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Ecology / Ecología

Assessing ecological traits of a secondary vegetation species in temperate forests of central Mexico: a case study

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Abstract
Background: Plant species used for reforestation purposes are idoneous if native, and also if they are present in regeneration sources. High germination percentages under different light conditions and a broad phenological pattern enhance adaptability to environmental heterogeneity. Preference for species responsive to mycorrhizal inoculation is recommended.
Questions: Is *Solanum pubigerum* a widespread species in temperate forests of central Mexico?, under which light conditions germination will be the highest?, how abundant is it in natural regeneration sources?, does inoculation with mycorrhizal fungi benefit its germination and growth?
Studied species / data description / Mathematical model: *Solanum pubigerum*/distribution in central Mexico forests, germination and viability percentages, reproductive phenology, growth dependence on AM fungi/ Kruskal-Wallis test, synchrony, Spearman correlations, ANOVA.
Study site and dates: *Abies religiosa* forest, Mexico City. August 2019 – July 2020.
Methods: Seeds of *S. pubigerum* were exposed to different light qualities, their viability was monitored for two years. Its presence in natural regeneration sources was registered. Reproductive phenology was recorded, and seedlings were inoculated with arbuscular mycorrhizal fungi to assess their growth.
Results: Highest seed germination occurred under white light conditions, with sustained viability after two years. The species was found in the seed bank across seasons, less abundantly in seed rain during the dry season. It exhibited extensive flowering and fruiting patterns. Mycorrhiza inoculation significantly boosted seed germination and growth.
Conclusions: *Solanum pubigerum* is suitable for reforestation in central Mexico forests due to its high germination percentages and mycorrhizal responsiveness.
Keywords: arbuscular mycorrhizal fungi, natural regeneration, shrubs, Solanaceae.

Resumen
Antecedentes: En la reforestación se recomiendan plantas nativas y en fuentes de la regeneración natural. Altos porcentajes de germinación bajo diferentes condiciones de luz y patrones fenológicos amplios incrementan la adaptación en ambientes heterogéneos. Se sugieren especies que tengan una dependencia a la inoculación con hongos micorrizógenos.
Preguntas: ¿*Solanum pubigerum* está ampliamente distribuida en bosques templados del centro de México?, ¿en qué condiciones lumínicas germina?, ¿está en fuentes de la regeneración natural?, ¿la micorriza beneficia su germinación/crecimiento?
Especie de estudio / descripción de los datos /Modelo matemático: *Solanum pubigerum*/distribución en bosques del centro de México, porcentajes de germinación y viabilidad, fenología reproductiva, dependencia micorrícica/Kruskal-Wallis, sincronía, correlaciones de Spearman, ANdeVA.
Sitio de estudio y fechas: Bosque de *Abies religiosa*, Ciudad de México. Agosto 2019 - julio 2020.
Métodos: Las semillas de *S. pubigerum* fueron expuestas a diferentes calidades de luz, su viabilidad fue monitoreada por dos años, así como su presencia en la regeneración natural. Se registró su fenología reproductiva, se inocularon plántulas con hongos micorrizógenos arbusculares para evaluar su crecimiento.
Resultados: La mayor germinación ocurrió en luz blanca, la viabilidad se mantuvo en altos porcentajes después de dos años. *S. pubigerum* está en el banco de semillas en ambas épocas, fue menos abundante en la lluvia de semillas en la época seca, con un patrón de floración-fructificación amplio. La inoculación micorrícica incrementó su germinación y crecimiento.
Conclusiones: *Solanum pubigerum* se recomienda para reforestar bosques del centro de México por sus germinación y respuesta a la micorrización.
Palabras clave: arbustos, hongos micorrizógenos arbusculares, regeneración natural, Solanaceae.

Temperate forests have been one of the most profoundly altered ecosystems in history due to agriculture, livestock, and urbanization (Contosta *et al*. 2022). Reforestation programs have been designed and implemented to conserve and restore temperate forests in central Mexico (Galicia *et al.* 2015). However, these programs often fail due to low seedlings´ survival rates (30-50 %). This is the result of several factors, such as poor planting techniques, inappropriate timing, and/or lack of knowledge about the site characteristics (Honey-Rosés *et al*. 2018), as well as lack of mycorrhizal inoculation (Perry *et al*. 1987). Additionally, some management practices, affect the success of reforestation such as weeding (removal of shrubs and herbs), which is carried out in this area to reduce competition between shrubs and tree seedlings/saplings, because it reduces soil moisture content (Martínez-Orea *et al*. 2019). By reforestation success the survival of a high percentage of the reforested plants is considered (Derhé *et al.* 2016). Shrubs, like trees, should also be included in reforestation practices as it enhances the biodiversity and resilience of forest ecosystems. Shrubs play a critical role in providing understory habitat for a variety of wildlife species, contributing to the structural diversity of forests, and facilitating nutrient cycling and soil stabilization (Xiao *et al.* 2017). They often serve as pioneer species that quickly colonize disturbed or degraded areas, paving the way for the establishment of a more diverse plant community (Günter *et al.* 2009, Xiao *et al.* 2017). In addition, their diverse root systems can be critical in preventing soil erosion, improving water retention, and contributing to the carbon sequestration potential of reforested areas. As such, their inclusion is a strategic component in the pursuit of sustainable and resilient reforestation initiatives.

Some shrubs species in temperate forests, such as *Baccharis* sp. act as nurse plants for conifer seedlings/saplings (Lu *et al.* 2017, Perea *et al*. 2022). Some other species of shrubs form corridors between temperate forest fragments (Martínez-Orea *et al*. 2020), positively influencing natural regeneration (van Hall *et al*. 2017, Argüelles-Moyao 2018), as reported for species such as *Ageratina glabrata* L. (Asteraceae) (Mendoza-Hernández *et al*. 2013). However, there is still a lack of research about the benefits of the use of other shrub species in reforestation.

