UNIVERSIDAD MICHOACANA
DE SAN NICOLÁS DE HIDALGO

Instituto de Investigaciones Agropecuarias y Forestales
Programa Institucional de Doctorado en Ciencias Biológicas
Opción: Ciencias Agropecuarias, Forestales y Ambientales

“Respuesta a la transferencia climática y evaluación de la diversidad química entre procedencias de *Abies religiosa* para estimar adaptabilidad al cambio climático”

TESIS

QUE PARA OBTENER EL GRADO DE

DOCTORA EN CIENCIAS BIOLÓGICAS

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Morelia, Michoacán, agosto 2023

"El mejor momento para plantar un árbol fue hace 20 años.

El segundo mejor momento es ahora".

- Proverbio chino

DEDICATORIA

A mis padres
Gracias por su confianza, paciencia y amor.
Son mi inspiración y mi principal fuente de apoyo.
Me enseñaron el valor de la perseverancia,
la determinación, y todos los principios que hoy me rigen.
Siempre tuvieron razón cuando decían:
"La educación es la mejor herencia que podemos dejarte"

A mis hermanos
Quienes también son mis confidentes, cómplices y amigos,
sus palabras de aliento siempre me impulsan.
Gracias por apoyarme de principio a fin,
sin ustedes no podría estar en donde ahora me encuentro.

A mis amados sobrinos
Espero que la ciencia también llegue a su mente y corazón.

¡Gracias a todos por ser y estar!

AGRADECIMIENTOS

Al Consejo Nacional de Ciencia y Tecnología por la beca otorgada para realizar los estudios de doctorado (733822). A la Universidad Michoacana de San Nicolás de Hidalgo por ser un pilar fundamental en mi educación. Al Instituto de Investigaciones Agropecuarias y Forestales por permitirme formar parte del Programa Institucional de Doctorado en Ciencias Biológicas.

Al Dr. Cuauhtémoc Sáenz Romero y al Dr. Roberto Lindig Cisneros, por su excelente dirección, sus enseñanzas, apoyo, tiempo y sobre todo su paciencia.

A la Dra. Mariela Gómez Romero, Dr. Leonel López Toledo y Dr. Erick de la Barrera Montppellier, por las acertadas observaciones que realizaron a lo largo del proyecto de investigación.

Al Dr. Arnulfo Blanco García, por facilitar la obtención de semillas de oyamel y las observaciones realizadas a lo largo de todo el proyecto.

Gracias a mis compañeros del Laboratorio de Genética Forestal, Vero, Esme, Ceci, Erika, Sinaí, Lupita, Nancy e Irenka por su apoyo en el establecimiento del experimento en campo y sus posteriores mediciones.

A Don Francisco Ramírez Cruz†, su esposa Doña Petra Cruz Cruz y familia del municipio de La Mesa en San José del Rincón, Estado de México, así como a Don Magdaleno Martin Antonio Campos y familia de Tlalpujahua de Rayón, Michoacán, que fueron parte fundamental para el establecimiento y mantenimiento de los ensayos de procedencias.

A la Bióloga Yolanda García Rodríguez encargada del Laboratorio de Ecología Química y Agroecología del Instituto de Investigaciones en Ecosistemas y Sustentabilidad de la UNAM, campus Morelia, por las facilidades prestadas para los análisis de cromatografía de gases.

A la Dra. Tarin Toledo Aceves por su apoyo y atenciones durante la Estancia de Investigación en el INECOL, Xalapa, Veracruz.

Por apoyo de financiamiento al proyecto a: Monarch Butterfly Fund, Forest Genetic Resources Working Group / North American Forest Commission / Food and Agricultural Organization of the United Nations (FGRWG-NAFC), United States Forest Service, American Forest, Programa PAPIT-UNAM (IG200221), Fondo de Conservación del Eje Neovolcánico y a la Coordinación de la Investigación Científica, UMSNH.

A mi compañero de vida, por todo su apoyo durante todo este trayecto. Gracias por recorrer este camino conmigo, por alentarme, escucharme, comprenderme, por estar presente, pero sobre todo por creer en mí, aun cuando yo misma dudé.

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RESUMEN GENERAL

El cambio climático ha tenido un impacto significativo en las coníferas, ya que las temperaturas más cálidas les han hecho perder su ventaja competitiva en climas más fríos, provocando una disminución de su área de distribución y de su población. Asimismo, las temperaturas más cálidas han provocado un aumento de las condiciones de sequía, que son perjudiciales para las coníferas por tener en general una alta susceptibilidad al estrés hídrico. Por lo anterior, el hábitat climático se está desplazando hacia sitios a mayores altitudes. Desafortunadamente, las coníferas son incapaces de migrar naturalmente al mismo ritmo con el que el cambio climático avanza. Para compensar este desajuste, la migración asistida, que consiste en trasladar plántulas o árboles jóvenes de coníferas originados de semilla de sitios con climas más cálidos a zonas en las que se encontrará su hábitat favorable en el futuro, de forma que se incrementen las posibilidades de supervivencia frente al cambio climático, surge como una estrategia alternativa de conservación de las especies. Primero, se tiene que evaluar la variación dentro de la especie y la distancia de transferencia climática óptima para que las plantas no mueran por estrés al frío en el presente. La transferencia climática es la diferencia entre su clima de origen y su clima de destino. Con la finalidad de determinar los impactos derivados del cambio climático sobre 11 poblaciones de *Abies religiosa* originadas de un gradiente altitudinal (3000 – 3550 m snm) dentro de la Reserva de la Biósfera Mariposa Monarca, se establecieron trasplantes recíprocos en sitios con altitudes contrastantes: 3400 m snm (para estimar viabilidad de migración asistida), 3000 m snm (sitio referencia) y 2600 m snm (para visualizar impactos del cambio climático). Se evaluaron diferentes variables de respuesta de acuerdo a la edad de la plantación. En el primer año se evaluó supervivencia, biomasa e incremento de diámetro basal; en el segundo año se evaluó proporción de supervivencia, incremento en altura e índice de productividad (supervivencia *x* biomasa). Los resultados para ambos años de prueba se evaluaron ajustando curvas de respuesta mediante modelos mixtos. En ambos casos, la relación entre la temperatura apta para el crecimiento (grados día, es decir, sumatoria de la temperatura diaria > 5 °C a lo largo de todo el año) y la precipitación disponible, expresada como índice de aridez, fue la variable climática que tuvo mayor significancia. Para la supervivencia del primer año de evaluación la temporada de lluvias (junio – octubre) fue crítica, mientras que para la biomasa fue la estación fría y seca (noviembre – febrero) y para el incremento en diámetro fue el índice

de aridez anual. Para el segundo año de evaluación, la distancia de transferencia climática de la temporada fría y seca (noviembre – febrero) fue la variable de clima que mejor explica a las tres variables de respuesta. Al final de los dos años del experimento, el sitio a 3400 m snm registró el mayor porcentaje de supervivencia de plantas (95 %) aunque un bajo desempeño en el crecimiento e índice de productividad, mientras que en el sitio a menor altitud se presentó un efecto muy negativo de la transferencia climática con 5.7 % de supervivencia, aunque las plantas que lograron sobrevivir presentaron el segundo mejor crecimiento en altura, pero no en productividad. Este último sitio (2600 m de altitud) resultó ser más árido, con 1.8 °C más de temperatura y con 14 % menos precipitación que el sitio de referencia. Por lo que la migración asistida hacia sitios con elevaciones mayores podría ser una acción de conservación viable para las poblaciones de oyamel frente al cambio climático.

Además, con plantas de las mismas procedencias, pero establecidas en una casa sombra en el Instituto de Investigaciones sobre los Recursos Naturales (INIRENA), en Morelia, Michoacán, se evaluó la presencia de compuestos fenólicos entre procedencias para determinar si existe un patrón altitudinal como respuesta a la radiación UV. Las muestras fueron medidas en un espectrofotómetro a 700 nm, obteniendo la media de absorbancia por procedencia. Con los resultados de absorbancia se realizó un análisis estadístico por medio de un modelo lineal generalizado con los parámetros ambientales altitud, irradiación, pendiente y aspecto, sin encontrar un patrón claro. Sin embargo, la relación significativa para aspecto e irradiación es significativa ($p = 0.0432$ y $p = 0.0496$, respectivamente). Es posible que estos resultados sean consecuencia tanto de factores genéticos, así como de variación estacional y de la ubicación de cada individuo dentro de la parcela.

También se realizó el análisis de la composición fitoquímica entre 14 procedencias de *Abies religiosa* provenientes de un gradiente altitudinal (2850 – 3540 m snm) del Cerro de San Andrés, Municipio Libre de Hidalgo, Michoacán, para determinar la asociación de perfiles químicos. Se estableció un jardín común dentro de casa de sombra en el INIRENA, se colectaron y analizaron acículas maduras por individuo por procedencia con cromatografía de gases–masas. Se realizaron índices de diversidad y análisis multivariados, obteniendo 32 compuestos. Con los resultados se concluyó que las poblaciones por debajo de 3350 m snm presentaron la diversidad fitoquímica más alta, pero en menores concentraciones, lo que parece ser una adaptación a la posible mayor abundancia y diversidad de insectos a menores altitudes. Además, se

determinaron tres asociaciones o grupos químicos por gradiente de elevación. Es importante que se consideren las asociaciones químicas dentro de los programas de manejo forestal con migración asistida, para mantener la capacidad de respuesta de las poblaciones a las diferentes condiciones de estrés que pudieran tener en el futuro.

Palabras clave: *Abies religiosa*, cambio climático, transferencia climática, trasplantes recíprocos, metabolitos secundarios, perfil químico.

GENERAL ABSTRACT

Climate change has had a significant impact on conifers, as warmer temperatures have caused them to lose their competitive advantage in colder climates, leading to a decrease in their distribution range and population. Also, warmer temperatures have led to increased drought conditions, which are detrimental to conifers due to their generally high susceptibility to water stress. Thus, climatic habitat is shifting to sites at higher altitudes. Unfortunately, conifers are unable to migrate naturally at the same rate as climate change is advancing. To compensate for this mismatch, assisted migration, which consists of moving conifer seedlings or saplings originated from seed from warmer sites, to areas where their favorable habitat will be found in the future in order to increase their survival in the face of climate change, has emerged as an alternative species conservation strategy. First, it is needed to know the within-species variation and the optimal climatic transfer distance so that plants on one hand escape from stress drought, but survive from cold stress at present. Climate transfer is the difference between its origin climate and its destination climate. In order to determine the impacts derived from climate change on 11 populations of *Abies religiosa* originated from an altitudinal gradient (3000 – 3550 m asl) within the Monarch Butterfly Biosphere Reserve, reciprocal transplants were established at sites with contrasting altitudes: 3400 m asl (to estimate feasibility of assisted migration), 3000 m asl (reference site) and 2600 m asl (to visualize climate change impacts). Different response variables were evaluated according to the age of the plantation. In the first year, survival, biomass and increase in basal diameter were evaluated; in the second year, survival ratio, height increase and productivity index (survival x biomass) were evaluated. Results for both test years were evaluated by fitting response curves using mixed models. In both cases, the relationship between temperature suitable for growth (degree days, i.e., sum of daily temperature > 5 °C throughout the year) and available precipitation, expressed as the aridity index, was the climatic variable with the greatest significance. For survival in the first year of evaluation the rainy season (June – October) was critical, while for biomass it was the cold and dry season (November – February) and for increase in diameter it was the annual dryness index. For the second year of evaluation, cold and dry season (November – February) climate transfer distance was the climate variable that best explained the three response variables. At the end of the two years of the experiment, the site at 3400 m asl registered the highest percentage of plant survival (95 %) although a low performance in growth and productivity index, while the site at the lower altitude presented a very negative effect of the climatic transfer with 5.7 % survival, although the plants that managed to survive presented the second-best growth in height, but not in productivity (when considering mortality). The latter site (2600 m of altitude) was found to be more arid, with 1.8 °C higher temperature and 14 % less precipitation than the reference site. Therefore, assisted migration to sites with higher elevations could be a viable conservation action for sacred fir populations in the face of climate change.

Additionally, with plants from the same provenances, but established in a shade house at the Instituto de Investigaciones sobre los Recursos Naturales (INIRENA), in Morelia, Michoacán, the presence of phenolic compounds between provenances was evaluated to determine if there is an altitudinal pattern in response to UV radiation. The samples were measured in a spectrophotometer at 700 nm, obtaining the mean absorbance per provenance. With the absorbance results, a statistical analysis was carried out by a generalized lineal model with environmental parameters such as irradiation, irradiation time, slope and aspect, without finding a clear pattern. However, the significant relationship for aspect and irradiance is significant ($p = 0.0432$ and $p = 0.0496$, respectively). It is possible that these results are a consequence of genetic factors, as well as seasonal variation and the location of each individual within the plot.

The analysis of the phytochemical composition among 14 provenances of *Abies religiosa* from an altitudinal gradient (2850 – 3540 m asl) of Cerro de San Andrés, Municipio Libre de Hidalgo, Michoacán, was also conducted to determine the association of chemical profiles. A common garden was established within the shade house at INIRENA, and mature needles were collected and analyzed per individual by provenance with gas-mass chromatography. Diversity indices and multivariate analysis were conducted, obtaining 32 compounds. With the results it was concluded that populations below 3350 m asl presented the highest phytochemical diversity, but in lower concentrations, which seems to be an adaptation to the possible greater abundance and diversity of insects at lower altitudes. In addition, three chemical associations or groups were determined by elevation gradient. Thus, it is important to consider chemical associations within forest management programs with assisted migration, in order to maintain the capacity of response of the populations to different stress conditions that may occur in the future.

Key words: *Abies religiosa*, climate change, climate transfer, reciprocal transplants, secondary metabolites, chemical profile.

INTRODUCCIÓN GENERAL

El cambio climático global se refiere a las modificaciones en los patrones climáticos y sus impactos asociados sobre los ecosistemas (Jia et al. 2019), causado por el aumento de la concentración de gases de efecto invernadero en la atmósfera como dióxido de carbono (CO_2), metano (CH_4), óxido nitroso (N_2O) y los clorofluorocarbonos (CFC), que para el año 2020 tuvieron un aumento del 49% en comparación a la década centrada en 1990 (National Oceanic and Atmospheric Administration 2022). A su vez, este aumento de gases de efecto invernadero ha generado el incremento en la temperatura superficial de la Tierra (incluyendo superficies continentales y océanos), entre 1.06 a 1.1 °C desde 1880 (National Oceanic and Atmospheric Administration 2023; National Aeronautics and Space Administration 2023), siendo los últimos nueve años los más calientes, y se espera que para 2024 la temperatura llegue hasta 1.5 °C de incremento en relación al período de referencia, o período base preindustrial 1880–1920 (Hansen et al. 2023). El océano también se ha calentado, pero en su caso, el calentamiento es más lento que en la superficie, porque el agua tarda más en absorber o liberar el calor (Hansen et al. 2010), aun así, el deshielo de los glaciares y el incremento del nivel del mar no deja de ser motivo de preocupación (Dutton et al. 2015).

El clima es la base moldeadora de los ecosistemas del mundo, pues la variación en la temperatura y la precipitación anual explican gran parte de los patrones observados en la distribución de los biomas (Hansen et al. 2001). La tasa de cambio para compensar la temperatura con la altitud es de ~ 0.5 °C por cada 100 m de elevación (Körner 2007; Jump et al. 2009; Sáenz-Romero et al. 2010). Los cambios derivados del cambio climático como las modificaciones en la frecuencia e intensidad de las precipitaciones (Dore 2005; Murray-Tortarolo 2021), la intensidad y duración de sequías severas, así como el incremento en la frecuencia y severidad de incendios (Westerling et al. 2006), han tenido un efecto negativo muy significativo, induciendo un desacoplamiento entre las especies y las condiciones climáticas a las que se han adaptado (Pearson y Dawson 2003; Castellanos-Acuña et al. 2015). Los cambios más drásticos sobre la vegetación mayormente observados y proyectados son las modificaciones del rango de distribución natural de las especies (Peñuelas et al. 2007), lo que implica una reducción o desplazamiento de su hábitat propicio (Sáenz-Romero et al. 2010; Gómez-Pineda et al. 2020), lo que a su vez genera cambios en su fenología y en la temporada de crecimiento de las poblaciones (Parmesan 2006). Estos cambios ocurrirán mucho más rápido que el ocurrido desde la última glaciación (Neilson et al. 2005).

Como una medida de conservación y restauración de ecosistemas, la "migración asistida, colonización asistida, reubicación gestionada o translocación gestionada", está tomando cada vez más fuerza (Leech et al. 2011; Vitt et al. 2016). Propuesta por primera vez por Peters y Darling (1985), la migración asistida trata del proceso de trasladar intencionalmente poblaciones, en respuesta anticipada al cambio climático, a sitios donde será más probable que sobrevivan y prosperen (Dumroese et al. 2015; Sáenz-Romero et al. 2020a). Este tema es controversial por cuestiones éticas, económicas, políticas, pero principalmente ecológicas pues rompe con los modelos tradicionales de conservación (Minteer y Collins 2010). Sin embargo, los partidarios a favor de esta estrategia, establecen que la migración asistida se ha vuelto una necesidad frente a los crecientes cambios en las condiciones climáticas, principalmente para especies con menor capacidad de adaptabilidad y de movimiento (Vitt et al. 2010; Williams y Dumroese 2013a), para así minimizar la pérdida de la biodiversidad y de servicios ecosistémicos (Williams y Dumroese 2013b) reduciendo el riesgo de extinción (Vitt et al. 2010).

La variación genética existente entre poblaciones de una misma especie se encuentra determinada por el ambiente en el que se ha desarrollado y adaptado (Rehfeldt 1980, 1994). Esta variación entre poblaciones es imprescindible para realizar migración asistida, para que las especies cuenten con un reservorio genético que les permita adaptarse a las condiciones climáticas proyectadas debidas al cambio climático (Sáenz-Romero et al. 2020b). Para estos efectos, el uso de ensayos de procedencias o de jardines comunes permite observar el desarrollo de las plantas bajo un mismo ambiente, beneficiando la expresión de caracteres regulados genéticamente (Mátyás 1996), proporcionando información sobre adaptación local y la plasticidad fenotípica de la especie (Benito-Garzón y Fernández-Manjarrés 2015). Algunos caracteres que se encuentran regulados por los genes son el crecimiento y la producción de compuestos químicos. Dentro de la composición química en coníferas, los grupos de metabolitos secundarios más importantes por su rol de defensa contra agentes externos son los terpenos y los compuestos fenólicos (Lundborg et al. 2016).

Las coníferas de alta montaña serán particularmente sensibles a los efectos producidos por el cambio climático (Chiu et al. 2022), debido a su distribución geográfica restringida (Parmesan

2006; Dakhil et al. 2021) y a su baja tasa de migración natural de aproximadamente cuatro metros por década, altitudinalmente hablando (Pauli et al. 1996; Sánchez Salas et al. 2011). Para lograr una migración exitosa, las especies dependen de su eficiencia reproductiva (viabilidad de semillas), dispersión, establecimiento y por último el crecimiento hasta su etapa de madurez reproductiva (Neilson et al. 2005), que en coníferas requiere de muchas décadas. Los efectos más evidentes del cambio climático sobre las poblaciones de coníferas, se han reflejado principalmente en el límite altitudinal inferior del rango de distribución natural de las especies, conocido como límite xérico (Mátyás et al. 2010; Mátyás 2010; Gea-Izquierdo et al. 2014). En la zona del límite xérico, el clima es cada vez más cálido durante la temporada de secas, propiciando el aumento de estrés por sequía en los árboles (Allen et al. 2015; Hammond et al. 2022), favoreciendo el brote de insectos descortezadores (Scheller et al. 2018). Incluso los inviernos más cálidos y húmedos aumentan la incidencia en el ataque de patógenos (Venäläinen et al. 2020). Todo lo anterior acrecienta considerablemente las tasas de mortalidad de las masas forestales a nivel mundial (Allen et al. 2010).

Los efectos derivados del cambio climático están afectando directamente a las poblaciones de *Abies religiosa* (Kunth) Schltdl. et Cham en México, mejor conocido por su nombre común, oyamel. Actualmente estos bosques ocupan entre el 0.1 y 0.5 % de la superficie forestal en México (Manzanilla-Quiñones et al. 2019). Sin embargo, desde 1970 se ha revelado que estos bosques sufren declinación forestal por el alto nivel de ozono y de dióxido de azufre en la atmósfera (Granados Sánchez y López Ríos 2001; González-Medina et al. 2010), contribuyendo a la reducción de biomasa en los árboles (Flores-Nieves et al. 2011). Además, se ha registrado la disminución en la densidad de árboles debido al aumento de cortas fitosanitarias por insectos descortezadores como *Scolytus mundus* y *Pseudohylesinus spp* (Manzo-Delgado et al. 2014; López-Gómez et al. 2015), debido principalmente a que las temporadas de sequía más frecuentes, prolongadas y cálidas favorecen su mayor incidencia (Gómez-Pineda et al. 2022).

Los bosques de oyamel se distribuyen como manchones aislados (Rzedowski 1978), dentro de la Faja Volcánica Transmexicana de México, principalmente desde los 19° N a los 20° N entre los 2400 y 3600 m snm (Rzedowski 1978; Sáenz-Romero et al. 2012), en sitios con temperaturas medias anuales entre 7 y 15 °C con precipitaciones anuales superiores a los 1000 mm (Rzedowski 1978). Los bosques de oyamel además, presentan un alto valor ecológico que radica principalmente en su capacidad de proveer importantes servicios ecosistémicos como el secuestro de CO_2 (Fragoso-López et al. 2017), generación de suelo orgánico (Peña-Ramírez et al. 2009), recarga de mantos acuíferos (Bolaños-Sánchez et al. 2021) y su importante papel como refugio de la biodiversidad, de las cuales, la especie más conocida es la mariposa monarca (*Dannaus plexippus* L.), la cual permanece cinco meses en los Santuarios ubicados en la Reserva de la Biósfera Mariposa Monarca para realizar su hibernación (Vidal y Rendón-Salinas 2014). También representa un importante valor económico, ya que es la segunda especie más aprovechada de país, después del género *Pinus*, contribuyendo con 2.8 % de producción de madera anual en México (Flores 2019).

Debido a la gran importancia de los bosques de oyamel, el objetivo de este trabajo fue determinar los impactos del cambio climático sobre la supervivencia, crecimiento, biomasa y productividad de distintas procedencias de *Abies religiosa*, así como analizar su diversidad química para sugerir lineamientos que puedan servir en la toma de decisiones para la implementación de estrategias de migración asistida con la finalidad de conservación y restauración de los bosques de oyamel.

HIPÓTESIS

- Las poblaciones de *Abies religiosa* originadas de la Reserva de la Biósfera Mariposa Monarca, presentarán efectos negativos a mayor distancia de transferencia climática, en crecimiento en altura, diámetro basal, supervivencia, producción de biomasa e índice de productividad, tanto en la dirección de la transferencia hacia sitios más cálidos y secos, como a sitios más fríos y húmedos.

- Las poblaciones de *Abies religiosa* del Cerro San Andrés, Municipio Libre de Hidalgo, Michoacán, recolectadas a menores altitudes presentaran la mayor diversidad y abundancia de terpenos que las procedencias de la parte intermedia y alta del gradiente altitudinal.

- Las poblaciones de *Abies religiosa* originadas a mayor altitud dentro de la Reserva de la Biósfera Mariposa Monarca, tendrán una presencia mayor de compuestos fenólicos como mecanismo de protección ante la radiación UV.

OBJETIVOS

General

Estimar el impacto de los efectos del cambio climático sobre la supervivencia, crecimiento, biomasa y productividad de distintas procedencias de *Abies religiosa* mediante el uso de trasplantes recíprocos en jardines comunes a altitudes contrastantes (2600, 3000 y 3400 m snm). Así como analizar la diversidad química y concentración de metabolitos secundarios (terpenos y fenoles), para sugerir lineamientos que puedan servir para la toma de decisiones, en la implementación de estrategias de migración asistida, con la finalidad de conservación y restauración de los bosques de oyamel.

Específicos

- Estimar el impacto de la distancia de transferencia climática sobre la supervivencia, incremento en diámetro y biomasa de 11 procedencias de *Abies religiosa* originadas en la Reserva de la Biósfera Mariposa Monarca (RBMM), a un año de su establecimiento en jardines comunes a altitudes contrastantes.

- Evaluar el cambio en el impacto de la transferencia climática sobre 11 procedencias de *Abies religiosa* originadas en la RBMM, a dos años de su establecimiento en jardines comunes a altitudes contrastantes sobre la supervivencia, incremento en altura e índice de productividad (supervivencia *x* biomasa).

- Determinar la diversidad y abundancia de terpenos presentes en 14 procedencias de *Abies religiosa* provenientes del Cerro San Andrés, Municipio Libre de Hidalgo Michoacán y establecidas en un jardín común en el Instituto de Investigaciones sobre los Recursos Naturales (INIRENA), para determinar si existe un patrón clinal altitudinal.

- Analizar la presencia de fenoles en 10 procedencias de *Abies religiosa*, originadas en la RBMM, desarrolladas en una casa de sombra en el INIRENA para determinar si existe un patrón clinal altitudinal en la producción de fenoles en respuesta a la radiación UV.

RESULTADOS

Capítulo I. Reciprocal common garden altitudinal transplants reveal potential negative impacts of climate change on *Abies religiosa* populations in the Monarch Butterfly Biosphere Reserve overwintering sites

forests

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Article

Reciprocal Common Garden Altitudinal Transplants Reveal Potential Negative Impacts of Climate Change on *Abies religiosa* Populations in the Monarch Butterfly Biosphere Reserve Overwintering Sites

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Abstract: Research Highlights: Reciprocal altitudinal transplants of *Abies religiosa* seedlings within the Monarch Butterfly Biosphere Reserve (MBBR) allow prediction of the impacts of climatic change, because they grow in sites with a climate that differs from that of their origin. Background and Objectives: Climatic change is generating a mismatch between the sites currently occupied by forest populations and the climate to which they have adapted. This study determined the effect on the survival and growth of *A. religiosa* seedlings of transfer to sites that were warmer or colder than that of the origin of their seeds. Materials and Methods: Eleven provenances of *A. religiosa*, collected along an altitudinal gradient (3000 to 3550 m a.s.l.), were assayed in common gardens in three sites of contrasting altitude: 3400, 3000 and 2600 m a.s.l. The results were evaluated by fitting a response curve with a mixed model. Results: The climate transfer distance for the seasonal balance between the temperature conducive to growth (degree days above 5 °C) and the available precipitation (a ratio expressed as dryness index) dominated the shape of the response function curve. The rainy season (June–October) dryness index transfer distance was critical for survival, while that of the cold and dry season (November–February) was critical for aerial biomass, and the annual index was critical for the increase in basal diameter. The effect of climatic transfer distance is much more negative (triggering about 45% mortality) when transfer is toward warmer and dryer sites (at 400 m lower in altitude, +1.9 °C warmer and 16% less precipitation), than when shifting toward colder and wetter sites (400 m higher in altitude, resulting in 95% survival). Conclusions: The projected higher temperatures and lower precipitation due to climatic change will undoubtedly cause severe mortality in young *A. religiosa* seedlings. A 400 m shift upwards in altitude to compensate for climatic change (assisted migration) appears to be a feasible management action.

Keywords: climate transfer distance; dryness index; climate change; assisted migration; *Abies religiosa*; survival; mortality; biomass; basal diameter; Monarch Butterfly Biosphere Reserve

Citation: Cruzado-Vargas, A.L.; Blanco-García, A.; Lindig-Cisneros, R.; Gómez-Romero, M.; Lopez-Toledo, L.; de la Barrera, E.; Sáenz-Romero, C. Reciprocal Common Garden Altitudinal Transplants Reveal Potential Negative Impacts of Climate Change on *Abies religiosa* Populations in the Monarch Butterfly Biosphere Reserve Overwintering Sites. *Forests* **2021**, *12*, 69. https://doi.org/10.3390/f12010069

Received: 9 December 2020
Accepted: 4 January 2021
Published: 9 January 2021

1. Introduction

In the near future, climatic change will generate warmer climates and displace the climatic zones towards the poles and towards higher altitudes in mountainous regions [1].

Given that, as the result of a long process of evolutionary adaptation, species and their populations are distributed in localities that are generally coupled to a climate suitable for them, climatic change will generate a decoupling between the populations and the climate to which they are adapted [2,3], influencing phenological changes, population and growth dynamics [4] and reproduction [5]. Stress induced by this decoupling favors the frequency and intensity of the development of pests and diseases [6–10] and defoliation through drought stress [11], inducing a severe process of forest decline, which is already underway globally [12], especially at the xeric limit (lower altitudinal or south in the northern hemisphere) [13,14].

