
**UNIVERSIDAD NACIONAL AUTÓNOMA DE MÉXICO**
**POSGRADO EN CIENCIAS BIOLÓGICAS**
INSTITUTO DE ECOLOGÍA
ECOLOGÍA

# EFECTO DEL ACONDICIONAMIENTO MÁTRICO EN LA GERMINACIÓN Y LA LONGEVIDAD DE SEMILLAS RECALCITRANTES Y/O INTERMEDIAS

**TESIS**

QUE PARA OPTAR POR EL GRADO DE:

**DOCTOR EN CIENCIAS BIOLÓGICAS**

PRESENTA:

**ÁNGEL GABRIEL BECERRA VÁZQUEZ**

**TUTORA PRINCIPAL DE TESIS: DRA. ALMA DELFINA LUCIA OROZCO SEGOVIA**
**INSTITUTO DE ECOLOGÍA, UNAM**

**COMITÉ TUTOR:**
**DRA. SOBEIDA SÁNCHEZ NIETO**
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**FES IZTACALA, UNAM**

**CIUDAD UNIVERSITARIA, CIUDAD DE MÉXICO. ABRIL 2024**


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COORDINACIÓN GENERAL DE ESTUDIOS DE POSGRADO
COORDINACIÓN DEL POSGRADO EN CIENCIAS BIOLÓGICAS
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ASUNTO: Oficio de Jurado

**M. en C. Ivonne Ramírez Wence**
**Directora General de Administración Escolar, UNAM**
**P r e s e n t e**

Por medio de la presente me permito informar a usted que el Comité Académico del Posgrado en Ciencias Biológicas, en su sesión celebrada el **27 de noviembre de 2023** aprobó el siguiente jurado para el examen de grado de **DOCTOR EN CIENCIAS** del estudiante **BECERRA VÁZQUEZ ÁNGEL GABRIEL** con número de cuenta **404058476,** con la tesis titulada**: "EFECTO DEL ACONDICIONAMIENTO MÁTRICO EN LA GERMINACIÓN Y LA LONGEVIDAD DE SEMILLAS RECALCITRANTES Y/O INTERMEDIAS",** bajo la dirección del **DRA. ALMA DELFINA LUCIA OROZCO SEGOVIA**, Tutora Principal, quedando integrado de la siguiente manera**:**

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Sin otro particular, me es grato enviarle un cordial saludo.

**A T E N T A M E N T E**
**"POR MI RAZA HABLARÁ EL ESPÍRITU"**
Ciudad Universitaria, Cd. Mx., a 15 de marzo de 2024

**COORDINADOR DEL PROGRAMA**

**DR. ARTURO CARLOS II BECERRA BRACHO**

c. c. p. Expediente del alumno

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Para ti, Laura Alexandra Rengifo Correa:

Eres el ejemplo tenaz y ferviente del ser que se supera. Eres la fiel constancia del significado y acción de la palabra mujer. Mujer hecha *per se* sin victimismos, egocentrismos, favoritismos ni demás *ismos*. La que confía en sus capacidades para cruzar istmos y vencer oquedades y veleidades, sin sufragios de agendas ni naufragios en mares de afrentas y reprimendas. La que, cuando cae, se levanta con más ahínco para luchar por nuevas oportunidades. Continúas con tu preferencia por la cauta sencillez y con la poderosa discreción de tu capacidad analítica y sentimental. Tu digna resiliencia en un cúmulo de ciencia, inocencia y experiencia. Eres la que en cada acto y pensamiento deja reflexión y sapiencia, todo dentro del marco de su femenina esencia. Millones de granos de arena repartidos en mis pies y en mi conciencia: tu presencia en múltiples ausencias... Mucho he aprendido y seguiré aprendiendo por ti.

*Como la lumbre, la que arde si se atañe*

*a la piel sincera; así, sin recetas ni poetas.*

*Así arde tu verdad concreta que reconozco…*

*y disfruto.*

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*Aires cálidos los del mes de marzo.*
*Han pasado muchos por entre las hojas*
*que, secas ahora, caen como hiriente cuarzo.*
*Se detiene el reloj. Mis recuerdos despojas.*

*Cae también la gota del sudor amargo*
*que surca la sien, abre la piel con tierra*
*y siembra el latir del follaje en mi letargo.*
*Me quita la palidez. Mi nostalgia se aferra.*

*Rumbos agrestes, como en mis andanzas*
*por el rudo barro colorado de Ocuilapa.*
*Otros negros: el lodo tierno de la labranza*
*que a mi huella selló rumbo a Mirador Pilapa.*

*Esos marzos de selva cauta de humedad*
*o de sequía intensa en la soledad de la colina.*
*De Los Tuxtlas a Tuxtla sin ansiedad.*
*Caminos verdes con libertad genuina.*

*También el reloj se detuvo en la Estación,*
*en el corredor con miles de vigías.*
*En la hojarasca quedó el eco de mi pasión.*
*Me canta el recuerdo sin hipocresías.*

*Apago las luces de mis cornisas llenas de sales.*
*Bajo las cortinas mientras llueven memorias.*
*Se escuchan sones de pájaros y mortales.*
*El sueño llega y mañana… otra historia.*

No todo el mundo puede dejarse llevar por la corriente de la Historia. Algunos tienen que detenerse y recoger aquello que queda en sus orillas. No vivimos únicamente en nuestro propio tiempo. También llevamos con nosotros nuestra historia.

Jostein Gaarder, El mundo de Sofía

**INDICE**

**RESUMEN**

Las semillas de longevidad corta, denominadas recalcitrantes e intermedias, son difíciles o prácticamente imposibles de mantener viables y vigorosas por períodos de tiempo ≥ a dos años, ya sea aplicando métodos naturales o artificiales. Dada esta situación, se requieren de estrategias sencillas y accesibles que contribuyan a la solución del problema asociado a la longevidad de estas semillas en condiciones controladas, así como el conocimiento de la ecología de estas especies. El acondicionamiento es un tratamiento de hidratación-deshidratación controlada de semillas que las lleva a un avance en los procesos bioquímicos hacia la germinación, pero sin que ésta culmine. Con ello, el tratamiento incrementa el vigor germinativo y en ocasiones mejora la longevidad de la semilla. En el acondicionamiento natural, una variante del tratamiento, las semillas se entierran *in situ* durante un período específico. Durante este tiempo, las semillas queden sujetas a las oscilaciones de humedad, entre otros factores al interior del suelo, pero sin que ocurra la protrusión de la radícula en las mismas. En esta tesis se investigó el efecto del acondicionamiento natural en la germinación y la longevidad de semillas intermedias y recalcitrantes de cinco especies de bosque tropical perennifolio y caducifolio: *Chamaedorea glaucifolia*, *Cymbopetalum baillonii*, *Damburneya coriacea*, *Magnolia perezfarrerae* y *Ternstroemia tepezapote*. Inicialmente, se evaluó la sensibilidad de las semillas a la deshidratación, así como su comportamiento en almacén. Para ello, las semillas se almacenaron a temperatura ambiente o en cámaras de ambiente controlado a temperatura constante. El contenido de humedad de las semillas y su germinación se evaluaron al inicio y después de un período de almacenamiento; los parámetros germinativos obtenidos fueron el porcentaje de germinación, la tasa máxima, el tiempo medio de germinación y el *lag time* (tiempo necesario para el inicio de la germinación). Para evaluar la sensibilidad a la desecación en la semilla, se calculó el contenido crítico de humedad ($WC_{50}$) y el tiempo para alcanzarlo ($TWC_{50}$). Además de estas variables funcionales, las semillas se caracterizaron morfológica y anatómicamente (volumen, peso, cantidad relativa de endospermo, tamaño del embrión) y se determinó la relación entre las mismas con las características ecológicas y climáticas de su hábitat (época de dispersión, precipitación total y temperatura máxima media en el mes de recolecta de las semillas). Posteriormente, se evaluó el efecto del acondicionamiento natural. Para ello, las semillas se enterraron *in situ* durante un período variable de acuerdo con las características germinativas de cada especie. Luego de su desentierro, las semillas tratadas se almacenaron, junto con el control respectivo, a 15 °C. La germinación de semillas y sus contenidos de humedad se evaluaron antes y

después de un período de almacenamiento. Se realizó un análisis de proteínas totales en semillas acondicionadas y sus controles. *Chamaedorea glaucifolia* presentó semillas de tipo intermedio principalmente por su menor sensibilidad a la desecación ($WC_{50}$ de 4.6% y $TWC_{50}$ de 426 días) en comparación con el resto de las especies, las cuales resultaron recalcitrantes ($WC_{50}$ de 11–60% y $TWC_{50}$ de 9–150 días). La relación encontrada entre las características de sensibilidad a la desecación, las germinativas y las morfológicas con la precipitación y temperatura máxima en el mes de recolecta y con la época de dispersión indicó una influencia del ambiente en la longevidad: semillas grandes germinan rápido y son dispersadas en los meses calurosos (CP 1, 38% de la varianza), mientras que semillas con embrión pequeño son más sensibles a la desecación y son dispersadas en meses lluviosos (CP 2, 34% de la varianza). Por otro lado, pese a que existió interacción entre el efecto del acondicionamiento natural con el almacenamiento y/o el año de recolecta, todas las especies respondieron favorablemente al tratamiento. El acondicionamiento influyó principalmente en la rapidez de la germinación, es decir, el tiempo medio de germinación y el *lag time* disminuyeron en las semillas tratadas en comparación con el control. Este efecto se observó incluso después del almacenamiento en la mayoría de las especies y, en *M. perezfarreae* en todos los años evaluados, hubo una mayor viabilidad que en el control, i.e., mayor porcentaje final de germinación. El perfil de proteínas totales obtenido en las semillas acondicionadas, comparado con el control, mostró nuevas bandas, por ejemplo, una de 21 kDa en *Chamaedorea glaucifolia*, o bien bandas ausentes en todas las especies. Estos cambios en los perfiles estarían relacionados principalmente con el avance pregerminativo producido por el tratamiento El acondicionamiento natural produjo un efecto en la germinación similar al manifestado por semillas de longevidad larga, por lo que constituye una herramienta práctica con gran potencial en semillas recalcitrantes e intermedias, tanto para la conservación de las especies como para el conocimiento de su papel ecológico.

**Palabras clave:** acondicionamiento (priming), acondicionamiento natural, bosque tropical, enterramiento de semillas, especies tropicales, longevidad de las semillas, semillas intermedias y recalcitrantes

**ABSTRACT**

Short-lived seeds, called recalcitrant and intermediate, are difficult or practically impossible to maintain viable and vigorous for periods of time ≥ than two years, either by natural or artificial methods. This situation requires the development of simple and accessible strategies to solve the problem associated with their longevity under controlled conditions, and the understanding of the ecology of these species. Priming is a controlled hydration-dehydration treatment of seeds that leads to an advance in the biochemical processes towards germination, but without this occurring. This treatment increases germination vigor and sometimes improves the longevity of the seed. In natural priming, a variant of traditional priming treatment, seeds are buried *in situ* for a specific period. During this time, in the soil, the seeds are subject to fluctuations in humidity and temperature, among other factors, but without radicle protrusion occurring. The aim of this thesis was to investigate the effect of natural priming on the germination and longevity of intermediate and recalcitrant seeds of five species from evergreen and deciduous tropical forests: *Chamaedorea glaucifolia*, *Cymbopetalum baillonii*, *Damburneya coriacea*, *Magnolia perezfarrerae* and *Ternstroemia tepezapote*. Initially, the sensitivity of the seeds to dehydration was evaluated, as well as their storage behavior. To do this, the seeds were stored at room temperature or in controlled environment chambers at constant temperature. The moisture content of the seeds and their germination were evaluated at the beginning and after different storage periods. The germination parameters obtained were the final germination percentage, the maximum germination rate, the mean germination time and the lag time (time necessary for the start of germination) were the germination parameters. To evaluate the sensitivity to desiccation in the seeds, the critic water content ($WC_{50}$) and time to reach $WC_{50}$ ($TWC_{50}$) were calculated. In addition to these functional traits, the seeds were characterized morphologically and anatomically (volume, weight, relative amount of endosperm, embryo size), and the relationship between them with the ecological and climatic characteristic of their habitat (time of dispersal, total precipitation and mean maximum temperature in the month of seed collection) was determined. Subsequently, the effect of natural priming was evaluated. Seeds were buried *in situ* for a variable period according to the germination characteristics of each species. After unearthing, the treated seeds were stored along with their respective controls, at 15 ºC. Seed germination and water content were evaluated before and after a storage period. Also, an analysis of total proteins was carried out in primed seeds and their controls. *Chamaedorea glaucifolia* had intermediate storage behavior mainly due to its lower

sensitivity to desiccation ($WC_{50}$ of 4.6% and $TWC_{50}$ of 426 days) compared to the other studied species, which were recalcitrant ($WC_{50}$ of 11−60% and $TWC_{50}$ of 9−150 days). The relationship found between the traits of sensitivity to desiccation, germinative and morphological traits with the precipitation and maximum temperature in the month of seed collection and with the time of dispersal indicated an influence of the environment on longevity: large seeds germinate quickly and are dispersed in hot months (PC 1, 38% of the variance), while seeds with small embryos are more sensitive to desiccation and are dispersed in rainy months (PC 2, 34% of the variance). On the other hand, although there was an interaction between the effect of natural priming with the storage and/or with the year of collection, all species responded favorably to the treatment. Priming mainly influenced the speed of germination, that is, mean germination time and lag time decreased in the treated seeds compared to the control. This effect was observed even after storage in most species and, in *M. perezfarreare* in all years evaluated, there was higher viability than the control, i.e.., high final germination percentage. Protein profiles of natural priming seeds showed either new bands with respect to control, e.g., a band of 21 kDa in *Chamaedorea glaucifolia*, or absent bands in all species. These changes would be related to germination advances produced by the treatment. Natural priming produced an effect on germination similar to that manifested in long-lived seeds. Natural priming is a practical tool with great potential in short-lived seeded species, both for the conservation issues and for the knowledge of their ecological.

**Key words:** seed priming, natural priming, tropical forest, seed burial, tropical species, seed longevity, intermediate and recalcitrant seeds.

## INTRODUCCIÓN GENERAL

La longevidad de una semilla es una característica funcional que se refiere a su tiempo de vida desde que es dispersada de la planta madre hasta que muere o germina y también es denominada como persistencia o longevidad ecológica (Vázquez-Yanes y Orozco-Segovia, 1993; Long et al., 2015). La longevidad puede ser incrementada en condiciones artificiales, es decir, por medio del control de variables como la temperatura, la humedad y el oxígeno del ambiente de almacenamiento (Hong y Ellis, 1996) y se le conoce como longevidad potencial en condiciones óptimas o subóptimas. Comúnmente, las clasificaciones sobre longevidad de las semillas se refieren a la longevidad potencial. Por otro lado, un grupo de semillas que destaca de entre la diversidad mundial de plantas es el de las especies con semilla de longevidad corta tanto ecológica como potencial. En estas especies la estrategia reproductiva reside en una pronta germinación para formar un banco de plántulas, que disminuya la depredación en el banco de semillas (Vázquez-Yanes y Orozco-Segovia, 1993). Estas semillas presentan un elevado metabolismo desde que ocurre su dispersión, el cual sumado a los niveles altos de humedad en el ambiente contribuyen a su rápida germinación (Berjak y Pammenter, 2013). Esta es una de las razones por las que los bosques tropicales de México y el mundo albergan un elevado número de especies con este tipo de semillas (Tweddle et al., 2003; Hamilton et al., 2013). En este sentido, las problemáticas actuales en torno al desarrollo humano, como el cambio de uso de suelo, la actividad industrial, entre otras, así como los potenciales efectos a largo plazo del cambio climático global tienen gran importancia en estas especies vegetales (Warren et al., 2013; Pritchard et al., 2022). El cambio en el patrón de precipitación y el advenimiento de un incremento en las temperaturas promedio, entre otros factores, son los principales riesgos que comprometen la continuidad del gremio ecológico de especies con semillas de longevidad corta (O'Brien et al., 2013; Fernández et al., 2023). En todas las especies, ortodoxas o recalcitrantes, la necesidad de prolongar su viabilidad ha conducido, desde los orígenes de la humanidad, a recurrir a estrategias prácticas como lo es el almacenamiento de semillas. Sin embargo, el almacenamiento de semillas de longevidad corta conlleva dificultades dada sus características fisiológicas (Pritchard et al., 2022; Fernández et al., 2023). Entonces, ¿qué estrategia podría contribuir a la conservación de las especies vegetales con esta característica funcional? Para abordar esta pregunta, es necesario primero revisar los fundamentos fisiológicos de la longevidad de una semilla.

La longevidad de las semillas está determinada por varios factores, de entre los cuales el factor genético destaca en primer lugar. La semilla pasa por una serie de modificaciones genéticamente programadas en su estructura y función durante su desarrollo en la planta madre; este desarrollo está definido por tres etapas: histodiferenciación, acumulación de reservas y finalmente maduración y secado (Kermode y Finch-Savage, 2002; Bewley et al., 2013). Los cambios que inducen tolerancia a la desecación y que además influyen en la longevidad de la semilla ocurren durante la parte final de la segunda etapa y también en la fase de maduración y secado. Estos cambios consisten en la reducción casi total en el contenido de agua, aumento en la biomasa, aumento en la síntesis de ácido abscísico, síntesis de compuestos osmoprotectores tales como oligosacáridos, síntesis y renovación de sistemas antioxidantes, así como la expresión de proteínas protectoras de biomoléculas, e.g., dehidrinas y proteínas de choque térmico (Buitink et al., 2002; Bewley et al., 2013), entre los más importantes. De esta forma, el citoplasma celular de las semillas adquiere un estado físico (i.e., *glassy state*) en el que la viscosidad celular se incrementa. Con este cambio físico se reduce la velocidad de difusión de las moléculas y la velocidad de las reacciones químicas; además, se reduce la probabilidad de congelamiento i.e., la formación de cristales de hielo que dañen la estructura y función celular. La etapa final en el desarrollo de la semilla (en inglés, *after-ripening*) continúa incluso después de que la misma se ha separado de la planta madre al ocurrir la dispersión, aunque esto es menos común en especies con semillas de vida corta. Las semillas que completan la etapa de maduración y secado son conocidas como de vida larga, las cuales son capaces de permanecer viables quiescentes o latentes, hasta el momento en el que se presentan las condiciones ambientales aptas para la germinación i.e., temperatura, luz y humedad. Esta característica funcional de las semillas de vida larga permite la formación de un banco temporal o persistente de semillas en el suelo (Vázquez-Yanes y Orozco-Segovia, 1993; Long et al., 2015). Además del factor genético, otros factores que intervienen en la longevidad de la semilla durante su desarrollo son la presencia de latencia en las mismas, el microclima del sitio, el tipo de suelo, la radiación solar, la competencia, la depredación, entre otras (Vázquez-Yanes y Orozco-Segovia, 1993; Pritchard et al., 2022). Estos factores determinan el grado de estrés asociado con la muerte y supervivencia de las semillas durante su permanencia en el suelo (Fig. 1), por lo que también son importantes en la determinación de la longevidad ecológica de las semillas en circunstancias específicas. Tanto la eliminación o manipulación de algunos de estos factores durante su almacenamiento *ex situ*, en condiciones controladas o semicontroladas, como una

condición morfo-funcional sana de las semillas al momento de la dispersión, permite a las semillas de vida larga extender y alcanzar su mayor longevidad potencial (Vázquez-Yanes y Orozco-Segovia, 1993).

