



Arbuscular mycorrhizal fungi and edaphic factors modulate flowering and fruiting of secondary vegetation species in a temperate forest

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Received: 30 August 2024 / Revised: 21 April 2025 / Accepted: 7 May 2025
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Abstract

Arbuscular mycorrhizal (AM) fungi enhance plant flowering and fruiting by improving nutrient and water uptake. The aim of this study was to evaluate how AM fungal colonization percentage and edaphic factors, modulate flowering and fruiting in secondary vegetation (SV) species with different biological traits, in a temperate forest. We conducted the research in four sites at two elevation intervals in a temperate forest in Mexico, focusing on three SV species. We recorded flower, fruit and AM fungal colonization percentages during the rainy and the dry season. Soil was collected from the rhizosphere of each SV species to determine organic matter (OM), pH, relative soil moisture (RSM), available phosphorus (Ava-P) and nitrogen (Ava-N). The AM fungal colonization, flower and fruit percentages showed interspecific and temporal variations. Structural equation models showed that flowering was positively influenced by RSM in *Acaena elongata* and negatively influenced by AN in *Solanum pubigerum*. Fruiting was positively influenced by AM fungal colonization and negatively by AP in *A. elongata*, with a relevant indirect effect of OM. In *S. pubigerum*, the effect of AM fungal colonization was slightly positive, while AN and pH had a greater effect on fruiting. While in *Ageratina glabrata* the effect of AM fungal colonization on fructification was not evident. The study highlights the critical role of AM fungal colonization and soil properties such as OM, pH and N in flower and fruit production, which depend on the biological traits of the host plant species.

Keywords Disturbed vegetation · Mutualistic · Reproductive phenology · Sacred fir forests · Secondary forests · Soil parameters

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1 Introduction

Temperate forests are ecosystems that have been significantly impacted by human population expansion worldwide (Gilliam 2016). They exhibit considerable topographic, edaphic, and microenvironmental heterogeneity that supports high aboveground and belowground biodiversity (Keenan et al. 2015). These forests are of critical ecological, social, and cultural importance worldwide, and have a significant economic value (Keenan et al. 2015). However, anthropogenic activities have profoundly altered the composition and structure of forest communities, resulting in the extensive formation of secondary vegetation areas (Major et al. 2005; Zamora-Crescencio et al. 2018).

Secondary vegetation (SV) is characterized by an indeterminate floristic composition that varies with proximity to propagule sources for natural regeneration. Its structure is influenced by the intensity of disturbance, which can range from low (e.g., selective logging) to high (e.g., clear-cutting

or complete vegetation removal), their frequency (e.g., single events versus recurrent agricultural or grazing activities), and the time elapsed since the last disturbance, with longer recovery periods generally favoring the reestablishment of more complex and diverse vegetation structures (Castillo-Campos and Laborde 2004; Martínez-Orea et al. 2013). SV plays a critical role in the ecological succession of forest ecosystems (van Hall et al. 2017). The establishment of SV species buffers structural changes and increase edaphic stability through the development of root systems and the incorporation of organic matter (OM) into the soil (Post and Kwon 2000; van Hall et al. 2017). In addition, certain SV species act as nurse plants for conifer seedlings (van Hall et al. 2017). These traits make SV species essential for ecological resilience and resistance in temperate forests.

SV plant species exhibit strategies that facilitate their establishment in disturbed sites, such as greater adaptation to continuous environmental and edaphic changes (Grime 2006). The association of SV species with arbuscular mycorrhizal (AM) fungi is critical for their survival and growth, allowing these plants to efficiently obtain essential soil nutrients and water (van Hall et al. 2017; Xing et al. 2022). This mutualistic relationship is particularly important in temperate forests, which are characterized by pronounced rainfall seasonality and soils with acidic pH and low nutrient availability (Berg 2014). Furthermore, the association of AM fungi with these plant species helps to maintain the edaphic network of connections and interactions between them even after disturbance (van Der Heijden et al. 2015). Therefore, the persistence of mycorrhizal networks in the soil is essential for the ecological resilience of temperate forests (Simard 2018; Toju et al. 2018).

AM fungi are among the most abundant groups within soil microbial communities (Pérez et al. 2011), establishing obligate symbiotic relationships with approximately 70% of plant families (Brundrett and Tedersoo 2018). These fungi enhance the transfer of water, macronutrients (Koide 1991; Smith and Read 2008), and micronutrients such as zinc (Watts-Williams and Cavagnaro 2018), copper (Leyval and Joner 2001) and iron (Gao et al. 2007) to their host plants (Gosling et al. 2006). This symbiotic relationship significantly increases the competitive advantage of host plants, allowing them to thrive in nutrient-limited environments (Stanescu and Maherali 2017). In return, AM fungi receive photosynthetically fixed carbon (C) from their host plants and a suitable habitat (Smith and Smith 2011). This mutualistic relationship is critical for several ecological processes, including net primary production, nutrient cycling, carbon sequestration, and maintenance of soil quality (Hodge and Fitter 2010; Smith and Smith 2011).

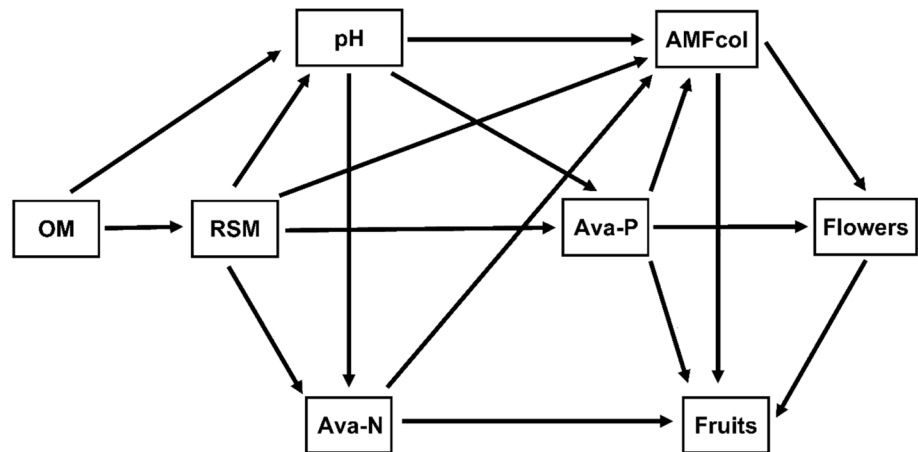
AM fungi can stimulate the production of reproductive plant structures by increasing the uptake of water and essential nutrients, primarily phosphorus (P) and nitrogen

(N) (Colla et al. 2008; Vega-Frutis and Guevara 2009). In addition, they influence the hormone production of their host plants (Shamshiri et al. 2012; Xie and Wu 2018; Zhang et al. 2009; Bennett and Meek 2020; Wang 2022). The availability of nutrients in the soil, combined with the C provided by the host plant, collectively regulate the effect of AM fungi on the production of reproductive structures in plants (Bennet 2020). This interaction can sometimes result in a fluctuating balance between mutualism and antagonism (Johnson 2010). It has been observed that association with AM fungi is enhanced under conditions of low soil nutrient availability, whereas it is reduced or inhibited under high nutrient availability (Janos 2007). This suggests that it is critical to consider the edaphic conditions that drive this mycorrhizal association.