Forest tree species that present a wide geographic distribution can present an important genetic differentiation among populations in terms of quantitative characters, such as adaptation to the diversity of environmental conditions [3,15,16]. This differentiation is expressed in different variables such as growth rate, seasonality of elongation of the apical bud and resistance to frost damage [17–20]. Provenance assays allow the expression of these differences by cultivating plants from different geographic origins (provenances) under the same environmental conditions [21]. In general, for forest populations, a pattern of variation of growth has been reported in which the provenances originating from the colder (higher altitude) part of the natural distribution express a lower potential for growth in height, but a greater resistance to frost damage while, in contrast, those from the low part of the altitudinal distribution (warmer) have greater potential for growth but are more susceptible to frost damage [22–24].

In Mexico, one of the most important species for biological conservation is *Abies religiosa* (sacred fir or oyamel), because it hosts the overwintering sites of the monarch butterfly (*Danaus plexippus* L.) for five months (November–March) [25]. The areas of the greatest extension of *A. religiosa* are found within the Trans-Mexican Volcanic Belt, at between 2400 and 3600 m a.s.l. and mainly between 19° and 20° in latitude North [26]. The Monarch Butterfly Biosphere Reserve (MBBR) is found within the Trans-Mexican Volcanic Belt, on the border between the states of Michoacán and Estado de México. The forests of *A. religiosa* can be strongly affected by climatic change—it will induce a decoupling between the populations of *A. religiosa* and the environment suitable for them, such that by the decade 2090, the models project that suitable climatic habitat for *A. religiosa* will have disappeared completely from the MBBR [26]. Previous studies have reported that *A. religiosa* presents a high percentage of non-viable seeds (63–79%) [27,28], in addition to very low recruitment and high mortality of sacred fir seedlings, necessitating the implementation of an active restoration program or other management actions [29]. Additionally, so that the populations of this species can remain coupled to their favorable climatic habitat, their upwards altitudinal displacement of between 300 and 500 m will be necessary by 2060 [30], due to the projected increases in temperature and diminution in precipitation. This could have a considerable effect on the overwintering of the monarch butterfly, in addition to severe environmental impacts. For these reasons, human-assisted displacement of seed sources towards higher altitudes (through reforestation programs) appears to be an option for consideration as a strategy of adaptive management to climatic change. This is because, while the populations of forest species can, and in fact currently are, migrating altitudinally towards higher altitudes through natural means, the speed at which they can accomplish this is lower than that necessary to remain coupled to the climate that favors them [31,32].

The results of two previous field tests indicate that it is feasible to conduct assisted altitudinal migration to above the provenances of *A. religiosa*, translocating the seed sources up to 400 m higher in altitude than their origin, but only when planted beneath the shade protection of local shrubs (such as *Baccharis conferta*) serving as nurse plants [33]. However, to date, no experiments have determined what would happen if young *A. religiosa* seedlings are exposed to greater temperatures and lower precipitation, as projected in scenarios of climatic change [26,34]. There is also evidence that there are serious difficulties in terms of the natural regeneration of young seedlings, because the warm and dry season of March–May is now more dry and hot than usual, causing a severe mortality of seedlings in

April [29]. Although an important genetic differentiation has been demonstrated among populations of *A. religiosa* for resistance to frost damage along altitudinal gradients [20], a significant genetic differentiation has not been demonstrated among populations for resistance to drought, for example, in the differential accumulation of solutes, such as the amino acid proline, that confer physiological resistance to drought [35,36].

Considering the above, the objective of this study was to evaluate the effects of climatic transfer (climatic difference between the site of origin and the site of plantation) on the survival and growth of young *A. religiosa* seedlings, through reciprocal transplants of eleven provenances of *A. religiosa* to common gardens at three contrasting altitudes. The climatic transfer was conducted by simulating three possible scenarios: (i) towards altitudes lower than the seed source origin (simulating a future climate of higher temperature and lower precipitation); (ii) towards higher altitudes (to confirm the viability of assisted migration); and (iii) to a site of intermediate altitude as a reference. The latter two sites were located in the core zone and on the edge of the buffer zone of the MBBR, respectively, in Estado de México, in central-western Mexico. The intention was to generate guidelines for decision-making in terms of the transfer of seed sources in a program of assisted migration, as well as to quantify the impacts of climatic change and the risks of failing to practice adaptive management in the face of climatic change.

2. Materials and Methods

2.1. Study Site

The study was conducted within the Monarch Butterfly Biosphere Reserve (MBBR), which forms part of the Trans-Mexican Volcanic Belt, and in Tlalpujahua de Rayón, Michoacán. The altitudes of the MBBR range from 2200 to 3640 m a.s.l. and the annual average temperatures range from 8 to 22 °C [37]. The vegetation is mainly comprised by forests of fir (*A. religiosa*), pine (*Pinus* spp.), oak (*Quercus* spp.), and cedar (*Cupressus* spp.) [38].

2.1.1. Seed Collection Sites

Cones were collected (in December 2017) from eleven provenances of *A. religiosa*, along an altitudinal transect (3000 to 3550 m a.s.l. in altitude; altitudinal difference of 50 m between sites), within the MBBR (Table 1). At each site, cones were collected at random from ten trees, at a distance of at least 30 m apart [39], in order to reduce the possibility of endogamy.

Table 1. Geographic location and some climatic variables (their acronyms as in Table 2) of *Abies religiosa* seed collection sites within the Monarch Butterfly Biosphere Reserve (MBBR).

Altitude (m a.s.l)	Lat. N	Long. W	MAT (°C)	MAP (mm)	MTCM (°C)	ADI Index	WDSDI Index	CDSDI Index	RSDI Index
3552	19.564	100.229	8.5	1107	6.2	0.034	0.152	0.160	0.029
3491	19.567	100.233	8.8	1094	6.5	0.035	0.161	0.176	0.030
3457	19.571	100.235	9.0	1089	6.7	0.036	0.165	0.184	0.031
3411	19.573	100.237	9.3	1079	7.0	0.038	0.173	0.199	0.032
3364	19.575	100.234	9.5	1065	7.2	0.039	0.183	0.206	0.033
3300	19.579	100.231	9.9	1048	7.5	0.041	0.191	0.222	0.035
3233	19.580	100.224	10.2	1029	7.8	0.043	0.201	0.233	0.036
3210	19.581	100.220	10.3	1022	8.0	0.044	0.204	0.238	0.037
3143	19.581	100.214	10.6	1001	8.3	0.046	0.216	0.250	0.039
3099	19.586	100.214	10.8	987	8.5	0.047	0.226	0.260	0.040
3003	19.595	100.210	11.3	969	8.9	0.050	0.253	0.303	0.041

Sites at altitudes from 3552 to 3300 m a.s.l and at 3099 m a.s.l. were collected at Ejido La Mesa. Sites from 3233 to 3143 m a.s.l. were collected at Ejido Buenavista Casablanca. Site at 3003 m a.s.l. was collected at Ejido Guadalupe Buenavista, Municipality of San José del Rincón, Estado de México.

Table 2. Climatic variables estimated for each provenance (seed origin, taken from [40]) and for each test site (taken from data loggers HOBOs and rainwater capture data).

Code	Unit	Definition
MAT	°C	Mean annual temperature
MAP	mm	Mean annual precipitation
GSP	mm	Growing season precipitation (total precipitation from April–September)
WDSP	mm	Warm and dry season (total precipitation March–May)
RSP	mm	Rainy season (total precipitation June–October)
CDSP	mm	Cold and dry season (total precipitation November–February)
MTCM	°C	Mean temperature in the coldest month
MMIN	°C	Mean minimum temperature in the coldest month
MTWM	°C	Mean temperature in the warmest month
MMAX	°C	Mean maximum temperature in the warmest month
DD5	°C	Degree-days > 5 °C
WDSDD5	°C	Warm and dry season (March–May degree days > 5 °C)
RSDD5	°C	Rainy season (June–October degree days > 5 °C)
CDSDD5	°C	Cold and dry season (November–February degree days > 5 °C)
ADI	index	Annual dryness index ($ADI = \sqrt{DD5}/MAP$)
GSDI	index	Growing season dryness index ($GSDI = \sqrt{GSDD5}/GSP$)
WDSDI	index	Warm and dry season (March–May) dryness index ($WDSDI = \sqrt{WDSDD5}/WDSP$)
RSDI	index	Rainy season (June–October) dryness index ($RSDI = \sqrt{RSDD5}/RSP$)
CDSDI	index	Cold and dry season (November–February) dryness index ($CDSDI = \sqrt{CDSDD5}/CDSP$)

2.1.2. Provenance Test Sites in Common Gardens

Three field provenance tests were established in common gardens (Table 3): (a) high altitude site at 3400 m a.s.l., to confirm the viability of altitudinally upwards assisted migration, within the core zone of the MBBR (at an approximate linear distance of 300 m from a monarch butterfly overwintering site); (b) intermediate altitude site at 3000 m a.s.l., on the edge of the buffer zone of the MBBR; and (c) low altitude site at 2600 m a.s.l., to quantify the effects of a warmer, drier climate than that of the seed origin (considering that the temperature changes by approximately 0.5 °C for each 100 m of altitudinal difference [34]).

Table 3. Location and some climatic variables (their acronyms as in Table 2) of sites of tests of provenances in common gardens.

Site	Altitude (m a.s.l.)	Lat. N	Long. W	MAT (°C)	MAP (mm)	MTCM (°C)	ADI Index	WDSDI Index	CDSDI Index	RSDI Index
Llano Grande [1]	3400	19.57	100.23	9.3	1076	7.0	0.026	0.198	0.041	0.026
La Mesa [2]	3000	19.58	100.18	11.3	951	8.9	0.056	0.537	0.251	0.044
Tlalpujahua [3]	2600	19.80	100.16	12.8	906	9.6	0.078	0.930	0.611	0.057

[1] Core zone of the Monarch Butterfly Biosphere Reserve (MBBR). [2] Ejido La Mesa, Municipality of San José del Rincón, Estado de México (near the buffer zone of the MBBR). [3] Michoacán state.

2.2. Production of Plants in Nursery

Once the seed was obtained from the mother tree, it was stratified at 4 °C for 14 days [39,41]. Each lot of seeds subjected to stratification comprised a similar quantity of seeds originating from each mother tree, so that the progenies were represented equally in the provenances.

Plant production was conducted for 14 months (May 2018–June 2019) in a shade-house (35% shade mesh) in the Instituto de Investigaciones sobre los Recursos Naturales (INIRENA), in Morelia city in Michoacán, Mexico. Plants were germinated in a seedbed and later transplanted into containers of 380 cm^3, with a mixed substrate comprising loamy soil, agrolite, vermiculite and peat moss (volume 60:15:15:10, respectively). Transplantation was conducted with an equal quantity of seeds germinated from each mother tree, so that they were represented equally in the provenances. To prevent differential growth among the provenances in the nursery as a result of possible environmental differences within the shade house, the plants were positioned in a completely random block design (6 blocks, 11 provenances, 54 plants per plot).

2.3. Test of Provenances in Common Gardens in the Field

In each field site (as in Table 3), three raised beds were constructed, with wooden frames of 5 × 1.5 × 0.4 m in length:width:height. The internal base of each raised bed was covered by a metal mesh (aperture size 3.5 cm) to prevent access by gophers. To ensure that the sites differed only in terms of the climate as a result of their altitude, and thus to evaluate the impact of the climatic transfer in each provenance, the raised beds were filled with the same forest soil, transported from a forest site dominated by *A. religiosa* at the intermediate altitude (3000 m a.s.l.), thus avoiding any confounding effect of the local soil. Finally, the raised beds were covered by a shade mesh (35%) at a height of 2.5 m, to simulate the protective effect of nurse plants [33] (Figure 1).

Figure 1. Raised beds of the provenance tests (common gardens) in the site at 2600 m a.s.l., October 2020, 15 months after transplantation. Each raised bed contained two complete randomized blocks.

The seedlings produced in the nurseries were transplanted to the raised beds in July 2019 (beginning of the rainy season), at age 14 months. In each site, six completely random blocks were established (11 provenances per block, five seedlings per provenance per plot in line, at a spacing of 0.2 × 0.2 m and with two blocks per raised bed. At the extremes of the raised bed, a protection strip was planted, comprising a mixture of seedlings of all of the provenances, in order to control the edge effect (Figure 1).

The trees (the seeds of which were collected at different altitudes) represented in the common garden tests by their seedlings are herein termed populations, while the location of origin of a population is called the provenance.

2.4. Measurements

For sixteen months, monthly or bimonthly evaluations of plant survival and height were conducted, with semestral evaluations of the basal diameter. The first evaluations of plant height and basal diameter were conducted six months after plantation, in the expectation that the plants would be well established by that time. The final measurement was taken at the end of the growing season of the following year (November 2020). Plant height was measured with a 1 mm precision up to the tip of the apical bud, supporting the ruler on a bar of aluminum, which was in turn supported on the edge of the wooden frame (to avoid measurement errors due to the irregular surface of the substrate). Basal diameter was measured at the base of the stem with a digital vernier (KNOVA®, Cupertino, CA, USA) at a 0.1 mm precision.

The increase in seedling height and basal diameter was obtained by subtracting the first measurement (December 2019) from the final measurement (November 2020). Survival was estimated as the percentage of live plants per plot. Substrate moisture content was measured with a W.E.T (water content, electrical conductivity and temperature) sensor of type W.E.T-2 (Delta-T-Devices, Cambridge, UK) as well as gravimetrically, by measuring the wet and dry weights of soil samples.

2.5. Estimation of Aerial Biomass

Following the methodology of Martinez-Luna, et al. [42], the aerial biomass of the seedlings in the common garden field assay was estimated using a regression formula from the measurements of total height and basal diameter. The formula was fitted by measuring (through destructive harvest) seedling height, basal diameter and dry weight of the stem, branches, and foliage (dried for 48 h at 65 °C in a Shel Lab drying oven (model FX4) and weighed on a Mettler Toledo analytical balance, model AB204-S). Five seedlings of diverse sizes were selected for this harvest from each of the 11 assayed provenances (harvested from a surplus lot of plants). The regression was fitted using Proc Reg of SAS [43,44], from which the following parameters were obtained:

$$Ba = -1.62490 + (0.03538 \times Ht) + (0.77238 \times Db) \tag{1}$$

where Ba is the aerial biomass (g), Ht is the total height (mm), and Db is the basal diameter (mm 0.1).

2.6. Climatic Data

Climatic data from the provenance of origin of the seeds were obtained for the reference period 1961–1990 through climatic spline models (based on [34]), available at the website [40]. The climate of the provenance is considered the recent historic climate to which the provenances have evolved and adapted [45]. The climate of the field assay sites, which was that experienced by the seedlings while growing in the field common gardens, was estimated using three data loggers HOBOs (temperature/external channel data logger; Onset Computer Corporation®, Bourne, MA, USA) per site, set to record temperature every 30 min. Monthly precipitation was measured by capturing rainwater, averaging the results from two rainwater traps per site.

The climatic variables estimated for both the provenances and the sites are described in Table 2, and are considered of physiological importance for representing the seasonal or annual balance between the available humidity and the temperature in terms of seedling growth [17,46]. The seasonal indices warm and dry season dryness index (WDSDI), rainy season dryness index (RSDI) and cold and dry season dryness index (CDSDI) aim to represent the seasonality of each of the *A. religiosa* study sites and are based on the seasonality observed in [33].

From the climate of the provenances and of the field test sites, the climatic or altitudinal transfer distance (CTD or ATD = climate or altitude of the test site − climate or altitude of the source of the provenance) was estimated in order to represent the impact of growing in a site with a climate (or altitude) that differed from that of the seed origin.

2.7. Statistical Analysis

A curve was estimated for each of the four response variables studied (survival, increase in basal diameter, increase in height, and aerial biomass of *A. religiosa* seedlings), to measure the effect of the climatic or altitudinal transfer (growing in a site with a climate or altitude that differed from that of the seed origin), using a mixed model in Proc Mixed of SAS [43,44], based on refs [16,46].

The fixed effects considered three components of the effect of climate as a selective force that produces a genetic differentiation among populations and/or a response of phenotypic plasticity (also considered as a genotype × environment interaction [15]): (a) the climate of the provenance (C); (b) the climatic transfer distance (D = climate of the test site − climate of the provenance); and (c) the interaction between climate of the provenance and climatic transfer distance (C × D). Random effects provided the variance that was not explained by the fixed effects; i.e., those derived from the experimental design: site, provenance, block and the interaction between the site and the provenance. The climatic transfer distance is a concept originally known as "ecological distance" [47]; more details regarding the reasoning of this model can be found in refs [16,48].

2.7.1. Mixed Model

The following mixed model was fitted, based on refs [16,46]:

$$Y_{ijkl} = \mu + \beta_0 + \beta_1 \mathrm{D_{ij}} + \beta_2 \mathrm{D_{ij}^2} + \beta_3 \mathrm{C_j} + \beta_4 (\mathrm{D_{ij}} \times \mathrm{C_j}) + \beta_5 S_i + \beta_6 P_j + \beta_7 B_k(S_i) + \beta_8 (S_i \times P_j) + e_{ijkl} \quad (2)$$

where Y_{ijkl} is the survival or aerial biomass or increase in basal diameter or increase in plant height, corresponding to the lth tree for the jth provenance in the kth block in the ith test site, and β_0 is the intercept. $\mathrm{D_{ij}}$ is the climatic transfer distance (difference between the value of a climatic variable in the test site and that of the provenance) for the jth provenance in the ith test site, $\mathrm{C_j}$ is the value of the climatic variable in the jth provenance, and $\mathrm{D_{ij}} \times \mathrm{C_j}$ is the interaction between the climatic transfer distance for the jth provenance in the ith test site and the climatic variable in the jth provenance. S_i is the effect of the ith test site, P_j is the effect of the jth provenance, $B_k(S_i)$ is the effect of the kth block nested within the ith test site, and e_{ijkl} is the error term.

2.7.2. Selection of Variables to Fit the Best Mixed Model

The climatic variables for use in the mixed model were selected using the following steps, independently for each response variable, based on refs [16,46]:

(1) Five climatic variables were selected that best described the climate of the provenance, estimating the Spearman correlations between the values of the climatic variables and the average value per provenance across sites of the response variables, and selecting those with the highest $|r|$ value.

(2) The climatic variables for the climatic transfer distance were selected by fitting a reduced mixed model, eliminating from the model (Equation (2)) the term of climate of the provenance ($\mathrm{C_j}$) and its respective interaction ($\mathrm{D_{ij}} \times \mathrm{C_j}$). The five climatic variables selected were those for which the model obtained the lowest (and thus the best) value of the Akaike information criterion (AIC) and, in addition, that necessarily presented the estimated parameter of the quadratic term both negative and significant, in order to ensure that it was biologically sound [15,16].

(3) Subsequently, 5 × 5 = 25 full "competing" models were run, which included all of the possible combinations of the five variables of the climate of provenance and the five variables of climatic transfer distance preselected in the previous two steps. The best model was selected based on the AIC value.

In total, 100 models (25 "competing" models for each of the four response variables) were run, in order to determine the best model for understanding the response of survival, increase in basal diameter, increase in plant height and aerial biomass.

Finally, from the regression parameters estimated for the fixed effects, quadratic curves of response to the climatic transfer were estimated for each response variable, in order to visualize the effect on survival and growth of growing in a site with a climate that differs to that of the origin of the populations.

3. Results

3.1. Climatic Variables That Best Explained the Climatic Transfer Distance Response Function

The survival response function was best explained (lowest AIC value = 1651.1) by the quadratic term of the rainy season dryness index transfer distance (RSDI_td^2; $p = 0.0112$), although the linear term was not significant ($p = 0.3542$). The other fixed effect terms, the climate of the provenance term selected (warm and dry season dryness index, WDSDI; $p = 0.0941$) and the interaction between climatic transfer distance and the climate of the provenance (RSDI_td × WDSDI; $p = 0.1273$), were also not significant. Regarding the random effect terms, only the block nested within the site was significant ($p = 0.0252$), contributing 21% of the total variance, where 100% is the sum of the contribution of the random terms only (Table 4).

Table 4. Analysis of the mixed model for seedling survival, biomass and increase in basal diameter. Akaike information criterion (AIC), estimated parameters (coefficients of regression for fixed effects and variances estimated for random effects), contribution to the total variance (of the random effects) and significance (p).

Parameter or Source of Variation	**Survival**			**Biomass**			**Increase in Basal Diameter**		
Fixed Effects	**Estimate**		***p***	**Estimate**		***p***	**Estimate**		***p***
Akaike Information Criterion	1651.1		—	2397.2		—	1917.3		—
Intercept	119.7		0.0115	2.4		0.0478	0.081		0.8824
Climate at seed source									
Warm and dry season dryness index (WDSDI)	−111.3		0.0941	6.8		0.0198	—		—
Mean temperature in the coldest month (MTCM)	—		—	—		—	0.19		0.0036
Climate transfer distance									
Rainy season dryness index (RSDI)	1426.8		0.3542	—		—	—		—
Cold and dry season dryness index (CDSDI)	—		—	8.7		0.0002	—		—
Annual dryness index (ADI)	—		—	—		—	143.5		<0.0001
(Climate transfer distance)^2									
RSDI^2	−55,955		0.0112	—		—	—		—
CDSDI^2	—		—	−10.6		<0.0001	—		—
ADI^2	—		—	—		—	−1291.2		<0.0001
Interaction climate seed source × Climate transfer distance	−10,202		0.1273	−20.6		0.0395	−14.7		<0.0001
Random Effects	**Variance**	**% ***	***p***	**Variance**	**% ***	***p***	**Variance**	**% ***	***p***
Site	0	0	1	0	0	1	0	0	1
Population	1.5	0.34	0.4412	0.042	3.10	0.1217	0.010	1.4	0.1364
Block (Site)	91.2	21.02	0.0252	0.097	7.19	0.0196	0.108	15.04	0.0095
Site × Population	0	0	1	0.019	1.43	0.2036	0	0	1
Error	341.0	78.64	<0.0001	1.195	88.28	<0.0001	0.598	83.55	<0.0001

* Contribution to total variance expressed as a percentage, where 100% is the sum of the contribution to the total variance of the random terms only.

The biomass response function was best explained (lowest AIC value = 2397.2) by the significant linear and quadratic term of the cold and dry season dryness index transfer distance (CDSDI_td, $p = 0.0002$; CDSDI_td^2, $p < 0.0001$; respectively). The climate of the provenance term selected (warm and dry season dryness index, WDSDI) was also significant ($p = 0.0198$), as was the interaction CDSDI_td × CDSDI ($p = 0.0395$). The random effect term that contributed most to the total variance (among the random effects) was block nested within site ($p = 0.0196$), contributing 7% of the total variance (Table 4).

The increase in basal diameter response function was best explained (lowest AIC value = 1917.3) by the significant linear and quadratic term of the annual dryness index (ADI) transfer distance ($p < 0.0001$). The climate of the provenance term selected (mean temperature of the coldest month, MTCM) was also significant ($p = 0.0036$), as was the interaction ADI_td × MTCM ($p < 0.0001$). The random effect term that most contributed to the total variance was block nested within site ($p = 0.0095$), contributing 15% of the total variance (Table 4).

The increase in seedling height response function was best explained (lowest AIC value = 5122.4) by RSDI, although, as with transfer distance, it was not significant (linear: $p = 0.23$; quadratic: $p = 0.3517$), as well as the climate of the provenance term selected (MMIN, $p = 0.1605$). Again, only block nested within site was significant ($p = 0.0096$) among the random terms, contributing 11% of the total variance (results not shown in Table 4 for simplicity).

In all of the four response variables analyzed, site was not significant, and the error term contributed the most among all the random terms (from 79% for survival to 88% for aerial biomass; Table 4).

3.2. Curves of Response to the Climatic and Altitudinal Transfer Distance

The predicted response curve for survival indicated that when the seed sources were moved toward warmer and dryer sites (toward lower altitude, right side of the curve in Figure 2a), there is a severe induced mortality. In sharp contrast, when the shift is conducted toward a colder and moister site (to higher altitudes, sites at 3400 m a.s.l.), survival is maintained mostly above 95%. This is even more evident when fitting a simple quadratic regression of survival against altitudinal transfer distance (Figure 3a), where exceeding the 400 m shift to lower altitudes (grossly equivalent to +2 °C, based on a lapse rate of 0.5 °C per 100 m [34]) causes significantly increased mortality. It should also be noted in Figure 2a that the maximum value of the response curve is displaced slightly towards the left side (colder and moister sites), either for the average species curve or for the extreme lower altitudinal population curve. This suggests that the optimum of survival for the species in general, and for the extreme lower population, would be a colder site than that occupied at present.

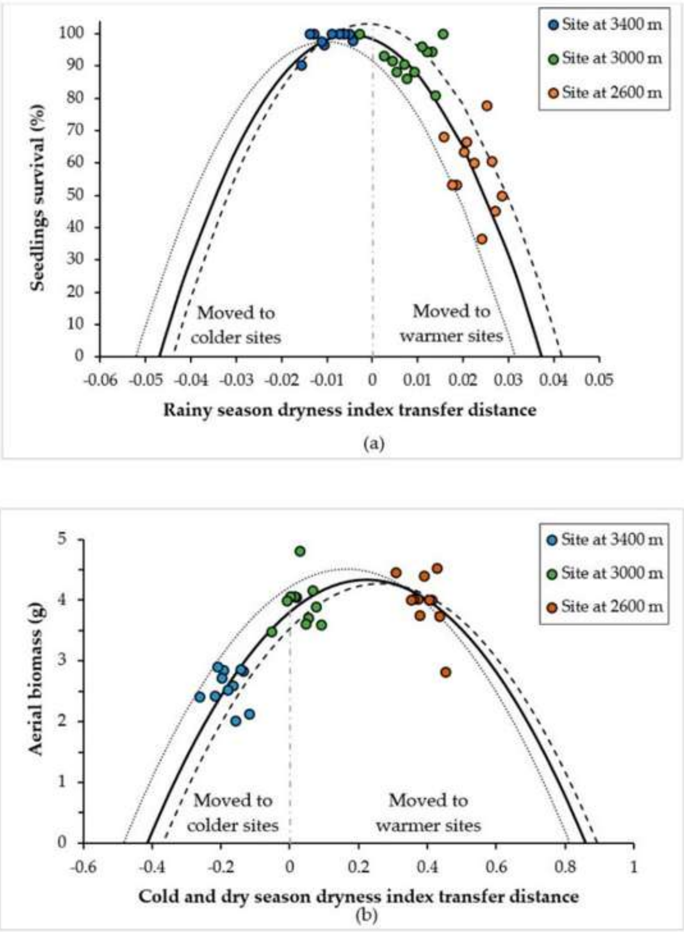


Figure 2. *Cont.*

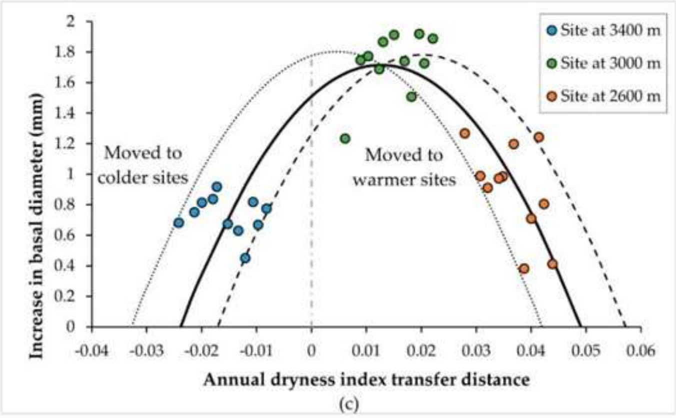


Figure 2. Seedling response to climatic transfer distance for: (**a**) seedling survival; (**b**) aerial biomass (dry weight); and (**c**) increase in basal diameter. Predicted response curves (constructed with the estimated parameters according to the fixed terms of the mixed model, as in Table 4), are indicated for the species (solid line) and for the populations of the coldest site (dashed line) and the warmest site (dotted line). Symbols are observed averages per population per site (the color distinguishes the particular test site). Respective climatic transfer distance on the *x*-axis signifies the transfer to drier and/or warmer sites if the values are positive, and transfer to cooler and/or moister sites if they are negative. Zero signifies a climate similar to that of the site of provenance.