Secondary vegetation species are those which establish after anthropogenic disturbances. These plant species (such as shrubs) establish a mutualistic relationship with arbuscular mycorrhizal fungi (AMF), allowing soil connective networks and interactions that can be maintained even after a disturbance, depending on its frequency, type, and magnitude (van der Heijden *et al*. 2015). This relationship represents an ecological strategy for plant establishment and survival in disturbed sites where environmental stress is high, as AMF buffer against environmental conditions that could be detrimental to plants (Smith & Read 2008). Shrub species are part of secondary forests, which have reached 50 % in Mexico (Honey-Rosés *et al*. 2018). In addition to the benefits mentioned above, shrub species should be considered suitable for reforestation purposes for several reasons. Some secondary vegetation species can grow in degraded sites as they are tolerant to changes in some environmental conditions after anthropogenic disturbance in temperate forests (Santibáñez-Andrade *et al*. 2015a, b, Bonilla-Valencia *et al*. 2022a, b). The presence of understory vegetation with a shrub layer is significantly diminished in well-conserved sites within temperate forests in central Mexico (Calderón de Rzedowski & Rzedowski 2005). However, in areas impacted by livestock and agriculture, species such as *Solanum pubigerum* Dunal (Solanaceae) become characteristic (Rojas-Zenteno *et al*. 2016).

A starting point in reforestation programs is to choose the idoneous plant species to be included. It is important to use native plant species present in regeneration sources, such as soil seed bank (Díaz-Espinosa & Vargas-Ríos 2009, Tiebel *et al.* 2018). Authors such as Gandolfi *et al*. (2007) mention that species used in reforestation should contribute to activate the ecological successional dynamics of the site. In terms of the requirements for the germination of their seeds, they should germinate in high percentages and under different types of light, since the sites for their introduction can be heterogeneous (Martínez-Orea *et al*. 2020). It is also important that the plant species used for reforestation establish mycorrhiza with soil fungi; certain species of Solanaceae that are part of the secondary vegetation, such as *Solanum nigrum* L., establish this type of symbiosis (Tarafdar & Rao 1997). It is also convenient that they show a wide phenological pattern, thus contributing to a constant availability of resources for pollinators and fruit dispersers (Buisson *et al*. 2017). The aim of this study is to analyze some ecological traits of *S. pubigerum* in order to propose it for reforestation programs in temperate forests of central Mexico. We expect *S. pubigerum* to be a species widely distributed in temperate forests of this area. Germination percentages will be the highest in white light conditions because it is a secondary vegetation species, the association with arbuscular mycorrhizal fungi will benefit its germination and growth. Also, as an idoneous plant species for reforestation, we expect to find it in regeneration sources such as seed rain and seed bank. A wide phenological pattern of *S. pubigerum* will allow the presence of resources (flowers and/or fruits) throughout the year for pollinators and fruit dispersers.

Materials and methods

Study site. This study was conducted in the *Abies religiosa* (Kunth) Schltdl. & Cham. forest of the Magdalena River basin (MRB) in Mexico City (Figure 1). This forest has an area of approximately 1,130 ha (Galeana-Pizaña *et al*. 2009). The climate is subhumid with summer rains and a mean annual temperature of 18 °C and a mean annual rainfall of 1,250 mm (García 2004). In this area there is a rainy season that extends from May to October and a dry season from November to April. The importance of this forest in terms of ecosystem services, among others, is that it can store an average of 163 Mg C ha^{-1} (Galeana-Pizaña *et al*. 2013). It has a high-water filtering capacity, contributing with 52 % of the total rainfall in the MRB (Jujnovsky *et al*. 2012). The soil type is humic Andosol (AN hu: WRB in IUSS Working Group 2015), with high amounts of available nitrogen (> 60 mg kg^{-1}), while the availability of phosphorus is 5.5-11 mg kg^{-1}. Since this forest is located in one of the biggest cities in the world, it is subject to constant anthropogenic disturbances that have affected in several ways the vegetation, soil quality (Santibáñez-Andrade *et al.* 2015a) and fungal structure. According to Santibáñez-Andrade *et al*. (2015a) conserved and anthropogenically disturbed areas conform this forest, depending on topographic heterogeneity and slope orientation.

Study species. Solanum pubigerum Dunal (= *Solanum cervantesii* Lag, and = *Solanum glabrum* Dunal (WFO 2023) = *Solanum cervantesii* subsp. *Glabrum* (Dunal) C.V. Morton ex S. Knapp & *et al*.) (IPNI 2023, Tropicos 2023) is a native shrub species, 1 to 5 m tall, with fleshy fruits of about 5 to 10 mm in diameter, black at maturity containing seeds of 3.5 mm long. It is found in temperate forests exposed to livestock, agriculture, urbanization, and deforestation (Hortelano-Moncada & Cervantes 2011, Jiménez-Hernández *et al.* 2023). It is also present in the valley of Mexico, at altitudes from 2,250-3,200 m asl. It has a wide ecological tolerance, being found from the driest to the moister parts of this region and it is found from central Mexico to El Salvador and Honduras (Calderón de Rzedowski & Rzedowski 2005). We consulted the following pages in the web to obtain the species distribution in the country: GBIF (2023) with data from DGRU (2023) (MEXU), FCME, CONABIO, ENCB-IPN and Naturalista (2023), herbarium material is the source for the building of these data bases (Figure 1).