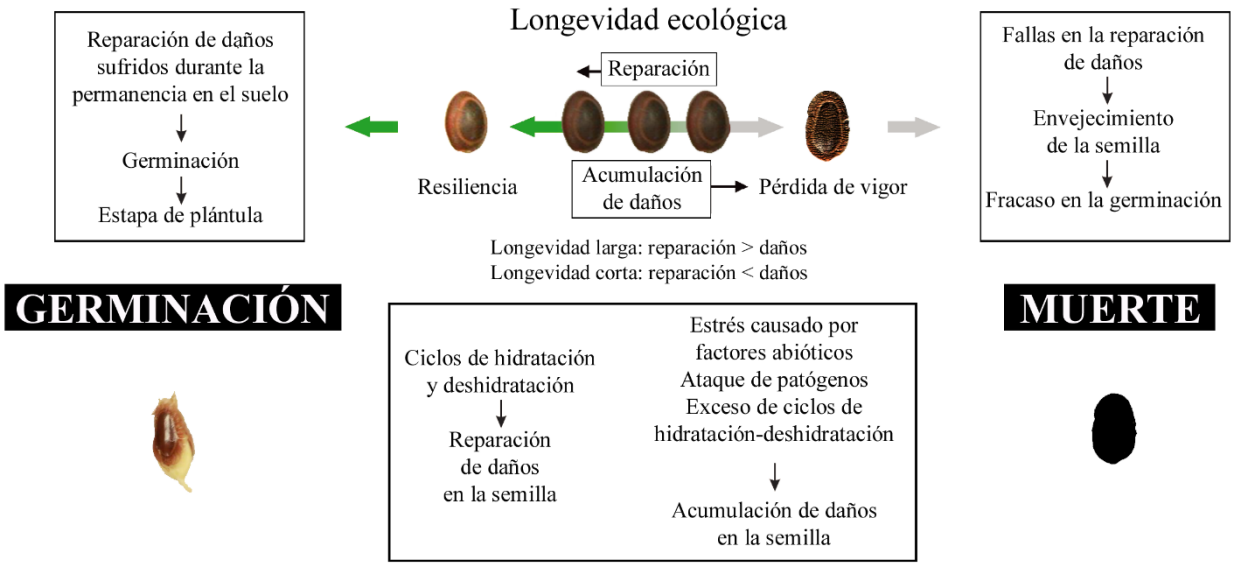


**Figura 1.** Longevidad ecológica, o persistencia, de la semilla mientras permanece en el suelo del bosque después de su dispersión. Modificada de Kranner et al. (2010) y Long et al. (2015). Imágenes de la semilla cortesía de Humberto Peraza-Villarreal.

Las semillas que han pasado por el programa de desarrollo completo adquieren tolerancia a la desecación y una longevidad potencial más extensa; y con base en su comportamiento en almacén se les denomina ortodoxas (Fig. 2a). Las semillas ortodoxas pueden almacenarse rudimentariamente, pero para optimizar su viabilidad a largo plazo (> 10 años), su contenido de humedad (%) debe reducirse hasta donde sea posible previo al almacenamiento. Posteriormente, las semillas se colocan en almacenamiento convencional, el cual requiere de una temperatura de −20 °C, un nivel de humedad ambiental reducido y buena aireación (Hong y Ellis, 1996). Para estas especies, las cuales representan alrededor del 75−92 % del total de espermatofitas en el mundo (Walters et al., 2013; Wyse y Dickie, 2017), existe un panorama alentador en torno a su conservación *ex situ*. En la actualidad existen numerosos bancos de germoplasma en el mundo que

albergan un número importante de especies de este tipo, tanto de importancia agrícola como científica (Breman et al., 2021; Trusiak et al., 2023).

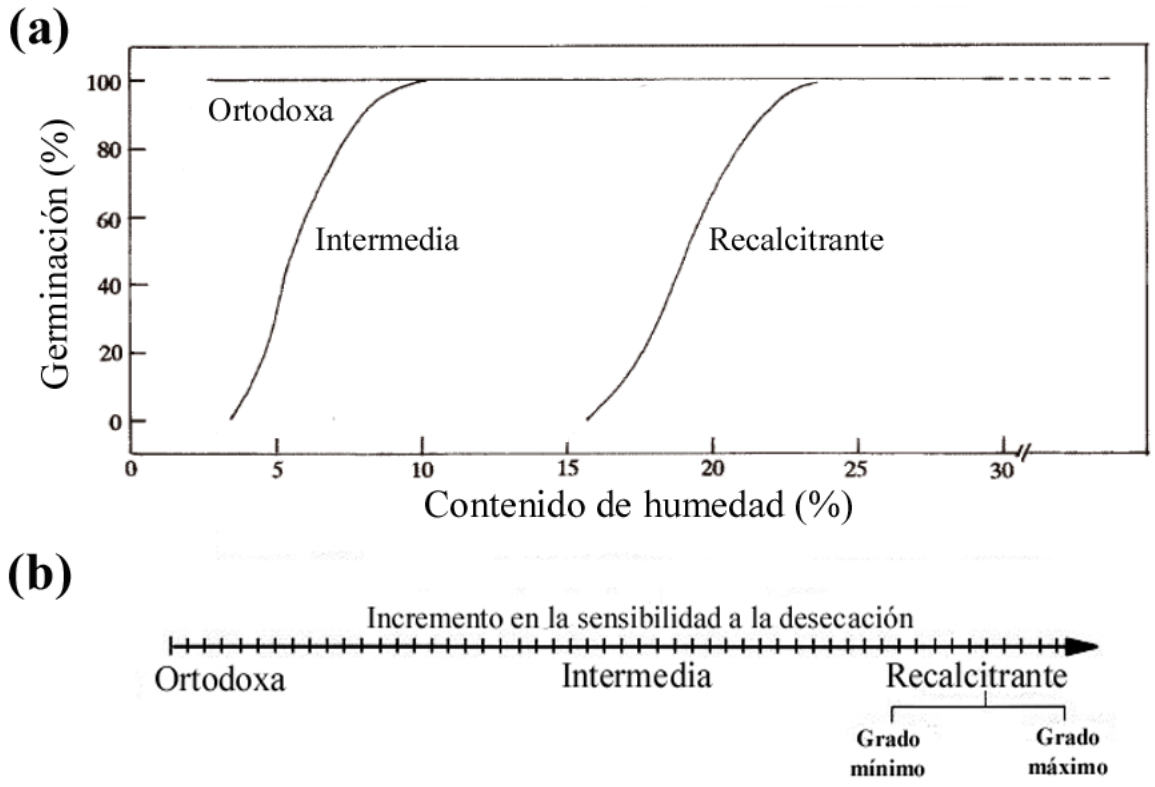


**Figura 2.** Categorías en el almacenamiento de las semillas en referencia a su longevidad potencial. En (a) se indica la relación entre el contenido de humedad de la semilla y su viabilidad (expresada con el porcentaje final de germinación). Mientras que en (b) se establece la existencia de un continuo en la respuesta a la desecación entre las diferentes categorías de almacenamiento de las semillas. Modificada de Hong y Ellis (1996) y Berjak y Pammenter (2000).

Ahora bien, ¿qué sucede con las semillas de longevidad corta? En estas semillas tanto la longevidad ecológica como potencial no son equiparables a la de las ortodoxas. La razón obedece a que en ellas no ocurren las modificaciones relacionadas con la tolerancia a la desecación y la longevidad, ya que son dispersadas justo en la parte final de la etapa dos del programa de desarrollo de la semilla (Kermode y Finch-Savage, 2002; Pritchard et al., 2022). Dada esta circunstancia en

estas semillas, las estrategias de almacenamiento convencional para las semillas ortodoxas resultan inadecuadas, por lo que se procede a clasificarlas en las categorías intermedia y recalcitrante (Fig. 2a; Hong y Ellis, 1996; Pence et al., 2022). Las semillas recalcitrantes son las menos tolerantes a la desecación debido a su alto contenido de humedad y a su elevada tasa metabólica y de respiración, razones por las que se recomienda su almacenamiento en condiciones ambientales que mantengan sus contenidos de humedad iniciales (de 10−170%, Farrant et al., 1985; Bonner, 2008) y que disminuyan su tasa metabólica hacia la germinación (Hong y Ellis, 1996; Berjak y Pammenter, 2013). Esto último se consigue si se reduce la temperatura, pero solo entre 10−15 °C, pues temperaturas más bajas incrementan el riesgo de daño celular por congelación (Berjak y Pammenter, 2013). En cambio, las semillas intermedias toleran una mayor disminución en su contenido de humedad base seca, siendo capaces de una disminución por debajo del 10%, pero no toleran niveles de deshidratación iguales a los de las ortodoxas. Adicionalmente, estas semillas resisten temperaturas cercanas al punto de congelación, pero responden negativamente a temperaturas por debajo de 0 °C, a diferencia de las ortodoxas (Hong y Ellis, 1996). Dado los múltiples factores que influyen en la tolerancia a la desecación, existe variabilidad dentro de las categorías intermedia y recalcitrante. Este hecho plantea que tanto la tolerancia a la desecación como la longevidad potencial de una semilla abarcan una escala continua cuyos extremos son el comportamiento ortodoxo y el recalcitrante (Berjak y Pammenter, 2000), mientras que el comportamiento intermedio se encuentra entre ambos (Fig. 2b; Vertucci y Farrant, 1995; Pence et al., 2022). No obstante, actualmente se mantiene el uso y utilidad de las tres categorías de almacenamiento (Pence et al., 2022; Trusiak et al., 2023). Esta tesis se centra en especies con semillas de tipo intermedio y recalcitrante, especies cuya conservación está comprometida precisamente por sus características funcionales, lo cual demanda en primera instancia determinar su comportamiento en almacén.

El almacenamiento de semillas surge como la opción práctica y económica más inmediata para la conservación *ex situ*. Sin embargo, como hemos señalado, el almacenamiento convencional de semillas de longevidad corta es inoperante para las de tipo recalcitrante. Si bien la criopreservación es un método en desarrollo con el potencial de ser una solución a largo plazo para esta categoría de semillas (Berjak y Pammenter, 2013; Pence et al., 2022), en la actualidad el almacenamiento convencional con las adecuaciones necesarias para semillas ortodoxas e intermedias continúa siendo la primera opción. La evaluación de las condiciones de temperatura ambiental, del contenido

de humedad de las semillas, etc., constituye la primera parte del protocolo al que se recurre para determinar la sensibilidad a la desecación y los requerimientos óptimos de almacenamiento (Berjak y Pammenter, 2013; Sommerville et al., 2018). Este protocolo es relevante en especies de las que no se cuenta con información previa (Rodríguez et al., 2000; Becerra-Vázquez et al., 2018). La determinación de la sensibilidad a la desecación es útil en los estudios de mejoramiento y manejo de diversas especies y cultivares de cacao (*Theobroma cacao*), limón (*Citrus limon*) y plátano (*Musa* spp.) (Haryati, 2019; Marques et al., 2019, Kallow et al., 2022).

Es posible evaluar la sensibilidad a la desecación de las semillas a través del monitoreo de los cambios en viabilidad y vigor a medida que transcurre el tiempo. Naturalmente, las semillas se deshidratan paulatinamente aun cuando permanezcan en condiciones óptimas durante el almacenamiento. Por lo tanto, un método práctico es la evaluación periódica y simultánea de la germinabilidad de las semillas y de su contenido de humedad durante el almacenamiento en condiciones ambientales controladas (Pritchard et al., 2004; van Treuren et al., 2013; Hay et al., 2023). De esta forma, es posible obtener un valor con el cual se estima cuantitativamente la tolerancia a la desecación, que también permite realizar comparaciones del efecto de las condiciones ambientales en ésta, al interior de las especies (diferentes temperaturas de almacenamiento, diferentes años de recolecta) y entre especies (King y Roberts, 1979). Una medida de la sensibilidad a la desecación lo constituye el contenido crítico de humedad ($WC_{50}$), el cual se define como el contenido de humedad al cual la viabilidad inicial de un lote de semillas decrece al 50% (King y Roberts, 1979; Hill et al., 2012). En la determinación del $WC_{50}$ deben tomarse en cuenta las variables que influyen en la respuesta a la desecación de una semilla, como son la procedencia y la madurez de la semilla, así como la temperatura de almacenamiento (Dussert et al., 2000; Oyerinde et al., 2023). Una vez determinado el $WC_{50}$, este valor puede correlacionarse con otras características morfológicas, anatómicas y funcionales de las semillas, e incluso con las características ambientales del hábitat de la especie en cuestión (Dussert et al., 2000; Hill et al., 2012). Esto permitiría obtener resultados que se relacionen con la historia natural de las especies. A la par, surge la necesidad de encontrar métodos que potencialmente mejoren la longevidad de semillas intermedias y recalcitrantes.

La gran diversidad de especies con semilla de comportamiento ortodoxo ha permitido estudiar en ellas el efecto de diversos tratamientos pregerminativos. Un método exitoso es el

acondicionamiento de semillas, endurecimiento o *seed priming*, en inglés. Este tratamiento tiene como objetivo activar los procesos fisiológicos y bioquímicos pregerminativos en las semillas sin que la radícula protruya (Rajjou et al., 2012; Ibrahim, 2019; Pagano et al., 2023). Esto se consigue por medio de una hidratación controlada, que permite que la semilla se embeba hasta alcanzar la fase II del proceso germinativo. Una vez transcurrido cierto tiempo en esta fase, se interrumpe la hidratación, evitando con ello la protrusión de la radícula en la fase III de imbibición (Fig. 3a). Posteriormente, la semilla se deshidrata para regresarla a su contenido de humedad inicial (Fig. 3a). Una vez hidratada y deshidratada la semilla, los procesos bioquímicos hacia la germinación, que comprenden la reparación de estructuras celulares y moleculares, restitución del sistema antioxidante, degradación y movilización de sustancias de reserva y síntesis de proteínas, RNA y DNA, permanecen en la semilla (Chen y Arora, 2013). Cuando la semilla se coloca de nuevo en condiciones óptimas para la germinación (humedad, luz y temperatura adecuada), ésta ocurre con mayor rapidez (Fig. 3, a y c). Incluso es posible que las plántulas provenientes de semillas acondicionadas incrementen su vigor en crecimiento y supervivencia (Sánchez et al., 2001; Peraza-Villarreal et al., 2018), pues el tratamiento induce la expresión de compuestos que confieren resistencia a altas temperaturas o al estrés hídrico (Chen y Arora, 2013). Gracias a sus efectos el acondicionamiento ha sido utilizado principalmente para el mejoramiento de la propagación y establecimiento de especies agrícolas de importancia económica (Sánchez et al., 2001). Adicionalmente, pese a su variabilidad, otro efecto del acondicionamiento es que las semillas mantengan por mayor tiempo su vigor durante el almacenamiento (Long et al., 2006; Butler et al., 2009). Todas estas ventajas han hecho que el acondicionamiento se emplee con los mismos objetivos en la conservación de especies silvestres.

De entre los diferentes tipos de acondicionamiento, el natural es un tipo de acondicionamiento mátrico que se lleva a cabo *in situ* en el ambiente propio de la especie. Este tratamiento ofrece una ventaja adicional a las semillas: mejora su vigor por medio de un método accesible y de bajo costo (Orozco-Segovia et al., 2014). El método consiste en enterrar a las semillas en el suelo de su hábitat natural durante un período similar al que transcurre desde su dispersión hasta su germinación (Fig. 3b; González-Zertuche et al., 2001). Durante esta etapa la semilla queda sujeta a las oscilaciones de humedad y temperatura que ocurren naturalmente en el suelo, las cuales producen ciclos de hidratación-deshidratación en la misma (Fig. 3b). Posteriormente, las semillas son exhumadas y deshidratadas a su contenido de humedad original. A nivel funcional el tratamiento vigoriza la

semilla incrementando la rapidez de la germinación (Fig. 3c) y, en ocasiones, el porcentaje de germinación (Orozco-Segovia et al., 2014).

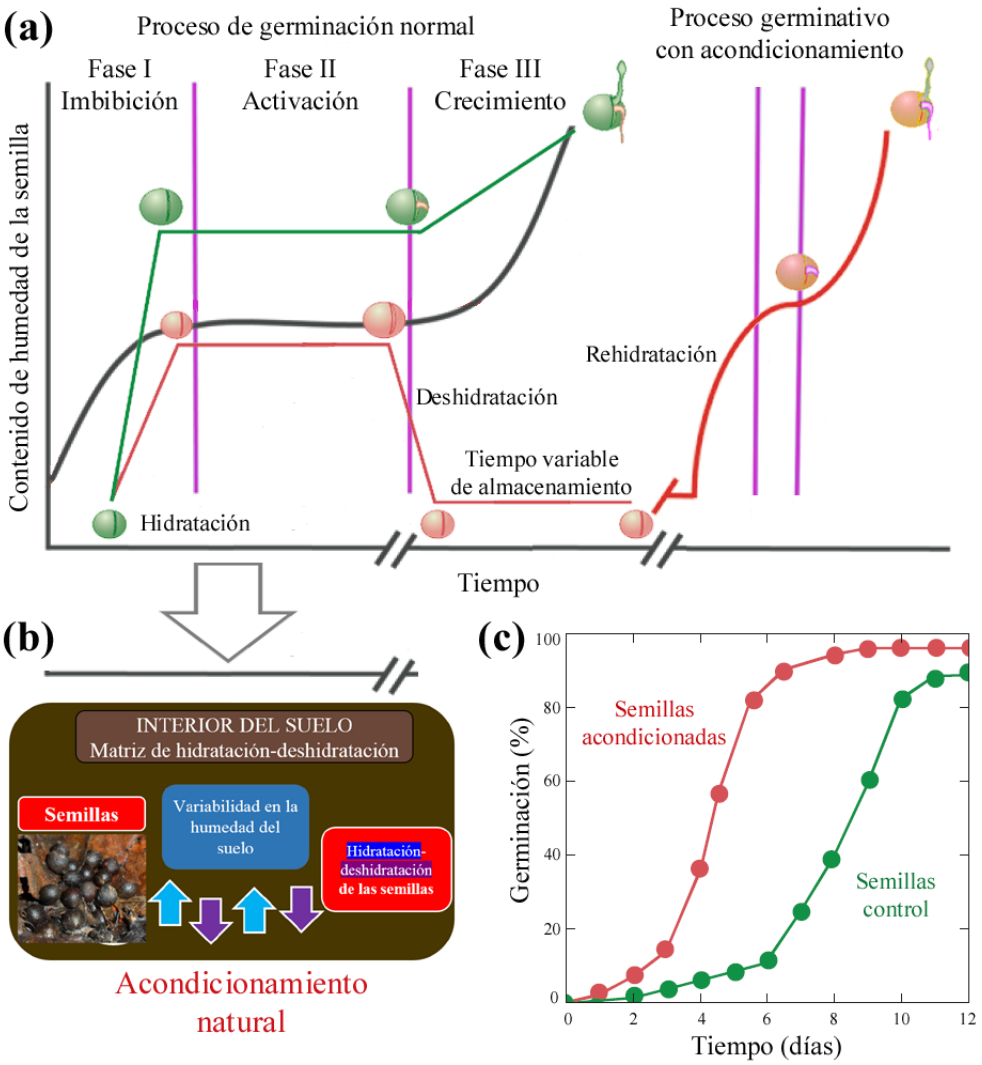


**Figura 3.** Tratamiento pregerminativo de acondicionamiento de semillas. (a) El fundamento del tratamiento en una semilla (curva en rojo) se ejemplifica considerándose como referente el proceso de germinación trifásico de hidratación (curva en negro) de una semilla sin tratamiento (control, curva verde). El acondicionamiento utiliza diversos medios para hidratar-deshidratar controladamente a la semilla; en el caso del acondicionamiento natural (b) la matriz de hidratación-deshidratación es el interior del suelo. En esta matriz las semillas permanecen durante un período similar al que transcurre desde su dispersión hasta su germinación. (c) Las semillas acondicionadas incrementan su rapidez de la germinación en la mayoría de los casos. Modificada de Rajjou et al. (2012) e Ibrahim (2019).

Adicionalmente, la vigorización de las semillas acondicionadas con este método mejora el desempeño de las plántulas en campo en comparación con semillas no tratadas, tanto en especies ortodoxas (Peraza-Villarreal et al., 2018), como recalcitrantes (Becerra-Vázquez et al., 2020). No obstante, el acondicionamiento natural se ha explorado poco en especies con semillas sensibles a la desecación. Al respecto, dos especies nativas de selva tropical con semillas recalcitrantes, *Cupania glabra* y *Cymbopetalum baillonii*, respondieron favorablemente al tratamiento (Becerra-Vázquez et al., 2020). Si bien las semillas de estas especies germinan rápido, una característica funcional común en las recalcitrantes, el acondicionamiento produjo una germinación aún más rápida y sincrónica. Este efecto del tratamiento sin duda repercutiría en las actividades de conservación y aprovechamiento de especies con semillas recalcitrantes e intermedias. Por ejemplo, dentro de las prácticas de reforestación es deseable contar con el mayor número de plántulas y con tallas adecuadas en el menor tiempo posible (Elliott y Kuaraksa, 2008; Martínez-Garza et al., 2013). Además, debe plantearse el efecto positivo del acondicionamiento natural en la longevidad de la semilla, como lo sugiere la presencia de compuestos relacionados con la resistencia al estrés hídrico presentes en semillas exhumadas (González-Zertuche et al., 2001; Long et al., 2011; Benítez-Rodríguez et al., 2014). Todos estos efectos del acondicionamiento natural en la fisiología de la semilla también abren la interrogante sobre si el tratamiento emula el endurecimiento que ocurre naturalmente durante el tiempo en el que las semillas permanecen en el suelo después de su dispersión (Long et al., 2015). Es decir, que el tratamiento influya en la historia de vida de una planta, particularmente en su etapa inicial.