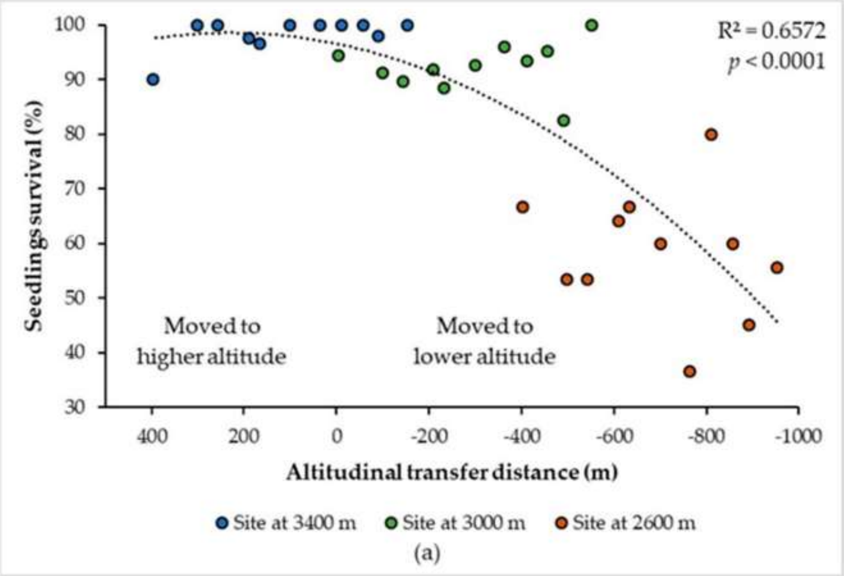
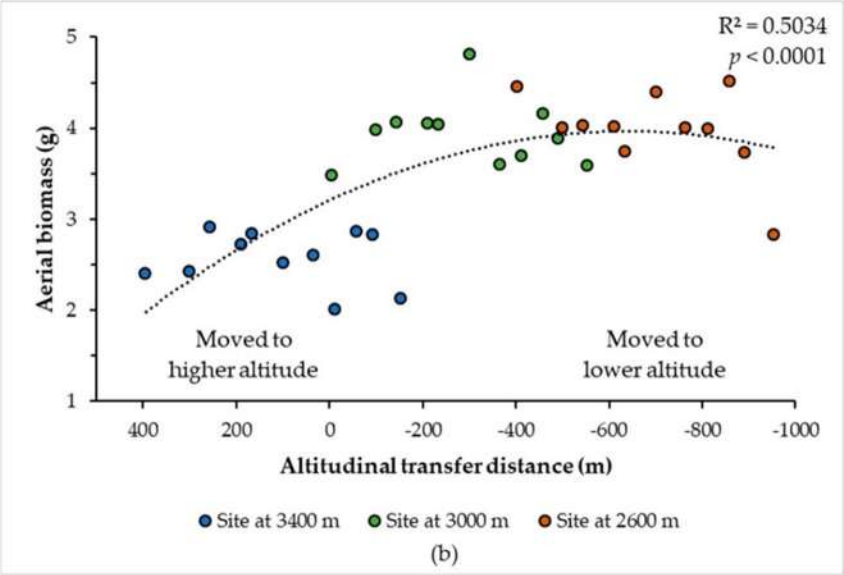


Figure 3. *Cont.*



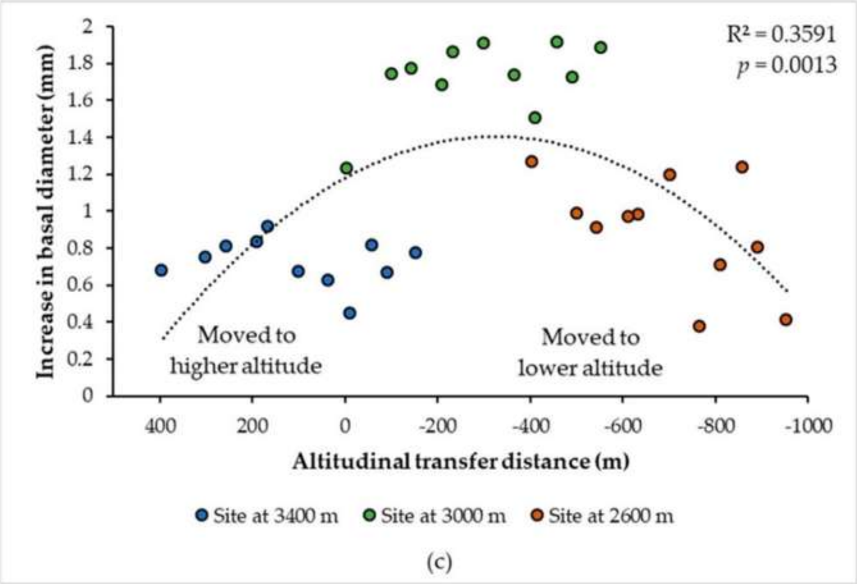


Figure 3. Estimated response curve (based on a quadratic regression) of the plants to the altitudinal transfer for: (**a**) survival; (**b**) aerial biomass (in dry weight); and (**c**) increase in basal diameter. Symbols indicate the average observed values per provenance per site. Symbol color denotes the particular assay site: blue, 3500 m a.s.l.; green, 3000 m a.s.l.; orange, 2600 m a.s.l. Positive values on the x-axis indicate movement towards sites of higher altitude, which are colder and moister, while negative values indicate movement towards sites of lower altitude, which are warmer and drier.

The predicted response curve for aerial biomass indicated that, when seed sources are moved toward warmer and dryer sites (to a lower altitude, right side of curve the in Figure 2b), there is little negative effect on the growth expressed as aerial biomass: the observed values fluctuated around the maximum predicted value of the curve (about 4.2 g of aerial biomass in dry weight). In contrast, when seed sources are shifted toward colder and moister sites, there is an important loss of accumulated aerial biomass. This becomes more evident when plotting the average biomass per population and per site against the altitudinal transfer distance (Figure 3b).

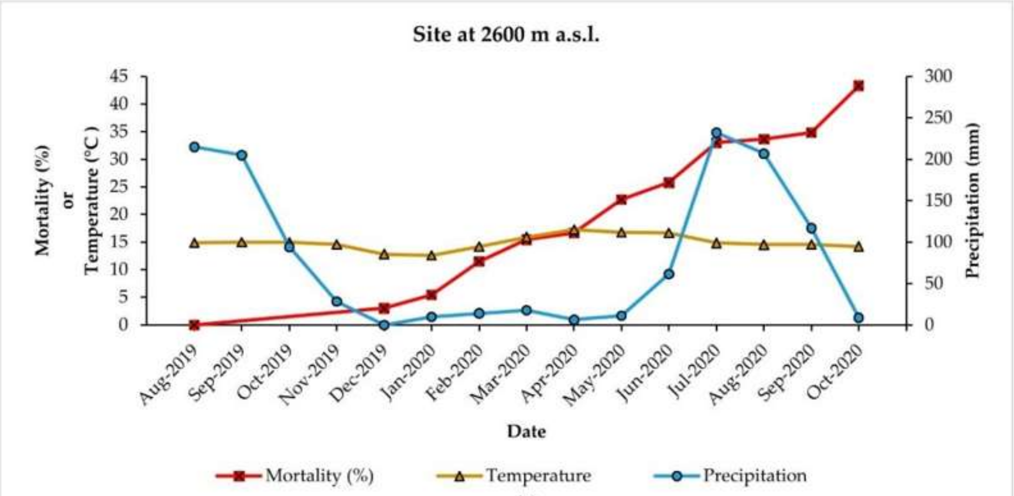
The predicted response curve for the increase in basal diameter indicated that the greatest growth occurs at a site that is warmer and dryer than that occupied by the populations at present, given that the curves are displaced to the right (Figure 2c). It is also clear that when they are moved toward colder and moister sites (left of the x-axis) or warmer and dryer sites (right side of the x-axis), relative to the optimum, they present a lower growth in basal diameter. The resulting curve regarding altitudinal transfer distance indicated clearly that the optimum growth, for all the provenances in general, was achieved at the site of intermediate elevation (3000 m a.s.l.; Figure 3c).

When comparing the average value across sites for the mean annual temperature and annual precipitation to the average value across sites of the reference period 1961–1990, it is evident that the sites have already increased in temperature (for the observed period) by an average of 0.58 °C, while precipitation has decreased by 27 mm (around 3%) (Table 5).

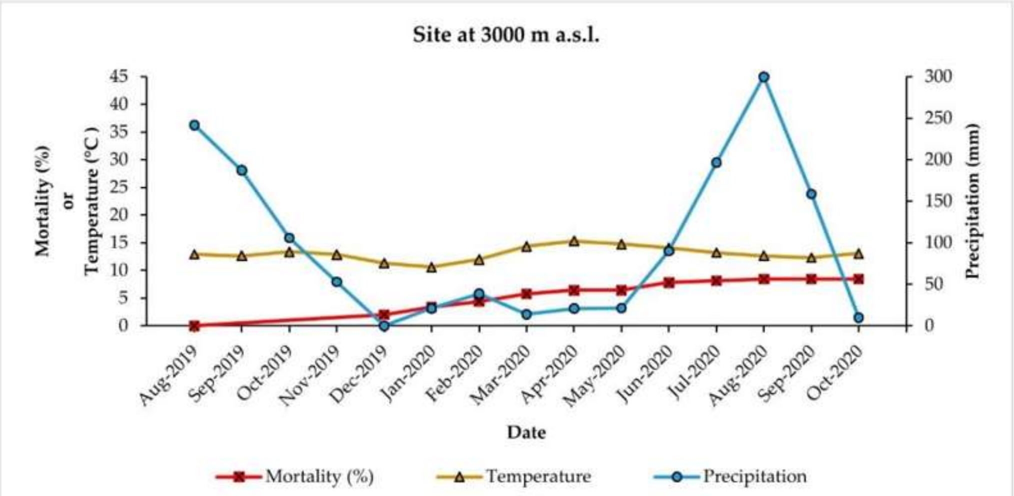
Table 5. Comparison of the most important climatic variables related to the survival response function, corresponding to the reference period 1961–1990 and the data obtained by averaging the test sites.

Climatic Variable	Reference Period 1961–1990	December 2019–November 2020	Difference
MAT	11.13 °C	11.71 °C	+0.58 °C
MAP	980 mm	952.7 mm	−27.3 mm

When plotting seedling mortality, monthly precipitation and mean temperature per site, and comparing among the sites (Figure 4), it is evident that precipitation differences among sites are not particularly large, but seem to have a meaningful effect, nevertheless. The low altitude site (2600 m a.s.l., 1407.4 mm total precipitation in 15 months; Figure 4a), had 16.5% lower total precipitation over the observed period than the site of intermediate altitude (3000 m a.s.l., 1685.14 mm; Figure 4b), while the site of elevated altitude (3400 m a.s.l., 1853.6 mm; Figure 4c) had 9.1% higher total precipitation than the intermediate site. The temporal patterning of the dry/rainy season seems to be similar across the three sites. Surprisingly, the apparently relative minor differences in mean temperature between the sites at 2600 m a.s.l. (14.9 °C averaging across all the 15 months observed) and 3000 m a.s.l. (13.02 °C; 1.9 °C of difference among those two sites), combined with the 16.5% difference in precipitation, seemed to be enough to trigger much higher mortality at the site at 2600 m a.s.l., particularly during the dry months, whether cold and dry (December–February) or warm and dry (March–May; Figure 4a). Moreover, an unexpected and relatively high mortality occurred just after the rainy season abruptly ended in October 2020 (Figure 4a), suggesting that several seedlings were already debilitated before the rainy season, perhaps subsequently expressing a drought stress legacy effect after the rainy season. In sharp contrast, seedling mortality at the site at 3400 m a.s.l., which was much colder and only 9.1% more rainy than the intermediate site, was nearly zero (Figure 4c).



(a)



(b)

Figure 4. *Cont.*

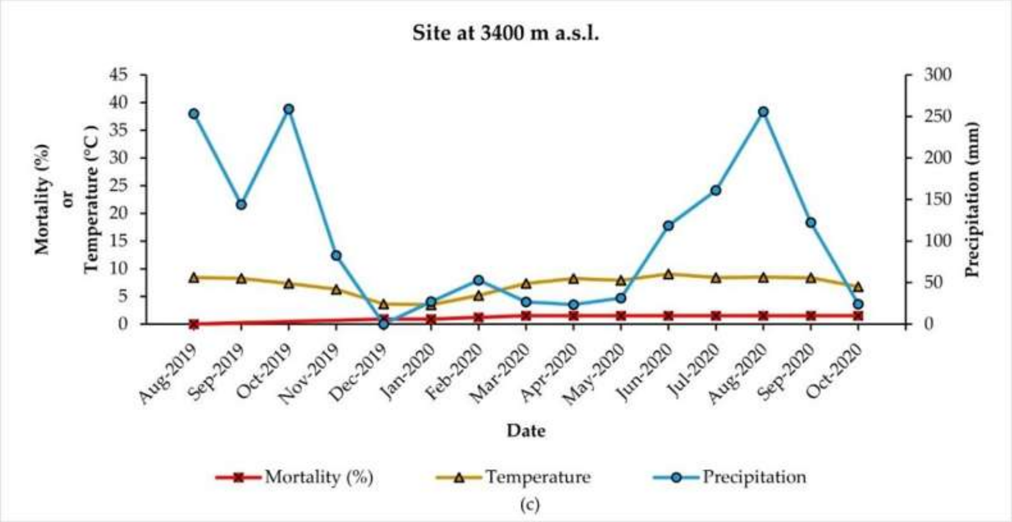


Figure 4. Mortality (%), monthly mean temperature (°C) and monthly precipitation (mm) in the period August 2019 to October 2020, for each test site, at (**a**) 2600 m a.s.l., (**b**) 3000 m a.s.l. and (**c**) 3400 m a.s.l.

4. Discussion

The most statistically significant climatic variables in terms of the response of survival and growth in *A. religiosa* seedlings to the climatic transfer distance were, in all cases, the balance between the temperature useful to growth (degree days above 5 °C) and available precipitation. That balance, expressed as dryness indices in our analysis, varied only in different seasons for the response variables: the rainy season (June–October) dryness index transfer distance was critical for survival, while that of the cold and dry season (November–February) was critical for aerial biomass, and the annual index was critical for the increase in basal diameter.

The effect of climatic transfer distance was much more negative (in terms of seedling survival) when transferring toward warmer and dryer sites, than when shifting to colder and moister sites. Apparently, there is a critical threshold that, when crossed, triggers mortality: a shift to a site 400 m lower in altitude, equivalent to +2 °C considering an overall lapse rate [34], or a difference of +1.9 °C based on the observed months, or a reduction in precipitation of 16%.

Most of the seedling mortality at the low altitude site (2600 m a.s.l.) occurred during the dry season (December–May). This is consistent with previous observations of a high mortality of emergent seedlings of natural regeneration in the core zone of the Monarch Butterfly Biosphere Reserve (MBBR) [29], and seems to support the projected decrease in climatic habitat suitable for *A. religiosa* inside the MBBR as a result of climatic change [26,30,49–51]. The apparent reason for the observed timing of this mortality is that the dry season was as dry as always, perhaps a little more so, but was warmer due to the ongoing climatic change. It was surprising to us that the relatively small differences in temperatures and precipitation between the sites at 2600 m a.s.l. and 3000 m a.s.l. made such a large difference to survival. Thus, the amount of residual moisture in the soil during the dry season, following the rainy season of the previous year, seems to be critical for survival of the young seedlings.

The high survival (around 95%) of the seed sources shifted to higher altitudes than their origin (to the site at 3400 m a.s.l.) confirms the viability of assisted migration to higher altitudes in the Trans-Mexican Volcanic Belt as a management measure for consideration, in order to reduce the projected impacts of climatic change, as shown in previous field tests [33].

Suitable climatic habitat projections for the 2060 decade, under intermediate greenhouse emission scenarios (RCP 6.0 watts/m^2), indicate that it is required to shift the *A. religiosa* seed sources upwards in altitude by between 300 and 500 m in order to realign the populations with the climate to which they are adapted [30]. Our evidence that is safe to move these sources upwards in altitude by 400 m therefore supports such a management proposal.

The mean temperature of the coldest month (MTCM) was the most important climate variable at the seed source for the increase in basal diameter. This indicates that genetic differentiation among populations for growth potential is shaped by the cold temperatures, at least in our case for those seedlings that survived the dry season. This is consistent with previous patterns of genetic variation among *A. religiosa* populations along altitudinal clines demonstrated in a common garden test under optimal conditions, where MTCM was also found to be the climatic variable that best explained the differences among populations [20].

Aerial biomass necessarily had to be measured, at the end of the observation period, in live plants that had survived the transfer toward warmer and dryer sites. It is therefore likely that those seedlings might have had some resistance or tolerance to the drought stress, and growing in a site with higher temperature (2600 m a.s.l.), they were able to grow more. In contrast, although the proportion of seedlings that survived in those that were transferred to a colder and moister site was higher (as in Figure 2a), growth was likely limited by the colder temperatures.

Implications for Management of Abies religiosa Inside the MBBR

The results indicate that increased temperatures and reduced precipitation due to climatic change would undoubtedly have a negative impact on the recruitment of *A. religiosa* seedlings at the lower altitudinal limits of the natural distribution range of the species, through increased mortality during the dry season (December–May). Active ecological restoration efforts in the MBBR must therefore consider how to increase the survival of planted *A. religiosa* seedlings in order to restore perturbed sites and ensure the maintenance of the monarch butterfly overwintering sites. In this context, perturbed sites at the low altitude of the natural range distribution of *A. religiosa* should have priority. Actions should include: (a) conducting reforestation using local shrubs (such as *Baccharis conferta* or other native understory species) as nurse plants for the *A. religiosa* seedlings [33]; and (b) conducting assisted migration to higher altitudes to realign *A. religiosa* populations with the climate to which they are adapted. Previous estimates regarding such realignment, targeting the climate projected for the 2030 decade, have suggested an upwards shift in altitude of 350 m [20]. The results of the present study suggest that it is safe to shift up to 400 m upwards in altitude, in terms of good seedling survival. Thus, an upward shift of between 350 and 400 m in altitude seems to be advisable. If such action is not undertaken, the lack of recruitment of natural regeneration seedlings is likely to continue as a result of the high mortality of young seedlings at lower altitudes (as demonstrated by ref [29]), impeding the natural renewal of adult *A. religiosa* trees within the MBBR, which will endanger the monarch butterfly overwintering sites in the long term.

5. Conclusions

The balance between the seasonal temperature available for growth (degree days > 5 °C) and the available precipitation is a determining factor for the permanence of *A. religiosa*, because the survival of young seedlings depends on the residual humidity of the soil during the dry season, following the rainy season of the previous year. A 2 °C increase in temperature and a 16% decrease in precipitation would imply a high mortality rate (~45%), negatively impacting the recruitment of naturally regenerating seedlings, mainly at the lower altitudinal limit of the natural distribution of the species. These results suggest that an upward movement of 400 m in altitude, through assisted migration, will have positive effects on the survival of *A. religiosa* seedlings, re-coupling the populations to the climate to which they are adapted and increasing the possibility of maintaining the overwintering sites of the monarch butterfly.

Author Contributions: C.S.-R., A.L.C.-V., A.B.-G. and R.L.-C. conceived the research project. A.B.-G. carried out the seed collection. A.L.C.-V., A.B.-G., M.G.-R. and C.S.-R. produced the seedlings and conducted the field experiments. A.L.C.-V. and C.S.-R. took measurements, determined the statistical analyses, and generated the response curves. R.L.-C., M.G.-R., L.L.-T. and E.d.l.B. provided important suggestions during the development of the project and for the data discussion. A.L.C.-V. and C.S.-R. led the writing of the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: Financial support was provided to C.S.-R. by The Monarch Butterfly Fund (Madison, WI, USA), the Mexican Fund for the Conservation of Nature and United States Forest Service USDA (funded by the U.S. Agency for International Development), the Fondo de Conservación del Eje Neovolcánico A.C., the American Forests (USA), the Forest Genetic Resources Working Group of the North American Forestry Commission (FAO) and the UMSNH Coordinación de la Investigación Científica; the Basic Research Fund of the Mexican Council of Science and Technology (CONACYT) (Ciencia Básica-2014-242985) was provided to A.B.-G.; the PAPIIT program (IN116218) of UNAM was provided to R.L.-C.; and a CONACyT graduate studies fellowship was provided to A.L.C.-V. (733822).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Acknowledgments: This paper is an undertaking of the Forest Genetic Resources Working Group (FGRWG)/North American Forest Commission/Food and Agricultural Organization of the United Nations. We thank Francisco Ramírez-Cruz (deceased), Doña Petra Cruz-Cruz, Francisca Ramírez-Cruz, and A. Belén Luis-Ramírez for providing numerous facilities for seed collection, seedling nursery production, common garden test planting, maintenance and measurements at Ejido La Mesa, Municipality of San José del Rincón, Estado de México; without their help, this study would not have been possible. Thanks also go to Esmeralda Navarro-Miranda, Cecilia Zamora-Sánchez, Erika Gómez-Pineda, Verónica Osuna-Vallejo, Adanely Jiménez-Villegas, J. Sinaí Ávalos-Montes, Patricia Herrejón-Calderón and other UMSNH students for assistance in the maintenance and measurement of the nursery and field experiments. Special thanks go to Gerald E. Rehfeldt (USDA—Forest Service, Moscow, Idaho) for advice on the experimental design and data analysis. Keith MacMillan provided assistance with the English writing.

Conflicts of Interest: The authors declare no conflict of interest.

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Capítulo II. Prediction of the impact of climate change on *Abies religiosa* and the feasibility of assisted migration

Prediction of the impact of climate change on *Abies religiosa* and the feasibility of assisted migration

Predicción del impacto del cambio climático en *Abies religiosa* y de la viabilidad de la migración asistida

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ABSTRACT

Climate change is generating a decoupling between populations and the places where suitable climate occurs. A field test of 11 provenances of sacred fir (*Abies religiosa* Kunth Schltdl. et Cham.), originating from an altitudinal gradient (3000–3550 m asl) within the Monarch Butterfly Biosphere Reserve, was established in three sites at contrasting altitudes: 3400 m asl (to estimate the feasibility of assisted migration), 3000 m asl (reference site) and 2600 m asl (to visualize the impacts of climate change). The evaluated response variables were: survival, height increase, and productivity index (survival *x* biomass). Climatic transfer distance (CTD = climate of planting site – climate of seed source) curves were fitted with mixed models. The climatic variable that best explained CTD was the dryness index of the cold dry season (November–February) for all response variables. Contrasting results were obtained for survival, height increase, and productivity index: at 3400 m asl, these values were 95%, 31 cm, and 6.4, respectively; at 3000 m asl, 91.6 %, 87 cm, and 14.7, respectively; and at 2600 m asl, 5.7 %, 63 cm, and 5.2, respectively. In the face of climate change, the assisted migration of sacred fir populations to higher altitudes could be a viable conservation management action for the Monarch butterfly overwintering sites. In contrast, at lower altitudes, massive mortality (94 %) of sacred fir seedlings is expected, with +1.8 °C and -14 % precipitation relative to the reference site, as occurred in this experiment.

KEY WORDS: climatic transfer distance, common garden, dryness index, height increase, productivity index, seedling survival.

RESUMEN

El cambio climático está generando un desacoplamiento entre las poblaciones y el lugar en que ocurre el clima que les es propicio. Se estableció un ensayo de 11 procedencias de oyamel (*Abies religiosa* Kunth Schltdl. et Cham.) originadas de un gradiente altitudinal (3000–3550 m snm) en la Reserva de la Biósfera Mariposa Monarca y plantadas en tres sitios de altitudes contrastantes: 3400 m snm (para estimar viabilidad de migración asistida), 3000 m snm (sitio referencia) y 2600 m snm (para visualizar impactos del cambio climático). Las variables de respuesta evaluadas fueron supervivencia, incremento en altura e índice de productividad (supervivencia *x* biomasa). Se ajustaron curvas de distancia de transferencia climática (DTC = clima sitio plantación–clima procedencia) con modelos mixtos. La variable climática que mejor explica a la DTC fue el índice de aridez de la temporada fría y seca (noviembre–febrero) para todas las variables de respuesta. Se obtuvieron resultados contrastantes de supervivencia, incremento en altura e índice de productividad: a 3400 m snm 95 %, 31 cm y 6.4; a 3000 m snm 91.6 %, 87 cm y 14.7; a 2600 m snm 5.7 %, 63 cm y 5.2, respectivamente. Ante el cambio climático, la migración asistida de poblaciones de oyamel a mayores altitudes podría ser una acción de manejo viable de conservación de los sitios de estancia invernal de la Mariposa Monarca. En contraste, a bajas altitudes se espera una mortalidad masiva de plántulas de oyamel (94 %) con +1.8 °C y -14 % de precipitación con respecto al sitio de referencia, como ocurrió en este experimento.

PALABRAS CLAVE: distancia de transferencia climática, jardín común, índice de aridez, incremento en altura, índice de productividad, supervivencia de plántulas.

INTRODUCTION

There is strong evidence that anthropogenic climate change is generating negative impacts on natural ecosystems, mainly changes in precipitation patterns and increases in the Earth's surface temperature (IPCC, 2014). These climate modifications are increasing the frequency and intensity of extreme droughts, generating massive mortality of forest stands (Allen et al., 2015; Brodribb et al., 2020; Hammond et al., 2022), mainly at the lower limit of the natural distribution of populations, known as the "xeric limit" (Mátyás, 2010; Mátyás et al., 2010). This is leading to a decoupling between local plant populations and the climatic conditions to which they have adapted over time (Chen et al., 2022; Jia et al., 2019). Decreasing water availability and rising temperatures cause metabolic stress on plants as they attempt to regulate their temperature (Girardin et al., 2016).

High mountain forests have become particularly sensitive to these effects (Brodersen et al., 2019; Rangwala & Miller, 2012) because the long-life cycle of trees does not allow for a rapid adaptation of populations to changing climatic conditions, as is eventually possible for other species with shorter life cycles (Brodribb et al., 2020; Lindner et al., 2010).

Sacred fir forests [*Abies religiosa* (Kunth) Schltdl. et Cham.] are considered forest relicts belonging to this forest type. In Mexico, they occupy between 0.1 % (Pineda-López et al., 2013) and 0.5 % (Manzanilla-Quiñones et al., 2019) of the forest area. Their populations are found in isolated and fragmented patches in locations with a humid and cold microclimate. They are often restricted to the top of mountains (between 2400 and 3600 m asl), in sites with high humidity (>1000 mm per year), mean annual temperatures of between 7 and 15 °C, and deep, moist, and well-drained soils (Madrigal-Sánchez, 1967; Rzedowski, 2006).

Given the extreme climatic conditions at the upper and lower limits of the sacred fir's altitudinal distribution, its populations present low seed viability (de Pascual Pola et al., 2003), low germination capacity (Ortiz-Bibian et al., 2019), low recruitment and natural regeneration rates (Guzmán-Aguilar et al., 2020), and defoliation due to drought stress (Flores-Nieves et al., 2011; Sáenz-Romero et al., 2020). Moreover, due to the expected effects of climate change, several modeling studies of the habitat suitable for the species have projected a drastic reduction of the habitat of between 77 % and 96.5 % (Gómez-Pineda et al., 2019; Pérez Miranda et al., 2014; Sáenz-Romero et al., 2012), determining that the climatic conditions suitable for sacred fir will be found even higher up in the mountains, occupying the climatic space where *Pinus hartwegii* Lindl. is currently found.

This has a negative impact on climate change mitigation efforts since forests are one of the main carbon sinks (Schoene & Bernier, 2012). Since the disturbance patterns resulting from global warming will be greater over the coming decades, it is important to highlight the importance of studying the climatic drivers of forest mortality (Seidl et al., 2017). That is, one can no longer necessarily think about how to return to or maintain forests in a historical condition, rather one must assess how to maintain forest health and influence the overall ecology of forest vegetation under the future climate (Sturrock et al., 2011). This can be achieved with the help of mechanisms such as altitudinal zonation (Ortiz-Bibian et al., 2017) and, eventually, assisted migration to higher elevations to realign species to a climate that will be favorable to them in the future (Sáenz-Romero et al., 2012). The present report is a follow-up at a higher age to an experiment at a younger age the results of which were first reported in Cruzado-Vargas et al. (2021).

OBJECTIVES

To determine the effect of the climatic transfer distance (climate of planting site – provenance climate) on survival rate, height increase, and productivity index in seedlings of eleven provenances of *Abies religiosa* (sacred fir), through reciprocal transplanting at test sites with contrasting altitudes: 3400 m asl to assess the feasibility of assisted migration to higher elevations: 3000 m asl as a reference site; and 2600 m asl to assess the impact of climate change at sites with higher temperatures and lower precipitation. This was conducted to help generate guidelines for making management decisions as a conservation measure and to contribute to efforts to adapt to the effects of climate change.

MATERIALS AND METHODS

Eleven collection sites for *Abies religiosa* (Kunth Schltdl. et Cham.) seeds were selected, with a 50 m altitudinal difference between sites along an altitudinal gradient from 3000 to 3550 m asl) located in the Monarch Butterfly Biosphere Reserve (MBBR), in Estado de México, Mexico (Table 1). Cones were collected from ten trees per site with a linear distance separation of at least 30 m between the trees to reduce the risk of endogamy.

Table 1. Geographic location of the *Abies religiosa* seed collection sites within the Monarch Butterfly Biosphere Reserve (MBBR).