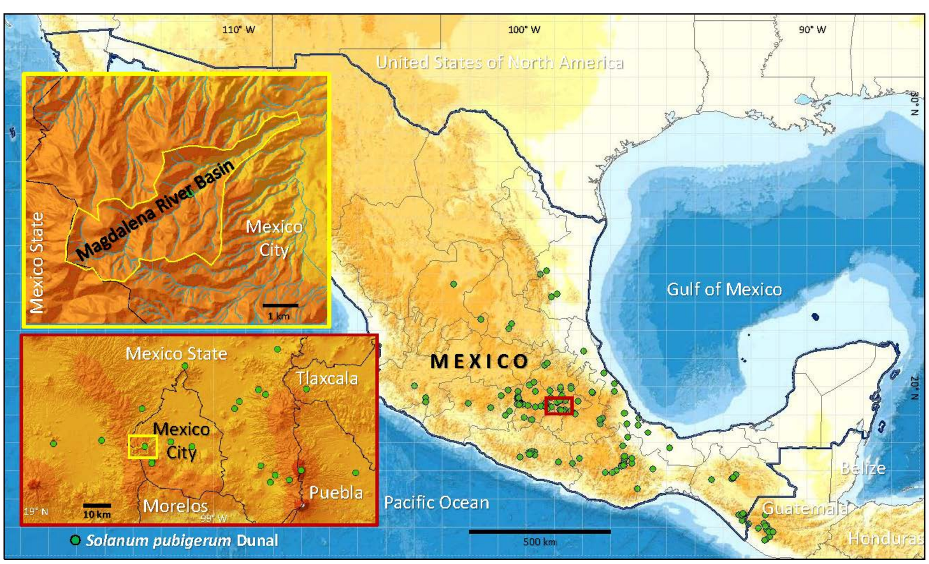

Figure 1. Geographic distribution of *Solanum pubigerum* Dunal in Mexico. The Magdalena river basin (MRB, study site) location (west Mexico City) is observed (Map: INEGI 2022, INEGI-MDM 2023, points: GBIF 2023).

According to Almeida-Leñero *et al*. (2014) *S. pubigerum* forms plant associations or subcommunities in fir and oak forests with species such as *Furcraea parmentieri* (Roezl) García-Mend. (Agavaceae), *Trisetum virletii* E.Fourn. ex Hemsl. (Poaceae), *Muhlenbergia cenchroides* (Humb. & Bonpl. Ex Willd.) P.M.Peterson (Poaceae). These authors characterize the forest sites where these species coexist as shallow in soil depth with low organic matter content in forest clearings with north, east and south slopes, at altitudes between 3,020-3,100 m, and soil pH values of 5.9-6.2.

Ecological traits of Solanum pubigerum. Germination.- Fruits of *Solanum pubigerum* were randomly collected from 30 individuals in July 2020, seeds were cleaned from the fleshy pulp, and they were disinfected in a 10 % sodium hypochlorite solution for 10 minutes. We stored a stock of seeds to use in other experiments (seed viability). For germination tests we sown seeds in Petri dishes with filter paper (50 seeds/petri dish) in different light qualities (darkness, white, far red, and red light) in a germination chamber (NuAire model I-36LL, Massachusetts, USA) at 22 °C/20 °C, 16/8 photoperiod (commonly used for temperate forest species germination of Solanaceae, Kew 2023). The light conditions were as follows (1) white light (WL; photon flux density (PFD) = 33.21 µmol $m^{-2}s^{-1}$, R:FR = 1.73; (2), red light (RL; PFF = 5.18 µmol $m^{-2}s^{-1}$, R:FR = 3.39), (3) far red light (FRL; PFF = 1.2 µmol $m^{-2}s^{-1}$, R: FL= 0.05), and (4) darkness (D). For the red-light treatment, Petri dishes were set inside a red plexiglass box (3 mm thick, 48 × 32 × 8 cm, Series 2424 Rohm and Hass, Mexico). For the far-red light treatment, Petri dishes were set in a red plexiglass box with a cover of blue plexi-glass (same dimensions of the red box, Series 2423). For the darkness treatment, Petri dishes were covered with aluminum foil. For the treatment under white light, the Petri dishes were just set inside the germination chamber equipped with fluorescent lamps (OSRAM of 17 watts and 60 % relative humidity). Every other day (and for 30 days) we registered germination (emergence of radicle) only for white light, germination t_0 (first germination) and t_{50} (germination of 50 % of the seed population) were also registered (Martínez-Villegas *et al.* 2018). Germination percentages were calculated for all light qualities.

Seed viability tests were carried out through the staining technique of tetrazolium (2,3,5 chlorine-triphenyl-tetrazolium) with seeds collected in 2020, one and two years (2021, 2022) after storage under dry/dark room conditions, at mean temperature of 22 °C.

Seed rain and seed bank.- To assess the presence of *S. pubigerum* in natural regeneration sources, we collected 45 samples of the top 8 cm of soil using a Gopher Auger Kit, 3-1/4", in the rainy season (July 2020) and in the dry season (February 2021). These soil samples were placed in a greenhouse at the study site (*Abies religiosa* forest) in plastic trays (25 × 15 × 6 cm^3) that were watered every other day. Every two weeks we quantified and identified the emerging seedlings or transplanted them to larger trays to allow their growth, if necessary for their identification. In the same site, we randomly placed 45 seed rain traps at the soil level for each season. The traps were circular (50 cm in diameter, 25 cm in deep), and made of metal and cloth mesh. From June 2020 to June 2021, and every two months, we collected the material deposited in the traps and quantified and identified the seeds.

Reproductive phenology.- In the field we randomly chose two sites in the *Abies religiosa* forest in the MRB (3,192-3,254 m asl). A soil sample of 200 g was collected at each site. These samples were then analyzed to record some variables: (i) organic matter (OM) content by wet digestion (Walkley & Black 1934); (ii) relative soil moisture (RSM) by measuring fresh and dry weight at 105 °C (Reynolds 1970); (iii) available phosphorus (PO_4^-) by $NaHCO_3$ extraction at 0.5 M and pH 8. 5 in a colorimetric determination (Olsen *et al*. 1954); (iv) available nitrogen (N), the sum of nitrate (NO_3^-) and ammonium (NH_4^+), using a KCl solution and analyzed by chromatography (KCl_2 technique); and (v) pH determined with a potentiometer in a 1:2 ratio in deionized water (true acidity), according to the NOM-02-SEMARNAT-2000 (DOF 2002) techniques.