In addition, AM fungal colonization percentage increases significantly during plant flowering and fruiting (Vega-Frutis and Guevara 2013). The development of reproductive structures requires increased resource allocation, which depends on both environmental conditions (Obeso 2002) and plant biological traits (Wolfe et al. 2005; Gange and Smith 2005; Bennett and Meek 2020; Rashidi et al. 2022). For example, plant species with different fruit types and sizes require different amounts of nutrients, leading to interspecific variation in AM fungal colonization (Bennett and Meek 2020). However, the direct relationship between AM fungal association and plant reproduction has not been fully explored for SV species. The availability of propagules, such as fruits and seeds, is essential for natural regeneration of temperate forests. Therefore, this research can improve our understanding of the key role of AM fungi in flowering and fruiting of SV plants, thus contributing to the natural regeneration processes of temperate forests. It also highlights the importance of promoting the association of AM fungi with SV plant species to support forest restoration and resilience.

Studies on the relationship between arbuscular mycorrhizal associations and edaphic conditions in temperate forests are scarce (Yokobe et al. 2018). Edaphic factors significantly influence mycorrhizal associations and fruit production in plants, highlighting their importance in different environments. For example, soil nutrient availability typically increases during the rainy season and decreases during the dry season (Roy and Singh 1994; Hartemink and Barrow 2023), which can simultaneously regulate AM activity and plant reproductive phenology (Vega-Frutis and Guevara 2013; Vázquez-Santos et al. 2019). This suggests a complex interaction between edaphic factors and biological processes above and below ground. Therefore, we propose a hypothetical model to clarify how the reproductive processes of SV plant species are directly or indirectly affected by AM fungal colonization percentage, as well as by site-specific edaphic factors such as pH, OM, moisture, and availability of P and N in a temperate forest (Fig. 1).

Fig. 1 Hypothetical model illustrating the effects of edaphic factors on AM fungal colonization percentage and subsequent effects on flowering and fruiting in secondary vegetation plant species. The arrows indicate the theoretical relationships between the variables: relative soil moisture (RSM), pH, available nitrogen (Ava-N) and phosphorus (Ava-P), organic matter (OM), AM fungal colonization percentage (AMFcol), and flower and fruit production (Flowers, Fruits)



Based on our model, we hypothesize that because the nutrient requirements of SV plant species differ among them due to their biological traits, phenological patterns will also differ, and nutrient requirements will increase during flowering and fruiting. Therefore, we expect to observe: (i) interspecific variations in flowering, fruiting and AM fungal colonization percentages and (ii) a positive relationship between AM fungal colonization percentage and plant flowering and fruiting. In addition, given the simultaneous interaction of edaphic and biotic factors, we expect to find an influence of soil pH, organic matter (OM), relative soil moisture (RSM), phosphorus (P) and nitrogen (N) availability on AM fungal colonization percentage, flowering, and fruiting.

The objective of this research was to evaluate how AM fungal colonization percentage and edaphic factors, modulate flowering and fruiting in secondary vegetation (SV) species with different biological traits, in a temperate forest. The specific objectives were: (i) to analyze the interspecific and temporal variations in flower and fruit production and AM fungal colonization percentage in *Acaena elongata*, *Ageratina glabrata*, and *Solanum pubigerum*, and (ii) to evaluate the joint effect of AM fungal colonization and edaphic factors on flowering and fruiting of the plant species using Structural Equation Models based on our hypothetical model.

2 Materials and methods

Study area This study was conducted in the temperate forest of *Abies religiosa* (Kunth) Schltdl. & Cham., in the Magdalena River basin (MRB), Mexico City. The weather is temperate subhumid with summer rains, with a mean annual temperature of 18 °C, mean annual precipitation of 1250 mm, and a thermal oscillation of less than 5 °C (García 1988). This forest exhibits a strong seasonality with the rainy season occurring from May to October and the dry season

from November to April (Delgadillo-Duran 2011). The predominant soil group is humic Andosol according to the WRB (2022). It is characterized as an acid soil dominated by allophanes, it is rich in OM, with a sandy-loamy texture and a high capacity to fix phosphates on the amorphous mineral clay surface, which limits their availability for plants (INEGI 2011; Galicia et al. 2016).

Sites selection Based on the differences in environmental factors along the elevation gradient in this area according to Oseguera-Olalde et al. (2022), we decided to establish four sites distributed at two elevation intervals in the *Abies religiosa* forest (Fig. 2). Sites 1 and 2 were located at the highest elevation (3192–3254 m a.s.l.), while sites 3 and 4 were established at the lowest (3027–3100 m a.s.l.). The criterion for selecting the sites was the coexistence of the three SV plant species under study. In each site, we marked seven individuals per plant species with reproductive structures and similar size, obtaining a total of 84 individuals ($N = 7 \text{ individuals} \times 4 \text{ sites}$). This forest has been frequently disturbed by anthropogenic activities such as deforestation, several silvicultural practices such as weeding, cattle ranging and recreational activities (Santibañez-Andrade et al. 2015).

Selected secondary vegetation plant species The selected plant species are three abundant shrubs in the secondary vegetation of the study forest (Fig. 3), (Santibañez-Andrade et al. 2015). *Acaena elongata* L. is a member of the Rosaceae family, reaching a height of 0.5–1 m, with serrated leaves 8–20 mm long. It is considered as an indicator of anthropogenic disturbance, it is dispersed by livestock and tolerates diverse environmental conditions, such as acidic to alkaline soils and low nutrient availability. *Ageratina glabrata* (Kunth) R.M. King & H. Rob., from the Asteraceae, it grows up to 2.5 m high with serrated leaves up to 8 cm long. It promotes plant regeneration in xerophytic thickets, it has white flowers and wind-dispersed achenes. Finally, *Solanum*