En este trabajo se presentan los resultados de una investigación sobre el efecto del acondicionamiento natural en semillas de cinco especies nativas de bosque tropical perennifolio y caducifolio del sureste mexicano: *Chamaedorea glaucifolia* H.Wendl. (Arecaceae), *Cymbopetalum baillonii* R.E.Fr. (Annonaceae), *Damburneya coriacea* (Sw.) Trofimov & Rohwer (Lauraceae, previamente *Nectandra coriacea*, Trofimov et al., 2016), *Magnolia perezfarrerae* Vázquez & Gómez (Magnoliaceae, previamente *M. mexicana*, Vázquez-García et al., 2013) y *Ternstroemia tepezapote* Schltdl. & Cham. (Pentaphylacaceae). Las especies seleccionadas cuentan con características morfológicas y funcionales que indican a priori una probable sensibilidad a la desecación y longevidad corta en sus semillas. Además, todas ellas cuentan con atributos de valor de uso, lo que posibilita tanto su utilización en actividades de restauración como su aprovechamiento sostenible (Dawson et al., 2014; Waiboonya y Elliot, 2020). Cabe resaltar que en

este tipo de especies se tiene una mayor urgencia de definir estrategias para su conservación. Si bien hoy en día existen trabajos orientados a determinar las características funcionales de las semillas sensibles a la desecación en México (Sánchez-Coronado et al., 2007; Garcias-Morales et al., 2021), aun se necesitan mayor número de estudios.

En el primer capítulo de esta tesis se presenta un estudio sobre las características de longevidad potencial en las semillas de las especies seleccionadas. Considerando las evaluaciones preliminares en todas ellas, estas especies presentan semillas de gran tamaño (0.1−0.5 g peso seco), germinación rápida y alto contenido de humedad (> 20% base seca). Adicionalmente, se consideraron características morfoanatómicas como el volumen, el peso, la cantidad relativa de endospermo y el tamaño del embrión, así como con datos referentes al clima en donde habitan. Dadas estas características, cabe esperar que una disminución en el contenido de humedad de las semillas afecte la respuesta germinativa en comparación con el control. Por consiguiente, el objetivo general fue examinar en todas las especies el efecto de la deshidratación en las semillas e identificar el comportamiento en almacén. Los objetivos particulares de este capítulo fueron: 1) evaluar el efecto tanto del tiempo como de la temperatura durante el almacenamiento en las características germinativas de las semillas de las especies en estudio, 2) evaluar si existen diferencias en la sensibilidad a la desecación en años de recolecta diferentes y 3) determinar si existe una relación entre la sensibilidad a la desecación y longevidad de la semilla con sus características morfológicas, funcionales y ecológicas, así como con características ambientales de su hábitat.

Dado que el acondicionamiento supone la activación de los procesos fisiológicos relacionados con la germinación, cabe esperar que en las semillas tratadas existan diferencias en la respuesta germinativa, en la viabilidad y vigor después del almacenamiento, así como en la expresión de proteínas respecto al control. Con base en la respuesta positiva de *Cymbopetalum baillonii* al acondicionamiento natural (Becerra-Vázquez et al., 2020), en el segundo capítulo de esta tesis se investiga la respuesta de semillas recalcitrantes e intermedias al acondicionamiento natural. Al respecto, se planteó como objetivo general evaluar el efecto del acondicionamiento natural en un mayor número de especies, considerando, al menos en algunas especies, el efecto del año de producción de las semillas en sus características funcionales. Los objetivos particulares fueron: 1) evaluar el efecto del acondicionamiento en los parámetros germinativos 2) determinar si el acondicionamiento natural influye en la longevidad de las semillas y 3) determinar si el

acondicionamiento natural produce diferencias en el perfil de expresión de proteínas de las semillas.

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**CAPÍTULO 1. Seed longevity of five tropical species from south-eastern Mexico: changes in seed germination during storage**

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*Research Article*

# Seed Longevity of Five Tropical Species From South-Eastern Mexico: Changes in Seed Germination During Storage

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**Abstract**

To design conservation strategies, the extent of plant richness of tropical forests needs to be characterized in terms of their seed longevity. In this study, we examined the potential seed longevity, that is, storage ex situ, of species from south-eastern Mexico: *Chamaedorea glaucifolia*, *Cymbopetalum baillonii*, *Magnolia mexicana*, *Nectandra coriacea*, and *Ternstroemia tepezapote*. Immediately after collection, seeds were stored at different temperatures (≤23°C). We evaluated seed germination after different storage durations. Seed water content (WC) was determined for each period. Seed desiccation sensitivity was determined as $WC_{50}$, which is the WC at which the initial seed viability decreases to 50%; further, the time required to reach $WC_{50}$ was also determined. Subsequently, we analyzed the relations between seed functional traits with other morphological and functional traits, along with the weather characteristics of their respective habitat. All of the studied species had short-lived seeds; they exhibited desiccation sensitivity after storage with differences across the species. Additionally, *C. baillonii* exhibited differences in seed desiccation sensitivity across 2 years of seed collection. Interaction was observed between storage time and storage temperature: Seeds exhibited less deterioration at 15°C in *C. glaucifolia* and *C. baillonii* and at 5°C in *M. mexicana* and *N. coriacea*. Seed storage behavior is discussed in this article. Finally, a relationship determined between germination traits, and seed WC, embryo size, endosperm amount, and rain and temperature patterns in the month of seed dispersal explained the limited longevity of the studied species.

**Keywords**

native plants, seed longevity, storage, seed desiccation sensitivity, tropical forest

## Introduction

Plants with short-lived desiccation-sensitive seeds form an important biological group in tropical forests. They account for ~15% to 19% of global plant species richness (Wyse & Dickie, 2017). However, in tropical environments, they constitute ~50% (Tweddle, Dickie, Baskin, & Baskin, 2003). Generally, these seeds are large and have a low seed coat ratio (ratio of endocarp and seed coat mass to dispersal unit mass) and high water content (WC) at dispersal (Vázquez-Yanes & Orozco-Segovia, 1993; Hamilton, Offord, Cuneo, & Deseo, 2013). Further, they maintain a high metabolic rate even after their dispersal from the mother plant (Berjak & Pammenter, 2008), so they generally germinate at a fast rate. These reasons explain their short potential longevity (lifespan under optimal environment storage conditions;

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Vázquez-Yanes & Orozco-Segovia, 1993). Therefore, they are also classified as recalcitrant seeds, or they may be classified as intermediate seeds if they can survive considerable dehydration, but fail to survive conventional subfreezing storage temperature (Hong & Ellis, 1996). Storage difficulties coupled with potential environmental modifications due to climatic change and land use turnover make these species the central concern in terms of the conservation and restoration of tropical forests (O'Brien, Philipson, Tay, & Hector, 2013). Thus, it is necessary to evaluate the potential longevity and degree of seed desiccation sensitivity in tropical species as a first step toward defining their optimal ex situ management and susceptibility to habitat change.

Once short-lived desiccation-sensitive seeds are dispersed, their initial WC begins to decrease. At a certain point, the water deficit of the seeds triggers physiological, structural, and molecular damages at the cell level (Farrant, Berjak, & Pammenter, 1985; Obroucheva, Sinkevich, & Lityagina, 2016). These damages are reflected by a loss of vigor, which is a property that is expressed by the rate and the uniformity of seed germination (Rajjou et al., 2012). In this manner, the desiccation sensitivity of the seed can be identified with the determination of its "critical water content" or $WC_{50}$ (King & Roberts, 1979) as well as the time elapsed to reach this value. The $WC_{50}$ is the WC at which the initial seed viability decreases to 50% (Hill, Edwards, & Franks, 2012). Recalcitrant seeds have a $WC_{50}$ in the range 25% to 20% and intermediate seeds ranged between 10% and 5% (Hong & Ellis, 1996). In comparison, orthodox seeds can reach a WC < 5% without a reduction of their initial viability (Hong & Ellis, 1996). Conversely, variability in $WC_{50}$ is related to the morphological and physiological traits of seeds (Hill et al., 2012) and with the weather characteristics of the habitat (Dussert et al., 2000; Rodríguez, Orozco-Segovia, Sánchez-Coronado, & Vázquez-Yanes, 2000). Moreover, $WC_{50}$ can vary according to the dehydration rate of seeds. A slow dehydration rate allows seeds to remain in a range of WC that allows germination metabolism to occur, but this causes an accumulation of metabolic and structural damage that increases desiccation sensitivity. Therefore, $WC_{50}$ will be high during a fast dehydration rate (Farrant et al., 1985; Berjak & Pammenter, 2008). During storage, seed dehydration rate is slow compared with the fast dehydration techniques (e.g., using silica gel or an air fan). However, slow drying can provide us information regarding what occurs in the habitat and where slow drying conditions are present (Vázquez-Yanes & Orozco-Segovia, 1994).

The dehydration rate can be modified by external factors, such as relative humidity and temperature (Berjak & Pammenter, 2008). Reduction of the temperature slows down the metabolic activity, prevents germination, and reduces rate of water loss and cell damage of seeds (McDonald, 2004). Nevertheless, the high WC and metabolic activity of short-lived seeds render them sensitive to chilling damage even at temperatures above 0°C, that is, arrest of the enzymatic reactions and structural damage of cell membranes (Tommasi, Paciolla, Concetta de Pinto, & De Gara, 2006). Further, if the temperature drops to 0°C, freezing damage can occur (Hong & Ellis, 1996). Thus, the recommended temperature for storage of tropical recalcitrant seeds is between the optimum for germination and the temperature at which no chilling damage occurs (≥10°C; Hong & Ellis, 1996). Additionally, these seeds can exhibit interannual variability in chilling sensitiveness (Berjak & Pammenter, 2008).

Mexico has tremendous plant diversity, but knowledge regarding the seed biology of most of the wild tropical species is extremely limited (Vázquez-Yanes, Batis-Muñoz, Alcocer-Silva, Gual-Díaz, & Sánchez-Dirzo, 2001). Determination of seed longevity and the desiccation sensitivity of wild species can increase the probability of tropical forest conservation (Hamilton et al., 2013; Wyse & Dickie, 2017) and being able to determine the effects of future climatic changes on the habitat of various species (O'Brien et al., 2013). In our research, we evaluated the seed longevity of five species from tropical forest in south-eastern Mexico. This research concentrates on the following questions: (a) What are the effects of storage time on seed germination? (b) What are the effects of storage temperature on seed longevity? (c) Does the desiccation sensitivity of seeds vary between years of collection? (d) What was the storage behavior of the studied species? (e) How are seed traits related to seed longevity, that is, $WC_{50}$ and the time required to reach $WC_{50}$ and other functional and morphological traits of seeds, in relation to the environmental factors of the habitat of the studied species?

## Material and Methods

### *Seed Collection and Study Site*

Fruit collection was done during the dispersal seasons of 2015 and 2016, in two localities with tropical forest, in south-eastern Mexico. One of them was the tropical rain forest at the UNAM Tropical Biology Station, localized in San Andrés Tuxtla (18°34′5″ N, 95°04′26″ W; 155 m asl). The second locality was the transition region between the tropical rain forest and the dry forest in Ocozocoautla, Chiapas, within the confluence area of the Central Depression of Chiapas and the North Mountains situated in this state (16°51′18″ N, 93°23′47″ W, 904 m asl). This site constitutes a part of the buffer area of the El Ocote Biosphere Reserve. The annual mean precipitation is 4,725 mm for Los Tuxtlas and 1,100 mm for El Ocote. The annual mean temperature is 24°C for

Los Tuxtlas and 23.4°C for El Ocote (Instituto Nacional de Estadística, Geografía e Informática, 2003; Soto & Gama, 1997; Gutiérrez-García & Ricker, 2011).

### Study Species

The goals of this research were addressed with five subcanopy species: *Chamaedorea glaucifolia* H.Wendl. (Arecaceae), *Cymbopetalum baillonii* R.E.Fr. (Annonaceae), *Magnolia mexicana* DC. (Magnoliaceae), *Nectandra coriacea* (Sw.) Griseb (Lauraceae), and *Ternstroemia tepezapote* Cham. & Schltdl. (Pentaphylacaceae). The taxonomic status of these species is in accordance with The Plant List (2013). These species are shade tolerant and inhabit mature forests (Standley & Steyermark, 1946, 1949; Coates & Estrada, 1988; Becerra-Vázquez, Ramírez-Marcial, & Holz, 2011). Additionally, these species have local utility for the human settlements and potential economic value (Escobar-Ocampo & Ochoa-Gaona, 2007). All these species, except the understory palm *C. glaucifolia* that has a seed covered by the fruit pericarp (Corner, 1976) as its dispersal unit (henceforth, we will consider it as a seed), are trees. We collected seeds of *C. baillonii* in Los Tuxtlas, Veracruz, and seeds of *C. glaucifolia*, *M. mexicana*, *N. coriacea*, and *T. tepezapote* in El Ocote. Seeds characteristics and other biological and ecological traits of the studied species are presented in Figure 1 and Table 1.

### Processing of Fruits and Seeds

We collected fruits of the studied species during the dispersal season of 2016 except for *C. glaucifolia* which seeds were collected in 2015. Additionally, in 2016, we collected seeds of *C. baillonii* and *M. mexicana* during the dispersal season of 2015. The dates of collection are shown in Table 1. We collected fruits directly from at least 10 mature trees. Immediately after collection, fruits were deposited in either plastic containers or black plastic bags and covered with a soil layer taken from the study area to avoid seed dehydration. After 2 days, fruits were taken from the recipients in a dark room ($22 \pm 0.9$°C, $50 \pm 3.2$% RH). Seeds inside fleshy fruits were cleaned in the laboratory. Because fruits of *M. mexicana* are dry and woody, we placed them on a table in a dark room until fruit dehiscence occurred. Seeds with an aryl were cleaned by gentle rubbing on a fine steel mesh.

### Morphological Traits of Seeds

We measured the length (L), width (W), and thickness (T) of recently collected seeds (RC-seeds, $n = 30$) with an electronic vernier caliper (accuracy = 0.01 mm). Subsequently, seed volume (V) was calculated with the formula for obtaining the volume of an ellipsoid (Cerdà & García-Fayos, 2002) as follows: $V = 1.333 \times \pi \times (L/2) \times (W/2) \times (T/2)$.

We measured the fresh weight of individual seeds ($FW_{t1}$) with an electronic analytical balance (model A-200DS, precision 0.001 g, Fisher Scientific, Fairlawn, NJ). Subsequently, individual seeds were dried in an oven (model 107801, Boekel Industries, Inc. Philadelphia, PA) at 80°C for 48 hr to avoid seed combustion, due to the low ignition point of seed lipids. Seeds were weighed again ($DW_{t1}$), and the initial water content ($WC_{t1}$ of RC-seeds) was calculated. The seed WC was determined

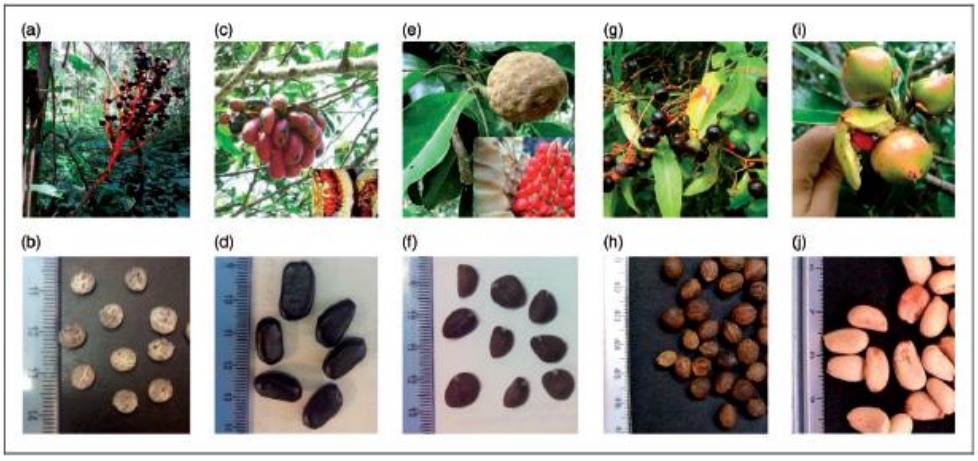


**Figure 1.** Fruits (upper row) and seeds (bottom row) of the studied species. (a and b) *Chamaedorea glaucifolia*, (c) and d) *Cymbopetalum baillonii*, (e and f) *Magnolia mexicana*, (g and h) *Nectandra coriacea*, and (i and j) *Ternstroemia tepezapote*. Image credit: A. G. Becerra-Vázquez.

**Table 1.** The Ecological Traits of the Studied Species, Environmental Traits of Their Habitat, and Seed Morphological Traits and Dates of Seed Collection and Season of Seed Dispersal.

| Species | Seed collection date | Season of seed dispersal[a] | Precipitation[b] (mm) | Max. Temp.[b] (°C) | Embryo size[c] | Endosperm amount |
|---|---|---|---|---|---|---|
| Cg | October 2015 | Late rainy season*** | 206.8 | 26.9 | Small[4] | High[4] |
| Cb | April 2016 | Late dry season** | 20.8 | 28.8 | Small[1,2] | High[1,2] |
| Mm | March 2016 | Mid dry season* | 0 | 27.1 | Small[1,3] | High[1,3] |
| Nc | September 2016 | Late rainy season* | 104.4 | 27.8 | Large[1,2] | Absent[1,2] |
| Tt | January 2016 | Early dry season* | 10.7 | 25.7 | Large[1,2] | Low[1,2] |

*Note.* Cg = *Chamaedorea glaucifolia*; Cb = *Gymbopetalum baillonii*; Mm = *Magnolia mexicana*; Nc = *Nectandra coriacea*; Tt = *Ternstroemia tepezapote*; Precipitation = total precipitation in the month of seed collection; Max. Temp. = maximum mean temperature in the month of seed collection; L, W, T = seed length, width, and thickness.

[a]Annual rain pattern in the collection sites (Instituto Nacional de Estadística, Geografía e Informática, 2003; Gutiérrez & Ricker, 2011). Dispersal dates from Becerra-Vázquez et al. (2011)*, Rodríguez et al. (2000)** and personal observations (A. G. Becerra Vázquez)*** in the study site.

[b]Total precipitation and mean of maximum temperature for the date of seed collections from Station 7319 and Torre CONAGUA-SMN – CONANP – IBUNAM (Comisión Nacional del Agua, 2017).

[c]Embryo and endosperm proportions are relative to seed size ([1]Corner, 1976; [2]Niembro-Rocas, 1989; [3]Alcántara-Flores, 2002; [4]Baskin & Baskin, 2014) and corroborated by personal observations (A. G. Becerra-Vázquez).

on dry basis ($WC_{db}$) as follows: $WC_{t1} = ((FW_{t1} - DW_{t1})/DW_{t1}) \times 100$ (Equation 1).

### Seed Storage

Seeds were stored in closed glass jars with three replicates for each species. Germination of stored seeds (St-seeds) was evaluated after different storage times depending on the species (storage times, t2 . . . tn). For *C. baillonii* and *M. mexicana*, we also evaluated the effect of the year of seed collection: 2015 (2015-S) and 2016 (2016-S) fruiting seasons. The jars were stored in laboratory conditions (23 ± 0.5°C, 50 ± 2.2% RH). To evaluate the effect of storage temperature, we maintained additional jars in growth chambers with controlled temperature (40 ± 5% RH, Labline Instruments Inc., Melrose Park, IL) at 15°C and at 5°C. In addition, *C. glaucifolia* and *C. baillonii* 2015-S were stored at 10°C. Seeds of *C. glaucifolia* were stored for 30, 101, and 426 days, seeds of *C. baillonii* for 30, 180, and 360 days (also 730 days for 2015 seeds), seeds of *M. mexicana* were stored for 25 and 210 days (and for 90 days only for 2015-S), seeds of *N coriacea* for 35, 90, and 180 days, and seeds of *T. tepezapote* for 16, 41, 251, and 365 days. All species differed in t2 . . . tn, according to the seed collection dates and the number of collected seeds.