In each site, we selected 30 *S. pubigerum* adult individuals of similar heights (1.50 m aprox.). We monthly registered the proportions of fruits and flowers of each individual using the phenological scale proposed by Fournier & Charpentier (1975), this was carried out during eight months (August 2019-March 2020).

The proportion of reproductive structures of each individual was determined based on their total cover. The total cover was obtained by measuring the canopy perpendicular diameters, we assumed that for each individual, cover is similar to the area of a circle, therefore it was calculated as follows:

Equation 1

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

Where C: cover, D1 = horizontal diameter, and D2 = vertical diameter.

Growth dependence on AM fungi.- From July 2019 to January 2020, we collected more fruits of our study species, and cleaned 200 seeds. We also randomly collected 25 kg of soil (20 cm of surface soil) in our study area. Soil was homogenized and divided into two equal parts. One was used as inoculum in the treatments “+AMF” (with mycorrhiza), supposing that there were enough spores, colonized fragments of roots and mycelium. Vázquez-Santos *et al.* (2019) examined the diversity and spore count of AMF in 80 soil samples from the study site. The spore density was reported to be 70 spores/50 g of soil. The study identified 29 morphospecies of AMF belonging to nine families and ten genera. The genus *Acaulospora* was predominant, accounting for 77.7 % of all spores, followed by *Ambispora* (9.07 %), *Claroideoglomus* (4.96 %), *Funneliformis* (3.8 %), and others including *Archaeospora*, *Diversispora*, *Glomus*, *Rhizophagus*, *Sclerocystis*, and *Scutellospora*, each accounting for less than 2.5 %. The other part of the soil was pasteurized at 100 °C in a vertical autoclave for one hour; this procedure was repeated for three consecutive days for the same time and temperature, in order to eliminate AMF propagules and was used for treatments “-AMF” (without mycorrhiza).

In the forest, we carried out a greenhouse experiment during July 2021. We collected more fruits and cleaned 80 seeds from the fleshy pulp, we disinfected them with sodium hypochlorite 10 % for 10 minutes. They were set for germination in pots with 250 g of soil (pasteurized and non-pasteurized soil). Pots were organized in two blocks, maintaining them separated in order to prevent contamination between treatments (+AMF and – AMF) (n = 60, 2 treatments × 6 individuals × 5 harvests). We carried out five harvests, extracting six individuals per treatment each month, we measured radicle size, shoot size and seed size using a caliper. Seed vigor was determined by the sum of shoot and radicle sizes. A pool of 20 seeds was weighed on an OHAUS Adventurer® balance – 420 g × 0.001 g. After obtaining these measurements, seedlings were dried in a Riossa® oven, at 72 °C for 24 hours (until reaching constant weight), we obtained the root dry and stem weights and with an analytic scale OHAUS®. With these values, we obtained the total weight value.

Simultaneously, we extracted the roots from six individuals of each treatment (6 individuals × 2 treatments × 5 harvests), during the same months in order to calculate the AMF colonization through the method proposed by McGonigle *et al*. (1990). We extracted the fine roots to process them by the Koske & Gemma (1989) method through trypan blue (0.05) staining. Observations were carried out using an optic microscope (NIKON®) (20× and 40×). We registered fields colonized by hyphae (aseptated), arbuscules (fungal structures similar to little trees), vesicles and spores. Colonization percentages were obtained as follows:

Equation 2

$$\text{AMF Colonization} = \frac{\text{Number of colonized fields}}{\text{Number of observed fields}} \times 100$$

Where the total number of observed fields was at least 80.

Mycorrhizal dependence index (IRM).- This index estimates the effect that AMF have on plants total dry weight (TDW). It compares the results of two treatments in which the difference is the presence of mycorrhizae and the values range between - ∞ and 100 % (Plenchette *et al*. 1983), and it expresses the difference in dry weight of plants with and without mycorrhizae in terms of dry mass of the plant with mycorrhizae in percentage.

Equation 3

$$\text{IRM} = \left(\frac{\text{TDWm (g)} - \text{TDW nm(g)}}{\text{TDW m}}\right) \times 100$$

Where: TDWm = Total dry weight of the plant with mycorrhiza and TDWnm = Total dry weight of the plant without mycorrhiza.

Data analysis. Germination.- Germination percentages were obtained for each quality light type. Germination rate and lag time were obtained as well. A Kruskal-Wallis test was carried out with the germination values between light types (STATISTICA 8.0: StatSoft 2007). Germination values in each petri dish were transformed with arcsin function of the square root (Zar 1999), they were then related to time using the exponential sigmoid function $y = \frac{\text{a}}{1 + ((\text{b}) * (\text{xc}))}$ to calculate lag time and germination rate (Table Curve 2D, Systat 2023).

Reproductive phenology.- The synchrony indexes (Z) values were calculated according to Augspurger (1983) through the following equation:

Equation 4

$$Xi = \left(\frac{1}{n-1}\right)\left(\frac{1}{fi}\right)\sum_{j=i}^{n} ej \neq i$$

Where: Xi = individual synchrony index, n= number of individuals in the population, fi = number of days in which the individual I has a phenological event and ej = number of days in which both individuals i and j have a phenological event in common.

Later, with the individual's synchrony index, we obtained the population synchrony index (Z) with the equation proposed by Augspurger (1983).

Equation 5

$$Z = \left(\frac{1}{n}\right)\sum_{j=i}^{n} Xi$$

Where: Z = population synchrony index, n is the number of individuals in the population and Xi = synchrony index per individual obtained with equation 4. When Z = 1 it indicates complete synchrony, whereas Z = 0 implies that there is not synchrony (Augspurger 1983, Bonilla-Valencia *et al*. 2017a, b).