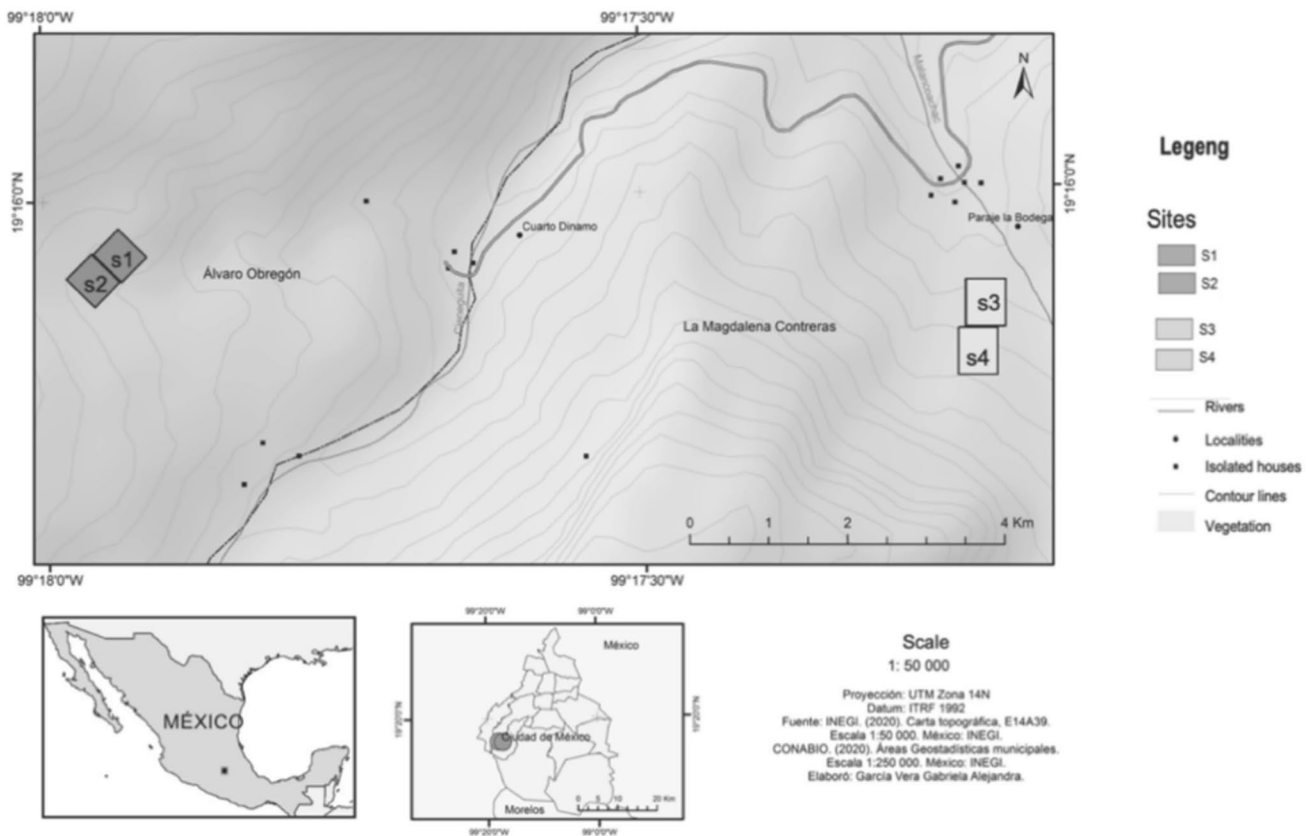


Fig. 2 Geographic location of the selected sites within the *Abies religiosa* forest of the Magdalena River basin, Mexico City. S1 = Site 1: 2350 m², S2 = Site 2: 2270 m², S3 = Site 3: 2731 m², S4 = Site 4: 2709 m².

pubigerum Dunal, it belongs to the Solanaceae family, 1.5–3.5 m height and whole or wavy leaves, it has fleshy fruits dispersed by animals (Calderón and Rzedowski (2005).

These species are abundant in the understory (Santibañez-Andrade et al. 2015), as they are also found in natural regeneration sources such as seed rain and seed bank (Martínez-Orea et al. 2013, 2024). They differ in flower and fruit types, seed size, and reproductive phenology patterns (Table 1). These differences in their biological traits allow us to suppose they exhibit a differential allocation of resources, since the amounts of nutrients and water allocated to the development of flowers, fruits, and seeds can vary among them (Obeso 2002).

Flowering and fruiting For all species, the reproductive phenology was measured using the phenological scale of Fournier and Charpentier (1974), which assigns values from 0 to 100 to represent the percentages of flowers and fruits. The phenophases recorded were: i) flowers and ii) fruits. Regular observations ensured consistent tracking of reproductive structures across sites and time. The values were obtained from August 2019 to March 2020. The percentage of reproductive structures for each individual was calculated

by weighting the recorded percentages observed in the field by the actual canopy cover of each plant. Cover values were obtained by measuring the perpendicular diameter of each individual. We assumed that the cover for each individual was similar to the area of an ellipse, which was calculated using the following equation:

$$C = \pi \left(\frac{D1 + D2}{4} \right)^2 \quad (1)$$

where: C = cover, $D1$ = horizontal diameter, $D2$, vertical diameter, and π

Evaluation of the AM fungal colonization percentage Twenty-five fragments of fine roots were collected from the marked individuals at each site, in different areas of their rhizosphere, to avoid root system damage. Two samplings were carried out during each season: rainy season (August and October 2019), and dry season (December 2019 and February 2020). The total number of samples was 336 (7 individuals $\times$ 3 species $\times$ 4 sites $\times$ 4 collections). Roots were processed according to the method of Koske and Gemma (1989), which consists in placing roots in a KOH (10%)

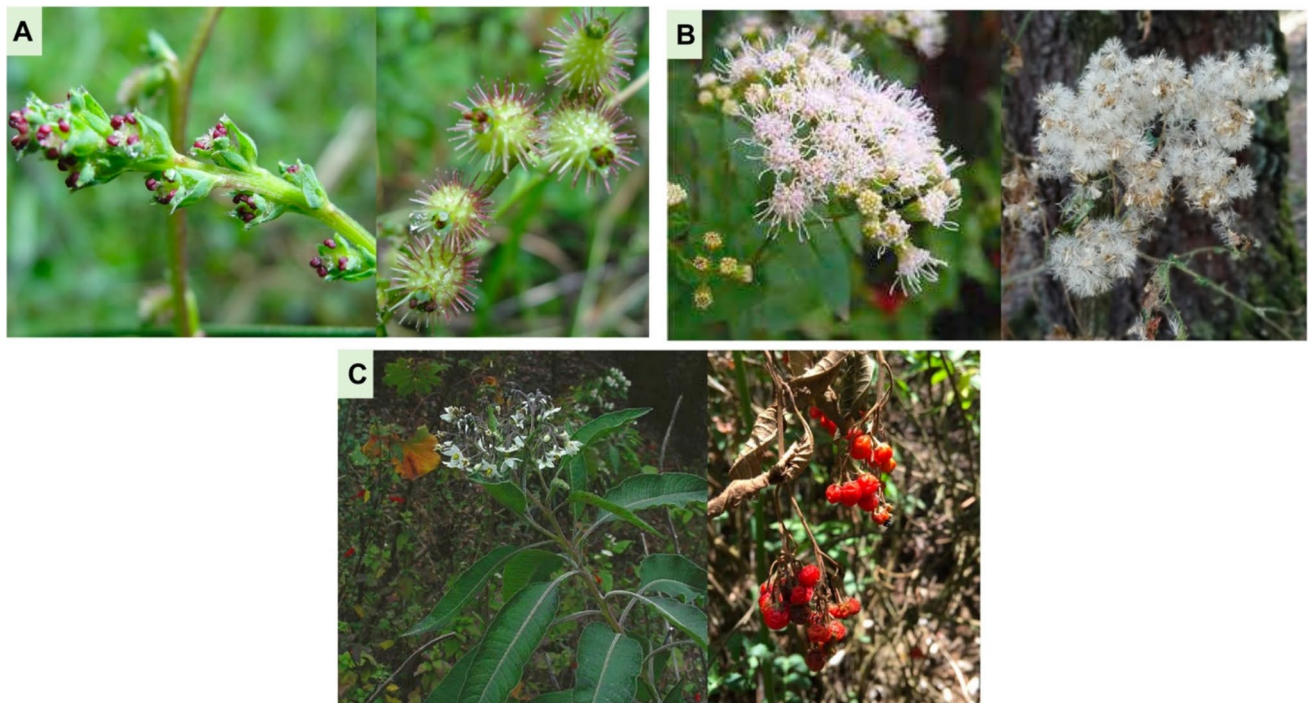


Fig. 3 Study plant species with their reproductive structures. **A** *Acaena elongata*, **B** *Ageratina glabrata*, and **C** *Solanum pubigerum*, all located in the *Abies religiosa* forest of the Magdalena river basin, Mexico City, Mexico

Table 1 Biological traits of the three secondary vegetation plant species within the *Abies religiosa* forest in the Magdalena River basin, Mexico City, Mexico

Plant species	Flowers		Fruits		Seeds	Reproductive phenology	
	Type	Spolin	Type	SDisper		Flower	Fruit
<i>Acaena elongata</i>	Inconspicuous	Anf	Achene	Exz	0.17 ± 0.03	C	C
<i>Ageratina glabrata</i>	Tubular	Anf/Mel	Achene	An	0.002 ± 0.00015	Dry	Rainy
<i>Solanum pubigerum</i>	Open-star shaped	Cant	Berry	Endz	0.014 ± 0.0007	Dry	Dry

*Spolin = pollination syndrome, Anf = anemophily, Mel = melittophily, Cant = cantharophily, SDisper = dispersal syndrome, Exz = exozoochory, An = anemochory, Endz = endozoochory, C = continuous

solution for 24 h, then in HCl (10%) for 10 min and finally in trypan blue (0.05%) for 24 h. We randomly selected 25 root fragments 2 cm long, which were mounted on slides in polyvinyl lactoglycerol (PVLG).