Altitude (m asl)	Latitude (N)	Longitude (O)
Seed collection sites		
3552	19° 33' 52.6"	100° 13' 47.4"
3491	19° 34' 04.1"	100° 13' 59.5"
3457	19° 34' 17.1"	100° 14' 08.2"
3411	19° 34' 25.0"	100° 14' 13.8"
3364	19° 34' 31.7"	100° 14' 03.8"
3300	19° 34' 46.2"	100° 13' 53.6"
3233	19° 34' 50.9"	100° 13' 26.6"
3210	19° 34' 52.6"	100° 13' 15.5"
3143	19° 34' 53.0"	100° 12' 53.1"
3099	19° 35' 12.0"	100° 12' 52.9"
3003	19° 35' 42.6"	100° 12' 37.5"
Field establishment sites		
3400	19° 34' 23.2"	100°14' 08.4"
3000	19° 35' 18.2"	100° 10' 46.0"
2600	19° 48' 29.6"	100° 10' 52.7"

In the field, three test sites were established in common gardens at contrasting altitudes: 3400 m asl (MBBR core zone), to assess the feasibility of conducting assisted migration; 3000 m asl (MBBR buffer zone margin), as a reference site, similar to the current optimum for the species; and 2600 m asl (Tlalpujahua de Rayón, Michoacán), to assess the impact of increased temperatures due to the predicted effects of climate change (Table 1).

At each trial site, three raised growing beds were established (wooden structure of 5 m in length x 1.5 m in width x 0.4 m in height. All beds at the three sites contained the same loam substrate obtained at 3000 m asl). The common substrate and the dimensions of the raised bed were intended to prevent plant roots from coming into contact with the local soil, thus excluding any confounding effect of the particular soil at each site and enabling assessment of the influence of climatic transfer distance alone on the growth and survival of the provenances. Subsequently, plants produced in a shade house at Instituto de Investigaciones sobre los Recursos Naturales (INIRENA), Morelia, Michoacán, were taken to the field during the rainy season (July) of 2019 and used to establish six randomized complete blocks (two blocks per raised bed) at each field site: 11 provenances per block, five seedlings per provenance per plot in line, with a spacing of

0.2 x 0.2 m. At the head of each raised bed, a plot of seedlings from mixed provenances was established to counter the bank effect. The raised beds were covered with a 35 % shade net to mimic the protective effect of nurse plants since, without such protection, very low survival would be expected based on data from previous field trials of nurse plant associations (Carbajal-Navarro et al., 2019).

In December 2019, initial plant height measurements were taken with a precision of ±1 cm, and measured from a metal bar placed on the wooden frame of the raised bed as a base to the tip of the plant apical bud. The bar was used to reduce measurement error due to the irregular surface of the substrate. Basal diameter was measured at the root collar with a digital vernier (TRUPER). Subsequently, plant height and survival were measured bimonthly, and basal diameter was recorded every six months. The final measurements were taken in November 2021 [end of the growing season, and approximately 2.5 years (28 months) after planting]. With this information, the height increase was obtained from the difference between the final and initial measurements, and the survival ratio was obtained per provenance per block and then per provenance per site.

To determine the aerial biomass of each plant in the field, an estimation was made through a destructive analysis of five seedlings per provenance that had been kept in the shade house at INIRENA, i.e., 55 seedlings in total. Total height (from the root collar to apical bud) and basal diameter (at the root collar) were obtained from each plant. The plants were then sectioned, weighed fresh (Mettler Toledo analytical balance), and placed in paper bags. The stem, branches, and foliage of each plant were placed separately in each bag, put in a drying oven (Shel Lab) at 65 °C for 48 h (until reaching constant weight) (Domec et al., 2010; Song et al., 2015), and the dry weight recorded. With these data, and based on the methodology of Martínez Luna et al. (2020), a fitted regression was performed using the PROC REG procedure of SAS (SAS Institute, 2014, 2015), obtaining the following parameters:

$$Ba = -1.62490 + (0.03538 \times Alt) + (0.77238 \times Db) \tag{1}$$

Where:

Ba is aerial biomass (g)

Alt is total height (cm)

Db is basal diameter (mm)

With the aboveground biomass and survival ratio data, an average was obtained per provenance per block per site, and a productivity index was calculated based on the methodology of O'Neill et al. (2008) and Sáenz-Romero et al. (2021), in which: survival ratio per provenance per block (values from 0 to 1) was multiplied by the average aboveground biomass per provenance per block (in grams dry weight).

For estimation of the climatic transfer distance, climate data were obtained through spline models based on Sáenz-Romero et al. (2010) and available at the website http://charcoal.cnre.vt.edu/climate/ (Moscow Forestry Sciences Laboratory, 2023). The climate of the provenance was considered as the historical climate in which the populations have developed; i.e., the reference period 1961-1990. To obtain the climate of the test sites, temperature loggers (HOBO Pendant Temperature Data Logger UA-001-08) were installed and rainfall was measured monthly with rain traps strategically placed in each test site. Thirteen climate variables were estimated for the climates of provenance (1961-1990) and of the test sites (2019-2021). Using these climate data, a series of mixed models were tested to determine which climate variables (Table 2) had the greatest impact on survival ratio, height increase, and productivity index. Such models have been used similarly by Cruzado-Vargas et al. (2021); Leites et al. (2012), and Sáenz-Romero et al. (2017). The structure of the mixed models was as follows:

$$Y_{ijkl} = \beta_0 + \beta_1 \mathrm{CP}_j + \beta_2 \mathrm{DTC}_{ij} + \beta_3 \mathrm{DTC}_{ij}^2 + \beta_4 (\mathrm{CP}_j \times \mathrm{DTC}_{ij}) + \beta_5 S_i + \beta_6 B_k(S_i) + \beta_7 P_j + \beta_8 (S_i \times P_j) + e_{ijkl} \quad (2)$$

Where:

Y_{ijkl} is the survival, height increase, or productivity index corresponding to the l-th plant of the j-th provenance in the k-th block added in the i-th assay site

β_0 is the intercept

$\beta_1 - \beta_4$ are the parameters of the fixed effects

$\beta_5 - \beta_8$ are the parameters of the random effects

CP_j is the value of the climate of the j-th provenance

DTC_{ij} is the climatic transfer distance; i.e., the difference between the value of the climatic variable at the test site and that of the climate of the provenance, for the k-th provenance in the i-th assay site DTC_{ij}^2 is the climatic transfer distance squared

CP_{ij} x DTC_{ij} corresponds to the interaction between the climate value of the $j^{\text{-th}}$ provenance and the climatic transfer distance of the $j^{\text{-th}}$ provenance in the $i^{\text{-th}}$ assay site

S_i is the effect of the $i^{\text{-th}}$ assay site. B_k (S_i) is the effect of the $k^{\text{-th}}$ block added in the $i^{\text{-th}}$ assay site

P_j is the effect of the $j^{\text{-th}}$ provenance

(S_i x P_j) is the interaction between the $i^{\text{-th}}$ assay site and the $j^{\text{-th}}$ provenance

e_{ijkl} is the error term

Table 2. Climatic variables estimated for provenances and test sites. Source: Cruzado-Vargas et al. (2021).

Code	Unit	Definition
MAT	°C	Mean annual temperature
MAP	mm	Mean annual precipitation
GSP	mm	Growing season precipitation (total precipitation April–September)
WDSP	mm	Total warm and dry season (March–May) precipitation
RSP	mm	Total rainy season (June–October) precipitation
CDSP	mm	Total cold and dry season (November–February) precipitation
MTCM	°C	Mean temperature in the coldest month
MMIN	°C	Mean minimum temperature in the coldest month
MTWM	°C	Mean temperature in the warmest month
MMAX	°C	Mean maximum temperature in the warmest month
DD5	°C	Degree-days > 5 °C
WDSDD5	°C	Warm and dry season (March–May) degree days > 5 °C
RSDD5	°C	Rainy season (June–October) degree days > 5 °C
CDSDD5	°C	Cold and dry season (November–February) degree days > 5 °C
ADI	index	Annual dryness index ($\mathrm{ADI} = \sqrt{\mathrm{DD5}}/\mathrm{MAP}$)
GSDI	index	Growing season dryness index ($\mathrm{GSDI} = \sqrt{\mathrm{GSDD5}}/\mathrm{GSP}$)
WDSDI	index	Warm and dry season (March–May) dryness index (($\mathrm{WDSDI} = \sqrt{\mathrm{WDSDD5}}/\mathrm{WDSP}$)
RSDI	index	Rainy season (June–October) dryness index (($\mathrm{RSDI} = \sqrt{\mathrm{RSDD5}}/\mathrm{RSP}$)
CDSDI	index	Cold and dry season (November–February) dryness index (($\mathrm{CDSDI} = \sqrt{\mathrm{CDSDD5}}/\mathrm{CDSP}$)

To select the mixed models that best described the response of the dependent variables, a series of steps were followed as described below. a) With the climate variables for the climate of provenance, a Spearman correlation analysis was performed between the climate variables of the source of provenance and the mean value per provenance of the response variables, selecting those with the value closest to ±1. b) A reduced model was fitted between the climate variables for the climatic transfer distance; i.e., the term for the climate of provenance was dropped. The climate variables from the DTC with a significant p-value and with the lowest Akaike Information Criterion (AIC) value were then selected. c) Mixed models were run using the climate provenance and climatic transfer distance variables selected in the previous steps (a) and (b), and those with a negative and significant value of the quadratic term (Leites et al., 2012), as well as the lowest AIC, were selected. d) A total of 75 models were run, of which one was selected for each response variable with the lowest AIC value.

Mixed models for the survival ratio analysis were performed in RStudio software version 2022.07.2 Build 576 (RStudio Team, 2020). The glmer function of the lme4 package (Bates et al., 2015) was used and the "binomial" family was selected since the variable type is binary (0 = dead and 1= alive). Given the binomial glmer predict in logits, we then transformed the response to exponential values following the formula $y = 1/(1+1/\exp x)$ to enable fitting of the curves to the range between 0 and 1. The final model was then used to predict the response for: i) the average for the species (average across all populations), ii) the population originating at a higher altitude and iii) the population originating at a lower altitude.

The PROC MIXED procedure of SAS (SAS Institute, 2014, 2015) was used for the height increase and productivity index.

RESULTS

For the three response variables (survival ratio, height increase, and productivity index) the response function best explained by the significance of the linear, quadratic, and lowest Akaike Information Criterion (AIC) terms was that of the climatic transfer distance of the dry and cold season dryness index (CDSDI: November–February). For the climate of provenance variables, this was the dryness index of the dry and hot season from March to May (WDSDI) for the survival ratio (Table 3) and the rainy season dryness index (RSDI: June–October; Table 4) for height increase and productivity index.

Table 3. Akaike Information Criterion (AIC) and estimated parameters of fixed effects for survival ratio with a binomial distribution.

Parameter of the source of variation	**Estimator**	***p***
AIC	223.32	
Intercept	6.1705	0.0022
Seed source climate		
Warm and dry season dryness index (WDSDI)	-12.38	0.1944
Climatic transfer distance		
Cold and dry season dryness index (CDSDI)	-0.599	0.8940
(Climatic transfer distance)^2		
(CDSDI)^2	-6.99	0.0073
Interaction climatic seed source × climatic transfer distance	-0.314	0.9826

Table 4. Akaike Information Criterion (AIC), estimated parameters of fixed effects, contributions to total variance (%) and significance of random effects.

Parameter of the variation source	**Height increase**			**Productivity index**		
Fixed effects	**Estimator**		***p***	**Estimator**		***p***
AIC	4432.7			556		
Intercept	32.36		0.1728	4.60		0.2951
Seed source climate						
Rainy season dryness index (RSDI)	759.39		0.0828	184.41		0.0465
Climatic transfer distance						
Cold and dry season dryness index (CDSDI)	276.62		<0.0001	39.14		<0.0001
(Climatic transfer distance)^2						
(CDSDI)^2	-115.64		<0.0001	-26.36		<0.0001
Interaction climatic seed source × climatic transfer distance	-4243.83		<0.0001	-548.55		0.0081
Random effects	**Variance**	**%***	***p***	**Variance**	**%***	***p***
Site	0	0	1	0	0	1
Provenance	17.6	3.6	0.0864	0.2397	2.6	0.3569
Block (Site)	16.2	3.3	0.1254	1.97	21.2	0.0579
Interaction Site x provenance	0	0	1	0.71	7.6	0.2117
Error	457.78	93.1	<0.0001	6.39	68.6	<0.0001

*Contribution to the total variance expressed as a percentage, where 100 % is the sum of the contribution to the total variance of the random

The predicted response curve for the survival ratio plotted with a regression fitted from the mixed model parameters (Fig. 1a) indicated that when plants were transferred to the cooler and wetter site at 3400 m asl (left side of the graph, 400 m ~ -2 °C based on a rate of change of 0.5 °C/100 m [Barry, 2008; Körner, 2007; Sáenz-Romero et al., 2010]), the survival ratio was high (0.95 = 95 %, range between 0 and 1). A high survival performance was also found at the site at 3000 m asl, where the average survival was 91.6 %. In contrast, at the site at 2600 m asl, which is warmer and drier, the survival rate was ~16 times lower than at the other two sites (5.7%). The flattened shape of the upper part of the survival curve is because the curves the range between 0 and 1 given the binomial character of the survival (0–1). The optimum survival value found lies between -0.5 and 0.6 of the x-axis climatic transfer distance values. In this range, the sites at 3400 and 3000 m asl presented the highest values, while the site at 2600 m asl was located in the extreme lower part of the right-hand side of the curve (much drier), which explains the very low survival ratio value. However, when comparing the mean annual temperature and mean annual precipitation values between the reference site at 3000 m asl and the lower site at 2600 m asl, it was observed that the latter is 1.8 °C warmer and also drier (with 14 % less precipitation). These two variables are probably responsible for the severe seedling mortality found at this site (94.3 %).

For the height increase (Fig. 1b), the response curve indicates that, at the 3400 and 3000 m asl sites, growth among the provenances per site was very homogeneous, with an average plant height of 31 cm at the 3400 m asl site, and 87 cm at the reference site (3000 m asl). At the 2600 m asl site, however, the (few) plants that survived presented heterogeneous growth; i.e., the maximum height recorded was 88 cm for the tallest plant and 26 cm for the shortest, with an overall average of 63 cm at the site.

Regarding the response function for the productivity index, which represents the balance between growth and survival, an average value of 6.4 was recorded at the site at the highest elevation, while the reference site had the highest productivity index (14.7). The site at the lowest elevation presented different and extreme values among the plants, but the average value was 5.2, which was the lowest value of the three test sites (Fig. 1c). Figure 2 shows the plant status (survival and growth) at the different test sites: a) the site of highest elevation (3400 m asl), b) the reference site (3000 m asl), and c) the site that simulated the projected effects of climate change (2600 m asl).

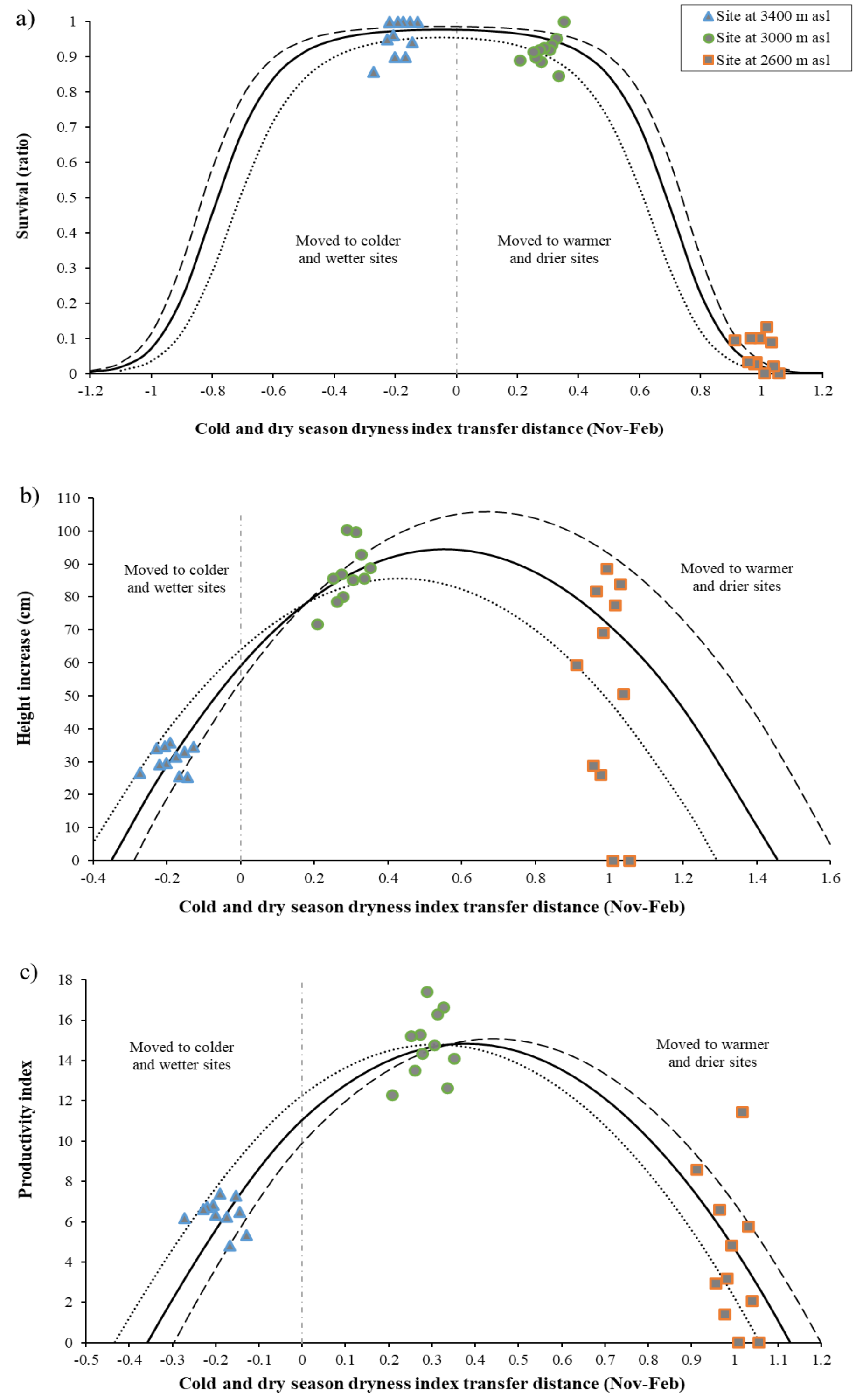

Figure 1. Response curves of plants from the different provenances to the climatic transfer of the cold and dry season dryness index (CDSDI: November–February) for: a) survival ratio; b) height increase; c) productivity index (survival ratio × aerial biomass). The curves were constructed with the parameters estimated using the fixed effects of the mixed model. Solid lines

represent the average for the species. Dashed lines represent the population originating at a higher altitude. Dotted lines represent the population originating at a lower altitude. The symbols represent the means per provenance, where their shape and color indicate the trial site: blue triangle site at 3400 m asl, green circle site at 3000 m asl, and orange square site at 2600 m asl. The respective climatic transfer distances on the x-axes signify a transfer to warmer and drier sites where the values are positive and a transfer to colder and wetter sites where negative. Zero denotes a climate similar to that of the source site.

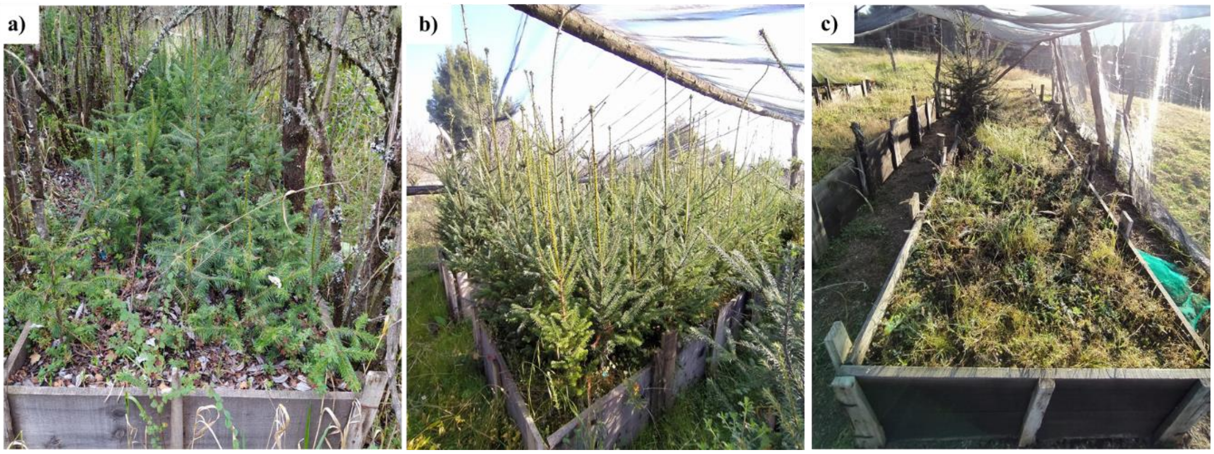

Figure 2. Provenances of *Abies religiosa* at approximately 2.5 years (28 months) after establishment (on planting, they had been in a greenhouse for one year; approximate age since seed – three years), in three provenance testing sites in common gardens at contrasting altitudes: a) 3400 m asl core zone of the MBBR; b) 3000 m asl edge of the MBBR buffer zone; c) 2600 m asl low altitude, outside the original distribution of the provenances (in this site, note the only surviving sacred fir plants at the top left of the raised growth bed).

Figure 3 represents the change in mean annual temperature (MAT) and mean annual precipitation (MAP) of the three sites within the historical period 1961-1990: a) the site at the highest altitude recorded a decrease in temperature of 2.4 °C, thus being even colder than the historical period, with an increase in mean annual precipitation of 15 %; b) the reference site at 3000 m asl recorded an increase in MAT of 1.7 °C, with respect to the historical value; i.e., it has become drier despite the increase in precipitation of 8.5 % that was recorded; c) the site at 2600 m asl has become ~2.1 °C warmer and also slightly drier (considering only the change in precipitation, but not the change in evapotranspiration due to the increased temperatures), with a loss of 1.25 % in the annual precipitation with respect to the historical value, even though the rainfall was heavier in the month of August.

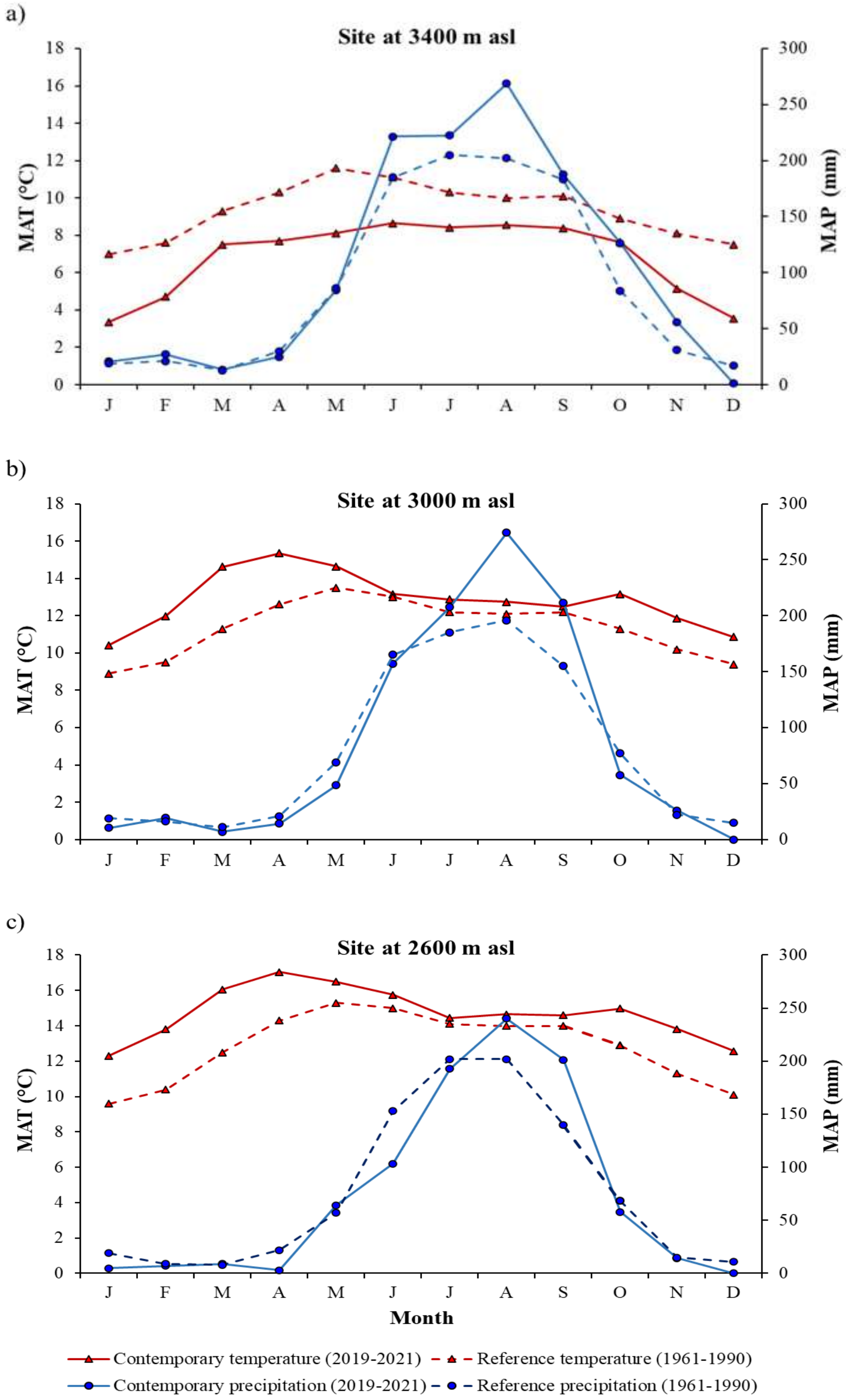

Figure 3. Mean annual temperature (MAT °C) and Mean annual precipitation (MAP mm) for each test site: a) 3400 m asl, b) 3000 m asl and c) 2600 m asl. Solid lines denote monthly mean values for the period August 2019 to November 2021 (during the common garden experiment). Dashed lines denote the mean monthly values for the historic reference period 1961-1990. Red lines refer to temperature and blue lines to precipitation.

DISCUSSION

The balance between temperature and precipitation in the cold and dry season (November–February); i.e., the CDSDI, proved to be the most significant climate variable of climatic transfer distance for the three response variables. Winter-spring precipitation is of vital importance for the survival, growth, and productivity of different conifers, including *Pinus cembroides* Zucc (Carlón Allende et al., 2018), *Pinus pseudostrobus* Lindl., and *Abies religiosa*, since the warm temperatures influenced by the El Niño phenomenon generate a decrease in precipitation during this season in central Mexico (Carlón Allende et al., 2016). It has also been found that the change in the periodicity and intensity of rainfall has caused the seasons to become extreme, with the wet season now wetter and the dry season drier. (Murray-Tortarolo, 2021).

At the site at 3400 m asl, a high survival rate (95 %) was recorded. This was probably related to the absence of extreme heat and the 51 % increase in precipitation during the cold and dry season (November–February) compared to the historical period (1961–1990). This availability of moisture most likely conferred a greater capacity for survival on the plants (Allen et al., 2015; Mátyás et al., 2018). At the site at 3000 m asl, the 22 % decrease in precipitation during the same November–February period, compared to the historical period of 1961–1990, did not negatively impact plant survival, possibly because precipitation during the previous rainy season (June–October) of the 2019–2021 trial period was higher (>15 %) compared to that of the historical period 1961–1990, thus providing the plants with sufficient soil moisture to survive the cold and dry season. At the 2600 m asl site during the November–February cold and dry season, there was an increase in temperature of ~3.5 °C and a decrease in precipitation of 8 % (compared to the historical 1961–1990 values), causing survival to be ~16.7 times lower than at the higher altitude site and ~16.1 times lower than at the 3000 m asl site.

It is important to highlight the value of the comparison of the effect of the balance between temperature and precipitation at the reference site at 3000 m asl, which presented the best performance of the plants in terms of growth, as well as high survival (91.6 %), compared to the site at 2600 m asl. Although the latter site at a lower altitude is outside the distribution of the origin of the provenances (>3000 m asl), the fact that it has both higher temperatures (1.8 °C) and lower precipitation (-14 %) than the reference site at 3000 m asl allows us to visualize the magnitude of the climatic impact to be expected with a temperature increase of less than 2 °C and a decrease in precipitation of only 14 %. This result indicates that such an increase in temperature and decrease in precipitation, which is a highly likely scenario for Mexico for the period between the 2060s and 2090s (Sáenz-Romero et al., 2010), will be sufficient to induce massive seedling mortality, such as was observed at the 2600 m asl site (94.3 % mortality).