Growth and dependence on AM fungi.- Spearman correlations were performed using the "corrplot" library (Friendly 2002) with a confidence level of 0.95 to evaluate the relationship between radicle size, shoot, seed vigor, seed size, seed weight, germination, and AMF colonization. In addition, with an analysis of variance (ANOVA), we estimated the effect that AMF have on root/shoot/total dry weight of plants through the index of response to mycorrhiza (IRM). Data were previously transformed (arcsin function) to meet the normality assumptions. Post-hoc Tukey's tests were carried out.

Results

Germination. The highest germination percentage of *S. pubigerum* seeds occurred under white light conditions (85.6 %). Percentages were similar between red light and darkness (52 and 69 % respectively). The lowest germination percentage corresponded to far-red light (5 %). The Kruskal-Wallis test showed significant differences between germination percentages of quality light types (χ^2 = 16.45, P = 0 0.0009). The maximum germination rate was 6.3 ± 0.21 (% day^{-1}), t_0 was 12.4 ± 1.24 days, and t_{50} 25 days. The seed viability values were 90 % at the collect year, which was maintained for 2021. Two years after seed collection and storage (2022) this value was 80 %.

Ten shrub species were registered in the seed bank, among them *S. pubigerum* represented 20 % (194 seedlings) of the seedlings in this regeneration source, with 50 % of the seedlings emerged in the soil collect corresponding to the dry season and 50 % to the rainy season. In the seed rain, among the 20 species of shrubs registered, *S. pubigerum* had 10 seeds (0.17 %) and only in the dry season.

Reproductive phenology. Solanum pubigerum showed a broad span of flowering mainly from October to February in both sites with high values of synchrony (Site 1, Z = 0.85; Site 2, Z = 0.73) between the sampled individuals. Between November and January, we registered the highest percentages of flowers, these correspond to the dry season (Figure 2).

In February, March and August, the highest percentages of fruiting were registered, mostly in site 1. The synchrony index values were lower between individuals compared to flowering, site 1 showed the highest value (Z = 0.65) compared to site 2 (Z = 0.31) (Figure 3).

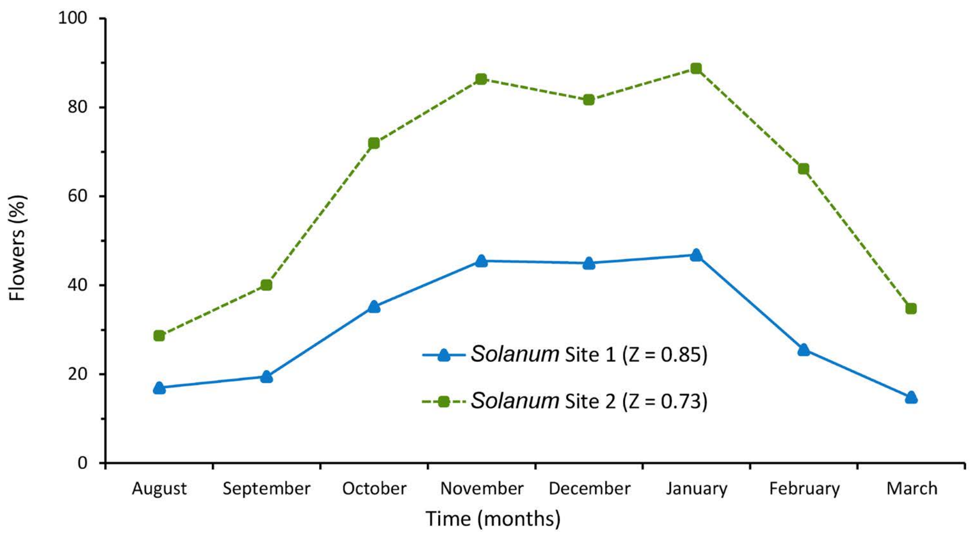

Figure 2. Percentages of flowers of the individuals of *S. pubigerum* in the Magdalena river basin, Mexico City, Mexico in two sites (*Solanum* S1 = site 1, *Solanum* S2 = site 2) of the *Abies religiosa* forest. Z = Synchrony index, values of Z near 1 indicate complete synchrony, and Z near to 0 imply synchrony absence.

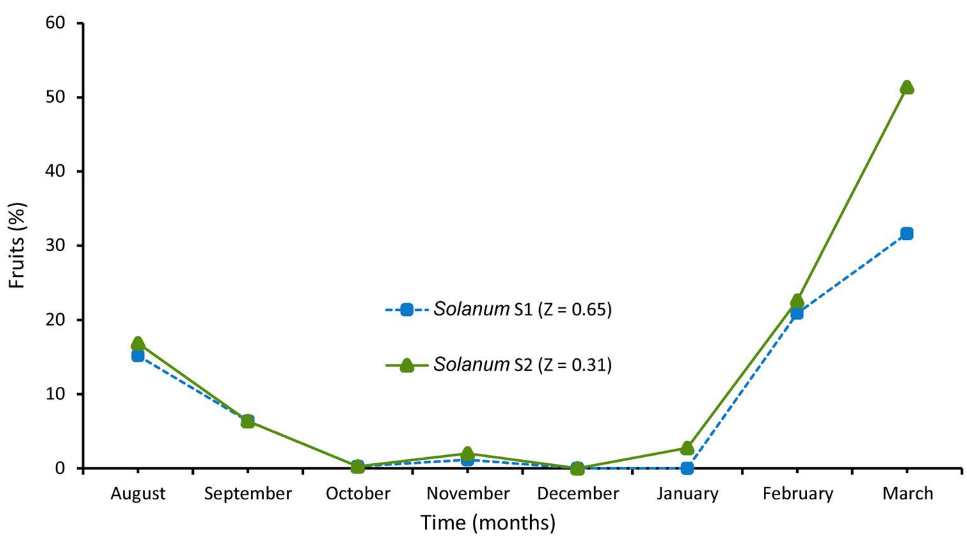

Figure 3. Percentages of fruits of the individuals of *S. pubigerum* in the Magdalena River basin, Mexico City, Mexico in two sites (*Solanum* S1 = site 1, *Solanum* S2 = site 2) of the *Abies religiosa* forest. Z = Synchrony index, values of Z near 1 indicate complete synchrony, and Z near to 0 imply synchrony absence.