The percentage of AM fungal colonization was estimated using the method proposed by McGonigle et al. (1990). The observations were directly made on the root fragments with a light microscope Nikon Eclipse E100 (20X and 40X). We recorded (20X) the colonized fields by aseptate hyphae, arbuscules (characteristic structures of AM fungi), vesicles and spores. The colonization percentages were obtained using the following equation:

$$\% \text{ AM fungal colonization} = \left(\frac{\text{Number of colonized fields}}{\text{Number of observed fields}} \right) (100) \quad (2)$$

where: the total number of observed fields was at least 90, all the fields that did not stain were deleted.

Soil analysis A mixed sample of 500 g of the mineral soil attached to the roots (the first 0–15 cm deep) of each SV individual was recollected at each site. These samples were analyzed for the registration of the following variables: (i) the percentage of organic matter percentage (OM) through wet digestion (Walkley and Black 1934); (ii) relative soil moisture (RSM), which was obtained through the registration of the fresh and the dry weight at 105 °C (Reynolds 1970); (iii) available phosphorus (PO_4^-) through a NaHCO_3 extraction 0.5 M and a pH value of 8.5 in a colorimetric determination (Olsen et al. 1954); (iv) available N, the sum of nitrate (NO_3^-) and ammonium (NH_4^+), this was carried out through the use of a KCl solution then analyzed

by chromatography (KCl₂ technique), and (v) the pH value was determined with a potentiometer in a ratio 1:2 in deionized water (actual acidity), using the NOM-02-SEMAR-NAT-2000 (2002) techniques.

Statistical Analysis We used the R software version 4.2.2 (R Core Team 2016) for the statistical analysis. To assess differences in AM fungal colonization percentages we performed Linear Mixed-Effects Models (LME) using the “nlme” package (Eq. 3, Pinheiro et al. 2016). For all models, the linear predictor coefficient was estimated using the maximum likelihood or restricted maximum likelihood (REML) method. The fixed factors were: species (*Acaena elongata*, *Ageratina glabrata*, *Solanum pubigerum*), season (rainy and dry), elevation (high and low), and the interaction among these factors. Sites and individuals were included as random effects in all models because they represent a random sample of the levels of each factor. Colonization percentages were transformed with arcsin (asen), to meet the normality assumptions of the model. Normality of the residuals was assessed using the Shapiro–Wilk test ($p > 0.05$). Statistical differences between the all-levels variation were evaluated with a mean multiple comparisons (*post-hoc* contrast) using Tukey’s method with a confidence level of 0.95.

$$\text{Response variable}_i = \beta_0 + \beta_1 \text{Season} * \beta_2 \text{Elevation} * \beta_3 \text{Species} + b_1(\text{Site/Individual}) + \epsilon \text{ rror}_i \quad (3)$$

where: β_0 = intercept, β_1 , β_2 , β_3 = parameters, b_1 = random factor variance, $\epsilon \text{ rror}_i$ = variation that cannot be explained.

To assess the differences in flowers and fruits percentages, we ran Generalized Linear Mixed-Effects Models (GLMERs) using the “lme4” library (Bates et al. 2014) and with a binomial error structure (Eq. 4). We kept the same fixed and random factors as described above. Differences between variation factors were tested with a model simplification and analysis of variance between the models (Bates et al. 2014).

$$\text{Response variable}_i = \beta_0 + \beta_1 \text{Season} * \beta_2 \text{Elevation} * \beta_3 \text{Species} + b_1(\text{Site/Individual}) + \epsilon \text{ rror}_i \quad (4)$$

where: β_0 = intercept, β_1 , β_2 , β_3 = parameters, b_1 = random factor variance, $\epsilon \text{ rror}_i$ = variation that cannot be explained.

To determine the differences between pH, RSM, OM, available N, and P, a two-way analysis of variance (ANOVA) was performed, considering seasonality and elevation as sources of variation. Edaphic factors were transformed using the neperian logarithm to meet the normality assumptions of the models. The normality of the residuals was assessed using the Shapiro–Wilk test ($p > 0.05$). Statistical differences were evaluated by means of multiple comparisons (*post-hoc*

contrast) using Tukey’s method with a confidence level of 0.95.

For *Acaena elongata* and *Solanum pubigerum*, we carried out a structural equation model (SEM) using the same causal diagram or hypothetical model shown in Fig. 1, to estimate the direct and indirect effects between OM, RSM, pH, P and N availability, AM fungal colonization percentages, flowering and fruiting. To test the hypothetical model on the joint effect of the AM fungi colonization and edaphic factors on the flowering and fruiting of SV plant species, SEM was fitted by the robust maximum likelihood method using the “piecewise SEM” library (Lefcheck 2016). Piecewise SEM is a route and confirmatory analysis that allows us to confirm the theoretical causal diagram proposed in Fig. 1. It consists of GLMERs that are evaluated one at a time. Since a piecewise SEM does not produce a global covariance, it does not require the assumption of multivariate normality (Lefcheck 2016). This feature makes it particularly suitable for datasets that do not meet this assumption, often due to complex data structures or the presence of mixed models.

In this study, GLMERs were included as part of the SEM to account for the hierarchical structure of the data. Random effects were added to account for variability at the site and individual plant levels, which allowed us to account for non-independence of observations within these groups.

In addition, we calculated the marginal and conditional R^2 values for each model (T3 SI). The marginal R^2 represents the proportion of variance explained by the fixed effects alone, while the conditional R^2 indicates the total variance explained by both fixed and random effects (Nakagawa and Schielzeth 2013). To evaluate the parsimony of the fitted structural models, the corrected Akaike Information Criterion (AICc) was calculated according to the formula proposed by Hurvich and Tsai (1989). This analysis allowed us to identify the most parsimonious model by considering both the model fit and its complexity, which is particu-

larly relevant given the limited sample size in this study. The AICc was calculated based on the number of estimated parameters (K) and the sample size (n), allowing a robust comparison between alternative models.

To assess which variables are conditionally independent, we used a Shipley’s directed separation test, which assesses whether the hypothesized model adequately captures the causal relationships between variables (Shipley 2000). Conditional independence implies that there are no missing

relationships between unrelated variables. The directed separation test is performed by combining all P values over the established base of a test statistic, Fisher C , using the following equation:

$$C = -2 \sum_{i=1}^K \ln(\pi_i) \quad (5)$$

where: π_i is the i th independence claim in a basis set consisting of k claims. C can then be compared to a chi-square distribution with $2k$ degrees of freedom. In this case, the P value for the chi-square is higher than the chosen significance threshold (typically $\alpha = 0.05$). pH, RSM, OM, available N and P were transformed using the neperian logarithm to satisfy the normality assumptions of the models.

