Growth of naturally regenerated *Abies religiosa* (Kunth) Schltdl. & Cham. seedlings in a nursery and genetic variation among provenances

Crecimiento de plántulas de regeneración natural de *Abies religiosa* (Kunth) Schltdl. & Cham. en vivero y variación genética entre procedencias

Ana L. Cruzado-Vargas1,2; Francisco J. Zamudio-Sánchez1,3; Gabriel A. Rodríguez-Yam1,3; Aglaen L. Carbajal-Navarro4; José A. Blanco-García4; Cuauhtémoc Sáenz-Romero5*

1Universidad Autónoma Chapingo, División de Ciencias Forestales. km 38.5 carretera México-Texcoco, C. P. 56230. Texcoco, Estado de México, México.
2Universidad Michoacana de San Nicolás de Hidalgo, Instituto de Investigaciones Agropecuarias y Forestales, Unidad San Juanito Ixtlucuaro. Av. San Juanito Ixtlucuaro s/n, col. Nueva Esperanza. C. P. 58337. Morelia, Michoacán, México.
3Universidad Autónoma Chapingo, Departamento de Matemática, Estadística y Cómputo. km 38.5 carretera México-Texcoco, C. P. 56230. Texcoco, Estado de México, México.
4Universidad Michoacana de San Nicolás de Hidalgo, Facultad de Biología. Gral. Francisco Múgica s/n, Ciudad Universitaria. C. P. 58030. Morelia, Michoacán, México.
5Universidad Michoacana de San Nicolás de Hidalgo, Instituto de Investigaciones sobre los Recursos Naturales. Av. San Juanito Ixtlucuaro s/n, col. Nueva Esperanza. C. P. 58337. Morelia, Michoacán, México.

*Corresponding author: csaenzromero@gmail.com; tel.: +52 (443) 271 9388.

Abstract

Introduction: The forests of *Abies religiosa* (Kunth) Schltdl. & Cham. in the Monarch Butterfly Biosphere Reserve (MBBR) serve as a refuge for the monarch butterfly.

Objective: To determine the viability of growing naturally regenerated *A. religiosa* seedlings in a nursery and the existence of genetic variation associated with the altitudinal gradient among populations.

Materials and methods: Recently naturally germinated seedlings were collected in an altitudinal transect (2 960 to 3 450 m) that covered six populations of the MBBR. The plants grew for 18 months in a nursery at 3 000 m.

Results and discussion: The average survival of *A. religiosa* was 75 %. The plants showed significant differences in survival \((P < 0.0001)\), height \((P = 0.0430)\) and basal diameter \((P < 0.0001)\) among provenances; the populations of the altitudinal extremes had the highest values.

Conclusion: Transplanting plants that naturally germinated in the forest into containers for nursery growth is feasible. The altitudinal pattern was atypical, since the populations of the altitudinal extremes had the best growth.

Keywords: sacred fir, altitudinal gradient; plant height; survival; basal diameter.

Resumen

Introducción: Los bosques de *Abies religiosa* (Kunth) Schltdl. & Cham. en la Reserva de la Biosfera Mariposa Monarca (RBMM) sirven como refugio para la mariposa monarca.

Objetivo: Determinar la viabilidad del crecimiento de plántulas de regeneración natural de *A. religiosa* en vivero y la y la existencia de variación genética asociada al gradiente altitudinal entre poblaciones.

Materiales y métodos: Se recolectaron plántulas recién germinadas de manera natural en un transecto altitudinal (2 960 a 3 450 m) que abarcó seis poblaciones de la RBMM. Las plantas crecieron durante 18 meses en un vivero a 3 000 m de altitud.

Resultados y discusión: La supervivencia promedio de *A. religiosa* fue de 75 %. Las plantas mostraron diferencias significativas en la supervivencia \((P < 0.0001)\), altura \((P = 0.0430)\) y diámetro basal \((P < 0.0001)\) entre procedencias; las poblaciones de los extremos altitudinales tuvieron los valores más altos.

Conclusión: El trasplante de plantas germinadas de manera natural en el bosque a envases para su crecimiento en vivero es viable. El patrón altitudinal fue atípico, ya que las poblaciones de los extremos altitudinales tuvieron el mejor crecimiento.

Palabras clave: oyamel; gradiente altitudinal; altura de planta; supervivencia; diámetro basal.