Prior to storage, seeds were treated with fungicide (Interguzan 30-30, pentachlorinenitrebenzene, and terametiltiuram disulphide). Subsequently, the jars were closed and sealed with a plastic film and placed in the storage site. The jars were reviewed and aerated periodically to prevent fungal contamination or avoid the increase in HR inside the jars due to the seed WC loss. After each storage time (t2 . . . tn), a sample of seeds were sown on agar, and another sample ($n \geq 4$) was utilized to determine the WC ($WC_{t2 \ldots tn}$). For this purpose, Equation (1) was used, with the seed FW and DW data for each t2 . . . tn ($FW_{t2 \ldots tn}$ and $DW_{t2 \ldots tn}$, respectively).

### Seed Germination

Before (RC-seeds) and after storage (St-seeds), the seeds were sown on plates of 1% agar (10 g/L agar/water; Bioxon, Becton Dickinson de México S.A. de C.V., México). The agar medium was placed in transparent plastic boxes (12 × 16 × 5.5 cm). Before sowing, seeds were disinfected with a 1% sodium hypochlorite solution and, subsequently, with a 0.2% fungicide solution (Interguzan 30-30, pentachlorinenitrebenzene and terametiltiuram disulphide). All the sown boxes (30 seeds per box) were placed in growth chambers (25°C, 12/12 h photoperiod). Germination took place when the radicle protruded. Germination was registered every third day until no germination took place. Seeds that did not germinate were fully covered with fungal infection or were rotten. We used three replications for species for each germination essay.

### Data Analysis

To compare the mean values for the different morphological traits of seeds (V, FW, DW, and WC), considering all the years of collection, we applied the Kruskal–Wallis test, because the assumptions required for analysis of variance (ANOVA) were not met with the available data. Tukey and Kramer's test constituted the post hoc comparison. These analyses were conducted with R software, version 3.2.3 (R Core Team, 2016).

For each species, we obtained the germination parameters relative to each germination test of RC-seeds and

St-seeds. First, the cumulative germination percentages were arcsine transformed and fitted to the exponential sigmoid curve $y = a / [1 + b^{(-cx)}]$ using the Table Curve 2D software, version 5.01 (AISN Software, Chicago, IL). All the fitted curves had $R^2 \geq 0.9$ and $p \leq .01$. From the fitted curve, we obtained the lag time (LT), maximum germination rate (MGR; i.e., maximum first derivative of the sigmoid curve), and mean germination time (MGT; i.e., time for maximum germination rate). These variables, along with the final germination (FG), were the germination parameters.

The mean values of the germination parameters of RC-seeds and St-seeds were compared, considering each storage time and each storage temperature (temperature). For this, we applied ANOVA tests, followed by the Tukey's test for post hoc comparisons. When the variance analysis assumptions were not met, the Kruskal–Wallis and post hoc Tukey and Kramer's tests were applied. To evaluate if there was any interaction between storage time and temperature, we employed a two-way ANOVA, and for *C. baillonii* and *M. mexicana*, a three-way ANOVA was conducted considering the year of seed collection. These analyses were conducted with *R*. Additionally, to determine the effect of temperature on seed germination (for 2015-S in *C. glaucifolia* and *M. mexicana* and for 2016-S in *N. coriacea* and *T. tepezapote* and for both years in *C. baillonii*), a multiple regression analysis with temperature and storage time as factors and the same intercept value (FG before storage or t1) was performed. We applied the square root or logarithm transformation to satisfy the assumptions of the regression analysis. Subsequently, ANOVA test was employed to determine the statistical significance of each slope values. This analysis was conducted with Statgraphics Centurion XVI version 16.1.03 (Statpoint Technologies, Inc.).

To evaluate seed longevity and desiccation sensitivity between species, we determined both $WC_{50}$, that is WC when the initial viability decreases to 50%, and the time required to reach $WC_{50}$ (in days), respectively. To determine $WC_{50}$, the values of seed viability (FG) and seed WC for each storage time (t2 . . . tn) were fitted to the functions indicated in Appendix B. In the case of *C. glaucifolia*, we determined the $WC_{50}$ from the fitted curve (FG vs. WC), because the seed FG achieved after 14 months of storage was higher than 50%. In addition, the time of storage at which seeds reach $WC_{50}$ was calculated using a regression analysis (WC vs. storage time). All fittings were done with Table Curve 2D. To enable comparisons of $WC_{50}$ and the time required to reach $WC_{50}$ values between species, we selected the seeds from the same year and stored at the same temperature. For this, we considered 2016-S (2015-S in *C. glaucifolia*) stored in laboratory conditions ($23 \pm 0.5$°C, $50 \pm 2.2$% RH, and only for *N. coriacea*, to avoid seed germination during storage, these seeds were stored at $15 \pm 0.5$°C, $40 \pm 5$% RH). The mean values of $WC_{50}$ and the time required to reach $WC_{50}$ were compared with ANOVA and followed for the Tukey's post hoc test. Also, we made comparisons between years of seed collection for both *C. baillonii* and *M. mexicana* (2015-S and 2016-S, respectively). Therefore, we applied the *t*-test or Wilcoxon rank sum test in cases of no normality and or homocestadicity of the data. Analyses were performed with *R*.

Finally, we performed a principal component analysis to explore the relationships between species in terms of (a) morphological traits, as seed DW, volume, initial WC, relative amount of endosperm, and embryo size, (b) functional traits, as seed $WC_{50}$, time required to reach $WC_{50}$, and initial values of LT, MGR, and MGT, and (c) ecological and environmental traits, as the season of seed dispersal and both mean maximum temperature and total precipitation for the month of seed collection (Table 1). Before the analysis, all variables were normalized. For this analysis, we included data for 2016-S of all species and 2015-S for *C. glaucifolia*. The analysis was also done with *R*.

## Results

### Morphological Traits of Seeds

The values for each morphological trait of the seeds of studied species are presented in Table 2. The volume ranged from $95.7 \pm 17.0$ to $386.9 \pm 87.6$ mm$^3$, FW from $0.16 \pm 0.026$ to $0.53 \pm 0.101$ g, and DW from $0.11 \pm 0.019$ g to $0.34 \pm 0.075$ g. *C. glaucifolia* had the smallest and lightest seeds, while *C. baillonii* had the largest and the heaviest seeds in terms of FW and DW. Seeds of *M. mexicana* had the lowest WC value ($19.6 \pm 5.63$%). For the other species, WC was > 36%; seeds of *T. tepezapote* had the highest value ($75.3 \pm 13.81$%). Seeds of *C. bailloni* collected in 2015-S were significantly heavier in FW than those from 2016-S ($0.62 \pm 0.057$ and $0.53 \pm 0.101$ g, respectively).

### Seed Storage

Before seed storage (t1), final germination (FG) ranged between 67% in *T. tepezapote* to 94% in *C. glaucifolia* (Table 3). The LT ranged from 8 days in *N. coriacea* to 26 days in *T. tepezapote* (Table 4). MGR ranged from 1.1% day$^{-1}$ in *C. glaucifolia* to 5.4% day$^{-1}$ in *C. baillonii* (2016-S; Table 3). MGT ranged from 22 days in *C. baillonii* (2016-S) to 89 days in the seeds of *C. glaucifolia* (Table 4).

After storage (St-seeds), both storage time and temperature exerted a significant effect on all germination parameters (FG, LT, MGR, and MGT) and in all species,

**Table 2.** Seed Morphological Traits ($n \geq 30$) of the Studied Species ($\bar{x} \pm SE$).

| Species | Year | L × W × T (mm) | Volume ($mm^3$) | FW (g) | DW (g) | $WC_{db}$ (%) |
|---|---|---|---|---|---|---|
| *Chamaedorea glaucifolia* | 2015 | 6.1, 5.5, 5.3 (±0.45, 0.30, 0.29) | 95.7 ± 17.07e | 0.16 ± 0.026e | 0.11 ± 0.019d | 36.3 ± 2.36c |
| *Cymbopetalum baillonii* | 2015 | 15.4, 8.7, 5.5 (±1.27, 0.69, 0.65) | 396.6 ± 64.57a | 0.62 ± 0.057a | 0.37 ± 0.057a | 68.6 ± 14.51ab |
| | 2016 | 14.9, 8.5, 5.7 (±1.27, 0.65, 0.75) | 386.9 ± 87.69a | 0.53 ± 0.101b | 0.34 ± 0.075a | 56.3 ± 16.26b |
| *Magnolia mexicana* | 2015 | 10.4, 8.4, 4.5 (0.69, 1.37, 0.39) | 211.6 ± 38.07c | 0.20 ± 0.038d | 0.16 ± 0.027c | 21.9 ± 4.05d |
| | 2016 | 10.5, 8.2, 4.6 (±0.74, 0.86, 0.53) | 212.9 ± 42.05c | 0.22 ± 0.039d | 0.18 ± 0.030c | 19.6 ± 5.63d |
| *Nectandra coriacea* | 2016 | 9.0, 8.0, 7.8 (±0.82, 0.67, 0.63) | 302.4 ± 73.02b | 0.39 ± 0.098bc | 0.25 ± 0.063b | 56.5 ± 5.84b |
| *Ternstroemia tepezapote* | 2016 | 10.6, 6.4, 4.7 (±0.95, 0.68, 0.77) | 167.8 ± 26.6d | 0.21 ± 0.04d | 0.12 ± 0.021d | 75.3 ± 13.81a |
| | | | $F_{(6,\ 224)} = 219.2$ | $\chi^2 = 435.04$ | $\chi^2 = 461.21$ | $\chi^2 = 493.7$ |

*Note.* L = seed length; W = seed width; T = seed thickness; Volume = seed volume; FW = fresh weight; DW = dry weight; $WC_{db}$ = water content dry basis. Lowercase letters indicate statistical differences inside each column (Tukey's test or Tukey and Kramer's test, $p < .0001$). Year of seed collection for species is indicated.

except *C. glaucifolia* (Tables 3 to 6). As the storage time increased, both FG and MRG decreased, and LT and MGT increased. There was a negative relation between the duration of storage and seed viability of all species and all storage temperatures (Figure 2). In contrast, in *C. glaucifolia*, only storage time had a significant effect on the MGT and MGR (Table 6). In this species, the MGT of St-seeds decreased with time, and the MGR increased after 101 days of storage, but after 426 days, the MGR decreased.

Results of multiple linear regression showed that slopes of linear regression between FG and storage time differed significantly between temperatures for each species, as occurred in all species except in *T. tepezapote* (Figure 2). This means that the storage temperature had an effect in seed longevity: Lower slope values indicate less seed deterioration (Tables 3 and 4, Figure 2). The St-seeds at 15°C showed slower deterioration than at 23°C, 10°C, and 5°C in *C. glaucifolia*, $F(3, 4) = 7.04$, $p < .05$, and in *C. baillonii* for both years of seed collection, $F(2, 3) = 11.13$, $p < .05$ for 2015-S and $F(2, 3) = 28.54$, $p < .01$ for 2016-S. The St-seeds at 5°C showed slower deterioration than at 23°C and 15°C in *M. mexicana*, $F(2, 3) = 17.78$, $p < .01$, and at 15°C in *N. coriacea*, $F(1, 2) = 7.15$, $p < .05$. Although slopes did not differ significantly for *T. tepezapote*, $F(1, 3) = 1.99$, $p > .05$, after 251 days, germination only occurred in seeds stored at 5°C (Table 3). Summary of multiple regression analysis is shown in Appendix A.

The multiway ANOVA tests applied to the mean values of all parameters confirm in some cases an interaction between the factors year, storage time, and temperature (Tables 5 and 6). Individually, the year of seed collection exerted a significant effect on the St-MGT in *C. baillonii* (Table 5). In this species, RC-seeds collected in 2016 had a shorter MGT than RC-seeds collected in 2015, but in 2015-S seeds, the St-MGT was shorter compared with RC-seeds (Table 4). Conversely, in *C. baillonii*, we found a triple interaction between the collection year, storage time, and temperature (Table 5). That is, 2016-S seeds stored for 180 days at 23°C showed the longest St-MGT; whereas, 2015-S did not exhibit significant differences (Table 4). On the other hand, the collection year had a double interaction with both storage time and temperature for all germination parameters of *C. baillonii* seeds, except St-LG (Table 5). After 180 days of storage at 15°C, the FG of 2016-S decreased. Also, after 30 days of storage at 23°C for both years, the MGT decreased in 2015-S, but in 2016-S, it increased. Finally, only 2016-S showed differences in St-LT and St-MGR in terms of temperature of storage (Tables 3 and 4).

An interaction between storage time and temperature also was observed for St-FG, St-LT, and St-MGT in seeds of *N. coriacea* and *T. tepezapote* (Table 6). Seeds of *N. coriacea* stored for 180 days at 5°C had a higher FG and shorter LT and MGT than seeds stored at 15°C, while in *T. tepezapote*, germination occurred only in seeds stored for 251 days at 5°C (Tables 3 and 4).

We found significant differences in $WC_{50}$ values between species, ANOVA, $F(4, 10) = 329.1$, $p < .0001$. Seeds of *C. glaucifolia* had the lowest $WC_{50}$, 4.6 ± 0.08% (Figure 3). *C. baillonii* and *M. mexicana* showed no difference between them in $WC_{50}$, with

**Table 3.** Final Germination and Maximum Germination Rate of Seeds ($\bar{x} \pm SE$) After Different Storage Times or Durations (t2 . . . tn) in Different Storage Temperatures (°C) for Five Species From Tropical Forests of Mexico.

| Storage time (days) | Final germination (%) | | | | Maximum germination rate (% × day$^{-1}$) | | | |
|---|---|---|---|---|---|---|---|---|
| | 23°C | 15°C | 10°C | 5°C | 23°C | 15°C | 10°C | 5°C |
| *Chamaedorea glaucifolia* (2015) | | | | | | | | |
| | $F = 18.66$, $df = 11.13$, $p < .0001$ | | | | $F = 4.206$, $df = 11.23$, $p < .01$ | | | |
| 0 (t1) | 94.4 ± 4.0a | | | | 1.1 ± 0.05c | | | |
| 33 | 92.2 ± 2.23a | 88.8 ± 2.22a | 81.1 ± 1.11ab | 95.5 ± 2.22a | 2.2 ± 0.17abc | 2.4 ± 0.07abc | 2.1 ± .037abc | 2.4 ± 0.04abc |
| 101 | 74.4 ± 1.11abc | 85.5 ± 1.11ab | 62.2 ± 9.68bcd | NE | 3.3 ± 0.23ab | 3.9 ± 0.93a | 2.7 ± 0.39abc | NE |
| 426 | 54.4 ± 2.93cde | 63.3 ± 5.77bcd | 27.7 ± 8.01e | 43.3 ± 5.77de | 1.6 ± 0.32bc | 2.4 ± 0.52abc | 0.7 ± 0.21c | 1.8 ± 0.42bc |
| *Cymbopetalum baillonii* (2015) | | | | | | | | |
| | $F = 20.12$, $df = 7.16$, $p < .0001$ | | | | $F = 0.898$, $df = 6.13$, $p > .05$ | | | |
| 0 (t1) | 84.2 ± 1.92a | | | | 4.4 ± 0.36 | | | |
| 30 | 64.4 ± 1.11ab | 67.7 ± 2.93a | 66.6 ± 8.81a | NE | 4.7 ± 0.40 | 3.6 ± 0.38 | 3.0 ± 0.42 | NE |
| 180 | 12.2 ± 8.88c | 63.3 ± 9.62ab | 33.3 ± 8.38bc | NE | 3.1 ± 0.63 | 8.0 ± 4.55 | 3.7 ± 0.84 | NE |
| 730 | NG | 5.5 ± 2.22c | NG | NE | NG | ND | NG | NE |
| *Cymbopetalum baillonii* (2016) | | | | | | | | |
| | $F = 105.1$, $df = 8.18$, $p < .0001$ | | | | $F = 18.03$, $df = 6.14$, $p < .0001$ | | | |
| 0 (t1) | 72.5 ± 7.40a | | | | 5.4 ± 0.41a | | | |
| 30 | 82.2 ± 2.93a | 85.5 ± 2.93a | NE | 91.1 ± 4.00a | 5.1 ± 0.15bc | 4.6 ± 0.36c | NE | 7.6 ± 0.69ab |
| 180 | 9.9 ± 3.33cd | 52.2 ± 7.77b | NE | 22.2 ± 2.93c | 2.5 ± 0.98cd | 3.1 ± 0.18cd | NE | 1.5 ± 0.21d |
| 365 | 3.3 ± 1.92d | 8.8 ± 1.11cd | NE | ND | ND | ND | NE | ND |
| *Magnolia mexicana* (2015) | | | | | | | | |
| | $F = 22.72$, $df = 3.8$, $p < .001$ | | | | $F = 0.996$, $df = 3.8$, $p > .05$ | | | |
| 0 (t1) | 88.8 ± 2.93a | | | | 1.92 ± 0.30 | | | |
| 25 | 18.0 ± 3.80b | 35.0 ± 12.58b | NE | NE | 0.8 ± 0.06 | 1.2 ± 0.47 | NE | NE |
| 90 | NG | NG | NE | 17.1 ± 4.36b | NG | NG | NE | 1.0 ± 0.78 |
| 210 | NG | NG | NE | NG | NG | NG | NE | NG |
| *Magnolia mexicana* (2016) | | | | | | | | |
| | $F = 47.52$, $df = 3.8$, $p < .0001$ | | | | $F = 3.108$, $df = 2.6$, $p > .05$ | | | |
| 0 (t1) | 77.7 ± 4.84a | | | | 1.9 ± 0.09 | | | |
| 25 | 2.2 ± 2.2c | 40.0 ± 6.93b | NE | 55.5 ± 2.93b | ND | 1.4 ± 0.29 | NE | 2.6 ± 0.50 |
| 210 | NG | NG | NE | NG | NG | NG | NE | NG |
| *Nectandra coriacea* (2016) | | | | | | | | |
| | $F = 47.31$, $df = 6.14$, $p < .0001$ | | | | $F = 4.523$, $df = 6.14$, $p < .01$ | | | |
| 0 (t1) | 82.2 ± 4.84abc | | | | 1.5 ± 0.16ab | | | |
| 35 | NE | 100.0 ± 0.00a | NE | 96.6 ± 3.33ab | NE | 1.3 ± 0.08b | NE | 1.4 ± 0.13ab |
| 90 | NE | 64.4 ± 4.44c | NE | 76.6 ± 5.09bc | NE | 1.3 ± 1.16b | NE | 2.4 ± 0.21a |
| 180 | NE | 13.3 ± 1.92d | NE | 64.4 ± 6.18c | NE | 1.8 ± 0.38ab | NE | 2.3 ± 0.26ab |
| *Ternstroemia tepezapote* (2016) | | | | | | | | |
| | $F = 11.25$, $df = 5.12$, $p < .001$ | | | | $F = 7.23$, $df = 5.12$, $p < .01$ | | | |
| 0 (t1) | 67.7 ± 5.55a | | | | 1.6 ± 0.11ab | | | |
| 16 | 23.3 ± 3.84c | 54.4 ± 11.11ab | NE | NE | 0.7 ± 0.02bc | 1.8 ± 0.37a | NE | NE |
| 41 | 27.7 ± 1.11bc | 53.3 ± 3.33ab | NE | NE | 1.3 ± 0.15abc | 1.7 ± 0.10a | NE | NE |
| 251 | NG | NG | NE | 18.8 ± 5.87c | NG | NG | NE | 0.6 ± 0.17c |

*Note.* NG = no germination, ND: no determined; NE = no evaluated. Lowercase letters indicate statistical differences (Tukey's test after ANOVA, $p < .05$, or Tukey and Kramer's test after Kruskal-Wallis, $p < .05$). Comparisons were done individually for each germination parameter for each species and for each year of seed collection of *C. baillonii* and *M. mexicana*.