It is important to clarify that *Ageratina glabrata* was not included in the evaluation of the models due to the extremely low fruiting observed in most individuals during the study. This limitation made it difficult to obtain consistent and meaningful data, potentially biasing the results and reducing the robustness of the analysis.

3 Results

Flower and fruit pattern Different patterns of flower and fruit production were observed in this study. *Ageratina glabrata* showed higher flower production during the dry season. *Acaena elongata* showed a continuous pattern of flower and fruit production throughout the year (Fig. 4). Finally, *Solanum pubigerum* had a higher flower and fruit production during the dry season (November–March).

Interspecific variation Significant differences were observed between species and in the species:season:elevation interaction for AM fungal colonization percentage, but not for elevation, according to the linear mixed effects models (Table 2). *Acaena elongata* did not show significant differences in AM fungal colonization percentage between the rainy season ($\bar{X} = 34.93$, s.d. $\pm 5.67\%$) and the dry season ($\bar{X} = 39.75$, s.d. $\pm 5.14\%$). *Ageratina glabrata* showed a higher percentage of AM fungal colonization during the

Fig. 4 Mean $\bar{X} \pm$ s.d. values of the flower and fruit percentages over time in three secondary vegetation species in the *Abies religiosa* forest of the MRB. **A** Flower phenology. **B** Fruit phenology in *Ageratina glabrata* (circle marker), *Acaena elongata* (triangle marker) and *Solanum pubigerum* (square marker)

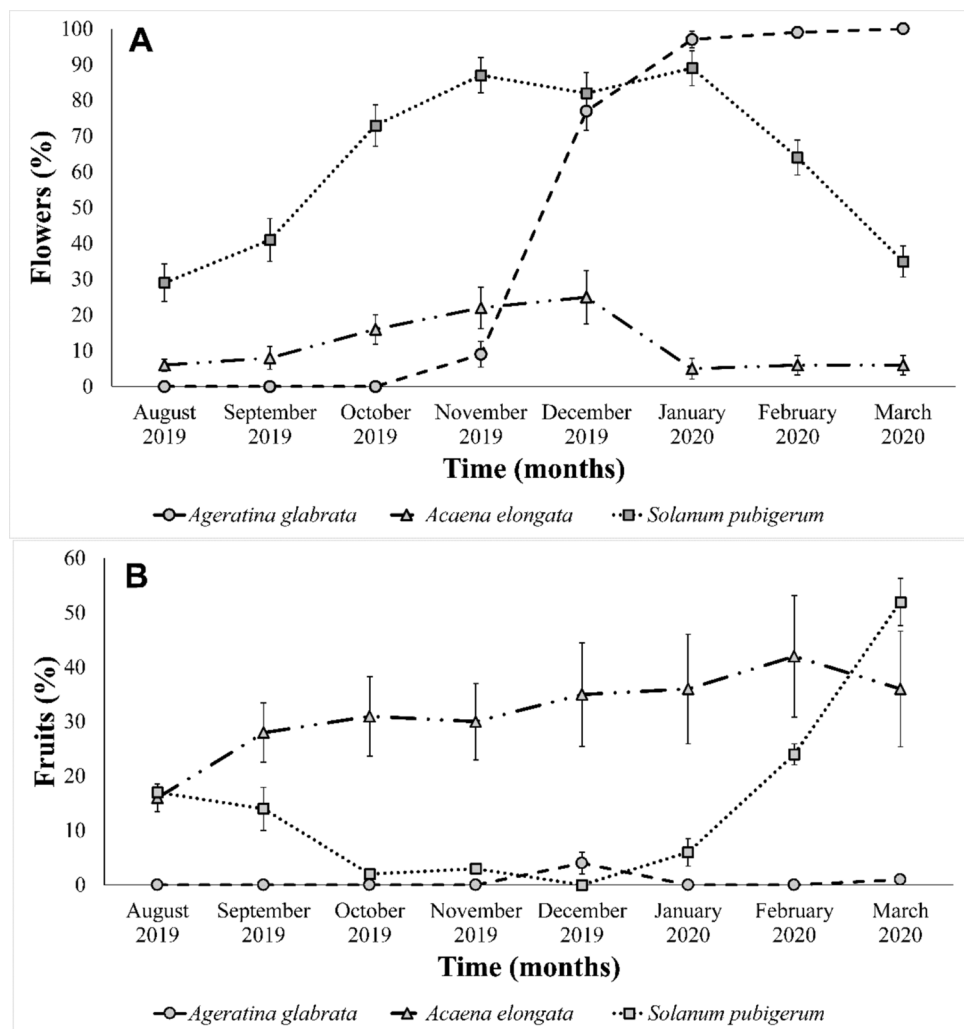


Table 2 Statistical summary of the mixed effects linear models (LME) implemented for AM fungal colonization, and Generalized Linear Mixed-Effects Models (GLMERs) for flower and fruit production and their interactions in host plant species of secondary vegetation within the temperate forest of *Abies religiosa* in Mexico City, Mexico

Source of variation	df	AMF coloniza- tion		df	Flowers		df	Fruits	
		F	P		X ²	P		X ²	P
Season	1, 176	0.23	0.63	1	4381.7	<0.01	1	47.41	<0.01
Elevation	1,2	0.23	0.67	1	0.1357	0.71	1	3.86	0.049
Host species	2,54	4.36	0.017	2	50.094	<0.01	1	12.75	<0.01
Season:elevation	1, 176	0.33	0.56	1	1.78	0.18	1	4.86	<0.01
Season: host species	2, 176	13.53	<0.01	2	3050.7	<0.01	1	8.16	<0.01
Elevation:host species	2,54	6.57	<0.01	2	0.003	0.96	1	1.31	0.19
Season:hosts:species:elevation	2, 176	0.25	0.777	2	3068.2	<0.01	1	301.3	<0.01

*Significant differences are marked with bolds

rainy season ($38.48 \pm 7.62\%$) compared to the dry season ($26.77 \pm 5.36\%$). Conversely, *Solanum pubigerum* showed a higher colonization percentage during the dry season ($36.05 \pm 6.03\%$) than during the rainy season ($32.37 \pm 5.03\%$) (Fig. 5).

Acaena elongata showed no differences in flower production between seasons. *Ageratina glabrata* showed the highest flower production during the dry season ($93.25 \pm 5.45\%$). *Solanum pubigerum* showed higher flower production

during the dry season ($67.57 \pm 12.04\%$) compared to the rainy season ($56.2 \pm 13.5\%$) (Fig. 5). The fruit production of *Acaena elongata* was $26.25 \pm 3.47\%$ in the rainy season and $37.25 \pm 3.16\%$ in the dry season.

The highest fruit production of *Solanum pubigerum* occurred in the dry season ($20.5 \pm 11.17\%$). Figure 1SI shows the variation of the percentages of colonization per fungal structure: hyphae, arbuscules and spores.