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Introduction

The number of monarch butterflies (*Danaus plexippus* L.) has decreased due to three main factors: degradation in hibernation zones in Mexico; reduction in breeding areas in the United States due to the loss of cottonwood plants (*Asclepias* spp.; host plants), as well as land-use change; and changes in climatic conditions (Brower et al., 2012). Reforestation of the Monarch Butterfly Biosphere Reserve (MBBR), on the borders of the states of Michoacán and Mexico, is important to increase the number of trees where the butterfly overwinters.

*Abies religiosa* (Kunth) Schltdl. & Cham. is the dominant forest species at overwintering sites. Based on our direct experience, nursery plant production of this species entails greater difficulty than plant production of the genus *Pinus*, due to the following reasons: (a) the female cones of *A. religiosa* are produced almost exclusively at the upper end of the crown, which makes collection difficult and expensive (Arriola et al., 2015; Nieto de Pascual-Pola, Musálem, & Ortega-Alcalá, 2003), especially when they are large trees; (b) *A. religiosa* usually has a high percentage of empty seeds, a low germination percentage (Iglesias-Andreu et al., 2010; Nieto de Pascual-Pola et al., 2003) and a short life under storage conditions (Arriola et al., 2015; Carrillo, Patiño, & Talavera, 1980; Nieto de Pascual-Pola et al., 2003; Salazar & Soihet, 2001); and (c) obtaining permits for the collection of *A. religiosa* seed in the MBBR’s core area is complicated. This last difficulty occurs because rule 62 of the MBBR Management Program (Comisión Nacional de Áreas Naturales Protegidas [CONANP], 2001) stipulates that during the monarch butterfly hibernation season, a period that coincides with the ripening of cones in November and December, it is prohibited to engage in activities that put the butterfly at risk. Additionally, when the seed is collected by personnel external to the communities and ejidos of the MBBR, a permit is required from SEMARNAT (Secretaría del Medio Ambiente y Recursos Naturales, 2018), which, in order to grant it, requests that the applicant demonstrate the consent of the communities (SEMARNAT-02-004 procedure). The consent document is prepared for each of the communities and ejidos in which seed is collected. This process is complex, since it is difficult to obtain the minimum legal quorum of the ejidal and communal assemblies, when the motive is only to give permission.

This situation has led to the production of nursery plants using seed from outside the MBBR’s core area, which implies that the plant does not adapt due to a lack of coupling between the climate of the seed collection site and the planting site (Castellanos-Acuña et al., 2018; Castellanos-Acuña, Lindig-Cisneros, Silva-Farías, & Sáenz-Romero, 2014; Johnson, Sørensen, St. Clair, & Cronn, 2004). As an alternative, the *ejidatarios* (ejido personnel external to the communities and ejidos of the MBBR, a permit is required from SEMARNAT (Secretaría del Medio Ambiente y Recursos Naturales, 2018), which, in order to grant it, requests that the applicant demonstrate the consent of the communities (SEMARNAT-02-004 procedure). The consent document is prepared for each of the communities and ejidos in which seed is collected. This process is complex, since it is difficult to obtain the minimum legal quorum of the ejidal and communal assemblies, when the motive is only to give permission.

Esta situación ha generado que la producción de planta en vivero se realicé con semilla ajena al área núcleo de la RBMM, lo cual implica que la planta no se adapte por falta de acoplamiento entre el clima del sitio de obtención de la semilla y el de plantación (Castellanos-Acuña et al., 2018; Castellanos-Acuña, Lindig-Cisneros,
members) and comuneros (joint land holders) of the MBBR have raised the possibility of rescuing naturally regenerated plants that, based on their practical experience, would otherwise die in the dry season following their germination. A thick layer of moss (>10 cm) is common in the area, which acts as a mechanical barrier, preventing the seedlings that germinate and emerge from reaching the soil (Rodríguez-Laguna, Razo-Zárate, Fonseca-González, Capulín-Grande, & Goche-Telles, 2015). In many cases, the seedlings do not survive the dry season of the following year (Madrigal-Sánchez, 1967; Manzanilla, 1974; Maraño et al., 2004), because the moss dehydrates (Ángeles-Cervantes & López-Mata, 2009; Chen et al., 2015) or acts as a competitor for the seedling of this conifer (Stuiver, Wardle, Gundale, & Nilsson, 2014).