12.7 ± 1.25% and 11.7 ± 0.29%, respectively (Figure 3). Seeds of *N. coriacea* and *T. tepezapote* had the highest $WC_{50}$, with 37.9 ± 1.96% and 59.1 ± 1.87%, respectively (Figure 3). In addition, the time required to reach $WC_{50}$ varied with the species, ANOVA, $F(3, 8) = 272.8$, $p < .0001$. Seeds of *M. mexicana* required the shortest time, 9.1 ± 0.70 days, followed by *T. tepezapote* seeds that required 17.7 ± 1.46 days (Figure 3). Seeds of

**Table 4.** Lag Time and Mean Germination Time of Seeds ($\bar{x} \pm SE$) After Different Storage Times or Durations (t2 . . . tn) in Different Storage Temperatures (°C) for Five Species From Tropical Forests of Mexico.

| Storage time (days) | Lag time (days) | | | | Mean germination time (days) | | | |
|---|---|---|---|---|---|---|---|---|
| | 23°C | 15°C | 10°C | 5°C | 23°C | 15°C | 10°C | 5°C |
| *Chamaedarea glaucifolia* (2015) | | | | | | | | |
| | $F = 3.407$, $df = 11.23$, $p < .01$ | | | | $\chi^2 = 32.134$, $df = 11$, $p < .001$ | | | |
| 0 (t1) | 22.6 ± 2.61ab | | | | 89.7 ± 2.71c | | | |
| 33 | 22.3 ± 1.17ab | 25.0 ± 1.79a | 26.0 ± 4.52a | 26.1 ± 0.65a | 55.4 ± 0.67a | 55.4 ± 0.67a | 57.1 ± 1.91a | 61.4 ± 2.61b |
| 101 | 14.0 ± 1.80ab | 18.2 ± 3.25ab | 18.0 ± 2.31ab | NE | 30.2 ± 0.52a | 34.7 ± 0.53a | 36.0 ± 0.64a | NE |
| 426 | 11.9 ± 0.98b | 18.7 ± 3.55ab | 25.0 ± 8.02ab | 23.3 ± 2.48ab | 40.9 ± 4.29a | 44.9 ± 0.56a | 53.4 ± 6.56a | 44.3 ± 0.98a |
| *Cymbopetalum baillonii* (2015) | | | | | | | | |
| | $F = 1.964$, $df = 6.13$, $p > .05$ | | | | $F = 4.611$, $df = 6.13$, $p < .05$ | | | |
| 0 (t1) | 19.6 ± 0.46 | | | | 35.6 ± 0.93a | | | |
| 30 | 16.4 ± 1.48 | 14.0 ± 1.40 | 11.4 ± 1.15 | NE | 27.1 ± 1.38b | 28.7 ± 0.79ab | 28.4 ± 0.70ab | NE |
| 180 | 20.0 ± 3.27 | 23.0 ± 5.98 | 18.4 ± 0.71 | NE | 26.4 ± 1.00b | 34.2 ± 2.69ab | 26.7 ± 0.82b | NE |
| 730 | NG | ND | NG | NE | NG | ND | NG | NE |
| *Cymbopetalum baillonii* (2016) | | | | | | | | |
| | $F = 12.7$, $df = 6.14$, $p < .0001$ | | | | $F = 10.96$, $df = 6.14$, $p < .001$ | | | |
| 0 (t1) | 11.5 ± 0.71a | | | | 22.1 ± 1.45cd | | | |
| 30 | 10.6 ± 0.93a | 7.5 ± 1.29a | NE | 9.8 ± 1.37a | 23.0 ± 0.68bcd | 22.0 ± 0.25bcd | NE | 20.2 ± 0.44d |
| 180 | 23.4 ± 2.50b | 13.3 ± 0.25a | NE | 11.8 ± 1.26a | 30.1 ± 0.76a | 26.6 ± 1.42ab | NE | 25.4 ± 2.08abc |
| 365 | ND | ND | NE | ND | ND | ND | NE | ND |
| *Magnolia mexicana* (2015) | | | | | | | | |
| | $F = 11.91$, $df = 3.8$, $p < .01$ | | | | $F = 2.815$, $df = 3.8$, $p > .05$ | | | |
| 0 (t1) | 18.56 ± 5.78c | | | | 54.18 ± 3.13 | | | |
| 25 | 58.2 ± 5.34a | 34.3 ± 3.07bc | NE | NE | 71.6 ± 4.62 | 58.0 ± 2.20 | NE | NE |
| 90 | NG | NG | NE | 42.2 ± 4.47ab | NG | NG | NE | 69.0 ± 8.06 |
| 210 | NG | NG | NE | NG | NG | NG | NE | NG |
| *Magnolia mexicana* (2016) | | | | | | | | |
| | $F = 9.396$, $df = 2.6$, $p < .05$ | | | | $F = 4.353$, $df = 2.6$, $p > .05$ | | | |
| 0 (t1) | 25.5 ± 1.46a | | | | 57.2 ± 0.55 | | | |
| 25 | ND | 36.3 ± 2.27b | NE | 34.1 ± 1.76b | ND | 60.4 ± 1.20 | NE | 54.1 ± 2.23 |
| 210 | NG | NG | NE | NG | NG | NG | NE | NG |
| *Nectandra coriacea* (2016) | | | | | | | | |
| | $F = 107.5$, $df = 6.14$, $p < .0001$ | | | | $F = 7.603$, $df = 6.14$, $p < .001$ | | | |
| 0 (t1) | 8.7 ± 0.83c | | | | 43.6 ± 1.45abc | | | |
| 35 | NE | 7.1 ± 0.01c | NE | 6.3 ± 0.79c | NE | 56.9 ± 4.74a | NE | 53.0 ± 8.37ab |
| 90 | NE | 13.0 ± 2.16bc | NE | 13.0 ± 1.63bc | NE | 48.2 ± 0.81abc | NE | 36.0 ± 0.81c |
| 180 | NE | 52.8 ± 2.20a | NE | 16.8 ± 1.94b | NE | 60.9 ± 0.73a | NE | 37.4 ± 2.20bc |
| *Ternstroemia tepezapote* (2016) | | | | | | | | |
| | $F = 10.7$, $df = 5.12$, $p < .001$ | | | | $F = 8.85$, $df = 5.12$, $p < .01$ | | | |
| 0 (t1) | 26.4 ± 1.13c | | | | 49.9 ± 1.65b | | | |
| 16 | 15.5 ± 1.12c | 16.8 ± 0.67bc | NE | NE | 42.8 ± 2.25b | 40.4 ± 2.33b | NE | NE |
| 41 | 29.6 ± 1.82ab | 18.4 ± 2.05bc | NE | NE | 48.3 ± 0.69b | 41.6 ± 1.36b | NE | NE |
| 251 | NG | NG | NE | 38.1 ± 8.52a | NG | NG | NE | 66.5 ± 7.50a |

*Note.* NG = no germination; ND = no determined, NE = no evaluated. Lowercase letters indicate statistical differences (Tukey's test after ANOVA, $p < .05$, or Tukey and Kramer's test after Kruskal-Wallis, $p < .05$). Comparisons were done individually for each germination parameter, for each species and for each year of seed collection of *C. baillonii* and *M. mexicana*.

*C. baillonii* and *N. coriacea* required 88.4 ± 9.27 and 150.9 ± 3.78 days, respectively (Figure 3). After 426 days, the seeds of *C. glaucifolia* showed a reduction in their FG from 100% to 57%; thus, they had the longest $WC_{50}$ (Figure 3). Functions used for the determination of $WC_{50}$ and time to reach $WC_{50}$ are shown in Appendix B.

We found significant differences in $WC_{50}$ between the 2 years for *C. baillonii* ($t = -20.55$, $p < .0001$).

**Table 5.** Results of the Multiway ANOVA on Effects of Year (Y), Storage Time (Sti), and Storage Temperature (ST) on Germination of *C. baillonii* and *M. mexicana* Seeds.

| | *Cymbopetalum baillonii* | | | | *Magnolia mexicana* | | | |
|---|---|---|---|---|---|---|---|---|
| Source of Variation | MS | *F* | *p* | *df* | MS | *F* | *p* | *df* |
| Final germination (%) | | | | | | | | |
| Y | 2 | 0.024 | .8774 | 1 | 101 | 0.945 | .3456 | 1 |
| Sti | 11525 | 135.99 | .0001 | 4 | 7053 | 65.822 | .0001 | 2 |
| ST | 1121 | 13.223 | .0001 | 3 | 2447 | 22.842 | .0001 | 2 |
| Y × Sti | 892 | 10.526 | **.001** | 2 | 32 | 0.301 | .5911 | 1 |
| Y × ST | 187 | 2.203 | .1469 | 1 | 327 | 3.05 | .0999 | 1 |
| Sti × ST | 849 | 10.023 | **.0001** | 4 | | | | |
| Y × ST × Sti | 30 | 0.35 | .5582 | 1 | | | | |
| Residuals | 2881 | | | 34 | 107 | | | 16 |
| Lag time (days) | | | | | | | | |
| Y | 259.84 | 17.55 | .0002 | 1 | 44.5 | 1.135 | .3015 | 1 |
| Sti | 201.93 | 13.639 | .0001 | 2 | 1084.2 | 27.669 | .0001 | 2 |
| ST | 53.28 | 3.598 | .0262 | 3 | 595 | 15.186 | .001 | 2 |
| Y × Sti | 9.7 | 0.655 | .5275 | 2 | 12.5 | 0.318 | .5799 | 1 |
| Y × ST | 62.87 | 4.246 | .0490 | 1 | | | | |
| Sti × ST | 18.4 | 1.243 | .3136 | 3 | | | | |
| Y × ST × Sti | 55.12 | 3.723 | .0642 | 1 | | | | |
| Residuals | 14.81 | | | 27 | 39.2 | | | 17 |
| Mean germination time (days) | | | | | | | | |
| Y | 336.2 | 55.362 | .0001 | 1 | 145.32 | 3.567 | .0761 | 1 |
| Sti | 64.2 | 10.575 | .001 | 2 | 146.51 | 3.596 | .0498 | 2 |
| ST | 16.9 | 2.791 | .0595 | 3 | 208.11 | 5.108 | .0183 | 2 |
| Y × Sti | 88.7 | 14.609 | **.0001** | 2 | 2.44 | 0.06 | .8096 | 1 |
| Y × ST | 64.7 | 10.658 | **.0029** | 1 | | | | |
| Sti × ST | 7.8 | 1.29 | .2980 | 3 | | | | |
| Y × ST × Sti | 26 | 4.274 | **.0484** | 1 | | | | |
| Residuals | 6.1 | | | 27 | 40.74 | | | 17 |
| Maximum germination rate (% × day$^{-1}$) | | | | | | | | |
| Y | 0.07 | 0.012 | .9149 | 1 | 2.5731 | 5.522 | .0311 | 1 |
| Sti | 21.46 | 3.725 | .0372 | 2 | 0.5744 | 1.233 | .3162 | 2 |
| ST | 3.74 | 0.648 | .5908 | 3 | 1.7047 | 3.659 | .0477 | 2 |
| Y × Sti | 34.08 | 5.915 | **.0074** | 2 | 0.0104 | 0.022 | .8829 | 1 |
| Y × ST | 3.27 | 0.567 | .4580 | 1 | | | | |
| Sti × ST | 10.96 | 1.903 | .1530 | 3 | | | | |
| Y × ST × Sti | 8.3 | 1.44 | .2405 | 1 | | | | |
| Residuals | 5.76 | | | 27 | 0.4659 | | | 17 |

*Note.* Interactions that were statistically-significant (*p* values) are indicated with values in bold. MS = mean square; *df* = degrees of freedom.

The 2015-S seeds had lower $WC_{50}$, compared with 2016-S seeds, with 4.8 ± 0.07%; but there was no difference in the time required to reach $WC_{50}$ ($t = 1.3098$, $p > .05$). No differences were found in *M. mexicana* between 2015-S and 2016-S with respect to its $WC_{50}$ ($t = 1.7132$, $p > .05$) and the time to reach $WC_{50}$ ($W = 0.0$, $p > .05$).

### *Relationships Between Biological, Ecological, and Environmental Traits*

The principal component analysis showed that Components 1, 2, and 3 accounted for 94% of the total variation (Figure 4, Appendix C). Component 1

**Table 6.** Results of the Two-Way ANOVA on Effects of Storage Time (Sti) and Storage Temperature (ST) on Germination of *C. glaucifolia*, *N. coriacea*, and *T. tepezapote* Seeds.

| | *Chamaedorea glaucifolia* | | | | *Nectandra coriacea* | | | | *Ternstroemia tepezapote* | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Source of variation | MS | *F* | *P* | *df* | MS | *F* | *p* | *df* | MS | *F* | *p* | *df* |
| Final germination (%) | | | | | | | | | | | | |
| Sti | 757.6 | 1.795 | .185 | 2 | 3623 | 68.47 | .0001 | 3 | 1215.2 | 11.25 | .001 | 3 |
| ST | 440 | 1.043 | .39 | 3 | 1867 | 35.29 | .0001 | 2 | 2408.3 | 22.294 | .001 | 1 |
| Sti × ST | 51 | 0.121 | .887 | 2 | 1141 | 21.57 | **.0001** | 1 | 23.1 | 0.214 | .6517 | 1 |
| Residuals | 422 | | | 27 | 53 | | | 14 | 108 | | | 12 |
| Lag time (days) | | | | | | | | | | | | |
| Sti | 81.31 | 2.878 | .0736 | 2 | 10.819 | 93.1 | .0001 | 3 | 0.644 | 15.027 | .001 | 3 |
| ST | 70.85 | 2.508 | .0801 | 3 | 5.538 | 47.66 | .0001 | 2 | 0.1218 | 2.841 | .1176 | 1 |
| Sti × ST | 0.96 | 0.034 | .9667 | 2 | 4.797 | 41.28 | **.0001** | 1 | 0.2379 | 5.551 | **.0363** | 1 |
| Residuals | 28.25 | | | 27 | 0.116 | | | 14 | 0.0429 | | | 12 |
| Mean germination time (days) | | | | | | | | | | | | |
| Sti | 3579 | 75.362 | .0001 | 2 | 0.0745 | 4.688 | .0181 | 3 | 0.14816 | 13.581 | .001 | 3 |
| ST | 47 | 0.992 | .412 | 3 | 0.3809 | 23.959 | .001 | 2 | 0.03263 | 2.991 | .1093 | 1 |
| Sti × ST | 3 | 0.07 | .933 | 2 | 0.0604 | 3.799 | **.0480** | 1 | 0.00582 | 0.533 | .4793 | 1 |
| Residuals | 47 | | | 27 | 0.0159 | | | 14 | 0.01091 | | | 12 |
| Maximum germination rate (% × day$^{-1}$) | | | | | | | | | | | | |
| Sti | 7.56 | 14.571 | .0001 | 2 | 0.5823 | 3.894 | .0324 | 3 | 0.6616 | 6.298 | .0082 | 3 |
| ST | 1.34 | 2.582 | .0741 | 3 | 1.5822 | 10.58 | .0057 | 2 | 1.4499 | 13.802 | .0029 | 1 |
| Sti × ST | 0.124 | 0.238 | .7897 | 2 | 0.3648 | 2.439 | .1233 | 1 | 0.3679 | 3.502 | .0858 | 1 |
| Residuals | 0.519 | | | 27 | 0.1496 | | | 14 | 0.1051 | | | 12 |

*Note*. Interactions that were statistically significant (*p* values) are indicated with values in bold. MS = mean square; *df* = degrees of freedom.

explained 38% of the variation and was represented for the positive loadings of seed DW, MGR, volume, and mean maximum temperature in the month of seed collection, followed by negative loadings of seed MGT and LT (Figure 4, Appendix C). Thus, large seeds germinate fast and are dispersed in the hottest months. Component 2 explained 34% of the variation and was represented for positive loadings in seed FG, the time required to reach $WC_{50}$, relative amount of endosperm, dispersal time, maximum mean temperature, and total precipitation in the month of seed collection, followed by negative loadings of seed WC, $WC_{50}$, and embryo size (Figure 4, Appendix C). Therefore, seeds with low WC, small embryos, and an abundance of endosperm tended to have less desiccation sensitivity, high viability (final germination), and were dispersed in wet months with high maximum temperatures. Component 3, which explained 21% of the variation, had positive loadings in seed LT and relative amount of endosperm, followed by negative loadings in seed WC, embryo size, dispersal time, and total precipitation in the month of seed collection (Figure 4, Appendix C). Thus, seeds with low WC, abundance of endosperm, small embryo, and those dispersed in dry seasons take more time to germinate.

## Discussion

All species had short-lived desiccation-sensitive seeds, because their vigor and viability decreased after storage. These functional traits, along with morphological and physiological traits, were in accordance to those reported for tropical species with short-lived desiccation-sensitive seeds (Pritchard et al., 2004; Hamilton et al., 2013), but we found variation in seed longevity between them. Indeed, we found that seed longevity was related to other functional and ecological traits, along with the prevailing weather conditions at the time of seed dispersal. Seed WC and internal structure are related to longevity (Hong & Ellis, 1996; Hill, Edwards, & Franks, 2010). Moreover, the amount of precipitation and temperature influence the seed development (Finch-Savage & Farrant, 1997).

Seeds of both *T. tepezapote* and *N. coriacea* had $WC_{50} > 30\%$, which signifies that both species might be recalcitrant, but they differ greatly in terms of the time required to reach $WC_{50}$ (17 and 150 days, respectively). However, $WC_{50}$ was determined in *T. tepezapote* seeds stored at 23°C, as was done in the other species studied, while we pointed out that *N. coriacea* seeds were stored at

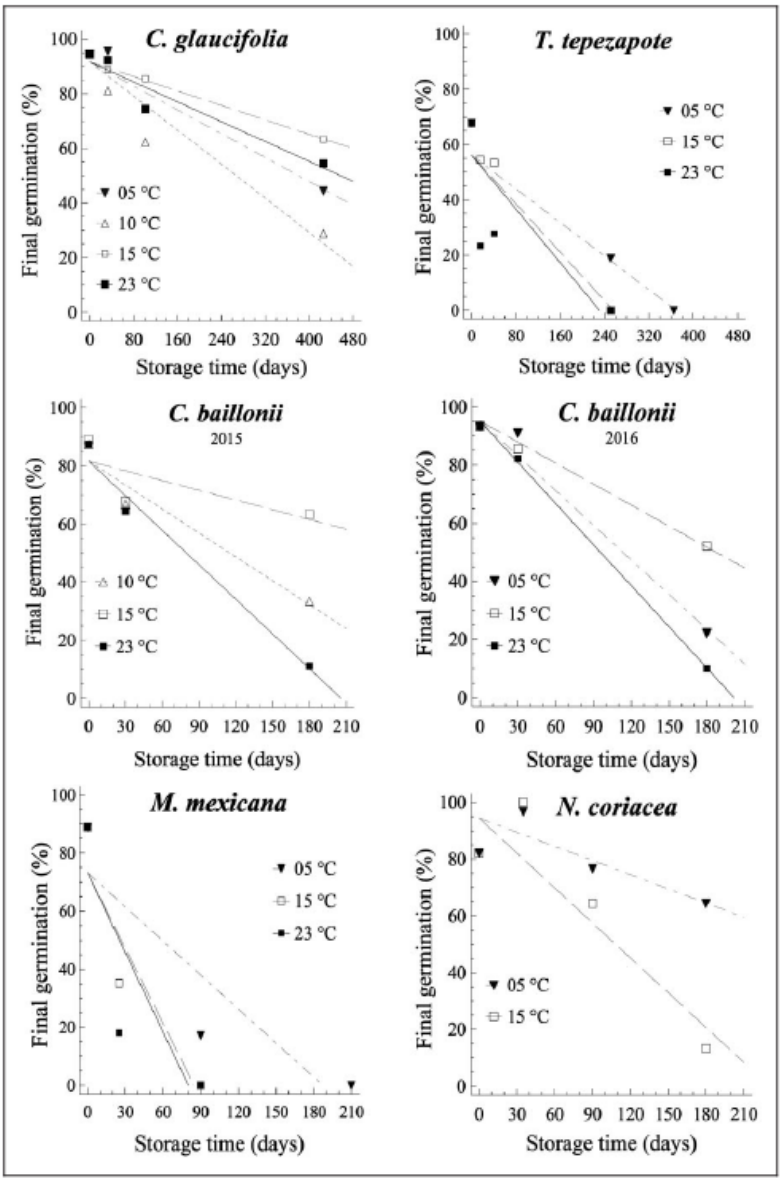


**Figure 2.** Final germination (%, $\bar{x} \pm SE$, $n$ = three replications) of seeds of the studied species after different storage times (t2 . . . tn) at different storage temperatures. The evaluation was done for seeds collected in 2015 (*Chamaedorea glaucifolia* and *Magnolia mexicana*), 2016 (*Nectandra coriacea* and *Ternstroemia tepezapote*), and those collected in both years, *Cymbopetalum baillonii*. Seeds were stored in closed glass jars at 23°C (room temperature, black square symbol), 15°C (white square), 10°C (up, white triangle), or 5°C (down, gray triangle). The values of multiple linear regression analysis are presented in Appendix A.

