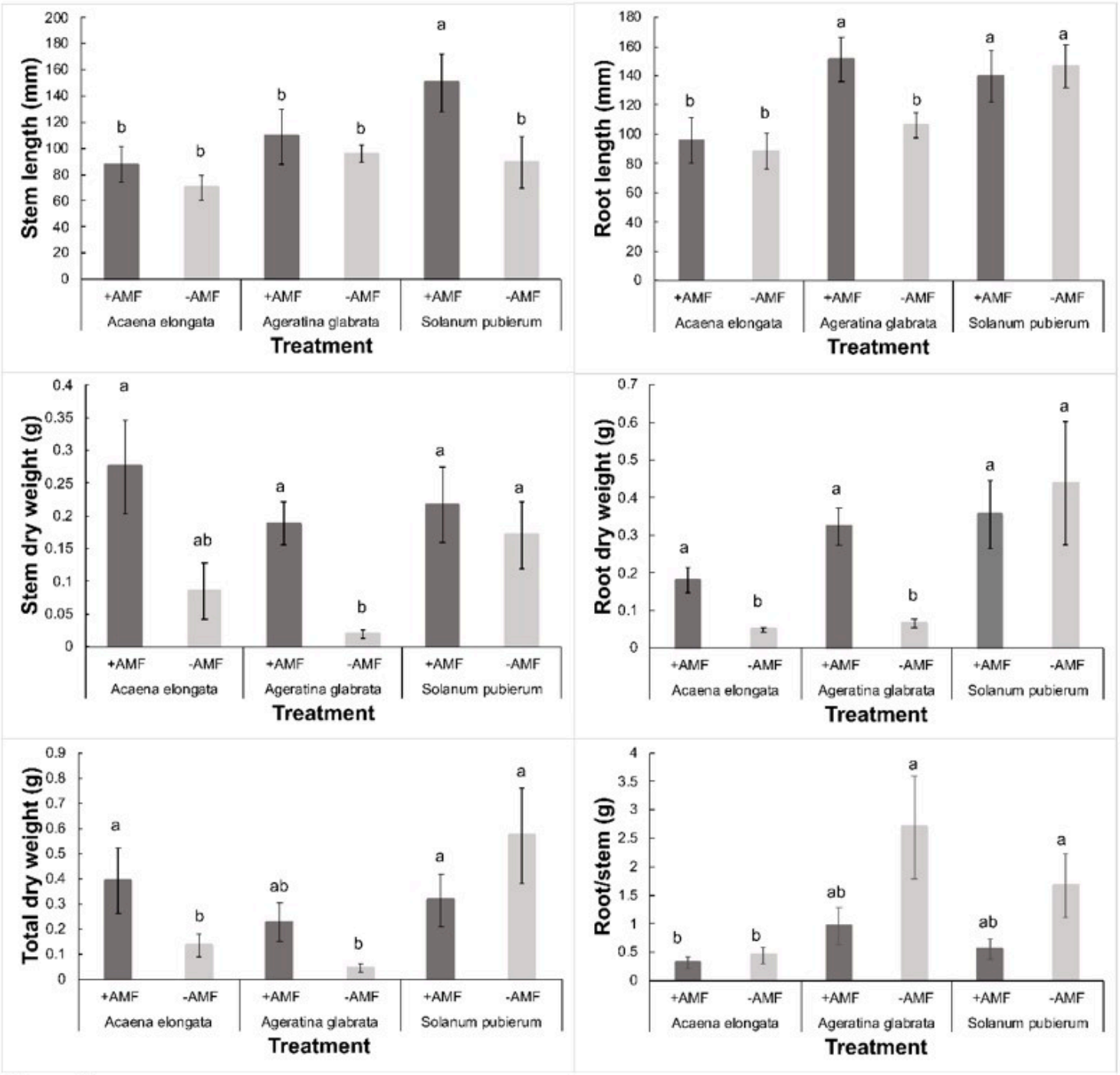

Figure 4

Figure 4

Mean values of growth parameters for the three species from secondary vegetation in a temperate forest under the treatments: with mycorrhiza (+AMF) and without mycorrhiza (-AMF). Different letters indicated statistical differences between treatments $p<0.05$.