In a sampling of A. religiosa populations in an altitudinal transect (2850 m to 3350 m) on San Andrés hill, 50 km west of the MBBR, Castellanos-Acuña et al. (2014) observed an altitudinal pattern of morphological variation in needles and cones, where higher-altitude populations showed longer needles and shorter cones than low-altitude populations. Generally, an assay of A. religiosa provenances collected along an altitudinal gradient indicates an important genetic differentiation between populations, considering that such expression can be quantified whenever the plants grow in the same environment (Sáenz-Romero, Beaulieu, & Rehfeldt, 2011; Sáenz-Romero et al., 2012; Sáenz-Romero, Ruiz-Talonia, Beaulieu, Sánchez-Vargas, & Rehfeldt, 2011). Populations originating at lower altitudes produce plants with greater height, their dominant bud elongates more, they cease growth later in the year and they accumulate higher stem and foliage biomass, but are more susceptible to frost damage than plants originating at higher altitudes (Ortiz-Bibian et al., 2017); however, such a pattern has not been confirmed in provenance trials with seed collected directly from the MBBR.

Based on the above, the objective of the present study was to determine the viability of producing A. religiosa in a nursery from naturally regenerated seedlings, and the genetic variation of quantitative traits among populations, associated with an altitudinal gradient.

Materials and methods

The recently germinated natural regeneration seedling was collected in an altitudinal gradient (2960 to 3450 m) located within the MBBR in the Casablanca, Guadalupe Buenavista and La Mesa ejidos, municipality of San José del Rincón, State of Mexico (Table 1). The seedlings germinated in situ in late spring and at the beginning of the rainy season in 2015 and were collected between the last week of October and the first week of November in 2016. The seedlings were collected in a nursery from naturally regenerated seedlings, and they accumulate higher stem and foliage biomass, but are more susceptible to frost damage than plants originating at higher altitudes (Ortiz-Bibian et al., 2017). The recently germinated natural regeneration seedling was collected in an altitudinal gradient (2960 to 3450 m) located within the MBBR in the Casablanca, Guadalupe Buenavista and La Mesa ejidos, municipality of San José del Rincón, State of Mexico (Table 1). The seedlings germinated in situ in late spring and at the beginning of the rainy season in 2015 and were collected between the last week of October and the first week of November in 2016. The seedlings were collected in a nursery from naturally regenerated seedlings, and they accumulate higher stem and foliage biomass, but are more susceptible to frost damage than plants originating at higher altitudes (Ortiz-Bibian et al., 2017).
first week of November of the same year. The selected seedlings correspond to sites where there is a thick layer of moss (> 5 cm, personal observation), which is produced by the large amount of moisture in these areas during the time when the natural regeneration of *A. religiosa* is established.

In each altitudinal site, the collection was done through a selection of seedlings still with a testa, cotyledonary leaves (without primary leaves), and a thin, pink and vigorous stem with a height less than 5 cm. The seedlings were moved from the field to the nursery in containers, where the roots with naturally attached soil were covered with an additional layer of local soil (Figure 1).

At the test site, weeds were removed and the nursery was covered with a 50 % mesh shadehouse to prevent predation by animals, dehydration of plants by direct exposure to the sun, and damage by strong winds or hail. Immediately after their collection, the seedlings were transplanted into polyethylene bags (8 x 7 x 17 cm) for a nursery that contained local soil (previously sifted) as substrate from sites dominated by *A. religiosa*.

The experimental design was six completely randomized blocks. In total, there were 200 seedlings from each of the six provenances, randomly distributed within the blocks in continuous plots of 36 seedlings, arranged in three rows of 12 individuals, except block number six, in which there were only 20 seedlings per provenance. Seedlings were placed at the ends of the nursery beds to avoid the edge effect. The six blocks were placed in three beds (two blocks per bed) to facilitate the measurement work with accessible corridors.