Table 1. Mean values ± standard error of temperature (tem), light, RSM (relative soil moisture), pH, electric conductivity (EC), organic matter (OM), phosphorus (P) and N (nitrogen), for two sites in the *Abies religiosa* forest in the Magdalena River basin, Mexico City, in the rainy and the dry season.

Site	Season	Tem	Light	RSM	pH	EC	OM	P	N
S1	Rainy	9.3	18.27	42.88	6.13	0.06	25.36	6.25	0.7
		± 0.09	± 0.94	± 3.34	± 0.12	± 0.01	± 4.24	± 0.83	± 0.06
	Dry	12.59	22.92	26.3	6.08	0.08	18.33	5.67	0.58
		± 0.72	± 3.81	± 0.99	± 0.11	± 0.01	± 1.52	± 2.94	± 0.06
S2	Rainy	10.07	15.59	42.54	6.16	0.08	21.31	5.8	0.63
		± 0.18	± 2.86	± 2.8	± 0.21	± 0.01	± 3.37	± 0.96	± 0.11
	Dry	12.78	24.87	30.74	6.10	0.08	18.92	5.69	0.58
		± 0.77	± 2.46	± 11.0	± 0.16	± 0.02	± 5.20	± 1.44	± 0.1

Germination, growth, and dependence on AM fungi. The germination of *Solanum pubigerum* seeds was significantly higher (89 %) in the treatment with mycorrhiza (+AMF), while for the -AMF treatment the maximum value reached was 59 % (Figure 4).

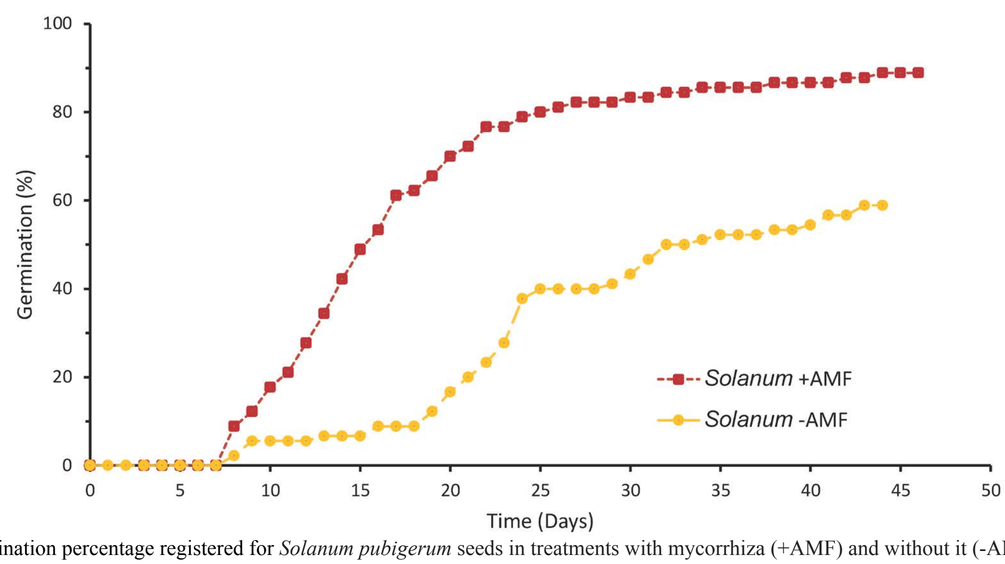

Figure 4. Germination percentage registered for *Solanum pubigerum* seeds in treatments with mycorrhiza (+AMF) and without it (-AMF).

A positive correlation between the AMF intraradical colonization percentage and radicle size, shoot (stem) biomass, seed vigor and accumulated germination was observed (Figure 5).

Arbuscular mycorrhizal (AMF) colonization influenced plant growth over five consecutive harvests, demonstrating increased root and shoot biomass in AMF-colonized plants. Root dry weight was significantly higher in the +AMF treatments, with a marked increase observed by the fifth harvest (Figure 6). Similarly, both total and shoot dry weights showed significant improvements in +AMF-treated plants.