For plant height increase, contrasting results were found among the test sites. For the site at the highest altitude, the height growth values were the lowest in the entire experiment. This is because, in conifers, the selection for high growth rates and that for cold hardiness are negatively correlated (Rehfeldt et al., 2018): growth decreases as cold increases and the growing season becomes shorter as elevation increases (Liu & El-Kassaby, 2018). Furthermore, temperatures of < 5 °C have been shown to limit root growth by decreasing transport of the enzyme H-ATPase, inhibiting different functions for cell growth (Alvarez-Uria & Körner, 2007), such that degree days (DD5; daily temperature occurring above 5 °C) have a great influence on increases in plant height. During the cold and dry season of November–February, the DD5 values differed significantly among sites; at 3400 m asl, 11 DD5 were calculated, at 3000 m asl there were 816 DD5, and at 2600 m asl there were 1031 DD5. This considerable difference in DD5 could lead to restricted growth at the higher altitude site while benefitting the plants at the other sites. It was also observed that the few plants at the 2600 m asl site that managed to survive until the following rainy season formed the second-highest average height increase among sites. It is likely that the populations found in the driest sites [at lower altitudes within the natural distribution of the species (Mátyás, 2010)] have a greater capacity for growth recovery following a period of drought stress than populations from wetter regions (located at higher altitudes). This is the case in populations of *Pinus pinaster* Ait. in the western Mediterranean region (Sánchez-Salguero et al., 2018) and in some forest species found more frequently in North America and Europe, such as *Pinus ponderosa*, *Pseudotsuga menziesii*, *Quercus alba*, *Picea glauca*, *Tsuga canadenis*, and *Taxodium distichum*, among others (Gazol et al., 2017).

Regarding the relationship between growth and survival, defined in this study as the productivity index, it can be assumed that the plants established on the site at 3400 m asl grew less in part because they designated a significant amount of resources obtained from photosynthesis for storage and defense against the low temperatures (Lusk & Jorgensen, 2013; Qin et al, 2022; Seiwa, 2007). This is a common response in shade-tolerant and slow-growing species such as *A. religiosa*, which allows them to survive longer but not increase their formation of biomass (Harsch & Bader, 2011). At the 3000 m asl site, a high productivity index was recorded, similar to survival-growth values for *Pinus ponderosa* Lawson & C. Lawson recorded in northern Arizona, where the highest survival and best growth were found in the intermediate range of the species altitudinal distribution (Dixit et al., 2021). As the following works indicate, the most favorable climatic conditions for the performance of *Abies religiosa* trees are found between 3050 and 3400 m asl: Ortiz-Bibian et al. (2019) and Guzmán-Aguilar et al. (2020) respectively showed that the highest number of viable seeds and the highest percentage of recruitment are found in the intermediate part of the natural distribution of this species. Similarly, Musule et al. (2016), reported that between 3100 – 3200 m asl, the anatomical structure of sacred fir wood features a lower content of hemicellulose (related to frost or drought resistance). As for the site at 2600 m asl, the productivity index was lower than the other test sites. Although the surviving plants at this site showed a good performance in terms of height increase, survival was too low and was insufficient to present a high value for the productivity index of the plants at this site. A study by Dixit et al. (2021) presented similar results, in which the survival of *P. ponderosa* seedlings was the poorest recorded at the lowest elevation site.

The results of this study seem to indicate that the site at 3000 m asl, is currently the site of ecological optimum for the provenances tested. The ecological optimum refers to the site where the species occurs most frequently and can be competitively exclusive (Rehfeldt et al., 2018). However, given the effects of climate change, it has been predicted that soon (by the decade centered on the year 2060), the optimal habitat for *Abies religiosa* will be 500 m higher in elevation, reaching 4000 m asl at its upper altitudinal limit (Gómez-Pineda et al., 2019). Considering that the altitudinal limit of the sacred fir in the MBBR is approximately 3550 m asl, assisted migration of populations of the species to other higher peaks of the Trans-Mexican Volcanic Belt, such as Nevado de Toluca, in the Estado de México, should be considered (Sáenz-Romero et al., 2012) as a vital strategy for conservation of the sacred fir forests, in addition to contributing to adaptation and mitigation of the effects of climate change.

CONCLUSIONS

The results of this study revealed that altitudinal movement towards cooler and wetter sites (up to ~2 °C cooler = 400 m altitudinal difference upwards) of *Abies religiosa* provenances will allow the balance between temperature and available moisture to be that required for plants to maintain their capacity for survival. Populations located at the lower limit of the distribution of

A. religiosa will increasingly suffer from water stress due to increasing temperatures, which will most likely be reflected in massive seedling mortality, as was recorded in this study (94.3 %) apparently induced by an increase in mean annual temperature of 1.8 °C and a decrease in precipitation of 14 % at the lowest altitude test site (2600 m asl).

Based on the results obtained in this study, it is recommended to implement assisted migration to higher altitude sites as a conservation measure for the species and as part of the effort to mitigate the expected effects of climate change.

ACKNOWLEDGMENTS

This project was an undertaking of the Forest Genetic Resources Working Group (FGRWG)/ North American Forest Commission (NAFC)/Food and Agricultural Organization of the United Nations. We thank the funding to CSR by the Monarch Butterfly Fund (Madison, Wisconsin, USA), the United States Forest Service (funded by the U.S. Agency for International Development, Washington D.C.), the American Forest (Washington D.C.), the Fondo de Conservación del Eje Neovolcánico A.C. (Zitácuaro, Michoacán), and the Coordinación de la Investigación Científica, Universidad Michoacana de San Nicolás de Hidalgo; to RLC by the program PAPIT-UNAM (IG200221). We thank to Francisco Ramírez-Cruz (deceased), Doña Petra Cruz-Cruz, Francisca Ramírez-Cruz, A. Belén Luis-Ramírez (Ejido La Mesa, Municipality of San José del Rincón, Estado de México), Magdaleno Martin Antonio Hernández Campos and his family (Tlalpujahua de Rayón, Michoacán) for providing access and assistence on the establishing, surveillance, maintenance and measurements of the field experiments; without their help, this project would not be possible. Thanks to Verónica Osuna-Vallejo (researcher "Cátedra" supported by the National Council of Science and Technology, CONACyT), Ma. Guadalupe Joaquín-Juan (National Research System Project assistant), Erika Gómez Pineda (CIGA-UNAM), Esmeralda Navarro-Miranda, Cecilia Zamora-Sánchez (graduate students), Adanely Jiménez-Villegas (undergrad student) and Jessica Simone Ambriz-Guerrero (Federal program "Jóvenes Construyendo el Futuro") and other students from the UMSNH by its help of field measurements. To the CONACyT for a doctoral fellowship to A.L. C.-V. (733822).

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Capítulo III. Phytochemical variation among sacred fir (*Abies religiosa* [Kunth] Schltdl. & Cham.) provenances in an altitudinal gradient

Scientific article https://doi.org/10.5154/r.rchscfa.2022.01.003

Phytochemical variation among sacred fir (*Abies religiosa* [Kunth] Schltdl. & Cham.) provenances in an altitudinal gradient

Variación fitoquímica entre procedencias de oyamel (*Abies religiosa* [Kunth] Schltdl. & Cham.) en un gradiente altitudinal

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Abstract

Introduction: A species' populations differ across its distribution due to selection pressure from the climate gradient.

Objective: To describe the phytochemical variation among 14 tree provenances in an altitudinal gradient of *Abies religiosa* (Kunth) Schltdl. & Cham. to determine if there are associations of chemical profiles among populations, expressed under a single environment.

Materials and methods: Seeds were collected from 165 trees from an altitudinal transect from 2 850 to 3 540 m in Cerro San Andrés, Municipio Libre de Hidalgo, Michoacán, Mexico. The plant was grown in a common garden trial; at 28 months, mature needles were collected per tree and analyzed by gas-mass chromatography. Data were analyzed with diversity indices, ANOVA and multivariate analysis (principal components, correspondences and cluster).

Results and discussion: Thirty-two compounds were identified, most of them terpenes. The populations showed differences in presence/absence of terpenes and in their concentration; above 3 350 m, diversity was lower, but with higher concentration. Three altitude groups were found based on the chemical profile of the populations: low = 2 850 to 3 300 m and 3 400 m; moderate = 3 350, 3 450 and 3 500 m; and high = 3 540 m.

Conclusions: The diversity and concentration of terpenes in *A. religiosa* varied depending on the altitude of populations. There are three chemical profiles that should be considered in the implementation of germplasm collection programs for reforestation and restoration.

Keywords: terpenes; secondary metabolites; chemical profile; diversity indices; multivariate analysis.

Resumen

Introducción: Las poblaciones de una especie son diferentes a lo largo de su distribución por la presión de selección del gradiente climático.

Objetivo: Describir la variación fitoquímica entre 14 procedencias en un gradiente altitudinal de *Abies religiosa* (Kunth) Schltdl. & Cham., para determinar si existen asociaciones de perfiles químicos entre poblaciones, expresados bajo un solo ambiente.

Materiales y métodos: Se recolectaron semillas de 165 árboles de un transecto altitudinal de 2 850 a 3 540 m en el cerro de San Andrés, Municipio Libre de Hidalgo, Michoacán, México. La planta se produjo en ensayo de jardín común; a los 28 meses se recolectaron acículas maduras por individuo y se analizaron por cromatografía de gases-masas. Los datos se analizaron con índices de diversidad, análisis de varianza y multivariados (componentes principales, correspondencias y conglomerados).

Resultados y discusión: Se identificaron 32 compuestos, la mayoría terpenos. Las poblaciones presentaron diferencias en presencia/ausencia de terpenos y en su concentración; arriba de 3 350 m, la diversidad fue más baja, pero con mayor concentración. Se encontraron tres grupos de altitud con base en el perfil químico de las poblaciones: baja = 2 850 a 3 300 m y 3 400 m; intermedia = 3 350, 3 450 y 3 500 m; y elevada = 3 540 m.

Conclusiones: La diversidad y concentración de los terpenos en *A. religiosa* variaron en función de la altitud de las poblaciones. Existen tres perfiles químicos que deben considerarse en el establecimiento de programas de recolecta de germoplasma para reforestación y restauración.

Palabras clave: terpenos; metabolitos secundarios; perfil químico; índices de diversidad; análisis multivariados.

Please cite this article as follows (APA 6): Cruzado-Vargas, A. L., García-Rodríguez, Y. M., Ortiz-Bibian, M. A., Espinosa-García, F. J., Antonio-López, P., Sáenz-Romero, C., & Lindig-Cisneros, R. A. (2022). Phytochemical variation among sacred fir (*Abies religiosa* [Kunth] Schltdl. & Cham.) provenances in an altitudinal gradient. *Revista Chapingo Serie Ciencias Forestales y del Ambiente, 28*(3), 427–445. doi: https://doi.org/10.5154/r.rchscfa.2022.01.003

Received: January 8, 2022 / Accepted: July 26, 2022

RCHSCFA

https://revistas.chapingo.mx/forestales

Introduction

Sacred fir (*Abies religiosa* [Kunth] Schltdl. & Cham.) forests are distributed in conifer-dominated upland areas, mainly between 2400 and 3600 m altitude, between 19° and 20° north latitude, along the Transmexican Volcanic Belt (Sáenz-Romero, Rehfeldt, Duval, & Lindig-Cisneros, 2012). These forests are of great ecological importance due to the ecosystem services they provide such as carbon sequestration, water uptake, and protection against erosion (Hernández-Álvarez, Reyes-Ortiz, Villanueva-Díaz, & Sánchez-González, 2021); they are also a wintering site for the monarch butterfly, *Danaus plexippus* L. (Oberhauser, Nail, & Altizer, 2015).

Sacred fir is an evergreen monoecious tree up to 40 m tall, with thick bark with scaly patches in adult trees and smooth in young trees. Its leaves are alternate, linear, sessile, straight, somewhat leathery, 19 to 70 mm long, with acute or rounded apex and oblong buds protected with resin (Rodríguez Trejo, Aparicio Lechuga, Lara Bueno, Uribe Gómez, & Ramírez Castell, 2021).

One of the main threats to sacred fir forests is climate change, because it will induce a decoupling between their populations and the climate suitable for them (Gómez-Pineda et al., 2020; Sáenz-Romero et al., 2012), which in turn will lead to increasing incidence of bark beetles, as high temperatures provide favorable conditions for the development of these insects (Gómez-Pineda et al., 2022; López-Gómez, Arriola Padilla, & Pérez Miranda, 2015; Rubin-Aguirre et al., 2015; Sosa Díaz et al., 2018).

Populations of the same species with a wide range of distribution are found in a diverse variety of environmental conditions that can be reflected in phenotypic variation (Premoli, Acosta, Mathiasen, & Donoso, 2012). This can be explained because of differential selection of genotypes under the various environmental conditions in an environmental gradient (Ignazi, Mathiasen, & Premoli, 2019). Understanding altitudinal patterns of genetic differentiation among populations is essential for the design of forest management measures that facilitate the adaptation of forest populations to projected climates (Sáenz-Romero et al., 2016). In mountainous regions, the altitudinal gradient is especially important for understanding richness patterns at both community and population levels (Muenchow, Dieker, Kluge, Kessler, & von Wehrden, 2018).

Provenance or common garden trials under open field conditions allow expression of genetic differences by growing plants from different geographical origins (provenances) under the same environmental

Introducción

Los bosques de oyamel (*Abies religiosa* [Kunth] Schltdl. & Cham.) se distribuyen en zonas altas dominadas por coníferas, principalmente entre 2400 y 3600 m de elevación, entre 19° y 20° de latitud norte, a lo largo de la Faja Volcánica Transmexicana (Sáenz-Romero, Rehfeldt, Duval, & Lindig-Cisneros, 2012). Estos bosques son de gran importancia ecológica debido a los servicios ecosistémicos que proporcionan como la captura de carbono, captación de agua y protección contra la erosión (Hernández-Álvarez, Reyes-Ortiz, Villanueva-Díaz, & Sánchez-González, 2021); además, son sitio de hibernación de la mariposa monarca *Danaus plexippus* L. (Oberhauser, Nail, & Altizer, 2015).

El oyamel es un árbol monoico siempre verde de hasta 40 m de altura, de corteza gruesa con placas escamosas en árboles adultos y lisa en árboles jóvenes. Sus hojas son alternas, lineares, sésiles, rectas, algo coriáceas de 19 a 70 mm de longitud, con ápice agudo o redondeado y yemas oblongas protegidas con resina (Rodríguez Trejo, Aparicio Lechuga, Lara Bueno, Uribe Gómez, & Ramírez Castell, 2021).

Una de las principales amenazas para los bosques de oyamel es el cambio climático, ya que inducirá un desacoplamiento entre sus poblaciones y el clima propicio para ellas (Gómez-Pineda et al., 2020; Sáenz-Romero et al., 2012), lo que a su vez provocará incidencia cada vez mayor de descortezadores, pues las temperaturas altas proveen condiciones favorables para el desarrollo de estos insectos (Gómez-Pineda et al., 2022; López-Gómez, Arriola Padilla, & Pérez Miranda, 2015; Rubin-Aguirre et al., 2015; Sosa Díaz et al., 2018).

Las poblaciones de una misma especie con un rango amplio de distribución se encuentran en una vasta gama de condiciones ambientales que se pueden reflejar en la variación fenotípica (Premoli, Acosta, Mathiasen, & Donoso, 2012). Esta puede explicarse como resultado de la selección diferencial de los genotipos bajo las diversas condiciones ambientales en un gradiente ambiental (Ignazi, Mathiasen, & Premoli, 2019). La comprensión de los patrones altitudinales de diferenciación genética entre poblaciones es fundamental para el diseño de medidas de manejo forestal que faciliten la adaptación de las poblaciones forestales a los climas proyectados (Sáenz-Romero et al., 2016). En las regiones montañosas, el gradiente altitudinal es de particular importancia para entender los patrones de riqueza tanto a nivel comunidades como poblaciones (Muenchow, Dieker, Kluge, Kessler, & von Wehrden, 2018).

Los ensayos de procedencias o jardín común en condiciones de campo permiten la expresión de las diferencias genéticas, mediante el cultivo de plantas conditions. In this type of studies, it is expected that the provenance best coupled to the climate of the trial site will have the best performance and the others will show differences, in part, related to the magnitude of the weather differences between the site of origin and the trial site (Cruzado-Vargas et al., 2021; Sáenz-Romero et al., 2017). A variant of the above are the common garden trials where optimal conditions are provided (as far as possible) to the set of provenances to favor the expression of their growth potential, and with this, quantify whether or not there is genetic differentiation between them. This can be achieved by establishing trials in open field sites located within the optimal climate space of the species, or, as in the case of this study, by providing favorable artificial conditions using shade netting and irrigation during the dry season (Ortiz-Bibian et al., 2017).

Defense and resistance mechanisms in conifers depend on genetic, biotic and abiotic factors and interactions. These mechanisms include physical defense traits and chemical compounds (Zas Arregui & Sampedro Pérez, 2015). Chemical defenses often take the form of sets of substances that defend plants against herbivores (including bark beetles) (Taft, Najar, Godbout, Bousquet, & Erbilgin, 2015), pathogens (Kopaczyk, Warguła, & Jelonek, 2020) and certain types of abiotic stresses such as high temperature (Materić et al., 2015).

Plants synthesize a large number of compounds, including secondary metabolites, many are volatile compounds that vary in composition and concentration between species and from one individual to another within the same species (García-Rodríguez, Bravo-Monzón, Martínez-Díaz, Torres-Gurrola, & Espinosa-García, 2012). Such variation also depends on the plant part being analyzed, the season of the year, geographical origin and other environmental factors (Kopaczyk et al., 2020). Secondary metabolites are synthesized in small quantities and not in a generalized way (some are produced only by one species, genus or family) (Pichersky & Raguso, 2018); they play defense, attraction or competition functions, and their effectiveness depends on the concentration (López-Goldar et al., 2018) and metabolism of the consumer: herbivore or pathogenic (García-Rodríguez et al., 2012).

Terpenes constitute the largest and most diverse group of secondary metabolites in plants (Kopaczyk et al., 2020) comprising more than 40 000 individual compounds (Abbas et al., 2017) and, together with phenolic compounds, are the main chemical defenses in conifers (Lundborg, Fedderwitz, Björklund, Nordlander, & Borg-Karlson, 2016). The most abundant terpenes in the pine family are α-pinene, β-pinene, myrcene, limonene, camphene, and 3-carene (Kopaczyk et al., 2020; Pokorska et al., 2012; Taft et al., 2015).

de diversos orígenes geográficos (procedencias) bajo las mismas condiciones ambientales. En este tipo de estudios se espera que la procedencia mejor acoplada al clima del sitio de ensayo tenga el mejor desempeño y las demás muestren diferencias, en parte, relacionadas con la magnitud de la diferencia climática entre el sitio de origen y el de ensayo (Cruzado-Vargas et al., 2021; Sáenz-Romero et al., 2017). Una variante de lo anterior son los ensayos de jardín común donde se proveen condiciones óptimas (en la medida de lo posible) al conjunto de procedencias con la finalidad de favorecer la expresión de su potencial de crecimiento, y con ello, cuantificar si existe o no diferenciación genética entre ellas. Esto se puede lograr estableciendo los ensayos en sitios de campo ubicados dentro del espacio climático óptimo de la especie, o bien, como es el caso de este trabajo, proporcionando de condiciones favorables artificialmente, a través del uso de malla-sombra y riego en temporada de estiaje (Ortiz-Bibian et al., 2017).

Los mecanismos de defensa y resistencia en las coníferas dependen de factores genéticos, bióticos, abióticos, así como de sus interacciones. Estos mecanismos incluyen caracteres de defensa físicos y compuestos químicos (Zas Arregui & Sampedro Pérez, 2015). Las defensas químicas a menudo se presentan como conjuntos de sustancias que defienden a las plantas contra herbívoros (incluidos los escarabajos de la corteza) (Taft, Najar, Godbout, Bousquet, & Erbilgin, 2015), patógenos (Kopaczyk, Warguła, & Jelonek, 2020) y ciertos tipos de estrés abiótico como las temperaturas altas (Materić et al., 2015).

Las plantas sintetizan gran cantidad de compuestos, entre ellos están los metabolitos secundarios que son compuestos volátiles variantes en composición y concentración entre especies y de un individuo a otro dentro de una misma especie (García-Rodríguez, Bravo-Monzón, Martínez-Díaz, Torres-Gurrola, & Espinosa-García, 2012). Dicha variación también depende de la parte la planta que se analice, la estación del año, origen geográfico y otros factores medioambientales (Kopaczyk et al., 2020). Los metabolitos secundarios se sintetizan en cantidades pequeñas y no de forma generalizada (algunos son producidos solo por una especie, género o familia) (Pichersky & Raguso, 2018); cumplen funciones de defensa, atracción o competencia, y su efectividad depende de la concentración (López-Goldar et al., 2018) y del metabolismo del consumidor: herbívoro o patógeno (García-Rodríguez et al., 2012).

Los terpenos constituyen el grupo más grande y diverso de metabolitos secundarios en las plantas (Kopaczyk et al., 2020) abarcando más de 40 000 compuestos individuales (Abbas et al., 2017) y, junto con los compuestos fenólicos, son las principales defensas químicas de las coníferas (Lundborg, Fedderwitz, The objective of this study was to determine whether the phytochemical diversity of *A. religiosa* varies among 14 populations from an altitudinal gradient (2 850 to the 3 550 m) established in a common garden trial. The hypothesis is that terpene diversity will be higher at lower altitudes, where there is higher herbivory and, therefore, greater selection pressure that would eventually favor terpene diversity as an adaptive response of forest populations (Pellissier et al., 2016).

Materials and Methods

Seed collection and plant production

Mature cones of *A. religiosa* were collected at 14 sites: 11 trees at each site separated by 50 m altitudinal difference in a transect from 2 850 to 3 550 m (Table 1) on Cerro San Andrés, Municipio Libre de Hidalgo, Michoacán, Mexico. Collection sites were well-preserved stands, dominated mainly by *A. religiosa* (89 % basal area) in the upper part of the mountain, combined with *Pinus hartwegii* Lindl. (*pino de las alturas*) and *Cupressus lindleyi* Klotzsch ex Endl. (cedar). In the intermediate part of the altitudinal transect, *A. religiosa* represents 60 % of the basal area of the tree stand in combination mainly with *Pinus pseudostrobus* Lindl. (*pino liso*), *C. lindleyi* and *Salix paradoxa* Kunth (*borreguito*). At the lowest part, *A. religiosa* represents 45 % in combination with *P. pseudostrobus*, *Pinus montezumae* Lamb (*pino blanco*), *Quercus obtusata* Bonpl. (white oak) and other leafy species such as *Arbutus*

Björklund, Nordlander, & Borg-Karlson, 2016). Los terpenos más abundantes en la familia de pinos son: α-pineno, β-pineno, mirceno, limoneno, canfeno y 3-careno (Kopaczyk et al., 2020; Pokorska et al., 2012; Taft et al., 2015).

El objetivo del presente trabajo fue determinar si la diversidad fitoquímica de *A. religiosa* varía entre 14 poblaciones provenientes de un gradiente altitudinal (2 850 a los 3 550 m) establecidas en un ensayo de jardín común. La hipótesis es que la diversidad de terpenos será mayor a menor altitud, donde hay mayor herbivoría y, por lo tanto, una mayor presión de selección que eventualmente favorecería la diversidad de terpenos como una respuesta adaptativa de las poblaciones forestales (Pellissier et al., 2016).

Materiales y métodos

Recolecta de semilla y producción de planta

Se recolectaron conos maduros de *A. religiosa* en 14 sitios: 11 árboles en cada uno de los sitios separados entre sí por 50 m de diferencia altitudinal en un transecto de los 2 850 a los 3 550 m (Cuadro 1) en el cerro de San Andrés, Municipio Libre de Hidalgo, Michoacán, México. Los sitios de recolecta fueron rodales bien conservados, dominados principalmente por *A. religiosa* (89 % de área basal) en la parte alta de la montaña, combinado con *Pinus hartwegii* Lindl. (pino de las alturas) y *Cupressus lindleyi* Klotzsch ex

Table 1. Geographical location of the cone collection sites of 14 provenances of *Abies religiosa* on Cerro San Andrés, Municipio Libre de Hidalgo, Michoacán, Mexico.

Cuadro 1. Localización geográfica de los sitios de recolección de conos de 14 procedencias de *Abies religiosa* en el cerro de San Andrés, Municipio Libre de Hidalgo, Michoacán, México.

Altitude (m)/Altitud (m)	Latitude (N)/Latitud (N)	Longitude (W)/Longitud (O)
3 540	19° 48' 01.0"	100° 36' 04.7"
3 500	19° 48' 03.6"	100° 36' 06.8"
3 450	19° 48' 06.3"	100° 36'10.3"
3 400	19° 48' 19.0"	100° 36' 13.5"
3 350	19° 48' 20.2"	100° 36' 13.8"
3 300	19° 48' 23.8"	100° 36' 18.6"
3 250	19° 48' 36.1"	100° 36' 16.3"
3 200	19° 48' 38.0"	100° 36' 20.6"
3 150	19° 48' 40.9"	100° 36' 26.4"
3 100	19° 48' 49.0"	100° 36' 31.2"
3 050	19° 48' 58.7"	100° 36' 30.6"
3 000	19° 49' 10.1"	100° 36' 28.4"
2 950	19° 49' 15.0"	100° 36' 05.8"
2 850	19° 49' 38.6"	100° 35' 35.0"

Source/Fuente: Ortiz-Bibian et al. (2017).

Figure 1. Common garden trial with 14 provenances of *Abies religiosa* in raised beds at the Instituto de Investigaciones sobre los Recursos Naturales de la Universidad Michoacana de San Nicolás de Hidalgo, Morelia, Michoacán, Mexico.

Figura 1. Ensayo de jardín común con 14 procedencias de *Abies religiosa* en camas elevadas en el Instituto de Investigaciones sobre los Recursos Naturales de la Universidad Michoacana de San Nicolás de Hidalgo, Morelia, Michoacán, México.

xalapensis Kunth (*madroño*) and *S. paradoxa*. The detailed description of the sites can be found in the study by Ortiz-Bibian et al. (2017).

The cones were dried in the sun for 15 days to extract the seeds and, subsequently, germinate them in a peat moss seedbed. The seedlings were transplanted into 380 cm^3 containers, where they developed for nine months. In March 2014, a provenance or common garden trial (Figure 1) was set up at the Instituto de Investigaciones sobre los Recursos Naturales de la Universidad Michoacana de San Nicolás de San Nicolás de Hidalgo (INIRENA-UMSNH) located in Morelia, Michoacán. The experiment had a six randomized complete block design with six seedlings per provenance (Ortiz-Bibian et al., 2017).

Sample collection

When seedlings were 28 months old, 2 g samples of mature needles were collected from 10 trees randomly selected from each of the 14 *A. religiosa* provenances present in the common garden trial to evaluate the chemical profile. Twelve hours before harvesting,

Endl. (cedro). En la parte intermedia del transecto altitudinal, *A. religiosa* representa 60 % del área basal del arbolado en combinación principalmente con *Pinus pseudostrobus* Lindl. (pino liso), *C. lindleyi* y *Salix paradoxa* Kunth (borreguito). En la parte más baja, *A. religiosa* representa 45 % en combinación con *P. pseudostrobus, Pinus montezumae* Lamb. (pino blanco), *Quercus obtusata* Bonpl. (encino blanco) y demás especies de hojosas como *Arbutus xalapensis* Kunth (madroño) y *S. paradoxa*. La descripción detallada de los sitios se puede consultar en el estudio de Ortiz-Bibian et al. (2017).

Los conos se secaron al sol durante 15 días para extraer las semillas y, posteriormente, germinarlas en almácigo de turba de musgo. Las plántulas se trasplantaron en tubetes de 380 cm^3, donde se desarrollaron durante nueve meses. En marzo de 2014 se montó un ensayo de procedencias o jardín común (Figura 1) en el Instituto de Investigaciones sobre los Recursos Naturales de la Universidad Michoacana de San Nicolás de Hidalgo (INIRENA-UMSNH) ubicado en Morelia, Michoacán. El experimento tuvo un diseño de seis bloques completos al azar con seis plántulas por procedencia (Ortiz-Bibian et al., 2017).

the trial was watered to field capacity. Samples were taken between 8 and 10 h in the morning in October 2016 with northern exposure and no signs of damage. Branches were taken from the middle part of the main stem.