The height from the seedling was assessed from the base to the tip of the main bud (mm) and the basal diameter (0.1 mm) of the stem were evaluated bimonthly and semiannually, respectively, for 18 months (December 2015 to December 2017) for a nursery that contained local soil (previously sifted) as substrate from sites dominated by *A. religiosa*.

| Provenance / Procedencia | Altitude (m) / Altitud (m) | N latitude / Latitud N | W longitude / Longitud O |
|--------------------------|---------------------------|------------------------|-------------------------|
| Provenance1 / Procedencia 1 | 3450                      | 19° 34’ 05.4”           | 100° 13’ 53.0”          |
| Provenance 2 / Procedencia 2 | 3350                      | 19° 34’ 28.0”           | 100° 13’ 05.5”          |
| Provenance 3 / Procedencia 3 | 3239                      | 19° 34’ 44.2”           | 100° 12’ 51.6”          |
| Provenance 4 / Procedencia 4 | 3157                      | 19° 35’ 24.0”           | 100° 12’ 31.2”          |
| Provenance 5 / Procedencia 5 | 3052                      | 19° 35’ 44.5”           | 100° 12’ 06.9”          |
| Provenance 6 / Procedencia 6 | 2960                      | 19° 36’ 56.0”           | 100° 11’ 13.3”          |

**Nursery trial site / Sitio del ensayo en vivero**

Poblado La Mesa | 3 000 | 19° 35’ 18.2” | 100° 10’ 46.1”

Growth and genetic variation of *Abies religiosa*

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2015 to May 2017), as well as the survival of each plant. The initial height or basal diameter was used as a covariate to rule out the effect of the initial size of the plant at the time of transplant to the nursery bag, because the exact germination date was not known.

An analysis of variance was performed using the SAS statistical package version 9.1 (SAS Institute, Inc. 2004) with the PROC GLM procedure, using the following statistical model:

\[
\text{Y}_{ij} = \mu + \alpha_i + \beta_j + \epsilon_{ij}
\]

where \(Y_{ij}\) is the observed value for the \(i\)th treatment and \(j\)th replicate, \(\mu\) is the overall mean, \(\alpha_i\) is the effect of the \(i\)th treatment, \(\beta_j\) is the effect of the \(j\)th replicate, and \(\epsilon_{ij}\) is the random error term.

Figure 1. Transplanting of recent Abies religiosa seedlings that emerged naturally in the field into containers in a community forest nursery in the La Mesa ejido, San José del Rincón, State of Mexico. (a) Seedling naturally emerged at sites with a thick layer of diverse moss and mulch. (b) Extraction of natural regeneration plant. (c) Transport of seedling from the collection site to the nursery; the roots were protected by the use of moist local soil, in addition to the naturally adhered soil. (d) Transplant into nursery bag with local soil. (e) Provenance test; each nursery bed (three) contains two blocks (six in total).

Figura 1. Trasplante de plántulas de Abies religiosa recién emergidas de manera natural en campo a envases en un vivero forestal comunitario del ejido La Mesa, San José del Rincón, Edo. de México. (a) Plántula emergida naturalmente en sitios con una capa gruesa de musgo y mantillo diversos. (b) Extracción de planta de regeneración natural. (c) Transporte de plántula del sitio de recolecta al vivero; las raíces fueron protegidas mediante el uso de tierra húmeda de monte, adicional a la adherida naturalmente. (d) Trasplante a bolsa de vivero con tierra de monte. (e) Ensayo de procedencias; cada cama de vivero (tres) contiene dos bloques (seis en total).
\[ Y_{ijk} = \mu + B_i + P_j + B_i \times P_j + S_{ijk} + E_{ijk} \]

where,
\[ Y_{ijk} \] = observation of the \( k \)-th individual in the \( j \)-th provenance of the \( i \)-th block
\( \mu \) = overall mean
\( B_i \) = effect of the \( i \)-th block
\( P_j \) = effect of the \( j \)-th provenance
\( B_i \times P_j \) = interaction between block and provenance
\( S_{ijk} \) = effect of covariate due to initial plant size
\( E_{ijk} \) = error.

Provenance and block were considered random effects; the same model was used for survival, but without a covariate. Additionally, a regression of means of the variables evaluated by provenance was made against the altitude of provenance, in order to determine the association between the growth potential and the altitudinal gradient.

**Results**

The average survival of *Abies religiosa* seedlings was 75 % (Figure 2) 18 months after establishment in the nursery and approximately two years after the possible date of germination in the field. Table 2 indicates that the provenances caused significant differences in the survival \((P < 0.0001)\), height \((P = 0.0430)\) and basal diameter \((P < 0.0001)\) of *A. religiosa*.