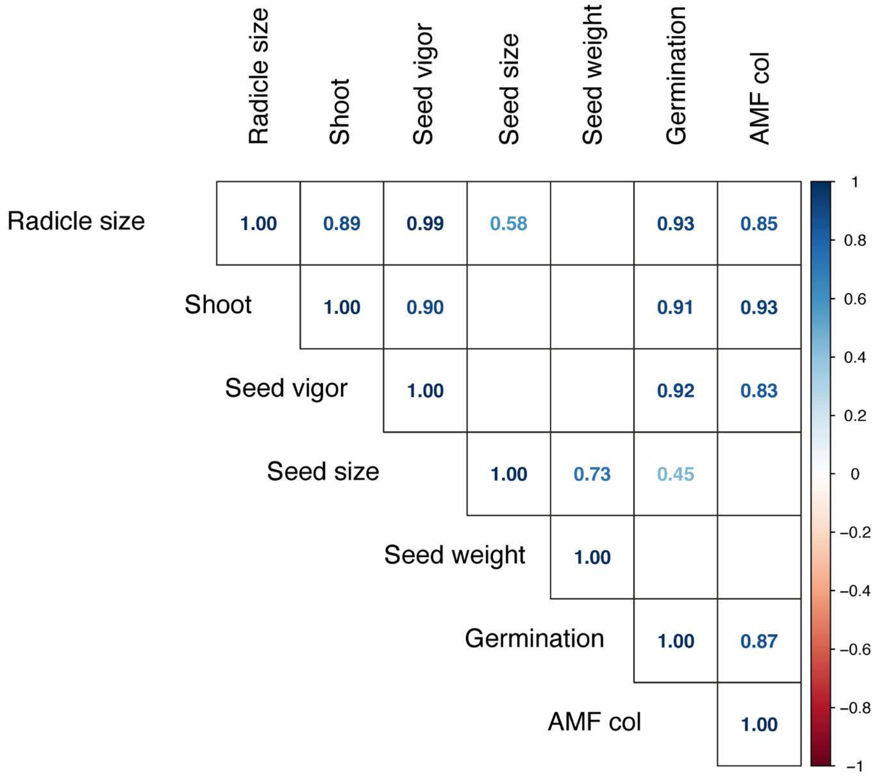

Figure 5. Spearman's correlations of seed features, germination, and AMF colonization for *Solanum pubigerum*. Statistically significant relations are shown ($P < 0.05$). Positive correlations in blue, negative ones in red.

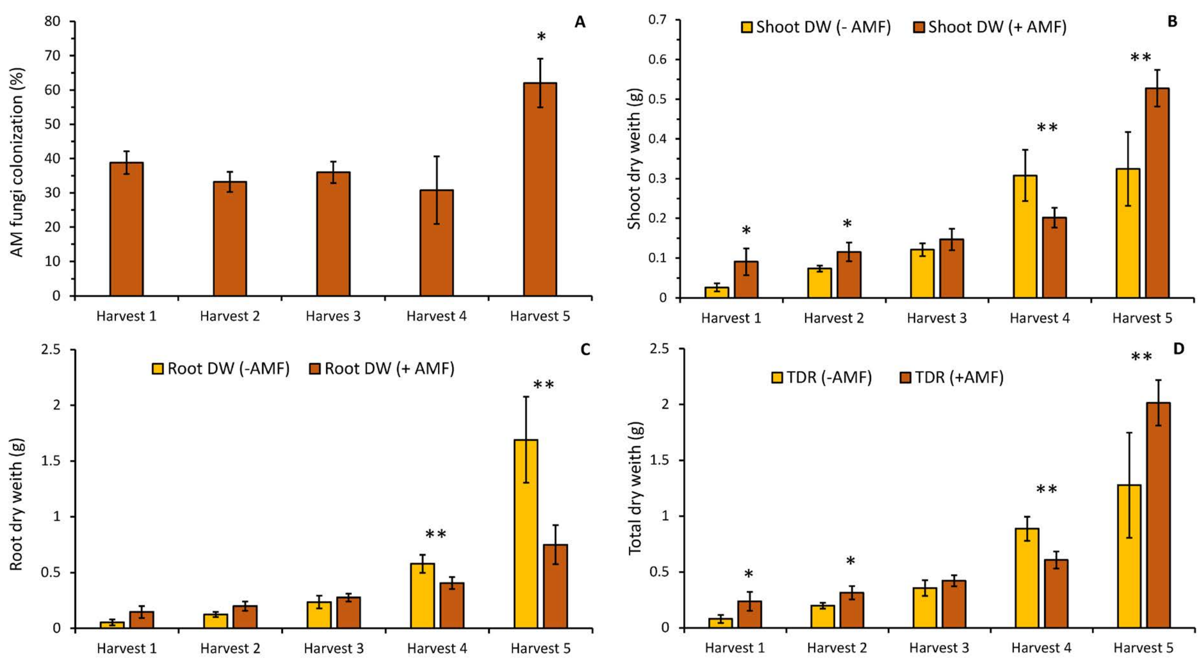

Figure 6. Effects of arbuscular mycorrhizal fungi colonization (A) on plant biomass over five harvests. B) Root dry weight, C) Total dry weight, and D) Shoot dry weight, comparing plants with (+AMF) and without AMF (-AMF). Statistically significant differences between treatments are indicated by asterisks (* = differences between mycorrhizal treatments, ** = differences in the interaction between mycorrhizal treatments and harvests).

Discussion

There are several aims and attributes that render certain species suitable for reforestation plans. In this regard, it is imperative to prioritize the use of native plant species. Within this context, the inclusion of native secondary vegetation species that naturally colonize disturbed forest sites becomes particularly advantageous. These species have demonstrated their remarkable tolerance to changing environmental conditions after forest disturbance, as previously reported for some Solanaceae species (Holl *et al*. 2000). Thus, the inclusion of secondary vegetation plant species, with a wide distribution, plays a crucial role in preserving the functionality of temperate forests by promoting both above-ground and below-ground interactions, for example, *S. pubigerum* can contribute to maintain pollinators such as beetles (van der Pijil 1982), and frugivorous bird species that feed on its fleshy fruits (Martínez-Orea 2011), and to the structuring and permanence of the community of arbuscular mycorrhizal fungi in its rhizosphere (Vázquez-Santos unpublished data).

The response of seeds of *S. pubigerum* to different light qualities, showed that most of them germinated under white and red light. Seeds with this response are favored under canopy gaps, where light incidence is high and direct (Orozco-Segovia & Sánchez-Coronado 2013) in temperate forests of central Mexico, it is these microsites where deforestation or weeding have occurred. Interestingly, a significant percentage of germination was also observed in darkness, indicating a certain level of indifference to light in a pool of seeds of this species (Orozco-Segovia & Sánchez-Coronado 2013), which makes the use of the seeds of this plant species as suitable for reforestation of a wide variety of heterogeneous microsites in their light environment. The seeds of several secondary vegetation species usually show high percentages of germination under far red light (730 nm) (Martínez-Orea *et al*. 2020), but this was not the observed behavior of the seeds of *S. pubigerum.* Far red light can be characteristic of disturbed sites, where extant vegetation still exists (sparse trees and shrubs). This is also the type of light that seeds under litter, or under a thin layer of soil receive (Yirdaw & Leinonen 2002).