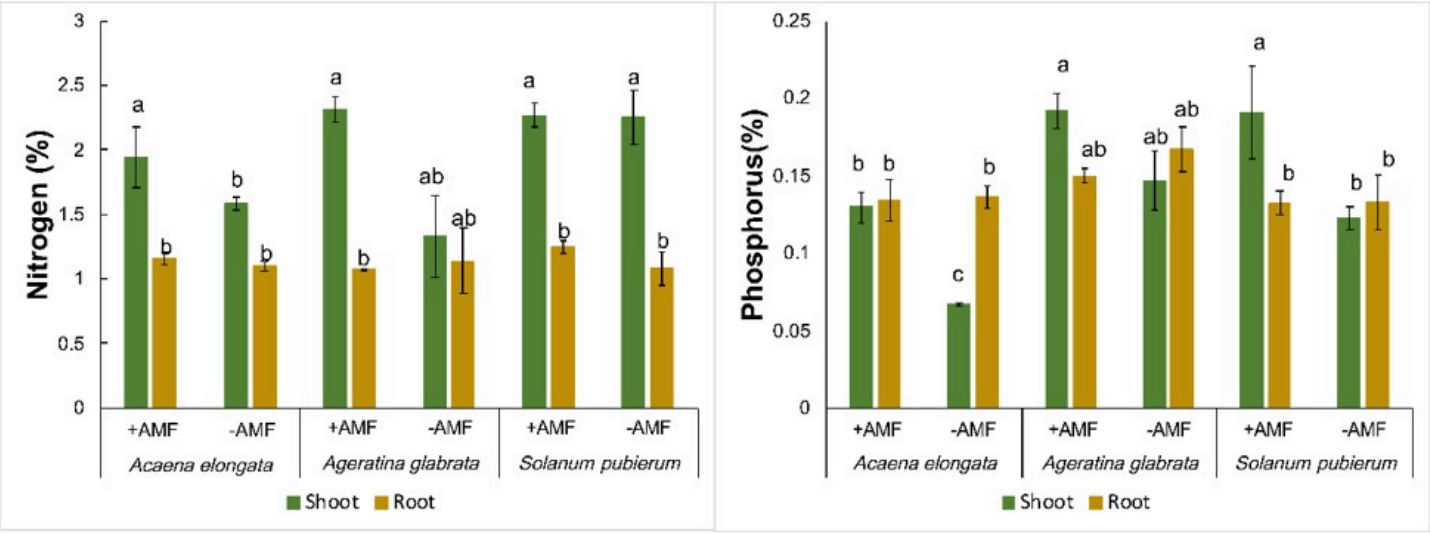

Figure 5

Figure 5

Nitrogen and Phosphorus content in the shoots and roots of *Acaena elongata*, *Ageratina glabrata*, and *Solanum pubigerum*, with (+AMF) and without (-AMF) arbuscular mycorrhizal fungi treatment. The bar graphs illustrate the percentage of Nitrogen (above) and Phosphorus (below) in both the shoots and roots.