According to Figure 3 and Table 3, the provenance with the most live plants was the one with the highest altitude (3450 m), being statistically similar to the lowest-altitude provenance (2960 m), while the lowest survival was an intermediate-altitude provenance (3157 m); regarding plant height, the 3350 m provenance had the highest value and was statistically similar to the provenances of the altitudinal extremes.

The altitudinal clinal pattern shown in Figure 4 indicates that the highest-altitude provenance had the greatest basal diameter and its value was statistically similar to that recorded in the 2960 m (lowest altitude) and 3350 m plants.

**Discussion**

The altitudinal clinal pattern of survival, height and basal diameter, in terms of the good performance of the higher-altitude provenances, is different from that reported for other conifers in Mexico, such as *Pinus pseudostrobus* Lindl. (Viveros-Viveros, Sáenz-Romero, López-Upton, & Vargas-Hernández, 2005) or *Pinus hartwegii* Lindl. (Loya- Rebollar et al., 2013). In these species, lower-altitude populations had larger basal

Se realizó un análisis de varianza mediante el paquete estadístico SAS versión 9.1 (SAS Institute, Inc. 2004) con el procedimiento PROC GLM, usando el modelo estadístico siguiente:

\[ Y_{ijk} = \mu + B_i + P_j + B_i \times P_j + S_{ijk} + E_{ijk} \]

donde,
\[ Y_{ijk} \] = observación del \( k \)-ésimo individuo en la \( j \)-ésima procedencia del \( i \)-ésimo bloque
\( \mu \) = media general
\( B_i \) = efecto del \( i \)-ésimo bloque
\( P_j \) = efecto de la \( j \)-ésima procedencia
\( B_i \times P_j \) = interacción entre bloque y procedencia (fuente de variación usado como término de error para determinar la significancia de bloque y procedencia)
\( S_{ijk} \) = efecto de la covariable debida al tamaño inicial de planta
\( E_{ijk} \) = error.

La procedencia y el bloque se consideraron efectos aleatorios; para la supervivencia se usó el mismo modelo, pero sin covariable. Adicionalmente, se hizo una regresión de medias de las variables evaluadas por procedencia contra la altitud de origen, a fin de determinar la asociación entre el potencial de crecimiento y el gradiente altitudinal.

**Resultados**

La supervivencia promedio de plántulas de *A. religiosa* fue de 75 % (Figura 2) a los 18 meses de su establecimiento en vivero y, aproximadamente, a los dos años desde la posible fecha de germinación en campo. El Cuadro 2 indica que las procedencias causaron diferencias significativas en la supervivencia \((P < 0.0001)\), altura \((P = 0.0430)\) y diámetro basal \((P < 0.0001)\) de *A. religiosa*.

De acuerdo con la Figura 3 y el Cuadro 3, la procedencia con más plantas vivas fue la de mayor altitud (3450 m), siendo estadísticamente similar a la altitud más baja (2960 m), mientras que la de menor supervivencia fue una procedencia de altitud intermedia (3157 m); con respecto a la altura de la planta, la procedencia de 3350 m tuvo el valor más alto y fue estadísticamente similar a las procedencias de los extremos altitudinales.

El patrón clinal altitudinal mostrado en la Figura 4 indica que la procedencia de mayor altitud tuvo el mayor diámetro basal y su valor fue estadísticamente similar al registrado en las plantas de 2960 m (altitud más baja) y 3350 m.
Table 2. Analysis of variance of the growth variables of six provenances of *Abies religiosa* from the Monarch Butterfly Biosphere Reserve.

| Source of variation / Fuente de variación | df / gl | Height / Altura | Basal diameter / Diámetro basal | Survival / Supervivencia |
|------------------------------------------|---------|-----------------|---------------------------------|--------------------------|
|                                          |         | MS / CM         | P                               | MS / CM                  | P                        |
| Block / Bloque                           | 5       | 4469            | 0.3959                          | 80.4                     | 0.5346                   | 0.3959                   | 0.3959                   | 0.5346                   | 0.53                   | 0.1133                   | ns                      |
| Provenance / Procedencia                 | 5       | 10 715          | 0.0430                          | 732.6                    | <0.0001                  | 2.26                     | <0.0001                  | ***                      |
| Block*Provenance / Bloque*Procedencia    | 25      | 4 227           | 0.0002                          | 97.5                     | 0.0011                   | 0.26                     | 0.2272                   | ns                      |
| Covariate / Covariable                   | 1       | 28 827          | <0.0001                         | 438                      | 0.0021                   | ---                      | ---                      | ---                      |
| Error                                    | 718     | 1 774           | 46                              |                          |                           |                          |                          |                          |