The lowest germination percentage under far-red light of *S. pubigerum* is probably related to its affinity for open/disturbed sites. Additionally, the use of this species in reforestation can be advantageous because its seeds can maintain their viability for longer time spans than recalcitrant seeds (León-Lobos *et al.* 2012). In situ soil seed banks have been frequently employed for revegetation, and most of the results points out that they are supplied by secondary vegetation species in open canopy temperate forests (Witkowski & Garner 2000).

Some of the other advantages of the use of this species in reforestation is that within the first 12 days (t_0) its seeds start to germinate and after 25 days the half of a seed pool have germinated (t_{50}). Previous studies have reported longer times for the germination of the seeds of other shrub species from temperate forests, that also have orthodox seeds, but they present a morphophysiological dormancy (Martínez-Orea *et al.* 2019), this, together with the advantages mentioned above, contribute to a good choice for their storage but in an artificial seed bank rather than in an *in situ* soil seed bank. The presence of *S. pubigerum* in extant vegetation, seed bank and seed rain (even in low abundances), are important traits to be considered for reforestation, because this contributes to fundamental ecological processes for natural regeneration, such as the movement of vertebrates (mainly birds) that feed on its fleshy fruits and disperse its seeds forming corridors that can connect forest fragments (Şekercioğlu *et al*. 2015).

Germination response of seeds can also be affected by other factors such as symbiotic interactions, as seen in our results. It has been proposed that AMF can play a crucial role in seed germination, thereby influencing natural regeneration of forests (Koide 2010, Smith & Smith 2011). In the case of *S. pubigerum*, the germination rate was significantly higher in the presence of AMF propagules (spores, previously infected roots). This finding suggests that these fungi may have the capability to induce seed germination (Koide 2010). The involvement of AMF in creating suitable microenvironments for germination is plausible, considering their role in soil micro and macroaggregate formation and the production of hyphal exudates (Lendzemo *et al*. 2009). However, van der Heijden (2004) suggests that AMF may also enhance seed germination. Thus, the participation of AMF in establishing favorable conditions for seed germination implies their potential contribution to the success of reforestation efforts. By facilitating the germination process and creating favorable microhabitats, AMF can enhance the establishment and survival of native plant species during the early stages of ecosystem recovery.

It is important to notice that the abundance of *S. pubigerum* in the seed bank was the same in both year seasons, which may be attributed to its wide/continuous phenological pattern: flowering during the dry season and fruiting during the rainy season, with high synchrony between individuals, mostly in flowering. Our results indicate that at least during eight months of the year, there are available resources for frugivores and pollinators in this forest from species such as *S. pubigerum*, which will allow the flow of genes between forest sites, and this is vital to accelerate regeneration/reforestation processes. This resource availability is a key factor in structuring communities, forests with different plant growth forms with less seasonal phenological patterns will provide more resources for animals (Garcia *et al*. 2014). This continuous phenological pattern of *S. pubigerum* indicates that these resources are available mostly all year either for pollinators (mostly beetles) or for birds that feed on the fleshy fruits and disperse the seeds. Plants with phenological continuous patterns possibly minimize a shortage on resources, therefore contributing to the maintenance of the functional diversity of the system.

The use of native species not only contributes to the preservation of local plant-soil-microbe interactions but also promotes the development of resilient systems. The potential role of AM fungi in ecological reforestation has been recognized long before the emergence of restoration ecology as a scientific field of study (Janos 1988). The positive growth response exhibited by *S. pubigerum* in the "+AM" treatment highlights the usefulness of the arbuscular mycorrhizal association in plants with potential applications for the reforestation of degraded sites, especially when applying this knowledge to secondary vegetation species. The efficiency of nutrient uptake is improved by AMF (Smith & Smith 2011). By colonizing the root system of *S. pubigerum*, these fungi contribute to the overall nutrient and water acquisition in the plant explaining the accumulation of biomass in this study species. Thus, in the context of reforestation, the inclusion of AMF can play a vital role in enhancing plant establishment and survival in degraded environments. These fungi not only provide nutrients to the plants but also aid in the improvement of soil structure and fertility, leading to increased resilience and productivity of restored ecosystems (Powell & Rillig 2018), and these experiments suggest that *S. pubigerum* enhances its germination and growth when colonized by AMF.

This work presents the results of some ecological traits of *S. pubigerum* that might make it suitable for reforestation plans. However, its uses as a nurse species for conifer seedlings (such as *A. religiosa*) still need to be assessed. Also, other attributes as its capacity to modify and improve the environment, should be researched. Further research with this species needs to clarify its tolerance to poor soil nutrient availability. Our results show that it establishes mutualisms with AMF, which contributes to the formation of soil aggregates and favors soil moisture, but it is also important to investigate if it favors a high belowground microbial diversity. With respect to the role of this species in terms of activating the ecological successional dynamics of this temperate forest, *S. pubigerum* is still found in extant vegetation coexisting with other shrub species such as *A. glabrata* and *Cestrum thyrsoideum* Kunth (Solanaceae) and in natural regeneration sources.

Even though there are 83 species of shrubs in the study site (and 5 correspond to Solanaceae), scarce research has been carried out with these species and their traits that make them suitable for reforestation in temperate forests. Therefore, our study represents a proposal for the study of other ecological traits of *S. pubigerum* and of other shrub species for reforestation.

Solanum pubigerum is a species broadly distributed in Mexico, with a favorable germination response, present in natural regeneration sources, with a continuous phenological pattern, and establishes an arbuscular mycorrhizal association which is positively reflected in its germination and growth. These ecological traits make of *S. pubigerum* a potential species for temperate forest reforestation.

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