From each sample, 1 g of needles was placed in a paper bag, kept in the drying oven at 80 °C for 24 h and weighed. The other gram was placed in an amber glass bottle containing 10 mL of reagent grade n-hexane, which remained at 5 °C in a refrigerator. Subsequently, the sample in n-hexane was ground in a porcelain mortar with 0.5 g of inert sand and 1 mL of tetradecane ($C_{14}H_{30}$) to a concentration of 1 mg·mL^{-1} as an internal standard. The extract was filtered through a funnel with 11 μm pore opening filter paper containing 0.5 g of reagent grade anhydrous sodium sulfate (Na_2SO_4) and stored in an amber vial at 5 °C until chemical analysis.

Chemical Analysis

Samples were analyzed by injecting 1 μL into a gas chromatograph equipped with a mass detector (Agilent 6890, MSD 5973), flow split (60.3:1) at 250 °C on a HP-5MS nonpolar capillary column (30 m x 25 mm x 25 μm), using helium as carrier gas with a flow rate of 1 mL·min^{-1}. The oven temperature program was initial ramp of 50-200 °C at 20 °C·min^{-1}, followed by another ramp of 200-280 °C at 15 °C·min^{-1} and a final ramp of 280-300 °C at 20 °C·min^{-1}. The mass spectrometer was run in electric ionization mode, flow rate of 1 mL·min^{-1}, ionization voltage at 70 eV, interface temperature at 280 °C and SCAN mode with a mass range of 50 to 550 m/z (García-Rodríguez, Torres-Gurrola, Meléndez-González, & Espinosa-García, 2016).

The concentration of compounds was calculated by the internal standard method, relating the area of each compound to the area of the tetradecane, whose concentration is known. Subsequently, the concentration was related to the dry weight of the sample (mg·g^{-1}). These data were used to calculate the chemical diversity of the provenances.

Spectra obtained on MSD ChemStation (Agilent Technologies Inc., 2004) were compared with those of the NIST/EPA/NIH Mass Spectrometer library, version 2.0 (Stein, Mirokhin, Tchekhovskoi, & Mallard, 2012). The identity of the compounds was validated with experimental retention rates by injecting 1 μL of two series of alkanes (C_8–C_{20} y C_{21}–C_{40}, Sigma Aldrich) with the same chromatographic method. The retention times of the alkanes were related to those of the compounds and compared with retention rates reported in the literature (Adams, 2007; Zenkevich, 2010).

Chromatograms were obtained for each sampled individual which, according to statistical analyses, were

Extracción de muestras

A los 28 meses de edad de las plántulas, se recolectaron muestras de 2 g de acículas maduras de 10 árboles seleccionados al azar en cada una de las 14 procedencias de *A. religiosa* presentes en el ensayo de jardín común, para evaluar el perfil químico. Doce horas antes de la recolección, el ensayo se regó a capacidad de campo. Las muestras se tomaron ente las 8 y 10 h de la mañana en octubre del 2016 con exposición norte y sin señales de daño. Las ramas se tomaron de la parte media del tallo principal.

De cada muestra, 1 g de acículas se introdujo en una bolsa de papel, se mantuvo en el horno de secado a 80 °C durante 24 h y se pesó. El otro gramo se colocó en un frasco de vidrio ámbar que contenía 10 mL de n-hexano grado reactivo, el cual permaneció a 5 °C en un refrigerador. Posteriormente, la muestra en n-hexano se molió en un mortero de porcelana con 0.5 g de arena inerte y 1 mL de tetradecano ($C_{14}H_{30}$) a una concentración de 1 mg·mL^{-1} como estándar interno. El extracto se filtró a través de un embudo con papel filtro de apertura de poro de 11 μm, que contenía 0.5 g de sulfato de sodio anhidro (Na_2SO_4) grado reactivo y se guardó en un vial ámbar a 5 °C hasta su análisis químico.

Análisis químico

Las muestras se analizaron inyectando 1 μL en un cromatógrafo de gases equipado con un detector de masas (Agilent 6890, MSD 5973), con división de flujo (split 60.3:1) a 250 °C en una columna capilar no polar HP-5MS (30 m x 25 mm x 25 μm), usando helio como gas acarreador con un flujo de 1 mL·min^{-1}. El programa de temperatura del horno fue: rampa inicial de 50-200 °C a 20 °C·min^{-1}, seguida de otra rampa de 200-280 °C a 15 °C·min^{-1} y una última de 280-300 °C a 20 °C·min^{-1}. El espectrómetro de masas se operó en modo de ionización eléctrica, flujo de 1 mL·min^{-1}, voltaje de ionización a 70 eV, temperatura de la interfase a 280 °C y modo SCAN con un rango de masas de 50 a 550 m/z (García-Rodríguez, Torres-Gurrola, Meléndez-González, & Espinosa-García, 2016).

La concentración de compuestos se calculó por el método de estándar interno, relacionando el área de cada compuesto con el área del tetradecano, cuya concentración es conocida. Posteriormente, la concentración se relacionó con el peso seco de la muestra (mg·g^{-1}). Estos datos se utilizaron para calcular la diversidad química de las procedencias.

Los espectros obtenidos en MSD ChemStation (Agilent Technologies Inc., 2004) se compararon con los de la biblioteca de Espectros de Masas NIST/EPA/NIH, versión 2.0 (Stein, Mirokhin, Tchekhovskoi, & Mallard, 2012). La identidad de los compuestos se validó con los índices de divided into three groups with an altitudinal interval of 200 m, so a chromatogram was selected from each altitudinal section to exemplify their phytochemical diversity (Figure 2).

Diversity indices

The alpha diversity, expressed through several diversity indices of the 14 provenances of *A. religiosa*, was estimated with the statistical analysis program PAST 4.03 (Hammer, Harper, & Ryan, 2001). Eleven diversity indices were obtained, of which the most informative (significant) were selected by ANOVA in the graphical interface of the free software R Rcmdr 4.0.2 (R Development Core Team, 2020). These were Fisher's alpha index and Menhinick's index which proved robust since it allows comparison of samples of different sizes.

Total diversity measured with the Menhinick index was based on the relationship between the total number

retención experimentales, inyectando 1 µL de dos series de alcanos (C_8–C_{20} y C_{21}–C_{40}, Sigma Aldrich) con el mismo método cromatográfico. Los tiempos de retención de los alcanos se relacionaron con los de los compuestos y se compararon con los índices de retención reportados en la literatura (Adams, 2007; Zenkevich, 2010).

Se obtuvieron cromatogramas para cada individuo muestreado que, de acuerdo con los análisis estadísticos, se dividieron en tres grupos con intervalo altitudinal de 200 m, por lo que se seleccionó un cromatograma de cada sección altitudinal para ejemplificar su diversidad fitoquímica (Figura 2).

Índices de diversidad

La diversidad alfa, expresada a través de varios índices de diversidad de las 14 procedencias de *A. religiosa,* se estimó con el programa de análisis estadístico PAST 4.03 (Hammer, Harper, & Ryan, 2001). Se obtuvieron 11 índices de diversidad, de los cuales se seleccionaron

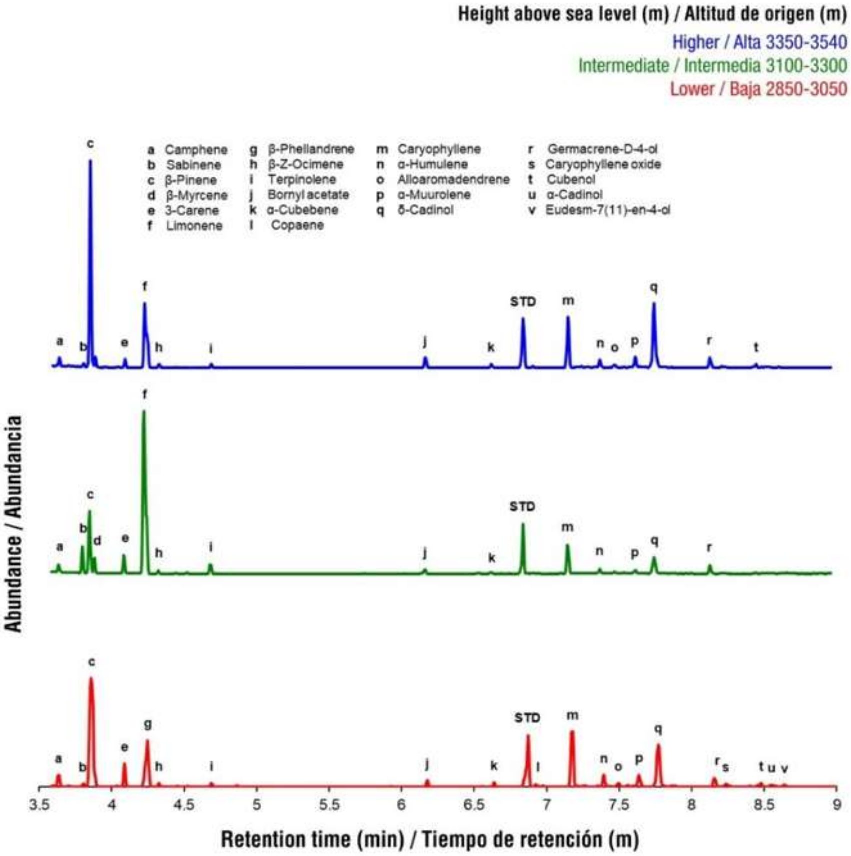

Figure 2. Representative chromatograms of the phytochemical diversity of *Abies religiosa* by height above see level from Cerro San Andrés, Municipio Libre de Hidalgo, Michoacán, Mexico.

Figura 2. Cromatogramas representativos de la diversidad fitoquímica de *Abies religiosa* por altitud de origen del cerro de San Andrés, Municipio Libre de Hidalgo, Michoacán, México.

of terpenes and the total number of trees (samples). Fisher's alpha index was used for the most common terpenes in most conifers (α-pinene, camphene, β-pinene, β-myrcene, 3-carene, limonene), which allows comparison of the level of richness between provenances, which vary in the level of abundance and depend on the number of individuals sampled. In both indices, each terpene was considered as a 'species' and the provenances as 'communities'.

Statistical Analysis

ANOVA and multivariate analyses were carried out using the SAS 9.4 TS 1 M3 statistical package (SAS Institute Inc., 2015). PROC GLM was used for the ANOVA and PROC STEPDISC for the stepwise analysis to select statistically significant ($P < 0.05$) and informative variables. A correlation analysis was also performed with PROC CORR to remove highly correlated variables and avoid collinearity problems, with the exception for terpenes commonly found in conifers such as α-pinene, camphene, β-pinene, β-myrcene, 3-carene, limonene (Kopaczyk et al., 2020; Pokorska et al., 2012; Taft et al., 2015). Subsequently, a matrix of averages was obtained for multivariate principal component, correspondence and cluster analyses performed with PRINCOMP, CORRESP and CLUSTER procedures, respectively.

Results

Table 2 shows the 32 compounds and their retention indices (RI) found in the needles of the 14 provenances of *A. religiosa*: 17 monoterpenes (RI 932-1 132), 10 sesquiterpenes (RI 1 345, 1 375-1 700), one phenyl propanoid (RI 1 196), one alkatetraene (RI 1 237) and three esters (RI 1 223, 1 287, 1 379).

Diversity indices

Figure 3 shows that provenances were significantly different from each other ($P \leq 0.0174$, $\alpha = 0.05$), according to the specific richness calculated with the Menhinick index. Simple linear regression reports a negative association between terpene diversity and height above see level ($P = 0.0016$, $R^2 = 0.5788$); that is, the number of terpenoids decreased as altitude increased. Tukey's mean comparison test showed three groups according to the number of terpenes in each provenance: 1) the 2 850 m provenance, different from the rest and where the highest terpene diversity is found; 2) the provenances between 2 950 and 3 300 m, including the highest altitude (3 540 m), which together present intermediate diversity; and 3) the provenances from 3 350 to 3 500 m, where the lowest terpene diversity is found regarding the distribution range studied.

los más informativos (significativos) mediante un análisis de varianza en la interfaz gráfica del *software* libre R Rcmdr 4.0.2 (R Development Core Team, 2020). Estos fueron el índice alfa de Fisher y el índice de Menhinick que resultó robusto dado que permite la comparación de muestras de tamaños diferentes.

La diversidad total medida con el índice de Menhinick se basó en la relación entre el número total de terpenos y el número total de individuos (muestras). El índice alfa de Fisher se utilizó para los terpenos más comunes en la mayoría de las coníferas (α-pineno, canfeno, β-pineno, β-mirceno, 3-careno, limoneno), el cual permite la comparación del nivel de riqueza entre procedencias, que varían en el nivel de abundancia y depende del número de individuos muestreados. En ambos índices se consideró a cada terpeno como una 'especie' y a las procedencias como las 'comunidades'.

Análisis estadístico

Se realizaron análisis de varianza y multivariados con el paquete estadístico SAS 9.4 TS 1 M3 (SAS Institute Inc., 2015). Para el análisis de varianza se usó el PROC GLM y para el análisis *stepwise* el PROC STEPDISC con el fin de seleccionar las variables estadísticamente significativas ($P < 0.05$) e informativas. También se hizo un análisis de correlaciones con PROC CORR, para eliminar las variables altamente correlacionadas y evitar problemas de colinealidad, a excepción de los terpenos encontrados comúnmente en las coníferas como α-pineno, canfeno, β-pineno, β-mirceno, 3-careno, limoneno (Kopaczyk et al., 2020; Pokorska et al., 2012; Taft et al., 2015). Posteriormente, se obtuvo una matriz de promedios para los análisis multivariados componentes principales, correspondencias y conglomerados realizados con los procedimientos PRINCOMP, CORRESP y CLUSTER, respectivamente.

Resultados

El Cuadro 2 presenta los 32 compuestos y sus índices de retención (IR) encontrados en las acículas de las 14 procedencias de *A. religiosa*: 17 monoterpenos (IR 932-1 132), 10 sesquiterpenos (IR 1 345, 1 375-1 700), un fenil propanoide (IR 1 196), un alcatetraeno (IR 1 237) y tres ésteres (IR 1 223, 1 287, 1 379).

Índices de diversidad

La Figura 3 muestra que las procedencias fueron significativamente diferentes entre sí ($P \leq 0.0174$, $\alpha = 0.05$), de acuerdo con la riqueza específica calculada con el índice de Menhinick. La regresión lineal simple indica una asociación negativa entre la diversidad de terpenos y la altitud de origen ($P = 0.0016$, $R^2 = 0.5788$); es decir, el número de terpenoides disminuye cuando

Table 2. Secondary metabolites identified in 14 provenances of *Abies religiosa* from Cerro San Andrés, Municipio Libre de Hidalgo, Michoacán, Mexico, ordered by retention time and retention index.

Cuadro 2. Metabolitos secundarios identificados en 14 procedencias de *Abies religiosa* del cerro de San Andrés, Municipio Libre de Hidalgo, Michoacán, México, ordenados por tiempo de retención e índice de retención.

RT/TR	RI/IR	RIexp/ IRexp	SM/MS	RT/TR	RI/IR	RIexp/ IRexp	SM/MS
3.45	932	942	α-Pinene*/ α-Pineno*	5.79	1 237	1 254	Pulegone/Pulegona
3.57	946	959	Camphene*/Canfeno*	6.10	1 287	1 298	Bornyl acetate/ Acetato de bornilo
3.74	969	981	Sabinene/Sabineno	6.64	1 345	1 376	α-Cubebene/ α-Cubebeno
3.78	974	987	β-Pinene*/ β-Pineno*	6.70	1 379	1 381	Geranyl acetate/ Acetato de geranilo
3.82	988	992	β-Myrcene*/ β-Mirceno*	6.78	1 375	1 397	Copaene/Copaeno
4.02	1 002	1 019	α-Phellandrene/ α-Felandreno	7.10	1 417	1 446	Caryophyllene/Cariofileno
4.09	1 008	1 027	3-Carene*/3-Careno*	7.33	1 452	1 482	α-Humulene/ α-Humuleno
4.13	1 020	1 032	o-Cymene/o-Cimeno	7.49	1 458	1 506	Alloaromadendrene/ Aloaromadendreno
4.16	1 024	1 036	Limonene*/Limoneno*	7.56	1 484	1 518	Germacrene D/ Germacreno D
4.24	1 025	1 047	β-Phellandrene/ β-Felandreno	7.57	1 500	1 519	α-Muurolene/ α-Muroleno
4.26	1 044	1 050	β-Z-Ocimene/β-Z-Ocimeno	7.69	1 522	1 539	δ-Cadinol/ δ-Cadinol
4.39	1 054	1 096	γ-Terpinene/ γ-Terpineno	7.77	1 574	1 552	Germacrene D-4-ol/ Germacreno D-4-ol
4.62	1 086	1 096	Terpinolene/Terpinoleno	8.18	1 582	1 619	Caryophyllene oxide/ Óxido de cariofileno
4.93	1 132	1 137	(*E*,*E*)-Cosmene/ (*E*,*E*)-Cosmeno	8.41	1 645	1 656	Cubenol/Cubenol
5.46	1 196	1 209	Estragole/Estragol	8.48	1 652	1 667	α-Cadinol/ α-Cadinol
5.63	1 223	1 232	Fenchyl acetate/ Acetato de fenchilo	8.65	1 700	1 695	Eudesm-7(11)-en-4-ol/ Eudesm-7(11)-en-4-ol

RT: retention time, RI: retention index, RIexp: retention index experimental, SM: secondary metabolite. *SM most commonly found in conifers.

TR: tiempo de retención, IR: índice de retención, IRexp: índice de retención experimental, MS: metabolito secundario. *MS más comunes en coníferas.

On the other hand, according to Figure 4, the provenances also showed significant differences among themselves ($P \leq 0.0083$), according to Fisher's alpha index. Similar to Menhinick's index, it was shown by simple linear regression that terpenoid diversity decreases as elevation increases ($P = 0.0013$, $R^2 = 0.5919$). Moreover, Tukey's comparison of means established three sets according to the diversity of terpenoids analyzed: 1) the provenance at 2 850 m having the highest diversity, 2) the provenances between 2 950 to 3 350 m, including the highest altitude provenance (3 540 m) with intermediate diversity, and 3) the provenances between 3 400 to 3 500 m with lower terpenoid diversity.

la altitud aumenta. La prueba de comparación de medias de Tukey indicó tres agrupaciones de acuerdo con el número de terpenos en cada procedencia: 1) la procedencia de 2 850 m, diferente del resto y en la cual se encuentra la mayor diversidad de terpenos; 2) las procedencias entre 2 950 y 3 300 m, incluyendo la de mayor altitud (3 540 m), que en conjunto presentan diversidad intermedia; y 3) las procedencias de 3 350 a 3 500 m, donde se encuentra la menor diversidad de terpenos con respecto al rango de distribución estudiado.

Por otra parte, de acuerdo con la Figura 4, las procedencias también mostraron diferencias significativas entre sí ($P \leq 0.0083$), de acuerdo con el índice de alfa de Fisher.

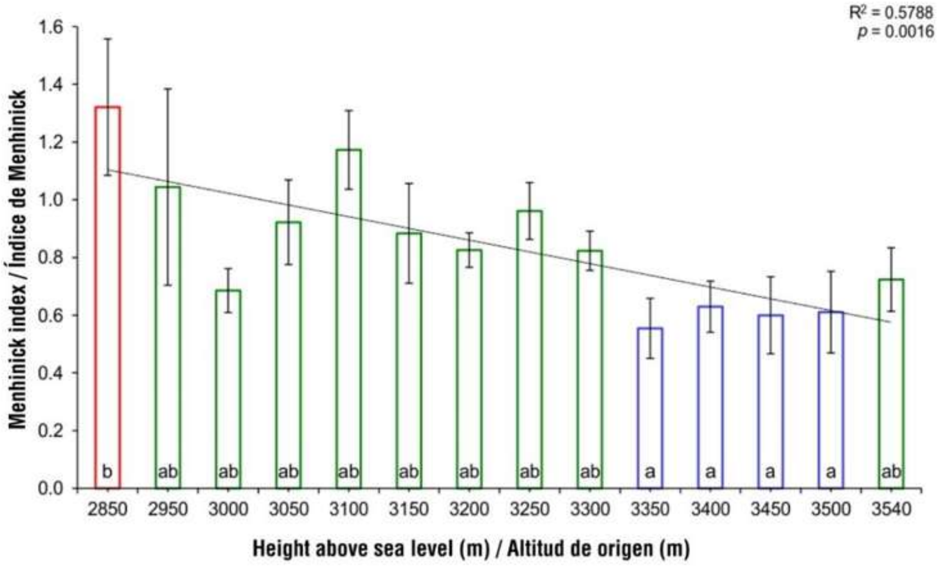

Figure 3. Variation of terpene diversity calculated with Menhinick's index for 14 provenances of *Abies religiosa* from Cerro San Andrés, Municipio Libre de Hidalgo, Michoacán, Mexico. The same color indicates belonging to a Tukey's mean clustering ($P = 0.05$).

Figura 3. Variación de la diversidad de terpenos calculada con el índice de Menhinick para 14 procedencias de *Abies religiosa* del cerro de San Andrés, Municipio Libre de Hidalgo, Michoacán, México. Un mismo color indica pertenencia a un agrupamiento de medias de Tukey ($P = 0.05$).

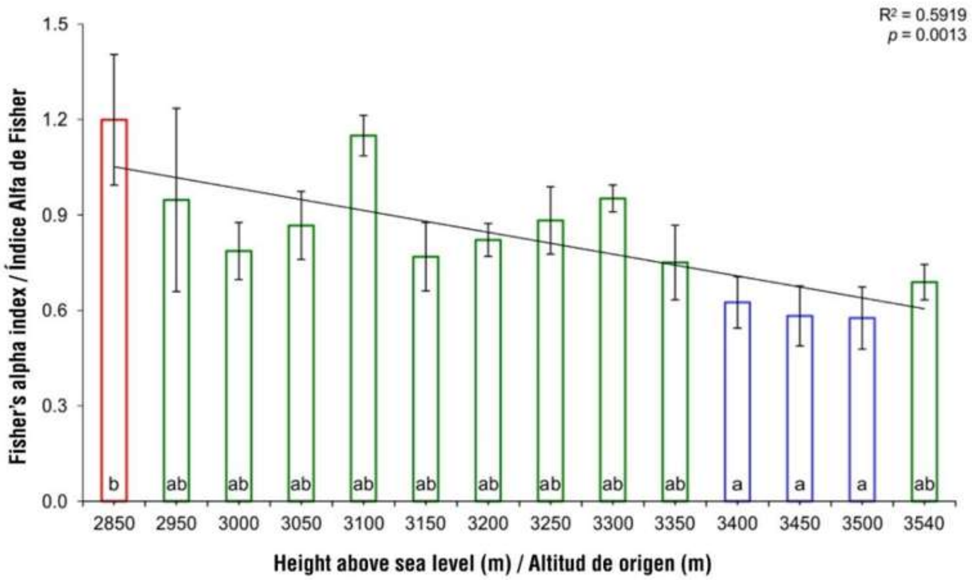

Figure 4. Variation of terpene diversity calculated with Fisher's alpha index for 14 provenances of *Abies religiosa* from Cerro San Andrés, Municipio Libre de Hidalgo, Michoacán, Mexico. The same color indicates belonging to a Tukey's mean clustering ($P = 0.05$).

Figura 4. Variación de la diversidad de terpenos calculada con índice alfa de Fisher para 14 procedencias de *Abies religiosa* del cerro de San Andrés, Municipio Libre de Hidalgo, Michoacán, México. Un mismo color indica pertenencia a un agrupamiento de medias de Tukey ($P = 0.05$).

Multivariate analysis

Significant differences between provenances were found by analysis of variance for sabinene, o-cymene, terpinolene and α-cubebene ($P < 0.05$) and highly significant differences for (E,E)-cosmene and eudesm-7(11)-en-4-ol ($P < 0.0001$). Stepwise analysis identified germacrene D, α-cubebene, germacrene D-4-ol and δ-cadinol ($P < 0.05$) and terpinolene ($P < 0.0001$) as more informative variables.

In addition to the terpenes selected with ANOVA and stepwise analyses, a correlation analysis was performed, removing terpenes highly correlated with each other, except for those commonly found in conifers. Thus, out of the 32 compounds identified, 22 were selected and principal component, correspondence and cluster analyses were performed. According to the principal component and correspondence analyses, three discrete groups were detected corresponding to altitudinal ranges of 200 m: 2 850 to 3 050 m, 3 100 to 3 300 m and 3 350 a 3 540 m.

Principal component analysis

The first five principal components had eigenvalues greater than 1.0 and explain 90 % of the total variance. The first component had an eigenvalue of 7.4 explaining 33.7 % of the total variance. Terpenes such as α-pinene, β-pinene, δ-cadinol and cubenol contributed strongly to the variance of this component, with eigenvectors greater than 0.3. The second component had an eigenvalue of 4.13 explaining 18.8 % of tbe total variation. The terpenes that contributed significantly to the variation were o-cymene, (*E,E*)-cosmene, pulegone and eudesm-7(11)-en-4-ol. The third component had an eigenvalue of 3.6 contributing 16.3 % of the total variance; the terpenes that had eigenvectors greater than 0.3 were limonene, β-phellandrene and eudesm-7(11)-en-4-ol. The fourth component had an eigenvalue of 2.85 which contributed 12.9 % of the variance; terpenes that had eigenvectors greater than 0.3 were camphene, sabinene and terpinolene. Finally, the fifth component had an eigenvalue of 1.88 contributing 8.5 % of the total variance; the terpenes α-phellandrene, germacrene D and cubenol contributed to this variation. Finally, the fifth component had an eigenvalue of 1.88 that contributed 8.5 % of the total variance; the terpenes α-phellandrene, germacrene D and cubenol contributed to this variation.

Figure 5 shows the distribution of the 14 provenances of *A. religiosa* according to the first three principal components (introduced with the prefix CP), which account for 68.6 % of the total variance explained. Populations below 3300 m (green squares and red triangles) are more closely related to each other than to

Similar al índice de Menhinick, se demostró mediante una regresión lineal simple que la diversidad de terpenoides disminuye conforme la elevación aumenta ($P = 0.0013$, $R^2 = 0.5919$). Asimismo, la comparación de medias de Tukey estableció tres conjuntos de acuerdo con la diversidad de los terpenos analizados: 1) la procedencia a 2 850 m que es la de mayor diversidad, 2) las procedencias entre los 2 950 a los 3 350 m, incluyendo la procedencia de mayor altitud (3 540 m) con diversidad intermedia y 3) las procedencias entre los 3 400 a los 3 500 m con menor diversidad de terpenos.

Análisis multivariados

Mediante análisis de varianza se encontraron diferencias significativas entre procedencias para sabineno, o-cimeno, terpinoleno y α-cubebeno ($P < 0.05$) y diferencias altamente significativas para (*E,E*)-cosmeno y eudesm-7(11)-en-4-ol ($P < 0.0001$). El análisis *stepwise* identificó a germacreno D, α-cubebeno, germacreno D-4-ol y δ-cadinol ($P < 0.05$) y a terpinoleno ($P < 0.0001$) como variables más informativas.

Además de los terpenos seleccionados con los análisis de varianza y *stepwise*, se hizo un análisis de correlaciones, eliminando los terpenos altamente correlacionados entre sí, a excepción de los que son comúnmente encontrados en coníferas. De esta forma, de los 32 compuestos identificados, se seleccionaron 22 y con ellos se realizaron análisis de componentes principales, de correspondencias y de conglomerados. De acuerdo con los análisis de componentes principales y de correspondencias, se detectaron tres grupos discretos que corresponden a rangos altitudinales de 200 m: 2 850 a 3 050 m, 3 100 a 3 300 m y 3 350 a 3 540 m.

Análisis de componentes principales

Los cinco primeros componentes principales tuvieron valores propios mayores de 1.0 y explican 90 % de la varianza total. El primer componente tuvo un valor propio de 7.4 que explica 33.7 % de la varianza total. Terpenos como α-pineno, β-pineno, δ-cadinol y cubenol contribuyeron fuertemente a la variación de este componente, con autovectores superiores a 0.3. El segundo componente tuvo un valor propio de 4.13 que explica 18.8 % del total de la variación. Los terpenos que contribuyeron de manera importante a la variación fueron o-cimeno, (*E,E*)-cosmeno, pulegona y eudesm-7(11)-en-4-ol. El tercer componente tuvo un valor propio de 3.6 que contribuye con 16.3 % de la varianza total; los terpenos que tuvieron autovectores superiores a 0.3 fueron limoneno, β–felandreno y eudesm-7(11)-en-4-ol. El cuarto componente tuvo un valor propio de 2.85 que contribuyó con 12.9 % de la varianza; los terpenos que tuvieron autovectores mayores de 0.3 fueron canfeno, sabineno y terpinoleno. Para finalizar, populations above that altitude (blue circles), according to the terpenes that define the first three components.