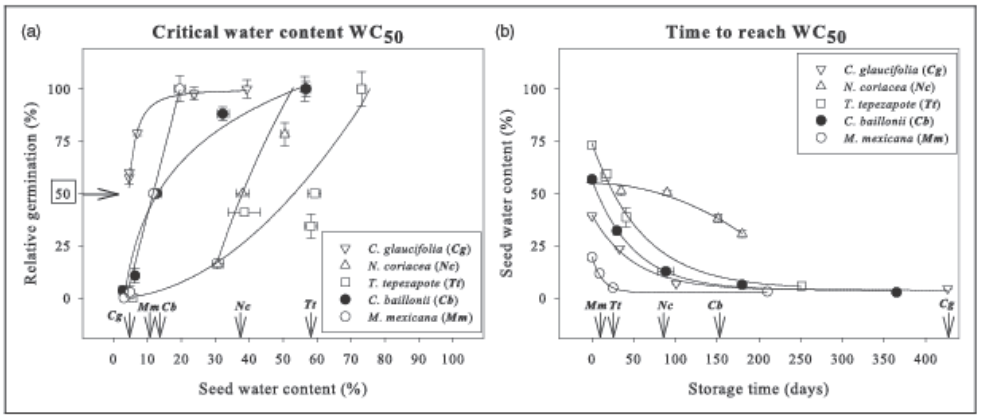


**Figure 3.** The determinations of the critical WC ($WC_{50}$, Panel A) and the time at which the seeds reached $WC_{50}$ (B) for the seeds of the species studied. The final mean seed germination ($\bar{x} \pm SE$, $n$ = three replications) was expressed as a relative percentage with respect to the initial seed germination before storage (germination at t1 = 100%). Seed water content was expressed in a dry weight basis. The arrow on the *x*-axis indicates the $WC_{50}$ value of seeds (A) or the time required to reach $WC_{50}$ (B) for each species (abbreviated names in bold and italicized letters).

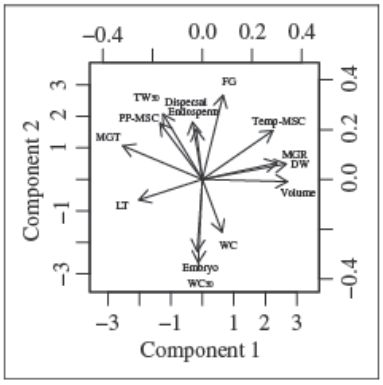


**Figure 4.** Relations between ecological, morphological, and functional, as well as environmental traits of the studied species, seeds, and habitat (tropical forest of Chiapas and Veracruz). Eigenvalues and eigenvectors are showed in Appendix C. FG = final germination; LT = lag time; MGR = mean germination rate; MGT = mean germination time; DW = seed dry weight; WC = seed water content dry basis; $WC_{50}$ = WC at which viability decreased by 50% from the initial viability; $TWC_{50}$ = time to reach $WC_{50}$; Endosperm = relative amount of endosperm in the seed; Embryo = embryo size; Volume = seed volume; PP-MSC = total precipitation in the month of seed collection; Temp-MSC = mean maximum temperature in the month of seed collection; Dispersal = season of seed dispersal.

15°C. This difference in storage temperature was because in 2014 seeds of *N. coriacea* with 78.8 ± 2.93% germination did not germinate after 90 days of storage at room environment (23 ± 0.5°C, 50 ± 2.2% RH; A. G. Becerra-Vázquez, personal observation, January, 2015). Thus, a storage temperature of 15°C may have entailed a longer time to reach $WC_{50}$. Therefore, the time required to reach $WC_{50}$ for seeds of *N. coriacea* at 23°C might be less than 90 days, while at 15°C seeds of *N. coriacea* had a FG of 13% after 180 days. Regardless of this, the $WC_{50}$ values of both species are consistent with those reported by Hong and Ellis (1996) for tropical recalcitrant seeds. In case of *T. tepezapote*, a closely related species *T. brasiliensis* has seeds with ecological longevity below 60 days (Pires, Cardoso, Joly, & Rodrigues, 2009). Inside the tropical forest, temperature could be almost constant above and beneath the litter (Vázquez-Yanes & Orozco-Segovia, 1994), closer to 23°C than to 15°C. Among the other *Nectandra* species, some are classified as recalcitrant (de Carvalho, Davide, Silva, & Carvalho, 2008).

Even though *M. mexicana* seeds had low $WC_{50}$ (11.7%), the short longevity of its seeds might be related to the anatomical and functional traits of both seeds and fruits. A small embryo is a trait of the family Magnoliaceae (Niembro-Rocas, 1989), and morphological dormancy is reported for species of *Magnolia* (Royal Botanic Gardens Kew, 2017). Nevertheless, in our study, seeds of *M. mexicana* that did not germinate

were rotten. Thus, its small embryos might be highly susceptible to dehydration, without entering into dormancy state, despite the large size of the surrounding endosperm (Alcántara-Flores, 2002). On the other hand, seeds of *M. mexicana* had lower $WC_{50}$, as in *C. glaucifolia* and *C. baillonii*, but *M. mexicana* seeds took shorter time to reach $WC_{50}$ (9 days) compared with both species (> 80 days). Therefore, *M. mexicana* seeds might be classified as recalcitrant. Seeds of *M. ovata*, a species closely related to *M. mexicana* (Figlar & Nooteboom, 2004), loose water rapidly during dehydration in silica gel (~10% from initial 100%), and after this dehydration, the FG decrease from 84% to 19% (José, Da Silva, Davide, Melo, & Toorop, 2011). Pupim et al. (2009) found that *M. ovata* seeds have a relatively low WC ($WC_{db} = 30\%$ calculated from $WC_{fb}$, according to Caddick (2005)). This species has a dehiscent dry fruit that exposes seeds to drying before dispersal, thus rendering them highly susceptible to dehydration (José et al., 2011). This was observed in field in *M. mexicana*.

Seeds of *C. baillonii* had low $WC_{50}$ (12%) and required 80 days to reach $WC_{50}$. Therefore, its seeds might be recalcitrant. Seeds of *C. baillonii* have been reported to be desiccation-sensitive (Rodríguez et al., 2000). As pointed out earlier, seeds of *M. mexicana* and *C. baillonii* exhibited no difference in their $WC_{50}$; but the fact that seeds of *C. baillonii* required a longer time to reach $WC_{50}$ than *M. mexicana* is interesting. Both species have small embryos and abundant endosperm (Niembro-Rocas, 1989). However, they differ in seed anatomy, because the seed coat of *C. baillonii* has an inner tegument with multiple folds, and these folds extend into the fissures present in the endosperm tissue, that is, ruminate endosperm (Niembro-Rocas, 1989). Also, *C. baillonii* seeds were larger than those of *M. mexicana*. However, a single factor such as seed size cannot predict longevity in desiccation-sensitive seeds (Hill et al., 2012), influencing partially the dehydration rate of the seed, as it occurs in some tropical species (Hill et al., 2010). Large seeds have a great seed surface to mass volume ratio (Cleri, 2016); thus, they dehydrate at a slower rate than small seeds. Therefore, seed structure and size might explain the differences in seed longevity of *C. baillonii*.

We found annual variation in desiccation sensitivity in seeds of *C. baillonii*: 2015-S had the lowest $WC_{50}$ compared with 2016-S. Weather conditions have influence on seed development. Indeed, RC-seeds from 2015 had higher FW than 2016. Seeds produced in different years can exhibit variation in their morphological and functional traits, as is the case of some tropical species (Sánchez-Coronado et al., 2007; Lamarca et al., 2016). Thus, weather conditions might have a similar effect during the development of the seeds of *C.baillonii*, because 2014 (the development year for seeds collected in 2015) had higher monthly mean maximum temperatures compared with 2015 (climatic data from Torre CONAGUA-SMN—CONANP—IBUNAM, Comisión Nacional del Agua, 2017). On the other hand, we found that seed vigor for *C. baillonii* increased after 30 days of storage for seeds collected in 2015 but not in 2016. This result agrees with that of Rodríguez et al. (2000), which found that a previous mild dehydration of the seeds of *C. baillonii* increased germination rate and final germination. The improving of germination velocity with the mild dehydration of seeds before germination is also found in other tropical species. (Eggers, Erdey, Pammenter, & Berjak, 2007; Rodríguez et al., 2000). During mild dehydration, seeds might end the seed maturation phase (maturation and dryness; Vertucci & Farrant, 1995) and maintain their active metabolism; so, if after this period they are placed in optimal germination conditions, seed germination rate might increase, as it occurs in recalcitrant seeds of *Avicennia marina* (Farrant et al., 1985).

Seeds of *C. glaucifolia* had the lowest value of $WC_{50}$ (4.6%) and required the longest time to $WC_{50}$ (~426 days). This seems to be closely related to the values reported for intermediate seeds (Hong & Ellis, 1996), so *C. glaucifolia* might have this storage behavior. Seeds of *C. elegans* lost >50% of their initial viability when WC of seed reach 16%; while in the less sensitive *C. microspadix* seeds, the loss of >50% of initial viability occurs when seeds reach WC of 7% (Carpenter & Ostmark, 1994). Therefore, seeds of *C. glaucifolia* had a desiccation response similar to that of *C. microspadix*. Conversely, seeds of *C. glaucifolia* are the smallest compared with the other studied species; however, they might have a high dehydration rate corresponding to their high surface/volume ratio (Cleri, 2016). A structural trait in seed of *C. glaucifolia* might be related to its longer time required to reach $WC_{50}$. The fruit and seed tissues (endocarp and hard endosperm) that surround its embryo (Corner, 1976) isolate it more than the seed coat does in the other studied species. Internal differences in water distribution along with physical changes in seed structure might explain the unexpected desiccation patterns in seeds, as is the case with other rainforest species (Hill et al., 2010). Finally, morphophysiological dormancy is common in palms (Baskin & Baskin, 2014), such as *Chamaedorea* spp (Carpenter & Ostmark, 1994). The removal of dormancy might require a seed life span longer than in quiescent seeds that have a faster germination.

### *Effect of Temperature in Seed Longevity*

The longevity of tropical desiccation-sensitive seeds can be extended by storage at ≥10°C (Hong & Ellis, 1996). In this study, longevity of *C. glaucifolia* and *C. baillonii*

seeds was longer at 15°C than at 23°C, 10°C, and 5°C. Nevertheless, seeds of *M. mexicana*, *N. coriacea*, and *T. tepezapote* exhibited slower aging at 5°C than at ≥15°C. Seeds of these three species have lipid reserves (Niembro-Rocas, 1989), like temperate red oaks with seeds that have a high lipid content (17.54 ± 4.43%, $n = 26$ species; data included from Bonner & Vozzo, 1987; Xia, Seal, Chen, Zhou, & Pritchard, 2010) and relatively longer viability than white oaks. Tropical species that can also be found in subtropical and even temperate habitats, such as *Calophyllum brasiliense* and *Persea americana*, can prolong their longevity at temperatures below 10°C (Gálvez-Cendegui, Peñaloza, Oyanedel, & Castro, 2017; Nery, Prudente, Alvarenga, Paiva, & Nery, 2017). Moreover, differences in lipid composition in seeds are related to differences in the chilling sensitivity of several species of *Cuphea*, a tropical genus with intermediate seeds (Crane, Miller, Van Roekel, & Walters, 2003). Thus, to clarify this variation, further research must include a biochemical analysis of seeds that considers phylogenetic affinity and the geographic distribution of species.

Seeds of the studied species, except *C. glaucifolia*, are probably recalcitrant. Since we did not follow the protocol to determine seed storage behavior (e.g., Hong & Ellis, 1996), further research is needed that includes the storage of seeds dehydrated to a specific WC and placed at temperatures above and below freezing. Moreover, a detailed determination of the presence of seed dormancy is required, as in *C. glaucifolia*, because it demonstrated a reduction in MGT after storage, which could indicate dormancy removal. The presence of dormancy could mask the degree of desiccation sensitivity in tropical seeds (Rodríguez et al., 2000).

### *Implications for Conservation*

The current deterioration of tropical forest requires the implementation of conservation strategies, but highly threatened species, such as those with desiccation sensitive seeds, clearly require special attention compared with other members of the plant community. Thus, knowledge about desiccation sensitivity in seeds is an essential step before designing in situ conservation and restoration programs for species with short-lived seeds (e.g., seedlings from seed in nurseries, direct seeding). In this study, we discovered that all species had short-lived desiccation-sensitive seeds. Therefore, this seed trait must be considered for conservation and restoration strategies of the species' habitat. In the same manner, the propagation and storage of seeds of these species must take into account their limited longevity and the temperatures with which the longevity can be extended.

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## Appendices

**Appendix A.** Results of Multiple Linear Regressions Between Final Germination, Storage Time (Sti) and Storage Temperature (ST) for the Studied Species.

| Species | Parameter | Estimate | Std. Error | t | p | ST | β |
|---|---|---|---|---|---|---|---|
| *C. glaucifolia* (2015) | Intercept (α) | 9.973 | 0.127 | 78.51 | .00001 | | |
| $R^2 = 0.959$ | sqrt(Sti) | −0.151 | 0.0166 | −9.07 | .00001 | 5°C | −0.151 |
| $F_{(4,14)} = 58.69$ | sqrt(Sti) × ST = 10°C | −0.065 | 0.0202 | −3.24 | .0087 | 10°C | −0.216 |
| $p = .00001$ | sqrt(Sti) × ST = 15°C | 0.058 | 0.0202 | 2.88 | .0162 | 15°C | −0.092 |
| | sqrt(Sti) × ST = 23°C | 0.027 | 0.0202 | 1.36 | .2022 | 23°C | −0.123 |
| *C. baillonii* (2015) | Intercept (α) | 81.607 | 3.4913 | 23.37 | .00001 | | |
| $R^2 = 0.943$ | Sti | −0.274 | 0.0482 | −5.69 | .0023 | 10°C | −0.274 |
| $F_{(3,8)} = 28.06$ | Sti × ST = 15°C | 0.163 | 0.0606 | 2.68 | .0433 | 15°C | −0.111 |
| $p = .0015$ | Sti × ST = 23°C | −0.122 | 0.0606 | −2.01 | .1003 | 23°C | −0.396 |
| *C. baillonii* (2016) | Intercept (α) | 95.185 | 1.7987 | 52.91 | .00001 | | |
| $R^2 = 0.99$ | Sti | −0.24 | 0.0248 | −9.69 | .0002 | 15°C | −0.24 |
| $F_{(3,\ 8)} = 175.89$ | Sti × ST = 23°C | −0.231 | 0.0312 | −7.39 | .0007 | 23°C | −0.472 |
| $p = .00001$ | Sti × ST = 5°C | −0.157 | 0.0312 | −5.02 | .004 | 5°C | −0.398 |

(continued)

**Appendix A.** Continued

| Species | Parameter | Estimate | Std. Error | *t* | *p* | ST | β |
|---|---|---|---|---|---|---|---|
| *M. mexicana* (2015) | Intercept (α) | 9.627 | 0.354 | 27.19 | .00001 | | |
| $R^2 = 0.984$ | sqrt(Sti) | −0.638 | 0.0472 | −13.5 | .00001 | 5°C | −0.638 |
| $F_{(3, 8)} = 103.19$ | sqrt(Sti) × ST = 15°C | −0.316 | 0.0737 | −4.28 | .0078 | 15°C | −0.954 |
| $p = .0001$ | sqrt(Sti) × ST = 23°C | −0.389 | 0.0737 | −5.27 | .0033 | 23°C | −1.027 |
| *N. coriacea* (2016) | Intercept (α) | 94.467 | 7.0043 | 13.48 | .00001 | | |
| $R^2 = 0.832$ | Sti | −0.41 | 0.0823 | −4.97 | .0042 | 15°C | −0.41 |
| $F_{(2, 7)} = 12.40$ | Sti × ST = 5°C | 0.244 | 0.0912 | 2.67 | .0441 | 5°C | −0.166 |
| $p = .0115$ | | | | | | | |
| *T. tepezapote* (2016) | Intercept (α) | 8.56 | 0.6246 | 13.7 | .00001 | | |
| $R^2 = 0.894$ | sqrt(Sti) | −0.481 | 0.0926 | −5.19 | .0013 | 15°C | −0.481 |
| $F_{(3, 10)} = 19.80$ | sqrt(Sti) × ST = 23°C | −0.075 | 0.1072 | −0.7 | .5052 | 23°C | −0.557 |
| $p = .0008$ | sqrt(Sti) × ST = 5°C | .107 | .0946 | 1.14 | .2915 | 5°C | −.373 |

*Note.* Year of seed collection is indicated after species name. Slopes values (β) are indicated.

**Appendix B.** Functions Used for the Determination of $WC_{50}$ and Time to Reach $WC_{50}$, Expressed as the Relation Between the Seed's Water Content (WC) and Seed Viability (FG) and WC and Storage Time (Sti).

| Species | FG vs. WC | | WC vs. Sti | |
|---|---|---|---|---|
| | Function | $R^2$ | Function | $R^2$ |
| *C. glaucifolia* | $y^{-1} = a + be^{-x}$ | ≥0.89 | $y = a + b^{-x/c}$ | ≥0.98 |
| *C. baillonii* | $y = a + b\ln x$ | ≥0.93 | $y = a + b^{-x/c}$ | ≥0.99 |
| *M. mexicana* | $y = a + bx$ | ≥0.90 | $y^{-1} = a + bx^{0.5}$ | ≥0.93 |
| *N. coriacea* | $y = a + b\ln x$ | ≥0.95 | $y = a + bx^2$ | ≥0.91 |
| *T. tepezapote* | $y = ax^b$ | ≥0.85 | $y = a + b^{-x/c}$ | ≥0.98 |

*Note.* $p$ values for all fittings were $< 0.05$.

**Appendix C.** Eigenvalues and Eigenvectors of Principal Component Analysis Done With Morphological and Functional Seed Traits Along With Ecological and Environmental Traits of the Habitat of the Studied Species.

| | Component 1 | Component 2 | Component 3 |
|---|---|---|---|
| Eigenvalue (%) | 2.32 | 2.20 | 1.72 |
| Percentage variation explained | 0.38 | 0.35 | 0.21 |
| Eigenvectors | | | |
| FG | 0.10 | 0.42 | −0.10045965 |
| LT | −0.31932204 | −0.10329617 | 0.36 |
| MGR | 0.38 | 0.08 | 0.17 |
| MGT | −0.39985941 | 0.17 | −0.02350418 |
| DW | 0.42 | 0.08 | −0.0481389 |
| WC | 0.10 | −0.26520745 | −0.27745038 |
| $WC_{50}$ | −0.02027922 | −0.41841439 | −0.20356546 |
| $TWC_{50}$ | −0.19807148 | 0.33 | −0.26254916 |
| Endosperm | −0.03109828 | 0.25 | 0.45 |
| Embryo | −0.02600443 | −0.36145248 | −0.3483421 |
| Volume | 0.43 | −0.0113558 | −0.04894734 |
| PP_MSC | −0.21306285 | 0.28 | −0.34563801 |
| Temp_MSC | 0.35 | 0.25 | −0.06838661 |
| Dispersal | −0.04966601 | 0.29 | −0.43476992 |

*Note.* FG = final germination; LT = lag time; MGR = mean germination rate; MGT = mean germination time; DW = seed dry weight; WC = seed water content dry basis; $WC_{50}$ = WC at which initial viability decreased by 50%; $TWC_{50}$ = time to reach $WC_{50}$; Endosperm = relative amount of endosperm in the seed; Embryo = embryo size; Volume = seed volume; PP-MSC = total precipitation in the month of seed collection; Temp-MSC = mean maximum temperature in the month of seed collection; Dispersal = season of seed dispersal.

## CAPÍTULO 2. Effects of natural priming on germination and potential longevity of short-lived seeds from Mexican tropical forests

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**Effects of natural priming on germination and potential longevity of short-lived seeds from Mexican tropical forests**

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**Abstract**

In the soil, the seeds undergo hydration-dehydration cycles naturally. After these cycles, the seeds are unearthed to avoid radicle protrusion, preserving the germinative advances acquired in the soil during natural priming. In long-lived seeds, natural priming produces physiological changes that induce vigor retention and restrain aging. However, few studies have been done in short-lived seeds. Here, we evaluated if natural priming (NP) has an effect on seed vigor and longevity for five Mexican species from tropical forests: *Chamaedorea glaucifolia*, *Cymbopetalum baillonii*, *Damburneya coriacea*, *Magnolia perezfarrerae* and *Ternstroemia tepezapote*. Seeds for each species were collected during their dispersal peaks, immediately cleaned from fruit debris, and then buried under a closed forest canopy site. The burial period was defined considering the germination behavior of each species. Seeds were unearthed once the NP period concluded; afterward, they were cleaned and stored in a temperature-controlled growth chamber during a period defined by the seed longevity of each species. Seed samples were taken for total protein analysis in each period. Seed germination was evaluated in an environmental chamber at 25 °C before and after storage. We found that NP promotes fast germination in all species before and even after storage compared with controls, which means that NP improved seed vigor and longevity. Protein profiles showed changes related with protein mobilization or degradation in NP seeds compared to control. Natural priming has the potential to deal with conservation and management practices in our species.