df = degrees of freedom (height and basal diameter = 718; survival = 1164); MS = mean square. *P ≤ 0.05; **P ≤ 0.01; ***P ≤ 0.001; ns = not significant. gl = grados de libertad (altura y diámetro basal = 718; supervivencia = 1164); CM = cuadrado medio. *P ≤ 0.05; **P ≤ 0.01; ***P ≤ 0.001; ns = no significativo.
Survival (%) / Supervivencia (%)
Survival (%) / Supervivencia (%)
Height (%) / Altura (%)
Altitude at origin (m) / Altitud de origen (m)
Height growth (mm) / Crecimiento en altura (mm)

Figure 3. Quadratic regression of survival and final height of six Abies religiosa provenances against altitude of origin. Different letters indicate significant difference between provenances, according to Tukey’s test ($P \leq 0.05$). Vertical bars indicate the standard error of the mean.

Figura 3. Regresión cuadrática de supervivencia y altura final de seis procedencias de Abies religiosa contra altitud de origen. Letras distintas indican diferencia significativa entre procedencias, de acuerdo con la prueba de Tukey ($P \leq 0.05$). Las barras verticales indican el error estándar de la media.

Table 3. Comparison of means of growth variables of six provenances of Abies religiosa at 18 months of establishment in a nursery.
Cuadro 3. Comparación de medias de las variables de crecimiento de seis procedencias de Abies religiosa a 18 meses de establecimiento en vivero.

| Provenance / Procedencia | Altitude (m) / Altitud (m) | Height (mm) / Altura (mm) | Basal diameter (mm) / Diámetro basal (mm) | Survival (%) / Supervivencia (%) |
|--------------------------|---------------------------|--------------------------|------------------------------------------|-------------------------------|
| 1                        | 3 450                     | 105.6 ab                 | 21.2 a                                   | 78.5 a                        |
| 2                        | 3 350                     | 117.8 a                  | 20.1 ab                                  | 67.5 abc                      |
| 3                        | 3 239                     | 97.6 ab                  | 16.7 bc                                  | 57.5 bcd                      |
| 4                        | 3 157                     | 88.5 b                   | 15.6 c                                   | 51 d                          |
| 5                        | 3 052                     | 87.5 b                   | 15.0 c                                   | 52 cd                         |
| 6                        | 2 960                     | 105.1 ab                 | 18.3 abc                                 | 71 ab                         |

LSD / DMS
25.6
3.9
16

Mean values with the same letters between provenances indicate non-significant difference (Tukey, $P \leq 0.05$). LSD = least significant difference.

Valores medios con letras iguales entre procedencias indican diferencias no significativas (Tukey, $P \leq 0.05$). DMS = diferencia mínima significativa.
diameters than higher-altitude ones, growing under optimal or suboptimal conditions. On the other hand, the data generated in the present study coincide with the good performance of early age (13-month-old) *P. hartwegii* seedlings, originating from seed of the populations of the upper altitudinal extreme (4050 m) of Cofre de Perote and grown in a shadehouse in Xalapa, Veracruz (Viveros-Viveros, Marín-Hernández, Aparicio-Rentería, & Sáenz-Romero, 2018).

It is important to emphasize that the results of Ortiz-Bibian et al. (2017), in which less growth is reported in the higher-altitude provenances, represent the growth potential (the genetically conditioned capacity to grow under optimal conditions), while the conditions of the present work were much more difficult, since the nursery was a very basic facility and located at 3000 m. Therefore, it is possible that a high provenance*environment interaction induces a

**Discusión**

El patrón clinal altitudinal de la supervivencia, altura y diámetro basal, en lo que se refiere al buen desempeño de las procedencias de mayor altitud, es diferente al reportado para otras coníferas en México, como *Pinus pseudostrobus* Lindl. (Viveros-Viveros, Sáenz-Romero, López-Upton, & Vargas-Hernández, 2005) o *Pinus hartwegii* Lindl. (Loya-Rebollar et al., 2013). En dichas especies, las poblaciones de menor altitud tuvieron mayor diámetro basal que las poblaciones de mayor altitud, creciendo en condiciones óptimas o subóptimas. Por otra parte, los datos generados en el presente trabajo coinciden con el buen desempeño de plántulas de edad temprana (13 meses) de *P. hartwegii*, originadas de semilla de poblaciones del extremo altitudinal superior (4050 m) del Cofre de Perote y crecidas en una casa de sombra en Xalapa, Veracruz (Viveros-Viveros, Marín-Hernández, Aparicio-Rentería, & Sáenz-Romero, 2018).