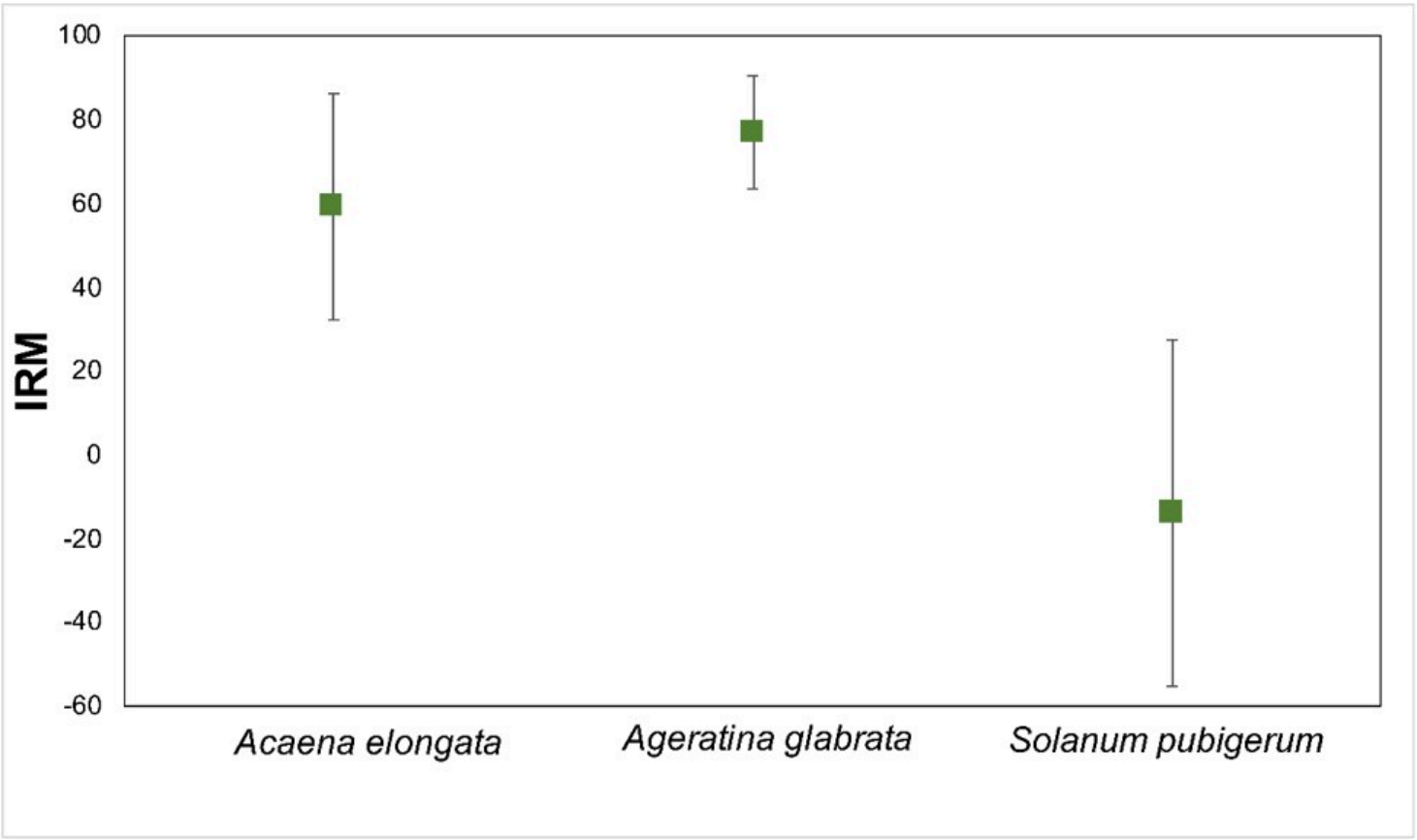

Figure 6

Figure 6

Mycorrhiza response index ($\bar{x}$ ± D.S) of the three secondary vegetation species in a temperate forest

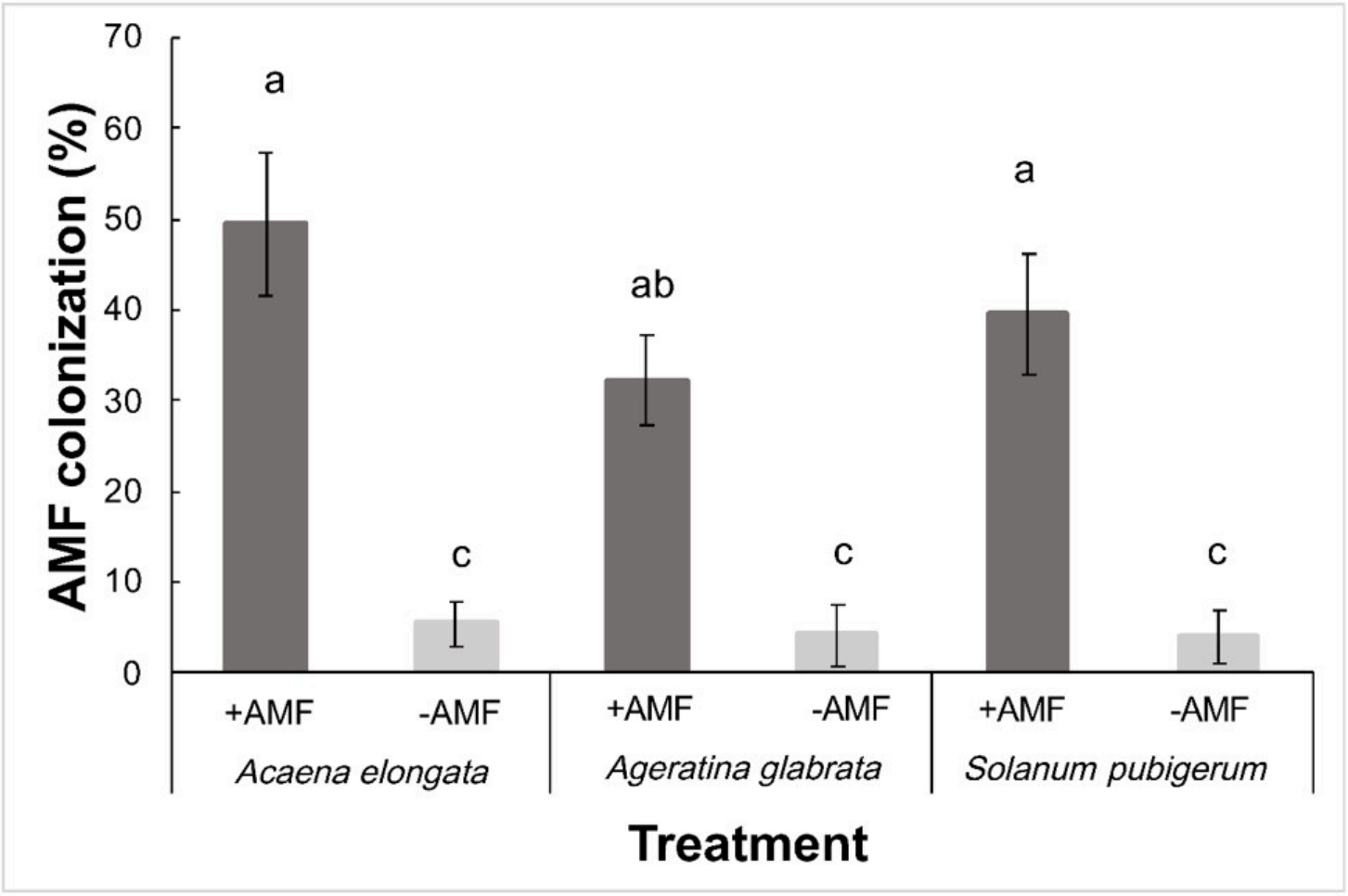

Figure 7

Figure 7

Mean values of AMF hyphae colonization percentage of the three species from secondary vegetation in a temperate forest. Different letters correspond to statistical differences $p<0.05$.