**Keywords**

Recalcitrant and intermediate seeds; seed desiccation sensitivity; seed burial; seed vigor; longevity and hydration memory.

## Introduction

The post-shedding physiological state of seeds determines their tolerance to desiccation, low temperatures (< 0 °C), and seed longevity. Based on their tolerance to desiccation and low temperatures, seeds are classified as orthodox or recalcitrant (Hong and Ellis 1996), but there is a continuum between these extremes (Vertucci and Farrant 1995; Berjak and Pammenter 2013). Orthodox seeds complete their development at the maturation drying phase, developing desiccation tolerance; and at shedding, they have low water content, and low metabolism rates and can be stored at subfreezing conventional temperatures (–20 °C) (Farrant et al. 1993; Vertucci and Farrant 1995; Kermode 1995). Thus, under optimal storage conditions, orthodox seeds can increase their longevity by >10 years (Hong and Ellis 1996). In contrast, recalcitrant seeds do not complete maturation up to the drying phase, so they are shed with a high water content and high metabolic rate, and they have low or no tolerance to desiccation and cannot be frozen (Vertucci and Farrant 1995; Kermode 1995). These seeds with a short lifespan cannot be successfully preserved under conventional storage for these reasons, especially if they have a tropical affinity (Wyse and Dickie 2017; Sommerville et al. 2021). Also, there are important physiological variations between species shed with recalcitrant seed attributes, even within the same species between years of seed production (Sánchez-Coronado et al. 2007; Lamarca et al. 2016). On the other hand, seed longevity is defined as the time that seeds remain viable and retain vigor under determined environmental conditions (Leprince et al. 2017). Seed vigor is defined as seed performance measured by the rate and homogeneity of germination (Finch-Savage and Bassel 2016). Seed longevity has been split into potential and ecological longevity: potential longevity refers to longevity expressed under optimal storage conditions, whereas ecological longevity refers to longevity expressed under the natural conditions of the habitat (Vázquez-Yanes and Orozco-Segovia 1993). In species habitat, seed viability is limited by predation,

pathogens, and the effects of environmental factors on them (Long et al. 2015). The wide difference between controlled and natural conditions is reflected in seed lifespan (Vázquez-Yanes and Orozco-Segovia 1990). Because of all these factors and processes, it is necessary to understand, from an ecological and physiological point of view, the effect of the soil microenvironment on seeds and its role in seed longevity.

Low-cost priming (seed hydration-dehydration treatments) has been successfully used to invigorate old or artificially aged seeds (Sánchez et al. 2001; Pedrero-López et al. 2015) and can also increase longevity (Long et al. 2006). Priming has been mostly used on orthodox seeds (Sánchez et al. 2001; Ibrahim 2019), but it has also been successfully applied to recalcitrant seeds (Castro-Colina et al. 2012; Becerra-Vázquez et al. 2020). Natural priming (NP) is a recently developed method that improves the seed vigor of Mexican wild tree species (Orozco-Segovia et al. 2014). In this method, the seeds are buried *in situ* to allow the natural solid matrix to regulate the water uptake by the seeds, favoring the occurrence of hydration-dehydration cycles. In order to avoid seed germination during burial treatment, its duration is calculated considering the seed germination lag time of each species and the time elapsed between the time of seed shedding and when the rainy season starts in the habitat of each particular species. After the set time has elapsed, seeds are unearthed, and, depending on the type of seeds (recalcitrant or orthodox), seeds are processed for germination or storage (Orozco-Segovia et al. 2014; Becerra-Vázquez et al. 2018). Natural priming induces the pregerminative metabolism in seeds, e.g., storage reserve mobilization and degradation, DNA and RNA repair and synthesis, improvement of antioxidant systems, and repair of cell membranes, as in conventional priming (Ibrahim 2019; Pagano et al. 2023). In orthodox seeds, once they are unearthed, dried, and stored, they retain these germination-related processes as a hydration memory (*sensu* Dubrovsky 1996). This effect has

been observed in tropical species from Mexican dry forests because their treated seeds show fast and synchronous germination, a higher seedling growth rate, and higher survival compared to non-buried seeds (Orozco-Segovia et al. 2014; Peraza-Villarreal et al. 2018). These results support the idea that hydration memory occurs naturally in orthodox seeds during their permanence in the seed bank (Leck et al. 2000; González-Zertuche et al. 2001). On the other hand, short-lived seeds do not form persistent seed banks. However, they can be buried transiently by secondary dispersers (Jansen et al. 2002) or can form transient seed banks, as in some tropical palm species (Gonçalves et al. 2020; Salvador et al. 2022). Recent studies suggest that hydric memory can also occur in desiccation-sensitive species. Seeds from *Cymbopetalum baillonii* and from *Cupania glabra*, both late-successional tropical rainforest species, treated with NP (i.e., buried for 12 days *in situ*), germinate faster than control seeds (Becerra-Vázquez et al. 2020). This effect on germination also occurs in the seeds of *Quercus rugosa*, a recalcitrant species, after their seeds were treated with hydropriming (Castro-Colina et al. 2012). Also, seeds of *Coffea arabica*, an intermediate-seeded species that were only pre-imbibed, maintain high vigor after 90 days of storage (Lima et al. 2001). Given the effects of global climate change and the few possibilities of storing recalcitrant seeds, it is necessary to develop low-cost methods that extend the life of recalcitrant seeds, and peasant communities can manage that in such a way that all recollected viable seeds germinate and produce a seedling that might be successfully used in restoration and conservation programs, as propose Kildisheva et al. (2020). Moreover, the occurrence of hydration memory in desiccation-sensitive seeded species encourages the assessment of NP in new species.

In addition to inducing hydration memory, the water regulation and avoiding radicle protrusion during priming treatment impose hydric stress (Chen and Arora 2012; Pagano et al.

2023). This effect is similar to that occurring naturally on forest soil, where water availability is not regular even in tropical forests (O'Brien et al. 2013). Stressful conditions in the forest soil can promote the plastic response of the long-lived seeds, i.e., biochemical and physiological mechanisms of damage reparation and its prevention in cells (Kranner et al. 2010), which in turn will increase seeds resilience to hydric stress and, therefore, improve their ecological longevity (Kranner et al. 2010; Long et al. 2011). In this sense, it is feasible to think that NP enables an improvement in the potential longevity of the seed. Although data concerning the effect of the transient permanence of seeds in or on soil *in situ* from short-lived seeded species is scarce (Tonetti et al. 2016; Marques et al. 2017; Gasparin et al. 2019), these suggest that their responses are like those of long-lived seeds in terms of improvements in their stress resistance. However, the effects of priming methods on the seed longevity of wild species are contrasting, probably due to a lack of studies or the variability in the results found (Wijayasinghe and Balestrazzi 2018). Regarding NP, seeds from Mexican species such as *Wigandia urens* and *Ceiba aesculifolia* show biochemical changes after the treatment that deal with a plastic response to stress (González-Zertuche et al. 2001; Gamboa-deBuen et al. 2006; Alvarado-López et al. 2018). Under this scenario, assessing whether NP influences seed longevity is encouraging, at least by evaluating the seed vigor after storage as a first step.

This study aimed to evaluate the effect of natural priming on the longevity of short-lived seeds from six Mexican tropical forest species. We evaluated the germination performance of unearthed (NP seeds) and control seeds (non-buried) before and after laboratory storage. We hypothesized that the temporal permanence of the seeds inside the soil produces changes related to restrained germination due to hydration and dehydration events, which will be reflected in their germination performance and protein profile. Compared to the control, we expect that seeds exposed to NP

might have:1) a higher germination rate and a shorter mean germination time and lag time, 2) a higher vigor and relatively longer viability after storage, and 3) differences in their protein profiles after storage treatments.

## Material and methods

*Study area*

We conducted the field work in two tropical forests in southeastern Mexico. The first one is in the "Los Tuxtlas" Tropical Biology Station, UNAM, located in San Andrés Tuxtla, Veracruz (18°34′5″ N, 95°04′26″ W; 155 m above sea level). The main vegetation in the area is tropical rain forest (Soto and Gama 1997). The second one is in Ocuilapa town, located in Ocozocoautla, Chiapas (16°51′18″ N, 93°23′47″ W, 904 m a.s.l.). The main vegetation in Ocuilapa is a transition between the northern tropical rain forest from El Ocote Biosphere Reserve and the southern tropical dry forest from the Central Depression of Chiapas (Escobar-Ocampo and Ochoa-Gaona 2007). The annual mean precipitation in Los Tuxtlas is 4725 mm and 1100 mm in Ocuilapa. The annual mean temperature in Los Tuxtlas is 24 °C and 23.4 °C in Ocuilapa (Instituto Nacional de Estadística, Geografía e Informática 2003; Soto and Gama 1997; Gutiérrez-García and Ricker 2011).

*The study species*

We focused this study on *Chamaedorea glaucifolia* H.Wendl. (Arecaceae), *Cupania glabra* Sw. (Sapindaceae), *Cymbopetalum baillonii* R.E.Fr. (Annonaceae), *Damburneya coriacea* (Sw.) Trofimov & Rohwer (Lauraceae, formerly *Nectandra coriacea*, Trofimov et al. 2016), *Magnolia*

*perezfarrerae* Vázquez & Gómez (Magnoliaceae, formerly *M. mexicana*, Vázquez-García et al. 2013), and *Ternstroemia tepezapote* Schltdl. & Cham. (Pentaphylacaceae). These wild species are used as livelihood resources by human settlements (building material, fuel, ornamental plant, food, medicinal), which, along with their biological and ecological traits, makes them suitable species for restoration in their native forests (e.g., Dawson et al. 2014; Waiboonya and Elliott 2020).

We collected mature seeds from each one of these species at the peak of seed dispersal (Table 1).

*General procedures*

We collected fruits from at least 10 healthy and mature trees from each species (Table 1). The fruits were placed in closed plastic containers previously filled with a soil layer from the study area to keep the fruits fresh until seed extraction. Immediately afterward, the fruits were taken to the laboratory and the seeds were extracted. Fleshy fruits of five species were opened directly by hand and/or using a knife in some cases, whereas woody fruits of *M. perezfarrerae* were kept in a dark place until their dehiscence. The seeds were manually cleaned by softly rubbing them with a fine steel mesh. Afterward, we determined seed water content ($n \geq 30$ per species). Individual seeds were weighed to obtain their fresh weight (FW) with an electronic analytical balance (model A-200DS, precision 0.001 g, Fisher Scientific, Fairlawn, NJ) and then dried in an oven (model 107801, Boekel Industries, Inc., Philadelphia, PA) at 80°C for 72 hours to avoid spontaneous seed ignition due to their high seed lipid content. Once the seed weight did not change, its dry weight (DW) was obtained. The water content on a dry basis of each seed (WC)

was calculated as follows: WC = ((FW – DW)/DW) × 100. This method was used to obtain seed WC subsequently.

*Natural priming treatment*

Natural priming was applied in Los Tuxtlas for *Cymbopetalum baillonii* and *Cupania glabra* and in Ocuilapa for the remainder of the four species included in this study. Clean seeds (n = 200) were put inside an aluminum mesh bag (13 × 20 cm) and then inside a plastic mesh bag (17 × 30 cm) to avoid seed predation along burial time. Three double bags with seeds per species were buried at 1 cm in depth in the forest soil at a site with a closed canopy. Burial duration was based on the germination lag time for each species (Rodríguez et al. 2000; Becerra-Vázquez et al. 2018): 68 days for *Chamaedorea glaucifolia*, 12 days for *Cupania glabra* and *Cymbopetalum baillonii*, 14 days for *D. coriacea*, 17 days for *M. perezfarrerae*, and 32 days for *T. tepezapote*. Natural priming was performed in the same year of seed collection for each species.

Once the burial period concluded, the bags were carefully unearthed, but first, the bags were covered with an aluminum paper layer to avoid light incidence and to retain soil with residual moisture inside the bag. The bags were carried out to the laboratory, placed in a dark room (22 ± 0.9 °C, 50 ± 3.2% RH), and then the seeds were cleaned, sieving them (steel mesh openings = 2.38 mm and 11.2 mm) to eliminate the remains of soil and litter. Finally, naturally primed seeds (NP seeds) were placed in storage conditions (see below).

*Storage treatment*

Seed germination was assessed in NP seeds and in non-buried seeds as control (3 replications, 30 seeds for replication, for treatment, and for species) to determine if NP affected seed longevity.

All seeds were submerged slightly in a 0.2% fungicide solution (Interguzan 30-30, pentachloronitrobenzene, and tetramethylthiuramdisulfide), dried with a paper towel and placed in glass jars of 0.5–1 l, depending on the seed size (approximately 200 seeds per jar). The jars with the seeds were closed with a stretch of plastic film. Jars were placed in an environmental chamber at 15 °C (40 ± 5% RH, Labline Instruments Inc., Melrose Park, IL, USA) for all species. In addition, the remaining seeds of *M. perezfarrerae* and *D. coriacea* were stored (both from the 2016 collection) in a chamber at 5 °C and the seeds of *Chamaedorea glaucifolia* at room temperature (23 ± 0.5°C, 50 ± 2% RH). All these selected temperatures are suitable for storing the seeds of the studied species (Becerra-Vázquez et al. 2018). Storage duration was defined considering the potential longevity reported for each species (Becerra-Vázquez et al. 2018, 2020) as follows (the year of seed collection evaluated is indicated in parentheses): 767 days for *Chamaedorea glaucifolia* (2015), 15 days for *Cupania glabra* (2012), 30 days for *Cymbopetalum baillonii* (2012, 2015, and 2016), 180 days for *D. coriacea* (2016), 25 days for *M. perezfarrerae* (2015, and 2016), and 41 days for *T. tepezapote* (2016). Additional storage evaluations were done after 426 days for *C. glaucifolia* (2015), 60 days for *C. glabra* (2012), and 180 days for *C. baillonii* (2016). Seed WC in all seeds was determined ($n \geq 3$, 3 seeds for replications) before and after storage.

Seed samples from NP and control seeds from each species were used for total protein analysis ($n \geq 9$). Samples were obtained before storage and after 426 days of storage for *Chamaedorea glaucifolia*, 180 days for *Cymbopetalum baillonii* (2016), 180 days for *D. coriacea*, and 25 days for *M. perezfarrerae* (2016). For *Cymbopetalum baillonii*, part of the seeds obtained after the indicated storage days (P1) were germinated, and then samples for protein analysis were obtained when the seeds had broken the seed coat (P2) and when the radicle

protruded (P3). All the seed samples were frozen with liquid nitrogen and stored at –80 °C. For comparison, we also consider the following seed samples: *Chamaedorea glaucifolia*, seeds collected in 2015 and stored for 426 days at 23 °C, and *D. coriacea*, seeds collected in 2016 and stored for 180 days at 5 °C. Also, we consider seeds from *Chamaedorea glaucifolia* buried at the same time and site as NP seeds but unearthed after 18 days, and seeds from *M. perezfarreae* that remained non-buried but were placed below the litter at the same time and site in 2016.

*Seed germination*

Seeds from all treatments were sown on 1% bacteriological agar medium (Bioxon, Becton Dickinson de México S.A. de C.V., México) contained in transparent plastic boxes (17 × 20 × 7 cm). Before sowing, the seeds were disinfected with a 1% sodium hypochlorite solution and subsequently with a 0.2% fungicide solution Interguzan 30-30 (pentachloronitrobenzene and tetramethylthiuram disulfide). The boxes were placed in an environmental chamber at 25 °C with a 12-h photoperiod. Seed germination occurred when the radicle protruded from the seed coat. Germination was registered each third day until no germination was registered for four weeks. Seeds that did not germinate were rotten. We conducted three replications per treatment in each species, with 30 seeds per replication. The experimental design was a priming factor with two levels (NP and control seeds) and a storage factor with two levels (stored and non-stored seeds). In *Cymbopetalum baillonii* and *M. perezfarrerae*, we considered the year of seed collections as the third factor, with three levels (years 2012, 2015, and 2016) and two levels (2015 and 2016), respectively.

*Total protein profile*

For protein quantification, total soluble proteins were extracted as follows: 1 g of seed was ground in a mortar and resuspended with 1ml of buffer solution Tris-HCl 0.1 M (pH 6.8) and 1% insoluble polyvinyl polypyrrolidone. This crude homogenate was centrifuged at 18 000x *g* at 4 °C for 5 min, and after that, the supernatant was extracted. The protein quantification was done following Bradford (1976). Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE 12%) was used to separate total soluble proteins. The protein samples (15 µg) were loaded into the gel and stained with Coomassie blue. We used either PS-101 (Jena Bioscience GmbH) or Amersham 17044601 (GE Healthcare) as molecular weight markers. Gel images were analyzed with Image Lab software version 5.2.1 (Bio-Rad Laboratories, Inc., USA).

*Data analysis*

We used one-way ANOVA to test for differences in seed water content between stored and non-stored seeds, applying the Tukey test as a post hoc test.

For seed germination analysis, we considered the following germination parameters: final germination percentage (FG), lag time (LT), maximum germination rate (MGR), and mean germination time (MGT). To obtain germination parameters, the cumulative germination percentage (for each replication essay for treatment for species) was arcsine transformed and fitted to an exponential sigmoid curve $y = a / [1 + b^{(-cx)}]$ with Table Curve 2D software, version 5.01 (AISN Software, Chicago, IL, USA). The fitted curves generated had $R^2 \geq 0.9$ and $p \leq 0.01$. In the fitted sigmoid curve, LT is the time elapsed until first seed germination occurs, MGR is the maximum first derivative value (slope value at the inflection point), and MGT is the time when MGR occurs.

We test the effect of seed treatment, storage, and their interaction with a two-way ANOVA test for each species. For *Cymbopetalum baillonii* and *M. perezfarrerae*, we also test the effect of the year of seed collection by performing three-way ANOVAs.

In addition to the multi-way ANOVA, and to obtain complementary information from data not included in the multi-way analysis, we performed a *t*-test to compare germination of seeds of *D. coriacea* from the 2015 collection (non-stored seeds), seeds of *Chamaedorea glaucifolia* stored for 426 days, and seeds of *Cymbopetalum baillonii* stored for 180 days.

All these analyses were done with R software, version 3.2.3 (R Core Team, 2017).

## Results

### *Seed water content*

The water content (WC) of NP seeds and control decreased after their storage for all species, but in some cases, it did not occur, as in *Cupania glabra* seeds after 15 days and *T. tepezapote* seeds after 41 days (Table 2). Between treatments, for *Chamaedorea glaucifolia*, NP seeds had higher WC than that of control only before storage; for *Cupania glabra*, NP seeds had lower WC than that of control after 15 days of storage, whereas there were no differences between seed treatments for both *D. coriacea* and *T. tepezapote* (Table 2). On the other hand, for *Cymbopetalum baillonii* seeds from 2015, initially NP seeds had lower WC than the control (55.7 ± 1.82 % and 70.2 ± 2.29 %, respectively), but after 30 days of storage, they had higher WC than the control (54.0 ± 2.44 % and 38.4 ± 4.21 %, respectively, Table 2). For seeds of this species from 2016, NP seeds had lower WC than control after 30 days of storage (41.6 ± 3.66 % and 50.3 ± 1.05 %, respectively, Table 2). However, after 180 days of storage, NP seeds had higher WC

than control (34.1 ± 1.23 % and 19.6 ± 0.37 %, respectively, Table 2). There were no differences between treatments, either non-stored or stored, in 2012. Whereas for *M. perezfarrerae* from 2016, NP seeds had higher WC than control both before (30.4 ± 0.94 % and 19.9 ± 1.08 %, respectively) and after 25 days of storage (18.0 ± 1.56 % and 10.6 ± 0.16 %, respectively), but there were no differences in both cases in 2015 (Table 2).

*Seed germination*

Natural priming, *per se*, affected at least two time-related germination parameters in five of six species, and in the six species the effect was produced by priming in interaction with storage (Tables 3–5). Also, in the case of *Cymbopetalum baillonii* and *M. perezfarrerae*, NP had a triple interaction with storage and the year of seed collection (Table 5). Namely, NP seeds showed higher vigor, i.e., seeds showed shorter lag time (LT) and mean germination time (MGT) than that of control seeds (Figures 1–3).