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**Figure 4.** Quadratic regression of final basal diameter of *Abies religiosa* by provenance against altitude at origin. Different letters indicate significant difference between provenances according to Tukey’s test (*P* ≤ 0.05). Vertical bars indicate the value of the standard error of the mean.

**Figura 4.** Regresión cuadrática de diámetro basal final de *Abies religiosa* por procedencia contra altitud de origen. Letras distintas indican diferencia significativa entre procedencias de acuerdo con la prueba de Tukey (*P* ≤ 0.05). Las barras verticales indican el valor del error estándar de la media.
different growth pattern. Additionally, it is necessary to evaluate the fact that the altitudinal pattern is not very pronounced (the difference between provenances of the altitudinal gradient is relatively minor), and that the last evaluation was made in May (in July they were planted in a field trial). There is a possibility that the pattern would have been different if the last evaluation had been made in November (2017), when the apical buds cease to grow and are at rest. Finally, the age of the plants can be considered too young to show a definite trend of their growth capacity in a nursery at 3000 m. It is therefore recommended that these results be treated with caution.

It is also recommended to conduct further research on the use of naturally regenerated plants for growth in a nursery, for reforestation purposes, in order to avoid costs in the collection of seed directly from the standing tree.

**Conclusions**

*A. religiosa* plants from the altitudinal extremes had the highest average survival, height, and basal diameter values. The average survival of 75% indicates that transplanting naturally regenerated plants, for later growth in a nursery, is viable. Therefore, the reintroduction in the field of the rescued naturally regenerated seedlings can be a complementary alternative to the usual reforestation programs, in order to achieve the ecological restoration of the overwintering *A. religiosa* stands of the Monarch butterfly.

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Es importante subrayar que los resultados de Ortiz-Bibian et al. (2017), en los que se reporta menor crecimiento en las procedencias de mayor altitud, representan el potencial de crecimiento (la capacidad genéticamente condicionada de crecer en condiciones óptimas), mientras que las condiciones del presente trabajo fueron mucho más difíciles, ya que el vivero es rústico y se encuentra a 3000 m de altitud. Por ello, es posible que una alta interacción procedencia*ambiente induzca un patrón de crecimiento diferente. Adicionalmente, es necesario valorar el hecho de que el patrón altitudinal es poco pronunciado (la diferencia entre procedencias del gradiente altitudinal es relativamente menor), y que la última evaluación se hizo en el mes de mayo (en julio se plantaron en un ensayo de campo). Existe la posibilidad de que el patrón fuera distinto si se hubiera hecho la última evaluación en el mes de noviembre (2017), cuando las yemas apicales cesan su crecimiento y se encuentran en reposo. Finalmente, la edad de las plantas puede considerarse demasiado juvenil para mostrar una tendencia definitiva de su capacidad de crecimiento en un vivero a 3000 m de altitud. Por lo anterior es recomendable que los presentes resultados se utilicen con cautela.

Se recomienda indagar más sobre el uso de la planta de regeneración natural y crecimiento en vivero, para establecer reforestaciones, con el fin de evitar costos en la recolección de semilla directamente del árbol en pie.

**Conclusiones**

Las plantas de *A. religiosa* provenientes de los extremos altitudinales tuvieron los valores promedio más altos de supervivencia, altura y diámetro basal. La supervivencia promedio de 75% indica que el trasplante de planta de regeneración natural, para su crecimiento posterior en vivero, es viable. Por ello, la reintroducción en campo de las plántulas rescatadas de regeneración natural puede ser una alternativa complementaria a los programas usuales de reforestación, para lograr la restauración ecológica de los rodales de *A. religiosa* de estancia invernal de la mariposa Monarca.

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