Correspondence analysis

The correspondence analysis showed a significant *P*-value (<0.0001) by means of the χ^2 statistic, where the sum of the first two dimensions is 61.4 % of the total variance explained. Figure 6 indicates the distribution of the colored provenances according to the altitudinal group they were assigned to against the first two dimensions yielded by the analysis. The graph shows that low and intermediate altitude sources are in the first quadrant (upper right quarter) and only two altitudes in the second (lower right quarter), which are associated with four of the compounds frequently found in conifers: β-pinene, limonene, camphene and 3-carene; terpenes that serve mostly for protection against pathogens. Higher altitude provenances are distributed in the second, third (lower left) and fourth quadrants (upper left) of the graph.

Cluster analysis

Figure 7 shows the dendrogram identified with cluster analysis according to Ward's clustering method, which statistically marks a cutoff at 0.1592 of semipartial R^2 based on the pseudo t^2 statistic. The dendrogram

el quinto componente tuvo valor propio de 1.88 que contribuyó con 8.5 % de la varianza total; los terpenos α–felandreno, germacreno D y cubenol contribuyeron a dicha variación.

La Figura 5 muestra la distribución de las 14 procedencias de *A. religiosa* en función de los tres primeros componentes principales (se presentan con el prefijo CP), los cuales representan 68.6 % de la varianza total explicada. Las poblaciones por debajo de los 3 300 m (cuadrados verdes y triángulos rojos) presentan mayor relación entre sí que con las poblaciones por encima de esa altitud (círculos azules), de acuerdo con los terpenos que definen a los tres primeros componentes.

Análisis de correspondencias

Por medio del estadístico χ^2 se obtuvo que el análisis de correspondencias presenta un valor *P* significativo (<0.0001), donde la suma de las dos primeras dimensiones es de 61.4 % de la varianza total explicada. La Figura 6 presenta la distribución de las procedencias con colores de acuerdo con el grupo altitudinal al que fueron asignados contra las dos primeras dimensiones que arroja el análisis. En el gráfico se observa que las procedencias de las altitudes baja e intermedia se concentran en el primer cuadrante (cuarto superior derecho) y solo dos altitudes en el segundo (cuarto

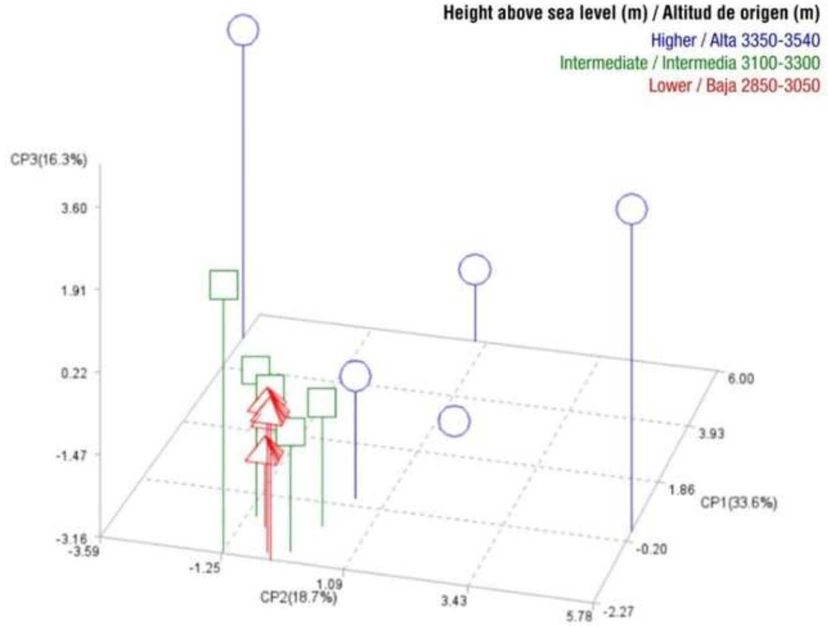

Figure 5. Dispersion of 14 provenances of *Abies religiosa* from Cerro San Andrés, Municipio Libre de Hidalgo, Michoacán, Mexico, according to terpenes defining the first three principal components (PC).

Figura 5. Dispersión de 14 procedencias de *Abies religiosa* del cerro de San Andrés, Municipio Libre de Hidalgo, Michoacán, México, en función de los terpenos que definen los tres primeros componentes principales (CP).

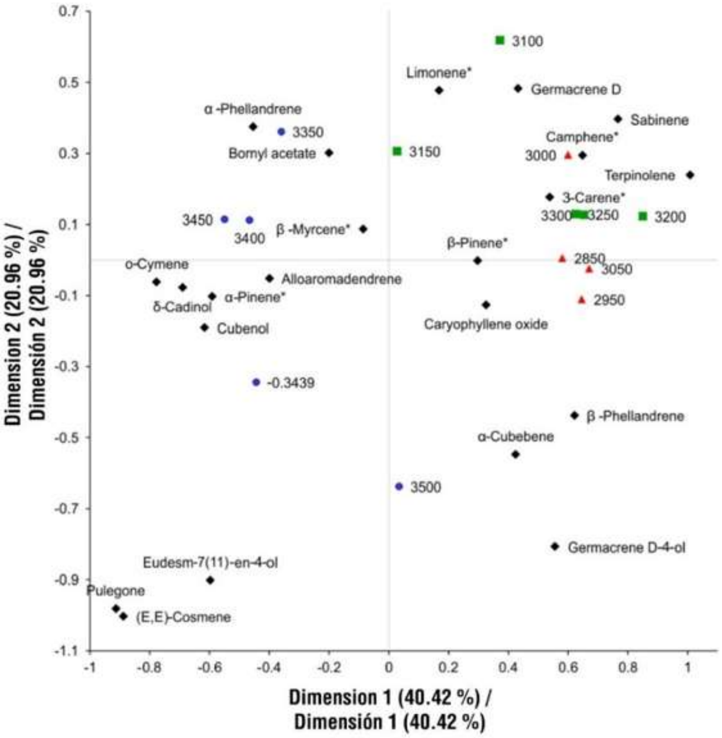

Figure 6. Distribution of *Abies religiosa* provenances according to the correspondence analysis. Red triangles correspond to low altitudes (2 850 to 3 050 m), green squares were assigned to intermediate altitudes (3 100 to 3 300 m) and blue circles represent high altitudes (3 350 to 3 540 m).

Figura 6. Distribución de las procedencias de *Abies religiosa* de acuerdo con el análisis de correspondencias. Triángulos rojos corresponden a las altitudes bajas (2 850 a 3 050 m), cuadrados verdes se asignaron a las altitudes intermedias (3 100 a 3 300 m) y los círculos azules representan altitudes elevadas (3 350 a 3 540 m).

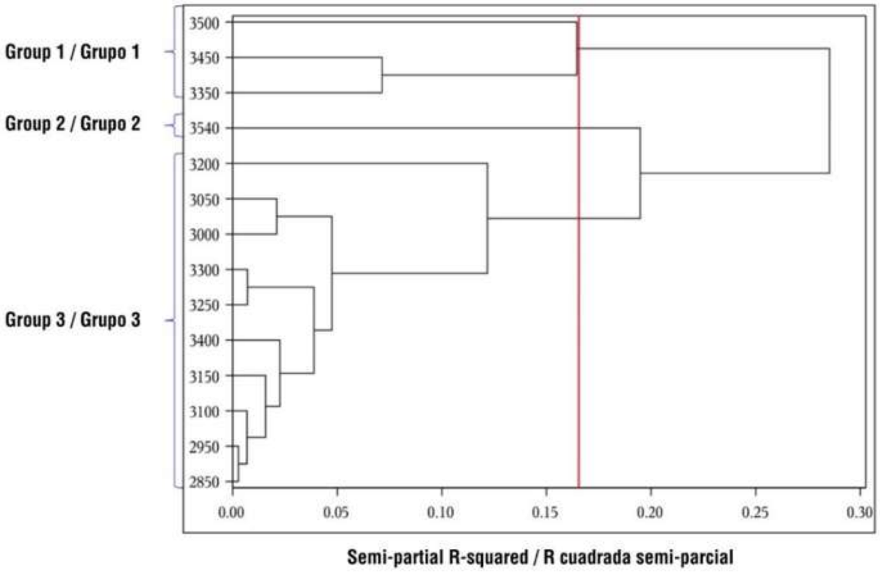

Figure 7. Cluster dendrogram of 14 provenances of *Abies religiosa* from Cerro San Andrés, Municipio Libre de Hidalgo, Michoacán, according to terpene diversity.

Figura 7. Dendrograma de agrupamiento de 14 procedencias de *Abies religiosa* del cerro de San Andrés, Municipio Libre de Hidalgo, Michoacán, en función de la diversidad de terpenos.

reflects an altitudinal clinal pattern; that is, the diversity and concentration of terpenes decrease as altitude increases. There are three groups formed: i) high altitude provenances (3 500, 3 450 and 3 350 m), ii) higher altitude provenance (3 540 m) and iii) low to intermediate altitude provenances (2 850 to 3 300 and 3 400 m).

Discussion

The needles of the 14 provenances of *A. religiosa* had 32 compounds, mostly terpenes, specifically monoterpenes. In conifer-dominated sites, monoterpenes are the major compounds in the emission of biogenic volatile organics (Iñíguez et al., 2014; Trowbridge et al., 2021). These metabolites play an important role in tree-insect interactions, because they are a central element of constitutive and inducible defenses of conifers and are an essential component of defensive resins as they are toxic to many herbivores (Pezet et al., 2013; Whitehill et al., 2019); oleoresin secretion is the main form of chemical and mechanical defense (Heredia-Bobadilla et al., 2014).

Several studies have shown that altitudinal variation and chemical diversity of chemical compounds are closely related. Mullin et al. (2021) determined that concentrations of monoterpenes (limonene and 3-carene) and diterpenes increase as elevation increases in populations of *Pinus contorta* Douglas ex Loudon (Lodgepole pine) (western Alberta, Canada). In contrast, Večeřová et al. (2021) found that, in *Picea abies* (L.) Karsten (spruce), the content of β-pinene, α-pinene, sabinene, and α-terpineol decreased substantially at higher altitude, and that the only positively associated monoterpene was 3-carene.

The indices in the present study indicate greater terpene diversity in the provenance at lower altitudes (2 850 m) and that as elevation increases, diversity decreases. This is probably because in the lower part of the species distribution, plants are more susceptible to pathogen attack (Rasmann, Pellissier, Defossez, Jactel, & Kunstler, 2014; Rodríguez-Castañeda et al., 2010); Moreira et al. (2014) indicate that altitude is a biotic driver of plant defense evolution. In this regard, several studies have shown that the incidence of herbivore damage, particularly bark beetles in conifers, is higher at lower altitudes when comparing both intraspecific and interspecific variability. For example, in intraspecific variability there is the case of the bark beetle *Ips confusus* Wood & Bright. that attacks *Pinus edulis* Engelm. (pinyon pine) (Santos & Whitham, 2010); in the interspecific, *P. contorta* is found on lower slopes and *Pinus albicaulis* Engelm (whitebark pine) on higher slopes, both continuously exposed to the complex formed by bark beetles and fungi, although

inferior derecho), las cuales se asocian a cuatro de los compuestos encontrados frecuentemente en coníferas: β-pineno, limoneno, canfeno y 3-careno; terpenos que sirven en mayor parte para la protección contra patógenos. Las procedencias de las altitudes más altas se distribuyen en el segundo, tercero (inferior izquierda) y cuarto cuadrantes (superior izquierda) del gráfico.

Análisis de conglomerados

La Figura 7 muestra el dendrograma generado con análisis de conglomerados de acuerdo con el método de agrupamiento de Ward, que estadísticamente marca un corte en 0.1592 de R^2 semiparcial con base en el estadístico pseudo t^2. El dendrograma refleja un patrón clinal altitudinal; es decir, la diversidad y concentración de los terpenos disminuyen conforme la altitud aumenta. Los grupos formados son tres: i) procedencias de altitudes elevadas (3 500, 3 450 y 3 350 m), ii) procedencia de mayor altitud (3 540 m) y iii) procedencias de altitudes bajas a intermedias (2 850 a 3 300 y 3 400 m).

Discusión

Las acículas de las 14 procedencias de *A. religiosa* tuvieron 32 compuestos en su mayoría terpenos, específicamente monoterpenos. En sitios donde predominan las coníferas, los monoterpenos son los compuestos mayoritarios en la emisión de orgánicos volátiles biogenéticos (Iñíguez et al., 2014; Trowbridge et al., 2021). Dichos metabolitos juegan un rol importante en las interacciones árbol-insecto, ya que son un elemento central de las defensas constitutivas e inducibles de las coníferas y son un componente esencial de las resinas defensivas al ser tóxicos para muchos herbívoros (Pezet et al., 2013; Whitehill et al., 2019); la secreción de oleorresina es la principal forma de defensa química y mecánica (Heredia-Bobadilla et al., 2014).

Diversos estudios han demostrado que la variación altitudinal y la diversidad química de los compuestos químicos están muy relacionados. Mullin et al. (2021) determinaron que las concentraciones de monoterpenos (limoneno y 3-careno) y diterpenos incrementan a medida que la elevación aumenta en poblaciones de *Pinus contorta* Douglas ex Loudon (pino Lodgepole) (oeste de Alberta, Canadá). En contraste, Večeřová et al. (2021) encontraron que, en *Picea abies* (L.) Karsten (abeto rojo), el contenido de β-pineno, α-pineno, sabineno y α-terpineol se redujo sustancialmente a mayor altitud, y que el único monoterpeno asociado positivamente fue 3-careno.

En el presente estudio, los índices indican que existe mayor diversidad de terpenos en la procedencia in the past *P. albicaulis* was intermittently exposed. In this ecosystem, chemical defense syndromes associated with pine monoterpenes are related to exposure history and thus elevation (Raffa et al., 2017).

Consistent with the diversity indices, principal component and correspondence analyses showed that terpene diversity is higher in provenances below 3 350 m and that they are mostly associated with limonene or 3-carene, which act as repellents for herbivore attack (Phillips & Croteau, 1999). Terpinolene, identified as part of a genotype of *Picea sitchensis* (Bong.) Carr. (Sitka spruce), confers resistance to white weevil (*Pissodes strobi* Peck) attack (Robert et al., 2010) and β-pinene found in *Pinus ponderosa* Douglas ex Lawson is synthesized in response to herbivory activity (Litvak & Monson, 1998).

On the other hand, the cluster analysis suggests association among provenances, except for group two, formed by the highest altitude provenance (3 550 m), which could have greater similarity with the populations of the lower part. This apparently atypical behavior of the provenance near the peak is possible because it receives pollen from the Michoacán slope on the west side of Cerro San Andrés and from the east side (Estado de México). This is consistent with patterns observed for species of the genus *Betula*, where terpenes have a dominant maternal heritability component, but with influence of pollen (Isidorov Stocki, & Vetchinikova, 2019). Furthermore, this atypical behavior of higher altitudinal provenances has been observed in *Pinus hartwegii* Lindl. at the peak of Pico de Tancítaro, for the composition of isozyme allele frequencies (Viveros-Viveros et al., 2010). The altitudinal clinal pattern for the diversity and concentration of the terpenes found is similar to that of *Pinus patula* Schiede ex Schltdl. & Cham. and its monoterpenes α-pinene, Δ-3-carene, β-phellandrene and limonene (Lockhart, 1990). Furthermore, the concentration of sesquiterpenes such as geranyl acetate and δ-cadinol decreased as altitude increased in *Pinus yunnanensis* Franch. (Yunnan pine, China) (Hengxiao et al., 1999).

It is important to consider that the results of the present study come from a common garden trial under optimal conditions for plant growth. Therefore, it is possible that terpene abundance could vary if the trial had been conducted under field conditions in the distribution area of *A. religiosa*, as a result of an interaction between genotype and environment; however, because all provenances grew under the same conditions, it is reasonable to assume that terpene diversity varied among populations as a consequence of genetic differences. Clinal patterns have been detected for height, diameter, and survival of this species (Castellanos-Acuña, Lindig-Cisneros,

originada a menor altitud (2 850 m) y que conforme la elevación incrementa, la diversidad disminuye. Esto se debe probablemente a que en la parte baja de la distribución de la especie, las plantas son más susceptibles al ataque de patógenos (Rasmann, Pellissier, Defossez, Jactel, & Kunstler, 2014; Rodríguez-Castañeda et al., 2010); Moreira et al. (2014) indican que la altitud es un impulsor biótico de la evolución de la defensa de las plantas. Al respecto, diversos estudios han mostrado que la incidencia de daño por herbívoros, en particular descortezadores en coníferas, es mayor en altitudes menores cuando se compara tanto la variabilidad intraespecífica como la interespecífica. Por ejemplo, en la intraespecífica se encuentra el caso del descortezador *Ips confusus* Wood & Bright, que ataca a *Pinus edulis* Engelm. (piñón colorado) (Santos & Whitham, 2010); en la interespecífica, *P. contorta* se encuentra en las partes bajas de laderas y *Pinus albicaulis* Engelm (pino de corteza blanca) en las altas, ambas expuestas continuamente al complejo formado por escarabajos descortezadores y hongos, aunque en el pasado *P. albicaulis* estaba expuesta de forma intermitente. En este ecosistema, los síndromes de defensa química asociados a los monoterpenos de los pinos están relacionados con la historia de exposición y, por lo tanto, con la elevación (Raffa et al., 2017).

De forma congruente a los índices de diversidad, los análisis de componentes principales y el de correspondencias demostraron que la diversidad de terpenos es mayor en las procedencias menores de 3 350 m y que están asociadas mayormente al limoneno o 3-careno que funcionan como repelentes para el ataque de herbívoros (Phillips & Croteau, 1999). En particular el terpinoleno, identificado como parte de un genotipo de *Picea sitchensis* (Bong.) Carr. (abeto de Sitka), confiere resistencia al ataque del gorgojo blanco (*Pissodes strobi* Peck) (Robert et al., 2010) y β-pineno encontrado en *Pinus ponderosa* Douglas ex Lawson se sintetiza en respuesta a la actividad por herbivoría (Litvak & Monson, 1998).

Por otra parte, el análisis de conglomerados sugiere asociación entre las procedencias, a excepción del grupo dos, formado por la procedencia de mayor altitud (3 550 m), que podría tener mayor similitud con las poblaciones de la parte baja. Este comportamiento aparentemente atípico de la procedencia cercana a la cumbre se debe posiblemente a que recibe polen de la vertiente de Michoacán del lado oeste del cerro de San Andrés y también del lado este (Estado de México). Esto es compatible con los patrones observados para especies del género *Betula*, donde los terpenos tienen un componente de heredabilidad materna dominante, pero con influencia de la fuente de polen (Isidorov Stocki, & Vetchinikova, 2019). Además, este comportamiento atípico de procedencias de mayor

Silva-Farias, & Sáenz-Romero, 2014; Ortiz-Bibian et al., 2017; Ortiz-Bibian et al., 2019), meaning that altitudinal variation should be considered in the management and conservation of forest species populations.

Conclusions

Diversity and concentration of terpenes in *Abies religiosa* varied according to the altitude of populations; above 3350 m, diversity was lower, but with higher concentration. The higher terpene diversity at lower altitudes, according to several studies, is a consequence of evolutionary pressures caused by herbivory. Both the diversity indices and the cluster analysis demonstrate the existence of three defined groups; that is, three differentiated chemical profiles that should be considered in the establishment of germplasm collection programs for reforestation and restoration, especially to adapt to the expected effects of climate change.

Acknowledgments

This document is an initiative of the Forest Genetic Resources Working Group of the North American Forestry Commission (FGRWG-NAFC), Food and Agriculture Organization of the United Nations. Funding is gratefully acknowledged to Dr. Cuauhtémoc Sáenz-Romero of the Monarch Butterfly Fund (Madison, Wisconsin, EUA), of the FGRWG-NAFC and the Coordination of Scientific Research of the UMSNH; Dr. Arnulfo Blanco-García of the Consejo Nacional de Ciencia y Tecnología (CONACYT, Fondo Ciencia Básica-2014-242985) and Dr. Roberto Lindig-Cisneros of PAPIIT-UNAM (AG 200221). To Gabriel Muñoz-Montoya (Queréndaro, Michoacán) and Miguel Ángel Silva-Farías (Servi-Ambiental El Bosque) for access and help in seed collection. To Nancy Izquierdo-Calderón for support in sample extraction and database processing. To Mariela Gómez-Romero, Leonel López-Toledo and Erick de la Barrera Montppellier for their comments on the manuscript. To CONACyT for granting a postgraduate scholarship (413133) and a mixed scholarship to the first author.

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altitud se ha observado en *Pinus hartwegii* Lindl. en la cumbre del Pico de Tancítaro, por la composición de frecuencias alélicas de isoenzimas (Viveros-Viveros et al., 2010). El patrón clinal altitudinal para la diversidad y concentración de los terpenos encontrados es similar al de *Pinus patula* Schiede ex Schltdl. & Cham. y sus monoterpenos α-pineno, Δ-3-careno, β-felandreno y limoneno (Lockhart, 1990). Asimismo, en *Pinus yunnanensis* Franch. (pino de Yunnan, China), la concentración de sesquiterpenos como el acetato de geranilo y δ-cadinol disminuyeron a medida que la altitud aumentó (Hengxiao et al., 1999).

Es importante considerar que los resultados del presente estudio provienen de un ensayo de jardín común en condiciones propicias para el crecimiento de las plantas. Por ello, es posible que la abundancia de terpenos pudiera variar si el ensayo se hubiera realizado en condiciones de campo en el área de distribución de *A. religiosa*, como resultado de una interacción entre el genotipo y el medio ambiente; sin embargo, debido a que todas las procedencias crecieron bajo las mismas condiciones, es razonable suponer que la diversidad de terpenos varió entre poblaciones como consecuencia de diferencias genéticas. Patrones clinales se han detectado para la altura, diámetro y supervivencia de esta especie (Castellanos-Acuña, Lindig-Cisneros, Silva-Farias, & Sáenz-Romero, 2014; Ortiz-Bibian et al., 2017; Ortiz-Bibian et al., 2019), lo cual implica que la variación altitudinal debe considerarse en el manejo y conservación de las poblaciones de especies forestales.

Conclusiones

La diversidad y concentración de los terpenos en *Abies religiosa* variaron en función de la altitud de las poblaciones; arriba de 3350 m, la diversidad fue más baja, pero con mayor concentración. La mayor diversidad de terpenos a menor altitud, de acuerdo con diversos estudios es consecuencia de presiones evolutivas causadas por la herbivoría. Tanto los índices de diversidad como el análisis de conglomerados demuestran la existencia de tres grupos definidos; es decir, tres perfiles químicos diferenciados que deben considerarse en el establecimiento de programas de recolecta de germoplasma para reforestación y restauración, en particular para adaptarse a los efectos esperados del cambio climático.

Agradecimientos

Este documento es iniciativa del Grupo de Trabajo sobre Recursos Genéticos Forestales de la Comisión Forestal de América del Norte (FGRWG-NAFC, por sus siglas en inglés), Organización de las Naciones Unidas para la Agricultura y la Alimentación. Se agradece el financiamiento al Dr. Cuauhtémoc Sáenz-Romero del

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Capítulo IV. Evaluación de compuestos fenólicos en acículas de distintas procedencias de *Abies religiosa* como defensa contra radiación UV

Evaluación de compuestos fenólicos en acículas de distintas procedencias de *Abies religiosa* como defensa contra radiación UV

RESUMEN

Actualmente, el cambio climático está afectando directamente las condiciones abióticas y bióticas de los ecosistemas forestales, induciendo ambientes de estrés cada vez mayores. Para poder subsistir frente a diferentes condiciones de estrés, las coníferas producen una variedad de compuestos químicos que forman parte del grupo de los metabolitos secundarios. Dentro de este grupo, los fenoles son muy importantes, ya que gracias a sus propiedades antioxidantes ayudan a las plantas a proteger sus células del daño oxidativo provocado por los radicales libres derivados de la radiación ultravioleta (UV) de la luz solar, así como de la defensa frente a herbívoros. Se evaluó la presencia de compuestos fenólicos mediante colorimetría en 10 plántulas de cada una de 10 procedencias de *Abies religiosa* originadas en un gradiente altitudinal (3100–3550 m) en la Reserva de la Biósfera Mariposa Monarca, y creciendo en un ensayo de procedencias en vivero. Se obtuvo una muestra de acículas de cada individuo y fue analizada con el método Folin-Denis para determinar el contenido de compuestos fenólicos mediante el cambio del pH de la muestra arrojando una coloración azul. Las muestras fueron determinadas en un espectrofotómetro a 700 nm, y se obtuvo una media de absorbancia por procedencia. Se realizó un análisis estadístico por medio de un modelo lineal generalizado y un análisis de agrupamiento entre la absorbancia contra altitud, irradiación, pendiente y aspecto de la ladera. No se encontró un patrón entre la absorbancia y los parámetros ambientales. Sin embargo, las relaciones absorbancia vs. irradiación ($p = 0.0496$) y absorbancia vs. aspecto ($p = 0.0432$) son ligeramente significativas. Es posible que esta variación sea consecuencia tanto de factores genéticos como de variación estacional además de la ubicación de cada individuo dentro de la parcela. Se recomienda caracterizar el ambiente lumínico de cada individuo y controlar la colecta de acículas tanto en términos estacionales como de ubicación de la muestra.

INTRODUCCIÓN

Las coníferas han desarrollado la capacidad de realizar una selección diferencial de su genotipo a lo largo de gradientes de elevación debido a la variación de las condiciones climáticas (Rehfeldt et al. 2017; Halbritter et al. 2018) y a su interacción con diferentes condiciones bióticas o abióticas (Abdala-Roberts et al. 2016). Estas variaciones han permitido que las coníferas desarrollaran estrategias de defensa efectivas: las constitutivas y las inducibles (Franceschi et al. 2005; Metsämuuronen y Sirén 2019; Kopaczyk et al. 2020).

Las defensas constitutivas están determinadas por la historia de vida de la especie, las adaptaciones genéticas de la población y pueden ser afectadas por las condiciones ambientales (Nybakken et al. 2021). Mientras que las defensas inducibles son aquellas que comprenden elementos estructurales y de defensa química que funcionan para la defensa, generalmente, contra agentes patógenos (Wallis et al. 2008), permitiendo que las plantas puedan invertir parte de su energía en defensa solo dónde y cuándo se necesite al percibir un agente externo (Franceschi et al. 2005). Los metabolitos secundarios, son los encargados proporcionar esta protección química a las plantas frente a cualquier daño (Franceschi et al. 2005; Stolter et al. 2010; Kabera et al. 2014). Los principales grupos fitoquímicos de los metabolitos secundarios son los fenoles, terpenos, alcaloides (Virjamo et al. 2020; Bhardwaj et al. 2021), glucósidos y saponinas (Velu et al. 2018), de los cuales, solo un tipo de compuesto predomina en las plantas (Szwajkowska-Michałek et al. 2020). Todos los compuestos mencionados son vitales para las plantas. Sin embargo, los compuestos fenólicos son los más importantes por sus propiedades antioxidantes, antimicrobianas y antisépticas (Velu et al. 2018).

Los compuestos fenólicos se derivan de la ruta metabólica de la vía del ácido shikímico (Kabera et al. 2014), son una clase de compuestos aromáticos que contienen un anillo de benceno con grupos hidroxilos simples o dobles (Swallah et al. 2020). Algunos compuestos fenólicos intervienen en la síntesis de carotenoides que participan en la absorción de energía durante la fotosíntesis, además se involucran en la síntesis de lignina que proporciona estructura a las plantas y confiere protección contra herbívoros (Rehman et al. 2012; Böttger et al. 2018). La radiación UV-B estimula la síntesis y acumulación de compuestos fenólicos (Morales et al. 2010), que protegen el medio celular de las plantas contra daños oxidativos (Warren et al. 2002; Raitanen et al. 2020).

Se ha demostrado que las acículas de coníferas como *Abies lasiocarpa* (Hooker) Nuttall y *Picea engelmannii* Carr. (DeLucia et al. 1992), son ricas en compuestos fenólicos solubles por lo que son capaces de absorber eficazmente la radiación de longitud de onda corta (Fischbach et al. 1999). Sin embargo, los niveles de tolerancia a la radiación UV gracias a los fenoles varían entre géneros, especies e incluso entre individuos (Jansen et al. 2001).

Los compuestos fenólicos han sido poco estudiados en coníferas de alta montaña, por lo que se sabe poco sobre la composición de los fenoles individuales en los gradientes ambientales (Stolter et al. 2010). Debido a esto, en este estudio se realizó el análisis del contenido de compuestos fenólicos en 10 individuos de 10 procedencias de *Abies religiosa* (Kunth) Schltdl. et Cham. originadas a lo largo de un gradiente altitudinal de la Reserva de la Biósfera Mariposa Monarca, para determinar la variación del contenido en compuestos fenólicos y evaluar si existe un patrón altitudinal.

OBJETIVO

Determinar las diferencias en el contenido de compuestos fenólicos a través de la absorbancia entre procedencias de *Abies religiosa* y evaluar si existe un patrón altitudinal.