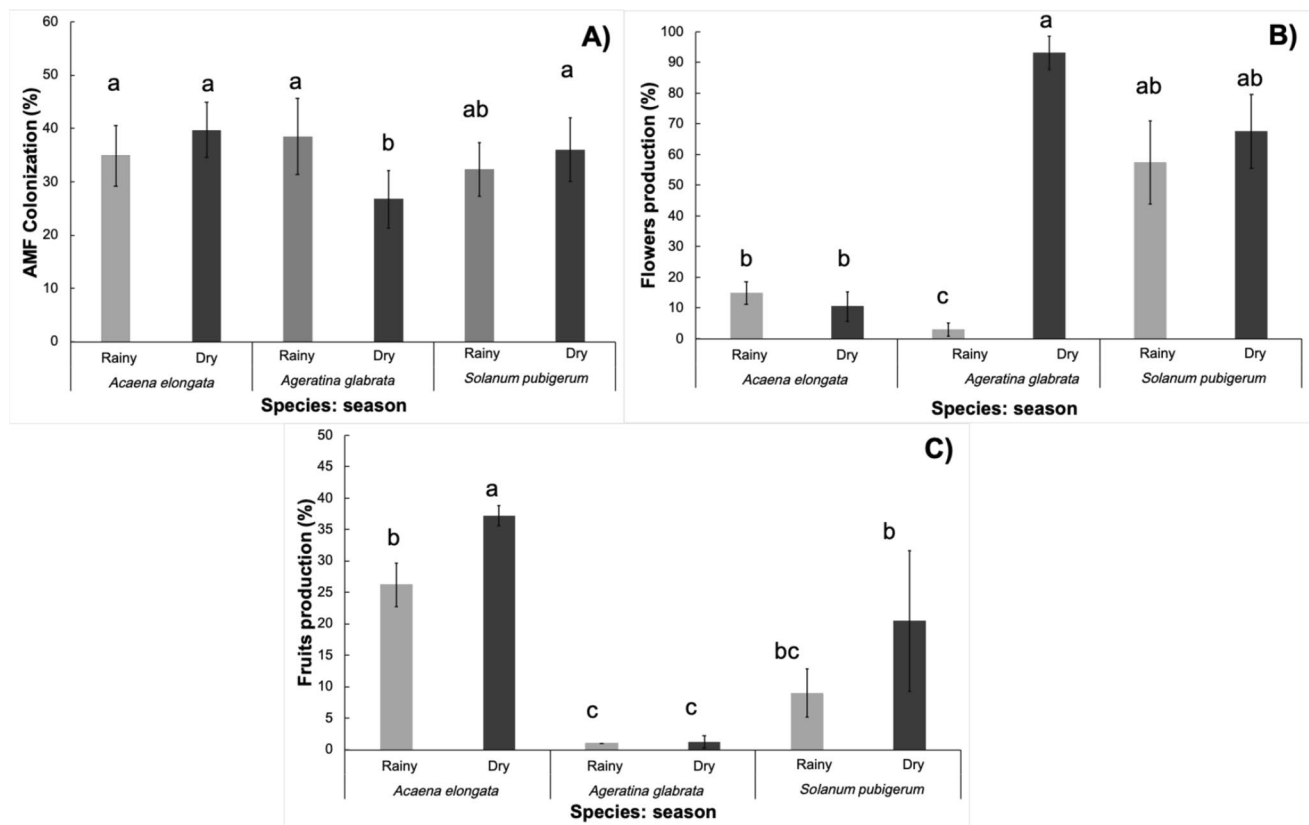


Fig. 5 Mean ($\bar{X}$) $\pm$ s. d. values of the AM fungal colonization percentage (A), flower (B) and fruit (C) production, for the species: season interaction. The light gray bars correspond to the rainy season, while the dark gray bars correspond to the dry season. Different letters indicate statistically significant differences $p < 0.05$ according to LME for AM fungal colonization percentage, and GLMER for flower and fruit percentage

Edaphic factors According to the ANOVA, edaphic factors showed significant differences related to the season, elevation and their interaction (Table 3). OM was higher at the highest elevation ($18.7 \pm 0.6\%$) compared to the lowest elevation ($17.8 \pm 1.7\%$). RSM was higher during the rainy season at the lowest elevation ($66.74 \pm 11.06\%$). Soil pH was more acidic during the rainy season at the lowest elevation (5.9 ± 0.001). Available phosphorus was highest during the dry season at the highest elevation (12.01 ± 2.29 ppm). Available nitrogen was higher during the rainy season regardless of the elevation (91.37 ± 8.45 mg kg⁻¹). The means $\pm$ SD (standard deviation) of OM, RSM, pH, available N and P are presented in Table 2SI.

Structural equation models: a particular case for *Acaena elongata* and *Solanum pubigerum* We observed a positive effect of the AM fungi on fruit production in both species. The magnitude of the effect was higher in *Acaena elongata* than in *Solanum pubigerum* (Fig. 6A and 6B). In the SEM for *Acaena elongata* (Fig. 6A), it was observed that fruit production was partially and positively influenced by the AM fungal colonization percentage, N availability and OM. However, available phosphorus had a negative effect on fruit production. While flower production was positively influenced by RSM. In *Solanum pubigerum* (Fig. 6B), fruit production was positively affected by AM fungal colonization percentage, although to a lesser extent than in *Acaena elongata*, and was also positively affected by pH and available nitrogen. pH also indirectly affected fruit production by reducing nitrogen availability. Flower production was negatively affected by available nitrogen. The calculated marginal and conditional R² values for each model were displayed in T3 SI.

4 Discussion

Our study highlights the influence of edaphic factors and AM fungal colonization percentage on flower and fruit production, although the strength of this combined effect varies among SV species, nevertheless, available N and P, OM, relative soil moisture, and pH had a more pronounced effect on flower and fruit production compared to AM fungal colonization in *Acaena elongata* and *Solanum pubigerum*. These plant species are contributing to the proliferation and persistence of AM fungal propagules in the soil following disturbance through previously colonized roots and rhizosphere-associated spores (Vázquez-Santos et al. 2025).

We observed interspecific and seasonal variations in the AM fungal colonization percentages among the three SV plant species. *Acaena elongata* showed similar and consistently high AM fungal colonization percentages in both seasons, *Ageratina glabrata* showed a lower colonization

Table 3 The statistical summary presents the results of a two-way ANOVA conducted on organic matter (OM), relative soil moisture (RSM), pH, available N, and available P in the temperate forest soil of *Abies religiosa* in Mexico City, Mexico

Source of variation		OM			RSM			pH			available N			available P		
	df	F	P	F	P	F	P	F	P	F	P	F	P	F	P	F
Season	1	51.2	<0.001	423.7	<0.001	218	<0.001	243.1	<0.001	243.1	<0.001	93.81	<0.001	93.81	<0.001	<0.001
Elevation	1	34.8	<0.001	15.26	<0.001	301	<0.001	19.31	<0.001	19.31	<0.001	531.8	<0.001	531.8	<0.001	<0.001
Season: Elevation	1	15.7	<0.001	1.84	<0.001	134	<0.001	0.045	<0.001	0.045	8.33	7.96	0.006	7.96	0.006	0.006