HIPÓTESIS

Ha: Como resultado de la adaptación de las poblaciones al gradiente altitudinal en el que se encuentran, existirán diferencias significativas entre poblaciones en la absorbancia/concentración de fenoles, siendo la procedencia de mayor altitud la que presenta mayor concentración como respuesta de protección a la radiación UV.

H0: No existirán diferencias significativas en la absorbancia de fenoles entre procedencias.

MATERIALES Y MÉTODOS

Colecta de semilla y producción de planta

En diciembre de 2017, diez sitios de recolecta de semillas fueron seleccionados a lo largo de un gradiente altitudinal (3100-3550 m) dentro de la Reserva de la Biósfera de la Mariposa Monarca, con una separación entre sitios de 50 m altitudinales. En cada sitio se recolectaron semillas de al menos 10 árboles. A cada población en adelante se le llamará “procedencia” (Cuadro 1).

Cuadro 1. Ubicación geográfica de los sitios de recolecta de semillas de *Abies religiosa* dentro de la Reserva de la Biósfera Mariposa Monarca (RBMM).

Altitud de procedencia (msnm)	**Latitud (N)**	**Longitud (O)**
3491	19° 34' 04.1"	100° 13' 59.5"
3457	19° 34' 17.1"	100° 14' 08.2"
3411	19° 34' 25.0"	100° 14' 13.8"
3364	19° 34' 31.7"	100° 14' 03.8"
3300	19° 34' 46.2"	100° 13' 53.6"
3233	19° 34' 50.9"	100° 13' 26.6"
3210	19° 34' 52.6"	100° 13' 15.5"
3143	19° 34' 53.0"	100° 12' 53.1"
3099	19° 35' 12.0"	100° 12' 52.9"
3003	19° 35' 42.6"	100° 12' 37.5"

En mayo de 2018, las semillas de cada procedencia fueron estratificadas a 4 °C por 14 días (Arriola et al. 2015). Pasado el tiempo de estratificación, las semillas fueron colocadas en charolas como almácigos (manteniendo la identidad de la procedencia) en un sustrato compuesto por polvilla, vermiculita, agrolita y peat-moss, en una proporción 6:1.5:1.5:1, respectivamente. La germinación de las semillas tardó aproximadamente dos semanas, después de las cuales, cada plántula recién emergida fue trasplantada a envases de vivero rígidos (tubetes) de 380 cm^3 con la mezcla de sustrato ya mencionada. Las plántulas pasaron tres años cinco meses en casa de sombra (sombra al 35 %) en el Instituto de Investigaciones sobre los Recursos Naturales (INIRENA, UMSNH), durante los cuales las plántulas fueron regadas continuamente. Para más información sobre la producción de planta ver Capítulo I.

Extracción fenólica

Preparación de Solución estándar

Se preparó una solución con 10 g de Ácido tánico ($C_{76}H_{52}O_{46}$) en 100 ml de agua destilada y se calentó a 70 °C hasta disolver.

Preparación de reactivo Folin-Denis

El reactivo Folin-Denis o Folin-Ciocalteu se basa en una reacción de transferencia de electrones (Appel et al. 2001; Ainsworth y Gillespie 2007), que funciona como oxidante expresado en términos de contenido fenólico. Se realizó la preparación del Reactivo Folin-Denis, con base en la metodología de Saxena et al. (2013). Algunas modificaciones fueron necesarias, debido a las cantidades de compuestos disponibles para la preparación. Se utilizaron 10 g de tungstato de sodio dihidratado ($Na_2WO_4 \cdot 2H_2O$) al 99 %, 2 g de hidrato de ácido fosfomolíbdico $H_3[P(Mo_3O_{10})_4] \cdot xH_2O$), 75 ml de agua destilada y 5 ml de ácido fosfórico (H_3PO_4). Esta disolución fue mezclada con un agitador magnético y calentada en una parrilla (Thermo Cientific) a 100-105 °C por 2 h. Una vez frío, se aforó con agua destilada en un matraz de 100 ml, para posteriormente dejar en refrigeración hasta su uso.

Preparación de acículas de *Abies religiosa*

A los tres años con cinco meses de edad de las plántulas, se cosechó 1 g de acículas frescas por plántula (10 individuos por procedencia). Posteriormente las acículas fueron colocadas en bolsas de papel, siempre manteniendo la identidad por procedencia e individuo, y se metieron a un horno de secado a 65 °C durante 24 h (Leski et al. 2010). Las acículas secas se trituraron mecánicamente en un molino para obtener un polvo fino para facilitar el análisis. Se pesó 0.1 g del polvo de tejido por individuo, en una balanza de precisión analítica Sartorius, y se agregó a un vaso de precipitado en donde se añadieron 7.5 ml de agua destilada. Esta mezcla fue calentada en una parrilla a 100 °C por 10 minutos. A continuación, la mezcla fue puesta en tubos de ensayo para centrifugarlos a 2000 rpm por 20 minutos, para acelerar la separación de las fases acuosa y orgánica. El sobrenadante de cada tubo se vació en diferentes matraces aforados de 10 ml, a los que se agregó 0.5 ml de Reactivo Folin-Denis y agua destilada hasta aforar. La solución resultante se dejó reposar por 30 minutos, para después, realizar la medición de absorbancia a 700 nm en un espectrofotómetro Genesys 20 (Thermo Scientific).

Curva de calibración

Se realizaron curvas de calibración probando diferentes periodos de calentamiento (5, 10, 15, 20, 25 y 30 minutos) a baño maría a una temperatura de 60 °C, y se midieron a 760 nm. Se seleccionó el periodo de calentamiento en donde las alícuotas se mantuvieran constantes, este fue el de 30 minutos. Se utilizaron nueve alícuotas para obtener la curva de calibración (Cuadro 2; Figura 1).

Cuadro 2. Alícuotas establecidas para la curva patrón.

Número de tubo de ensayo	Solución estándar (ml)	Folin-Denis (ml)	Agua destilada (ml)
1	0	0.5	8.5
2	1	0.5	7.5
3	2	0.5	6.5
4	3	0.5	5.5
5	4	0.5	4.5
6	5	0.5	3.5
7	6	0.5	2.5
8	7	0.5	1.5
9	8	0.5	0.5

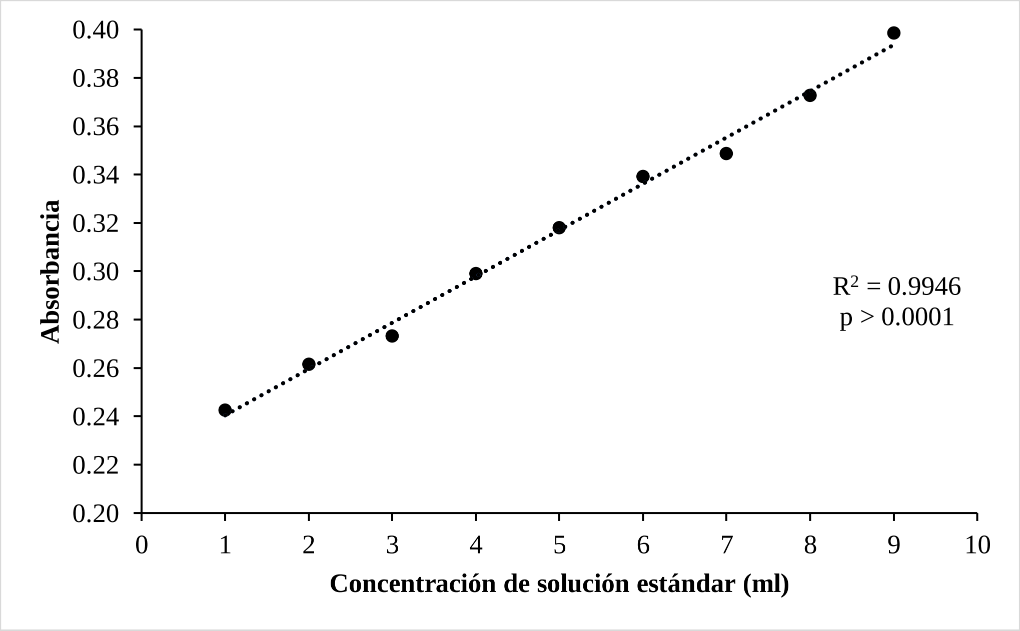

Figura 1. Curva patrón para los compuestos fenólicos que indica una respuesta lineal.

Obtención de los parámetros ambientales para cada procedencia

Se obtuvieron los valores de irradiación, pendiente y aspecto para el sitio de cada procedencia por medio del análisis MDE (Modelado de Elevación Digital) del Estado de México (INEGI) y el programa QGis 3.22. (QGIS Development Team 2023), con el cual se obtuvieron los parámetros ambientales: para la irradiación se utilizó la herramienta r.sun.insidout, para pendiente y aspecto se usó r.solpe.aspect.

El aspecto se refiere a la ubicación geográfica de los sitios de recolecta de acuerdo a su exposición en la ladera (puntos cardinales N, S, E, O). Mientras que la pendiente se refiere a la inclinación.

Análisis estadístico

Con los datos de absorbancia promedio por procedencia y lod parámetros ambientales (altitud, irradiación, pendiente y aspecto), se realizó un análisis estadístico con un modelo lineal generalizado (GLM) en el programa RStudio versión 2022.07.2 Build 576 (RStudio Team 2020). Además, se realizó un análisis de agrupación con distancia euclidiana, en donde se utilizaron los valores de absorbancia de cada individuo para generar un patrón de agrupamiento. Para el análisis estadístico se utilizó la absorbancia y no la concentración, porque la reacción del reactivo Folin-Denis con la disolución estándar de ácido tánico no fue satisfactoria a pesar de que la respuesta fue lineal. Esto debido a que las lecturas obtenidas de las muestras de las acículas muchas veces quedaban fuera del rango de la curva patrón para las concentraciones bajas (es decir, se obtenían valores negativos de la concentración en base al estándar).

RESULTADOS

Los parámetros ambientales irradiación y aspecto obtuvieron valores ligeramente significativos, p = 0.0496 y p = 0.0432, respectivamente (Cuadro 3); mapa de irradiación por procedencia Figura 2.

Cuadro 3. Análisis del modelo lineal generalizado de los parámetros ambientales en relación a la absorbancia para 10 procedencias de *Abies religiosa* de la Reserva de la Biósfera Mariposa Monarca.

Fuente de variación	Suma de cuadrados	Grados de libertad	Valor de F	*p*
Altitud	0.00015	1	0.4974	0.5121
Aspecto	0.00212	1	7.2385	0.0432
Irradiación	0.00195	1	6.6410	0.0496
Pendiente	0.00109	1	3.7175	0.1117
Residuales	0.00146	5		

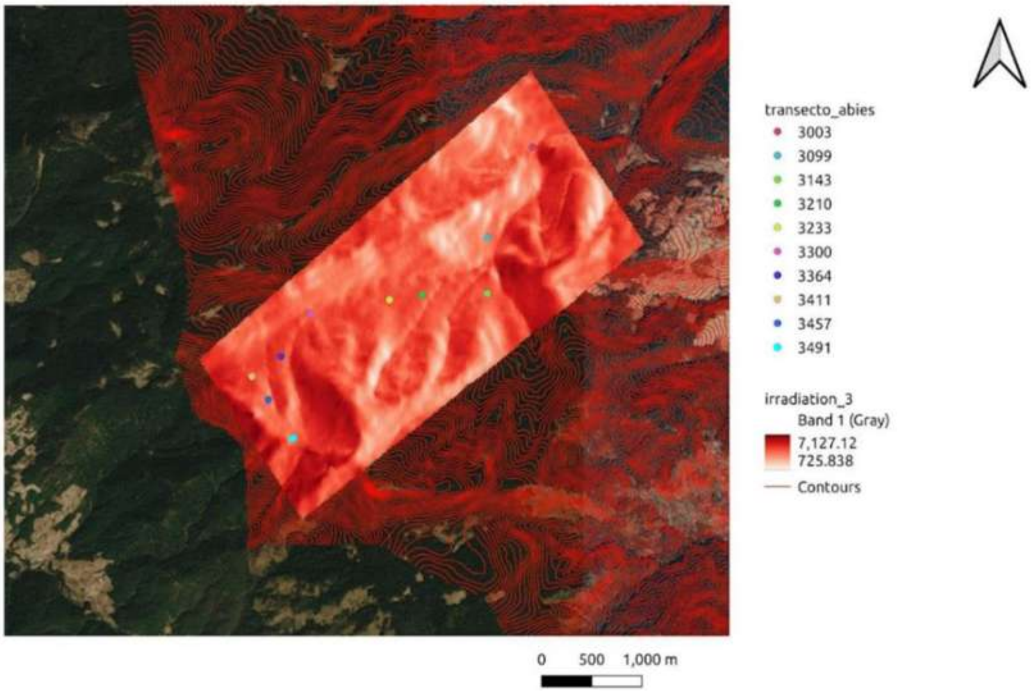

Figura 2 Mapa de irradiación de las 10 procedencias de *Abies religiosa* de la Reserva de la Biósfera Mariposa Monarca.

Con los resultados entre la absorbancia y los parámetros ambientales se determinó que la mayor absorbancia se encontró en altitudes entre los 3200 y 3300 m snm, mientras que el aspecto promedio se encontró entre los 0 y 45 °C, es decir la exposición en la mayoría de los sitios de recolecta de semilla fue Norte–Noreste y la pendiente de estos sitios se mantuvo por debajo los 12°. En cuanto a la irradiación, los sitios que se encontraron en las exposiciones Este–Sureste fueron las que recibieron la mayor cantidad de irradiación promedio (Figura 3).

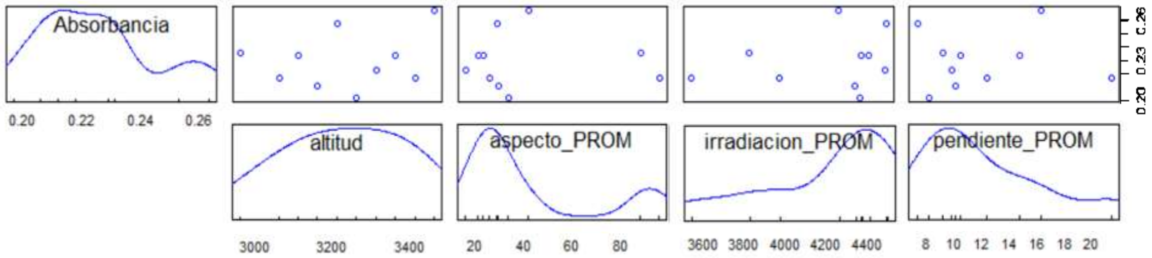

Figura 3. Comportamiento del valor promedio de las 10 procedencias de acuerdo a la absorbancia contra los parámetros ambientales: altitud, aspecto, irradiación, pendiente y tiempo de insolación.

El análisis de agrupamiento con distancia euclidiana entre individuos, no mostró un patrón determinante, ya que al menos un individuo de cada procedencia se agrupa con individuos de procedencias de altitudes completamente diferentes.

DISCUSIÓN Y CONCLUSIONES

Con los resultados de este estudio entre procedencias de *Abies religiosa* se encontró que la relación entre absorbancia vs. irradiación y absorbancia vs. aspecto es ligeramente significativa. Se tuvo que usar la absorbancia promedio por procedencia debido a la alta variación entre individuos dentro de cada procedencia. Sin embargo, no se encontró un patrón altitudinal claro en cuanto a la presencia de compuestos fenólicos. Es posible que la variación en la producción de compuestos fenólicos reflejada en la absorbancia sea consecuencia tanto de factores genéticos como de variación estacional (Kotilainen et al. 2010), la temporada de crecimiento (Kanerva et al. 2008), la posición del muestreo dentro de la planta (Metsämuuronen y Sirén 2019), e incluso de la variación de la luz incidente (Tegelberg et al. 2004). Estos resultados no concuerdan con lo encontrado con por (Raitanen et al. 2020), quienes detectaron que la radiación UV en las altitudes más altas indujo una mayor producción de compuestos fenólicos en coníferas como *Abies alba* Mill., *Pinus unicinata* Ramond ex A.DC. y *P. cembra* L. de los Alpes franceses, debido a que la radiación es mayor en elevaciones más altas y en latitudes más bajas (Jansen et al. 2001).

También es posible que los resultados encontrados tengan más que ver con que en las plantas existe la predominancia de algún tipo de compuesto químico ya sea alcaloide, compuestos fenólicos o terpenoides (Forkner et al. 2004; Szwajkowska-Michałek et al. 2020), que en el caso de *Abies religio*sa los terpenoides mostraron un patrón más definido (ver Capítulo III).

Es necesario tomar en cuenta que los resultados de este trabajo provienen de un ensayo de vivero en casa de sombra, por lo que en gran medida se puede asumir que las condiciones ambientales fueron iguales para todas las plantas, de forma que pudiera ser posible que si las muestras fueran obtenidas en condiciones naturales (en campo), los resultados fuesen más informativos debido a la interacción del genotipo y medio ambiente que pudiera expresar la producción de compuestos fenólicos en respuesta a la radiación UV, como sucedió en experimentos de *Pinus sylvestris* L. con exclusión de radiación UV en campo en el Parque Nacional de Pallas-Ounas Tunturi en la Laponia finlandesa, en donde Turunen et al. (1999) demostraron que la cantidad de flavonol 3-glicósido no acilado disminuía conforme disminuía la irradiación.

Para llevar a cabo una nueva evaluación sería recomendable caracterizar el ambiente lumínico de cada individuo, controlar la colecta de acículas tanto en términos estacionales, así como la ubicación dentro de la copa.

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DISCUSIÓN GENERAL

Los resultados obtenidos derivados de los Capítulos I y II de este trabajo, evidenciaron que las poblaciones de *Abies religiosa* son muy vulnerables al cambio climático, principalmente porque están sujetas al equilibrio entre la humedad disponible y la temperatura, especialmente de la temporada fría y seca (noviembre–febrero). Estos resultados coinciden con lo encontrado por Cerano Paredes et al. (2014) para árboles adultos de *A. religiosa*, para poblaciones de *Pinus hartwegii* Lindl. por Carlón Allende et al. (2021) y para *Abies jaliscana* (Martínez) Mantilla, Shalisko & A. Vázquez (Guerrero-Hernández et al. 2022), pues estos estudios demostraron que la precitación de la temporada de invierno es crítica para el crecimiento anual de estas especies. Por lo que la reducción de la precipitación más el incremento de la temperatura reducirán la productividad del bosque, siendo las poblaciones que se encuentran actualmente en el límite altitudinal inferior de su rango de distribución las primeras que sufrirán estrés por sequía (Mátyás et al. 2010; Mátyás 2010). Algo similar sucedió con las plantas del jardín común ubicado a 2600 m snm, en donde la supervivencia a dos años del establecimiento del experimento fue del 5.7 %, probablemente relacionado con una alteración en el metabolismo de las plantas intentando regular el desequilibrio entre el déficit de humedad disponible y el incremento de la temperatura (Girardin et al. 2016). Si bien, la literatura que describe el hábitat del oyamel establece que esta especie se distribuye entre los 2400 – 3600 m snm, el bajo porcentaje de supervivencia en este sitio concuerda con las proyecciones de reducción en la superficie climática apta para el oyamel (Sáenz-Romero et al. 2012; Pérez Miranda et al. 2014; Gómez-Pineda et al. 2020). En contraste, en el sitio de referencia a 3000 m snm se obtuvo una supervivencia de 91.6 %, y del 95 % cuando se realizó el movimiento de plantas 400 m más arriba, al sitio a 3400 m snm cerca de su límite de distribución superior, lo que permite sugerir que existe una correspondencia con la proyección de hábitat climático de Gómez-Pineda et al. (2020) en donde predice un desplazamiento del hábitat propicio para el oyamel a mayores altitudes, llegando hasta los 4000 m snm.

Los resultados para incremento en altura y diámetro, demostraron que el sitio de referencia es el que actualmente presenta las condiciones óptimas para el desarrollo del oyamel, pues en este sitio las plantas crecieron aproximadamente más del 25 % que el sitio a menor altitud por las condiciones de sequía. Para *A. religiosa* y *Pinus teocote* Schiede ex Schltdl. Vivar-Vivar et al.

(2021), encontraron que las condiciones de sequía durante el otoño e invierno se relacionan con anillos de crecimiento más estrechos, mientras que el clima más húmedo de la temporada de lluvias lo favorece. De forma similar en poblaciones de *Pinus edulis* Engelm. del oeste de EUA, la sequía promovió la reducción en la producción de semillas (Wion et al. 2020). Además, las plantas del sitio de referencia crecieron más del 50 % que el sitio a mayor altitud, porque el crecimiento disminuye conforme se avanza a la línea de árboles porque las condiciones climáticas son más extremas (Hoch y Körner 2009). Este patrón altitudinal coincide con otros estudios que involucran a *A. religiosa,* como Ortiz-Bibian et al. (2019), en el que determinaron que entre los 3000 y 3350 m snm hay una mayor viabilidad y germinación de semillas, y Guzmán-Aguilar et al. (2020), encontraron un mayor reclutamiento de plantas entre los 3050 y 3300 msnm. Además, cabe desatacar que la proyección de Gómez-Pineda et al. (2020), también coincide, pues su proyección indica que la mayor superficie climática apta para oyamel se encontrará entre los 3000 y 3100 m snm para la década centrada en 2060.

Para el índice de productividad, los resultados son similares al crecimiento en altura: las plantas establecidas en el sitio de referencia, fueron las que presentaron el índice de productividad más alto en comparación con los otros dos sitios de ensayo, mientras que, en el sitio a mayor altitud, este índice fue 43 % menor que el sitio a 3000 m snm. Este resultado coincide con el estudio de Dar et al. (2017), en donde analizaron la biomasa en un gradiente altitudinal de bosques de *Abies pindrow* Royle ex D. Don y *Pinus wallichiana* A. B. Jacks. en el Himalaya, concluyendo que la mayor cantidad de biomasa se encontró en la zona intermedia de la distribución de ambas especies, esto debido principalmente, a que la biomasa en las coníferas está fuertemente influenciada por el clima. En el sitio a menor altitud se encontró el índice de productividad más bajo, ocasionado por la alta tasa de mortalidad inducida por el aumento de la temperatura y la diminución de la precipitación.

De acuerdo a los resultados obtenidos en el Capítulo III, se encontraron 32 compuestos químicos, que en su mayoría fueron monoterpenos, en las 14 poblaciones de *Abies religiosa* dentro del gradiente altitudinal del Cerro de San Andrés ubicado en el Municipio Libre de Hidalgo, Michoacán. Se encontraron tres grupos o asociaciones químicas en intervalos altitudinales de 200 m: 2850 a 3050 m snm; 3100 a 3300 m snm; y 3350 a 3540 m snm. Además, se determinó que la diversidad de monoterpenos disminuyó conforme se incrementó la elevación, pero aumentó la concentración de aquellos compuestos que estuvieron presentes. Este resultado es similar a lo reportado para poblaciones de *Picea abies* (L.) H. Karst. originarias de República Checa (Večeřová et al. 2021), *Pinus flexilis* E. James y *P. ponderosa* Douglas ex C. Lawson de Colorado, EUA (Ferrenberg et al. 2017), así como en *Pinus contorta* Douglas de Alberta, Canadá (Mullin et al. 2021). Los monoterpenos, junto con los diterpenos, son los principales componentes de la resina en las coníferas y también se emiten como compuestos volátiles, que juegan un papel fundamental en la defensa contra la herbivoría (Celedon y Bohlmann 2019). Los monoterpenos mayormente asociados a las poblaciones de *A. religiosa* en altitudes menores a 3350 m snm, fueron 3-careno, terpinoleno, limoneno y β-pineno, los cuales son compuestos que están relacionados como respuesta al ataque por herbivoría en especies como *Pinus sitchensis* (Bong.) Carr. y *Pinus radiata* D. Don (Robert et al. 2010; Hall et al. 2011; Romón et al. 2017).

En cuanto a la evaluación de compuestos fenólicos en acículas de oyamel presentado en el Capítulo IV, se encontró una relación significativa entre la absorbancia y los parámetros ambientales aspecto e irradiación ($p = 0.0432$ y $p = 0.0496$, respectivamente), muy probablemente atribuido a que la mayoría de las procedencias presentaron exposición Norte, que es la exposición en la que regularmente se encuentra al género *Abies* (Cuevas Guzmán et al. 2008), y a que la tolerancia a la radiación UV de las plantas está relacionada con la producción de compuestos absorbentes de radiación como los compuestos fenólicos (Laposi et al. 2009). Además, se esperaba encontrar un patrón altitudinal como el de terpenos, en donde las procedencias de mayor altitud presentaran una mayor presencia de compuestos fenólicos como respuesta a la radiación UV (Brzezinska y Kozlowska 2008), debido a que la incidencia de la radiación es mayor en altitudes elevadas (Körner 2007). Sin embargo, este patrón no fue concluyente, posiblemente, este resultado se explica con el hecho de que la respuesta química en las plantas se relaciona con las propiedades del sitio (Stolter et al. 2010), y al estar estas en una casa de sombra, en gran medida se les propició condiciones ambientales similares, que posiblemente aminoraron la expresión de los compuestos fenólicos. En este sentido, Lavola (1998) evaluó tres procedencias de *Betula pendula* Roth *y B. resinifera* Britton en cámara de crecimiento, comparando plantas que fueron expuestas a radiación UV y plantas que no fueron expuestas, determinando que las plantas expuestas contenían mayores concentraciones de compuestos fenólicos. Por lo que es recomendable evaluar los compuestos fenólicos para *A. religiosa* en cámaras de crecimiento para regular la intensidad lumínica, además de realizar evaluaciones estacionales para determinar si existe variación o bien, tomar muestras de poblaciones con árboles adultos dentro de un gradiente altitudinal en campo.

En general, los resultados de este trabajo sugieren que la migración asistida a sitios de mayor elevación para las poblaciones de *A. religiosa* parece ser la acción más viable para su conservación. Sin embargo, tomando en cuenta que la Reserva de la Biósfera Mariposa Monarca tiene una altitud máxima de 3550 m snm, se debe considerar transferir poblaciones de oyamel a montañas con altitudes mayores dentro de la Faja Volcánica Transmexicana, como el Nevado de Toluca y Sierra Nevada en el Estado de México (Sáenz-Romero et al. 2012).

CONCLUSIONES GENERALES

Los resultados de este trabajo sugieren que las poblaciones de *Abies religiosa* son altamente vulnerables a los efectos del cambio climático, principalmente porque el desequilibrio entre precipitación – temperatura está generando una masiva mortalidad de árboles, especialmente en aquellas poblaciones ubicadas en el límite inferior de su distribución.

El incremento de la temperatura de 1.8 °C aunado a la disminución del 14 % de la precipitación, generaron una alta tasa de mortalidad (94.3 %) en el sitio establecido a 2600 m snm. En contraste, la mortalidad de las plantas establecidas en el sitio de referencia y en el sitio cercano al límite superior de distribución de la especie, fue menor al 9 y 5 %, respectivamente.

En cuanto al crecimiento e índice de productividad, el sitio a menor altitud fue el que presentó los valores con mayor variabilidad de crecimiento, teniendo plantas muy altas (88 cm) y otras muy bajas (26 cm). Siendo el sitio que presentó el índice de productividad más bajo (5.2) debido a la alta tasa de mortalidad. El sitio de referencia (a 3000 m de altitud), parecería ser actualmente el hábitat óptimo para la especie, ya que el crecimiento en altura promedio para el sitio fue de 87 cm mientras que el índice de productividad obtenido fue de 14.7; en el sitio a 3400 m snm, no se tuvieron los mejores valores de crecimiento, con 31 cm en promedio de crecimiento en altura y 6.4 para índice de productividad. Sin embargo, debido al desplazamiento del hábitat climático hacia altitudes mayores en las montañas, es de esperar la respuesta al crecimiento y productividad en la década centrada en 2060, sean similares a las observadas actualmente en el sitio de referencia.

Parte importante de la supervivencia de las poblaciones tiene que ver con su composición química, pues esta aporta información sobre la historia de vida de una especie, así como su relación con el entorno. De forma tal que las poblaciones ubicadas actualmente en los extremos altitudinales (tanto de menor como de mayor elevación), podrían tener un valor muy significativo para la respuesta de defensa frente a factores bióticos y abióticos, mediante la producción de diversos terpenos y de compuestos fenólicos. Mientras que los terpenos juegan un papel importante en la protección contra la herbivoría, los compuestos fenólicos sirven como absorbentes de radiación UV.

Debido a lo anterior, la migración asistida con un movimiento altitudinal de 400 m hacia sitios más elevados, parece ser una acción viable para la preservación de los bosques de oyamel de los impactos derivados del cambio climático global. Conservando la mayor variabilidad genética posible, que a la vez incluye la variabilidad de metabolitos secundarios.

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