*Significant differences are marked with bolds

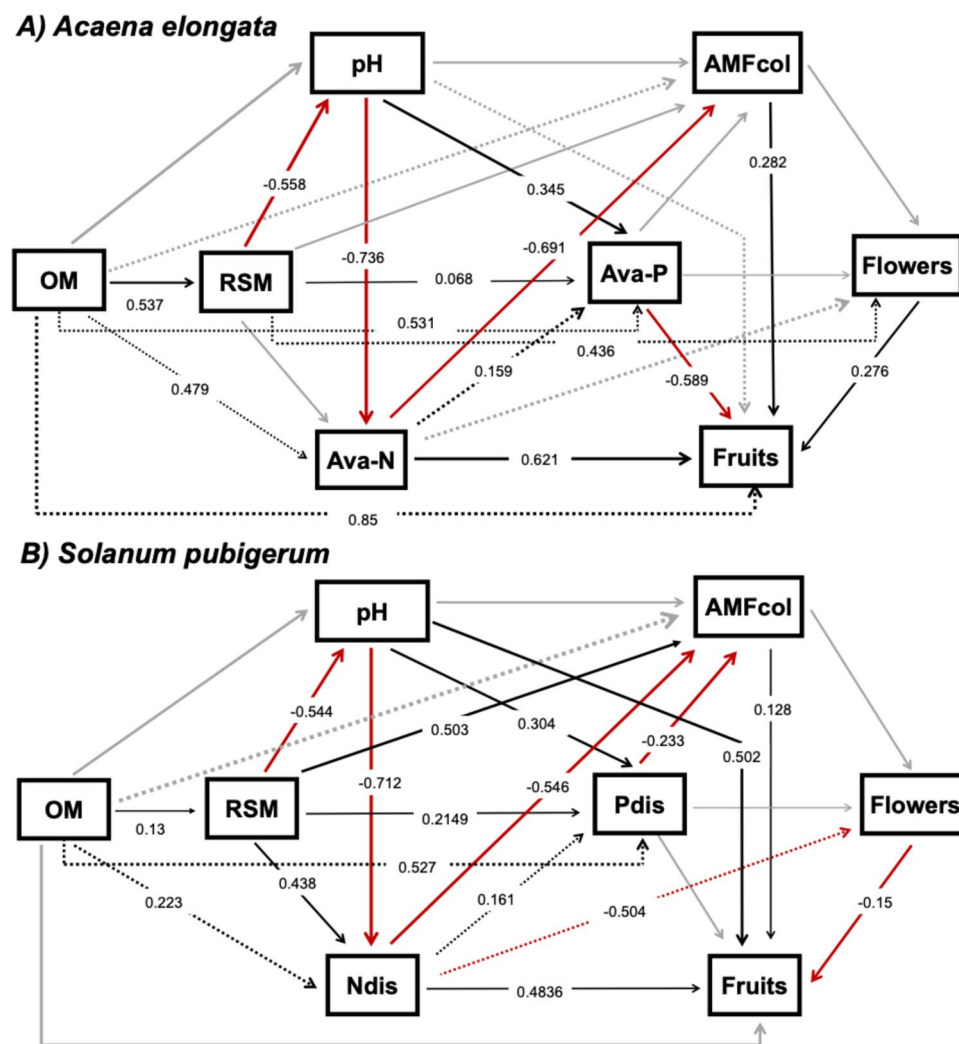


Fig. 6 Structural equation models (SEMs) showing the direct and indirect effects of AM fungal colonization on flower and fruit production of *Acaena elongata*. C value of Fisher = .307, $p = 0.858$, $df = 2$, AIC = 1327.293, AICc = 1547.293. **B** *Solanum pubigerum*, C value of Fisher = 6.925, $p = 0.31$, $df = 2$, AIC = 1416.64, AICc = 1673.312. Black solid arrows indicate positive relationships between the variables proposed in the theoretical model, while red solid arrows indicate negative relationships. Dashed arrows (black for positive, red for negative) indicate significant relationships that were not initially considered in the theoretical model but emerged during the analysis. Gray arrows represent non-significant relationships. The thickness of the arrows is proportional to the size of the effect (represent standardized path coefficients, between -1 and 1 , reflect the direct effect of one variable on another, the closer to ± 1 the stronger the coefficient), which is reported as the standardized effect size in the text boxes. OM = organic matter (percent), RSM = relative soil moisture (percent), pH, Ava-N = available nitrogen (ppm), Ava-P = available phosphorus (ppm), AMFcol = AM fungal colonization, flowers and fruits (production weighted with respect by plant cover)

and a significant decrease in the dry season compared to the rainy season, and *Solanum pubigerum* showed a higher AM fungal colonization percentage in the dry season. This variability can be attributed to the interplay between AM fungal colonization percentages and their functional role in nutrient supply, growth enhancement, germination and reproductive phenology of the host plant. It may also be influenced by the reproductive phenology of the AM fungi themselves. Also, variability may be influenced by the nutrient requirements of each plant species during flowering and fruiting, as well as by their biological traits (e.g., flower and fruit types and

sizes). AM fungi play a critical role in facilitating the uptake of essential nutrients, particularly nitrogen (N) and phosphorus (P), as was reflected in the SEMs (Smith and Smith 2012; Öpik and Davison 2016; Bennett and Meek 2020).

Acaena elongata showed no differences in flower production at the seasonal level, which is consistent with the continuous flower production reported in other studies (Pérez et al. 2011; Vázquez-Santos et al. 2019). We propose that the constant nutrient demand for flower production can explain this AM fungal colonization percentage pattern. A direct and statistically significant positive effect of AM fungal

colonization percentage on fruit production was observed in our SEM. A previous study by Vázquez-Santos et al. (2019) showed a significant negative correlation between AM fungal colonization percentage and flowering and fruit production in *Acaena elongata*. This can be explained by the fact that in this species there may be a trade-off between the resources allocated to its reproduction and those required to maintain the AM fungal associations. It highlights the importance of interspecific and contextual variation in how plants allocate their resources. It also shows the potential role of other factors, such as the reproductive phenology or carbon demand, in modulating this relationship, which warrants further investigation in future studies, including consideration of methodological intervention (sampling).

Ageratina glabrata showed higher flower production during the dry season when AM fungal colonization percentage was significantly lower. This suggests a negative relationship between these variables. However, the low availability of data limited a conclusive result. Nutrient requirements for reproductive allocation may be higher than the benefits provided by the mycorrhizal association during this process. As a result, the resources allocated to AM fungal activity in the roots decrease, increasing the resources available for flower development, making the association antagonistic (Johnson 2010; Vázquez-Santos et al. 2019). Maintaining the mycorrhizal association is energetically costly for the host plant, as the mycorrhiza consumes approximately 20% to 50% of the carbon fixed by photosynthesis, depending on the host plant species (Jakobsen and Rosendahl 1990). Thus, our results suggest that for *Ageratina glabrata*, the cost-benefit balance of the association with AM fungi is negative during the dry season, although it can also be explained by the low production of reproductive structures or a trade-off between resources allocated to reproduction and AM colonization. Long-term studies may confirm our suggestion also for flower and fruit production.

Solanum pubigerum showed higher flower and fruit production during the dry season, which corresponds to significantly higher AM fungal colonization. The increase in AM fungal colonization percentage during the dry season allows the plant to obtain the necessary nutrients and water for flowering and fruiting (Vega-Frutis and Guevara 2013). The structural equation model (Fig. 6B) revealed a partial positive correlation between AM fungal colonization and flower production, though soil variables like pH, organic matter, and available nitrogen had a stronger influence. The high AM fungal colonization percentage in the dry season is explained by the nutrient requirements of the host plant, as these fungi confer greater water stress tolerance to their host during dry periods (Treseder et al. 2004; Chaudhary et al. 2008). Thus, the arbuscular mycorrhiza of the three plant species is temporally related to the production of reproductive structures. While a positive correlation between flower

and fruit production and AM fungal colonization percentage is generally reported (Vega-Frutis and Guevara 2013; Bennett and Meek 2020), our results suggest that different correlation patterns between these variables may be related to specific biological traits and phenological patterns of the host plant. These patterns warrant further investigation through studies that quantify the specific influence of AM fungal colonization percentage on plant phenology, taking into account the ecological and biological traits of the host plants.

Consideration of the biological traits of host plants can help us understand the differences in the allocation of resources to reproduction. For example, dry fruit species produce significantly higher amounts of flowers and fruits than fleshy fruit species (Sutherland 1986). *Acaena elongata* and *Ageratina glabrata* are dry fruit species and showed higher flower and fruit production compared to *Solanum pubigerum*, a fleshy fruit species. Dry fruits are rich in lipids, fatty acids, and minerals, especially calcium and magnesium, and require less water for their production (Short and Epps 1976; Jordaan and Downs 2012). On the other hand, fleshy fruits require a high content of carbohydrates, proteins, and large amounts of water for their maturation (Cornejo-Tenorio and Ibarra-Manríquez 2007; Cortés-Flores et al. 2013). *Solanum pubigerum*, which has fleshy fruits, showed a positive correlation with AM fungal colonization percentage, likely due to the significant quantity and quality of nutrients and water required for their development. This relationship highlights the critical role of AM fungi in supporting the nutrient and water requirements for fleshy fruit production and needs to be explored.

It should be emphasized that the effect of AM fungal colonization percentage on flowering and fruiting may also be influenced by the biological traits of the symbiotic fungal species (Burkle and Zabinski 2022). Therefore, it is necessary to perform a detailed analysis of the AM fungal community structure in the host roots in future studies. This analysis would provide insight into the specific assemblages of AM fungal species associated with the rhizosphere of these SV species, and help assess how variations in AM fungal species turnover might influence flower and fruit production. Complementary experimental approaches, such as testing plant responses to colonization by specific AM fungal species, could further elucidate the functional roles of particular fungi species and their contributions to plant reproductive traits. Such insights could help to clarify the specific roles and interactions of different AM fungal species in relation to the reproductive processes of SV plant species.

AM fungal colonization percentage, flowering, and fruiting were influenced by the interaction of elevation and species. This suggests that an elevation gradient affect abiotic factors, AM fungal colonization and flower and fruit production in host plant species. For example, high-elevation sites

often have low nutrient availability due to factors such as low temperatures, short growing seasons, and slow decomposition rates of OM (Körner 2007; Sundqvist et al. 2014; Chen et al. 2017), as observed in this study. Under these conditions, plants may rely more heavily on mycorrhizal fungi for nutrient acquisition, resulting in higher AM fungal colonization percentage. In contrast, at lower elevation, warmer temperatures may promote more rapid decomposition of OM, resulting in increased nutrient availability (Chen et al. 2017). Therefore, plant dependence on mycorrhizal fungi may be reduced, resulting in lower AM fungal colonization percentage and higher flowering and fruiting (Laiolo and Obeso 2017).

Edaphic factors are known to be important modulators of AM fungal colonization (Wu et al. 2022). Similarly, flower and fruit production of temperate forest plant species is generally related to abiotic factors such as temperature, light, precipitation, and edaphic factors (Valdez-Hernández et al. 2010). However, although studies identify the variables that influence biological processes, there is a complex interaction between AM fungi, plants, and their environment (Zobel and Öpik 2014). Therefore, both biotic and abiotic factors should be included in an analysis to explain how soil processes affect aboveground processes. This integrated approach can help elucidate the intricate dynamics governing plant productivity and ecological interactions in temperate forests.

SEM analysis is a powerful tool for understanding the complex interactions that occur both above and below ground. The results obtained in this study through SEM are highly significant, indicating that the data obtained fit satisfactorily the proposed theoretical model. Relative soil moisture has an indirect effect on flower and fruit production because it affects soil pH and increases soil N availability. Fruiting was positively and directly affected by soil OM, available N, pH and AM fungal colonization. In contrast, flowering was directly affected by available N. The AM fungal colonization percentage is indirectly and negatively affected by RSM by modulating N and P availability. The magnitude of the effect between variables differed among plant species. In *Acaena elongata*, the magnitude of the effect of AM fungal colonization on fruiting was higher than in *Solanum pubigerum*. In *Solanum pubigerum*, available P negatively affected AM fungal colonization percentage, but this relationship was not observed in *Acaena elongata*.

Nutrient availability in the soil is one of the main factors controlling flowering and fruiting, as well as AM fungal activity in the roots (Smith and Smith 2012). Available N in the soil has been shown to stimulate fruiting (Wang et al. 2015), but does not affect flower production, resulting in a negative effect. However, the high rates of N mineralization reported at the study site (Facio-López 2015) may indicate that nitrogen is rapidly cycled and potentially immobilized by microbial activity or lost through processes such as

leaching. These dynamics, combined with plant-specific N requirements and temporal variation in N availability, could explain the observed patterns. Similarly, N and P concentrations are negatively related to the AM fungal colonization percentage (Smith and Read 2008; Horsch et al. 2023). This supports the idea that AM fungal colonization is favored when there are low concentrations of these nutrients in the soil.

In conclusion, our study shows that edaphic factors such as available P and N, RSM, pH and AM fungal colonization percentage play a partial role in the reproductive responses of SV plant species, with their influence varying among species and seasons. Specifically, the results show a partial influence of AM fungal colonization percentage, but a stronger influence of soil factors such as pH, OM, and available N on flower and fruit production in *Acaena elongata*. In contrast, the influence of AM fungal colonization was low for *Solanum pubigerum* and inconclusive for *Ageratina glabrata*. These results emphasize the predominance of edaphic factors and AM fungal colonization in shaping reproductive traits in SV plant species in temperate forests.

Nevertheless, the presence of arbuscular mycorrhizae in the studied plant species appears to facilitate resource acquisition for reproduction, especially under the challenging conditions of disturbed forest environments. Our results provide valuable insights into the interplay between reproductive allocation, edaphic factors, and AM fungal colonization. Further research is needed to elucidate the mechanisms driving these interactions, including the functional diversity of AM fungi species and their potential contributions to natural regeneration in temperate forests experiencing persistent disturbance.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s40415-025-01087-2>.

Acknowledgements We are grateful to Brenda Guerrero Bautista, Ariadna Peralta Valencia, and Irene Sánchez Gallen for their help in the field work and technical support in laboratory. This research is part of the doctoral studies of Y. Vázquez-Santos in the Posgrado en Ciencias Biológicas of the Universidad Nacional Autónoma de México. Y. Vázquez-Santos acknowledges Secretariat of Science, Humanities, Technology and Innovation (SECIHTI), Mexico (No. 818569) for scholarships to get te PhD.

Author contributions YVS, SCA, NM and FE: Conceptualization; YVS, YMO and SCA: Methodology and Fieldwork; YVS, SCS and EV: Data Collection and Data Analysis. YVS: Writing—Original Draft; YVS, NM, FE, YMO, SCA, SCS and EV Writing- Reviewing and Editing.

Funding This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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

Declarations

Conflict of 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.

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