Final germination percentage (FGP)

Storage in interaction with NP maintained significantly high FGP (>70%) in *Cymbopetalum baillonii* (2015) and *M. perezferrerae* (2015, 2016), without significant differences to non-stored seeds (Table 5; Figs. 2 and 3, respectively). Control seeds significantly reduced their FGP after storage in *C. baillonii* (<70%, 2015) and *M. perezfarrerae* (<40%, both years). In *Chamaedorea glaucifolia* and *D. coriacea*, both control and NP seeds (>75%) significantly reduced their FGP after storage (20–30%) (Tables 3 and 4, respectively; Fig. 1).

Maximum germination rate (MGR)

The interaction between storage and NP was significant for MGR (Tables 3–5). In non-stored seeds, MGR was significantly higher in NP seeds of *Cupania glabra* and *T. tepezapote* (Fig. 1). The same effect in the interaction between storage and year was found in *M. perezfarrerae* (2015 and 2016) (Fig. 3). After storage, NP seeds of *D. coriacea* had MGR significantly lower than that of NP non-stored seeds (Fig. 1). Stored control seeds of *Chamaedorea glaucifolia* had significantly higher MGR than that of non-stored seeds, both in control and NP seeds (Fig. 1). In *Cymbopetallum bailonii* (2012), NP significantly affects increasing MGR (Fig. 2).

Mean germination time (MGT)

In stored and non-stored seeds of *T. tepezapote* and *Cymbopetalum baillonii* (2012, 2015, 2016) and *M. perezfarrerae* (2015, 2016), NP seeds maintained significantly shorter MGT than in control seeds (Figs 1, 2 and 3, respectively). In *D. coriacea*, after storage, the MGT of both control and NP seeds was longer than that of non-stored NP seeds, but NP seeds maintained the pattern of having a shorter MGT than that of the control seeds (Fig. 1). In *Cupania glabra*, the MGT of stored control seeds was significantly shorter than that of non-stored control seeds (Fig. 1). After storage in *Chamaedorea glaucifoli*a, both control and NP seeds reduced their MGT (Fig. 1).

Lag time (LT)

Lag time decreased in five species as an effect of NP. The collection year did not affect the LT of *M. perezfarrerae* (Table 5; Fig 3).

Both stored and non-stored NP seeds of *Cupania glabra*, *T. tepezapote*, *Cymbopetalum baillonii* (2012, 2015, 2016) and *M. perezfarrerae* (2015, 2016) maintained short LT values

compared to controls (Figs. 1–3). In contrast, NP stored seeds of *Chamaedorea glaucifolia* significantly reduced their LT. Stored both NP and control seeds of *D. coriacea* increased significantly their LT compared to non-stored NP and control seeds, but stored NP seeds of this species had a shorter value than stored control seeds (Fig. 1).

*Additional germination tests*

In *Cupania glabra*, after 60 days of storage, only NP seeds germinated (FGP 16.6 ± 3.33 %, MGR 1.3 ± 0.07 % $day^{-1}$, LT 11.6 ± 1.52 days, MGT 24.9 ± 3.86 days). In *Cymbopetalum. baillonii* from 2016, after 180 days of storage, NP seeds had shorter lag time (4.5 ± 0.93 days) and mean germination time (14.4 ± 0.70 days) than control seeds (13.0 ± 0.44 days and 26.6 ± 1.42 days, respectively, *t*-test = 8.17, d.f. = 4, $P < 0.05$, and *t*-test = 7.59, d.f. = 4, $P < 0.05$, respectively). No differences were found in FGP (57.7 ± 5.55 % and 52.2 ± 7.77 %, respectively, *t*-test = −0.57, d.f. = 4, $P > 0.05$) and MGR (4.1 ± 0.31 $day^{-1}$ and 3.1 ± 0.18 $day^{-1}$, respectively, *t*-test = 8.17, d.f. = 4, $P > 0.05$). In *D. coriacea* from 2015, no differences in germination were found between NP and control seeds (FGP 92.2 ± 4.0 % and 81.1 ± 1.11 %, respectively, *t*-test = −2.65, d.f. = 4, $P > 0.05$; MGR 2.4 ± 0.12 $day^{-1}$ and 2.1 ± 0.12 $day^{-1}$, respectively, *t*-test = −1.46, d.f. = 4, $P > 0.05$; LT 4.0 ± 1.15 days and 5.8 ± 1.11 days, respectively, *t*-test = 1.13, d.f. = 4, $P > 0.05$; and MGT 29.3 ± 2.40 days and 31.0 ± 1.11 days, respectively, *t*-test = 0.64, d.f. = 4, $P > 0.05$). In *Chamaedorea glaucifolia*, no effects were found between NP and control seeds stored for 426 days (FGP 52.2 ± 5.87 % and 63.3 ± 5.77 %, respectively, *t*-test = 1.35, d.f. = 4, $P > 0.05$; MGR 1.4 ± 0.14 $day^{-1}$ and 2.3 ± 0.52 $day^{-1}$, respectively, *t*-test = 1.78, d.f. = 4, $P > 0.05$; LT 18.4 ± 3.09 days and 18.7 ± 3.5 days, respectively, *t*-test = 0.07, d.f. = 4, $P > 0.05$; and MGT 46.1 ± 3.09 days and 46.0 ± 0.98 days, respectively, *t*-test = − 0.03, d.f. = 4, $P > 0.05$).

*Total protein profile*

For all species evaluated, polyacrylamide gel analysis of proteins showed a similar band profile between all treatments. However, we found the following differences in each one of them. For *Cymbopetalum baillonii*, comparing non-stored, non-treated seeds with stored, treated, and non-treated seeds for 180 days at 15 °C, several bands were present in all treatments (Figure 4, lanes 1-7). One of them, a protein band of 31 kDa, a molecular weight like dehydrins, was more intense in control non-stored seeds (lane 7) and in control stored seeds (P1, lane 1). Generally, this last treatment showed higher intensity on each one of the protein bands than the rest of the treatments. In addition, a protein band of 63 kDa was absent in NP stored seeds, with a broken coat (P2, lane 4) and with radicle protrusion (P3, lane 6) and also absent in control stored seeds with radicle protrusion (P3, lane 5). A band of 40 kDa, like peroxidases, was not detected in control stored and NP stored seeds, both at P2 (lanes 3 and 4, respectively). A protein of 23.5 kDa, like heat shock proteins (HSP), was absent in NP stored seeds at P3 (lane 6), and a band of 21.5 kDa was not detected in NP stored seeds at P2 (lane 4).

For *Chamaedorea glaucifolia*, a protein band of 59 kDa was present in all treatments and was the most intense (Figure 5, lanes 1–7). Below this one, there were three protein bands between 59 and 40 kDa that were more intense in control non-stored seeds (lane 1), and some of those were absent in NP seeds buried for 18 days (lane 2) and in NP seeds stored for 426 days at 15 °C (lane 7). Also, a protein band of about 23 kDa was present in all treatments except for control non-stored seeds (lane 1). A protein band of 21 kDa was present only in NP seeds, both non-stored (lane 3) and stored for 426 days at 15 °C (lane 7), and in control seeds stored for 426 days at 23 °C (lane 4), whereas a protein band of 17.5 kDa, like HSP, was present on all treatments except NP seeds stored for 426 days at 23 °C (lane 5).

For *D. coriacea*, four protein bands between 66 and 20 kDa are present in all treatments, and a protein band of 55 kDa, like dehydrins, was the most intense in all treatments (Figure 6, lanes 1–7). However, a protein of 14 kDa, like HSP, was present in all treatments except control non-stored seeds from 2016 (lane 3), and a band of < 14 kDa was present in all treatments except control non-stored seeds from 2015 (lane 1). Also, a protein band of 27 kDa was absent in NP non-stored seeds from 2016 (lane 4) and control seeds from 2016 stored for 90 days at 15 °C (lane 5).

Finally, for *M. perezfarrerae*, five protein bands between 97 and 18 kDa are present in all treatments; a protein band of 38 kDa was the most abundant (Figure 7, lanes 1–6). However, control non-stored seeds had more protein bands than the rest of the treatments, where two bands of 83.6 and 18.4 kDa, respectively, were present only here (lane 1). Below the 18.4 kDa band, there were three bands, where the first one with the biggest molecular weight was in all treatments except control stored seeds at 5 °C (lane 2) and seeds below litter (lane 6), the next one was absent in stored NP seeds at 15 °C (lane 5), and the last one was absent in non-stored NP seeds (lane 4) and stored NP seeds at 15 °C (lane 5). Finally, a protein band of about 116 kDa was present on all treatments except for stored NP seeds at 5 °C (lane 3) and stored NP seeds at 15 °C (lane 5).

## Discussion

In general, the seed germination of all studied species was improved with natural priming. NP seeds germinated faster than non-treated seeds, as we found in the seeds of *Cymbopetalum baillonii* and *Cupania glabra* (Becerra-Vázquez et al. 2020). For the remaining species, there was no previous information. These results are consistent with the germination changes promoted and

maintained by priming (Ibrahim 2019) and with hydric memory (Dubrovsky 1996). This effect has been found in other short-lived seeded species (Lima et al. 2001; Ferrandis et al. 2011; Castro-Colina et al. 2012). The germination-related changes of NP seeds from our studied species seem to be confirmed by the protein profiles found in the species evaluated. Some protein bands in non-treated seeds were absent in NP seeds, both stored and non-stored, which could mean protein degradation (Wong et al. 2005), or new bands appeared on NP seeds, i.e., there was protein mobilization. These changes occur in orthodox seeds of wild species treated with NP (González-Zertuche et al. 2001; Benítez-Rodríguez et al. 2014; Alvarado-López et al. 2018). Further, we saw few changes in all species. Protein changes in germinated recalcitrant seeds show little change compared to non-germinated seeds (Wong et al. 2005; Lee et al. 2012; Romero-Rodríguez et al. 2019), maybe due to a lack of a quiescent phase in their metabolic activity after they are dispersed (Romero-Rodríguez et al. 2019). Nonetheless, this protein analysis gives us a window to further explore biochemical and molecular processes through proteomic studies, but this information about short-lived seeds is scarce (Romero-Rodríguez et al. 2019; Pence et al. 2022).

Natural priming seeds maintained higher vigor than the control after storage in our studied species. This result is remarkable because the seeds of our studied species have a recalcitrant behavior (Becerra-Vázquez et al. 2018); that is, their active metabolism is ongoing to germination once they are dispersed from the mother plant (except *Chamaedorea glaucifolia*, as we discuss later). They do not have enough desiccation resistance. We confirm this in our results because the viability of seeds decreases after storage in all species, whether they were treated or not, and both treated and non-treated seeds decrease in water content over time. Also, priming treatment might be considered to decrease the tolerance to desiccation on seeds causing their aging even more *a*

*priori*, but we found the opposite. Priming effects on longevity are variable (Finch-Savage et al. 2016; Gianella et al. 2020). On the one hand, priming can promote the reparation of cell and organelle membranes and molecules such as DNA, along with the activation and improvement of antioxidant systems, which in turn will be reflected in better resistance to hydric stress (Chen and Arora 2013; Ibrahim 2019; Pagano et al. 2023). Thus, primed seeds can increase their longevity. On the other hand, the ongoing metabolism of germination, which requires a great amount of oxygen, extra water input, and energy resources, produces a gradual decrease in desiccation tolerance (Bruggink and van der Toorn 1995; Śliwińska and Jendrzejczak 2002). Even desiccation-tolerant seeds have a threshold during their imbibition-germination processes that, if reached, causes their tolerance to be lost (Bruggink and van der Toorn 1995). As expected, this effect might be more critical for desiccation-sensitive seeds. The duality cost-benefit of priming puts the treatment at a debatable point. Natural priming was a beneficial tool for our studied species to maintain seed vigor after storage.

For *Chamaedorea glaucifolia*, NP effects were evident until 767 days, when treated seeds had a shorter lag time than control. However, this species had lower desiccation sensitivity than the rest, which deal with intermediate storage behavior (Becerra-Vázquez et al. 2018). Also, the seeds seem dormant because there was a decrease in lag time in both treated and non-treated seeds after storage (approximately 35 days on non-stored seeds versus 18 days on seeds stored for 426 days). Palm species commonly have morphophysiological dormancy (Baskin and Baskin 2014), which allows them to form transient seed banks (Orozco-Segovia et al. 2003). Seeds of *Chamaedorea glaucifolia* can be found buried in forest soil, and they can germinate (pers. obs.). For these reasons, testing NP once dormancy on seeds has been released is recommended to avoid misinterpretation of the results.

Seeds of *Cymbopetalum baillonii* and *T. tepezapote* with NP germinated quickly both before and after storage. However, we also found that priming improvement was expressed only on stored seeds, as in *Chamaedorea glaucifolia*, *D. coriacea*, and *M. perezfarrerae*. In *Cupania glabra*, the initial improvement in non-stored seeds was not maintained after 15 days, but after 60 days of storage, only natural primed seeds germinated, so we can conclude that the treatment is also expressed after storage. *Cupania glabra* had a higher seed desiccation sensitivity than the rest of the species, which perhaps caused a rapid aging rate that overcame the initial priming benefits. As we mentioned, the seeds of our studied species are more prone to aging during storage due to their high metabolism, so at some point, extra water supply was required (Pammenter et al. 1994; Pritchard et al. 2022). As this does not occur, a disruption of metabolism occurs, and highly reactive molecules are overproduced, which unbalance the antioxidant systems and cause damage to proteins, nucleic acids, and lipids (Pammenter et al. 1994; Kranner et al. 2010). Yet, aging effects in seeds due to damage to their DNA can be reflected in the seedling stage (Waterworth and West 2023). The NP effect was probably more conspicuous after storage because aging increased on seeds, so primed seeds maintain a higher level of resistance, i.e., protection and repair mechanisms, than non-treated seeds, which are reflected in their highest vigor. Seed invigoration by priming can be more notorious on aged seeds than in freshly or high-quality seed lots (Śliwińska and Jendrzejczak 2002; Butler et al. 2009). Nonetheless, there is a degree of seed aging at which priming will not be effective (Śliwińska and Jendrzejczak 2002; Gómez-Maqueo et al. 2022), which turns out to be more transcendental in short-lived seeds.

Despite interactions found between the treatment with storage, year, and even between these three factors in *Cymbopetalum baillonii* and *M. perezfarrerae*, we saw, in general, that NP increased seed vigor. For example, in *M. perezfarrerae*, after storage, NP seeds had higher

viability (final germination percentage) than control in all years evaluated, but interestingly, for *Cymbopetalum baillonii*, NP stored seeds had higher viability than control only in one year (2015) of three evaluated. Also, treated seeds of this species had a higher mean germination rate than the control, both before and after storage in 2012. In *M. perezfarreae*, NP seeds had a shorter germination time than the control, both before and after storage in seeds collected in 2016. These results could be due to the variations in functional traits of the seeds *per se* as an influence of the environment during their development. *Cymbopetalum baillonii* showed differences in their desiccation sensitivity between 2015 and 2016 seed collections, probably due to the rain pattern during the year of seed development, and *M. perezfarrerae* (formerly *M. mexicana*) did not have it (Becerra-Vázquez et al. 2018). Indeed, environmental factors influence desiccation sensitivity and other seed traits, such as water content (Sánchez-Coronado et al. 2007; Lamarca et al. 2016; Oyerinde et al. 2023). Related to this last trait, for *Cymbopetalum baillonii* from 2016, we found that their naturally primed seeds had higher water content than control only after 180 days of storage, and the same effect for *M. perezfarrerae* from 2016 after 25 days. However, we could not establish a relationship between the variation in desiccation sensitivity and the water content of treated and non-treated / stored and non-stored seeds with the interactions found in this study. Thus, we must be cautious about establishing a concrete reason for those results. However, the effect of priming seems to deal with the probable variation in the traits of seeds.

*Final remarks*

Natural priming increases seed vigor before and after storage. We do not evaluate germination far beyond the storage periods included here. However, we might suppose that vigorous seeds probably remain viable longer, as occurs with *Cupania glabra*. In some sense, vigor retention by

the seed after storage is an expression of its longevity (Leprince et al. 2017). Although we found protein bands with similar weights to stress-related compounds, such as dehydrins or small heat shock proteins (González-Zertuche et al. 2002; Lee et al. 2012; Liu et al. 2022), the protein profile did not give us enough information about the presence of stress-resistance-related compounds. Moreover, we know seed protection from desiccation can also be due to other compounds that protect or repair, such as oligosaccharides and antioxidant-ROS scavengers (Leprince et al. 2017; Zinsmeister et al. 2020). This point leads us to think that more than one kind of hydric stress resistance mechanism might be acting in treated seeds. All of them have been found in short-lived seeds (Lee et al. 2012; Romero et al. 2018).

It has been argued that the storage environment can mask the priming benefits (Gianella et al. 2020), but as we found, the seeds of our studied species maintained germination after storage, but also showed a decrease in viability. The chambers that we used to store seeds provide a stable temperature and moist environment (15 °C, 40 ± 5% RH), as is recommended for desiccation-sensitive seeds to reduce as much as possible their active metabolism toward germination (Hong and Ellis 1996; Pritchard et al. 2022). Indeed, we do not observe radicle protrusion during the storage of all species.

## Conclusion

Natural priming is an effective tool to increase seed vigor in our studied species, even after storage. Therefore, metabolic changes through germination occur and are maintained in the seeds of studied species. Several protection and prevention mechanisms are probably occurring on seeds to restrain seed aging effects. Seed dormancy might mask priming effects, so this factor should be considered. Hydration memory could be present as a trait that provides some degree of

resilience to our short-lived seed species to deal with the driest periods until they germinate, like long-lived species (Contreras et al. 2016), but more studies are needed to unravel it. Our results undoubtedly contribute to understanding the ecology and conservation of species with short-lived seeds, which are classified as exceptional species due to their storage difficulties in conventional seed banking (Pence et al. 2022).

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**Conflict of interest**

The authors declare that they have no conflict of interest.

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**Tables**

Table 1**.** Species from Ocuilapa, Chiapas (*) or from Los Tuxtlas, Veracruz, Mexico. Collection dates, localities, fruit type (FT) and number of fruits collected (NFC) are shown.

| Species | Collection date | Locality | FT / NFC |
|---|---|---|---|
| *Chamaedorea glaucifolia** | October 2015 | Ocuilapa | Fleshy / 1000 |
| *Cupania glabra* | April 2012 | Los Tuxtlas | Fleshy / 1000 |
| *Cymbopetalum baillonii* | April 2012, 2015, 2016 | Los Tuxtlas | Fleshy / 100 |
| *Damburneya coriacea** | September 2015, 2016 | Ocuilapa | Fleshy / 1000 |
| *Magnolia perezfarrerae** | March 2015, 2016 | Ocuilapa | Dry / 50 |
| *Ternstroemia tepezapote** | January 2016 | Ocuilapa | Fleshy / 1000 |

Table 2. Water content (mean ± SE) of seeds treated with natural priming (NP) and non-treated (Control) for six species from Ocuilapa, Chiapas (*) or from Los Tuxtlas, Veracruz, Mexico. Collection date, days of storage and ANOVA results for each species and collection years are indicated. Seeds were stored in glass jars at 15 °C.

| Species | Year | Storage (days) | Treatment | Seed water content (%) |
|---|---|---|---|---|
| *Chamaedorea glaucifolia** | 2015 | $F_{(5, 12)} = 2336$, $P < 0.001$ | | |
| | | 0 | Control | 37.2 ± 0.94 b |
| | | 0 | NP | 44 ± 1.02 a |
| | | 426 | Control | 4.3 ± 0.05 c |
| | | 426 | NP | 3.6 ± 0.07 cd |
| | | 767 | Control | 3.1 ± 0.06 d |
| | | 767 | NP | 3 ± 0.11 d |
| *Cupania glabra* | 2012 | $F_{(5, 12)} = 15.21$, $P < 0.001$ | | |
| | | 0 | Control | 72.1 ± 2.88 ab |
| | | 0 | NP | 69.9 ± 6.4 ab |
| | | 15 | Control | 78.4 ± 3.54 a |
| | | 15 | NP | 53.7 ± 7.57 bc |

| | | | | |
|---|---|---|---|---|
| | | 60 | Control | 31.2 ± 4.49 c |
| | | 60 | NP | 35.9 ± 4.02 c |
| *Cymbopetalum baillonii* | 2012 | $F_{(3, 8)} = 2.257, P > 0.05$ | | |
| | | 0 | Control | 67.9 ± 6.48 |
| | | 0 | NP | 70.6 ± 3.5 |
| | | 30 | Control | 66.6 ± 3.55 |
| | | 30 | NP | 55.5 ± 3.35 |
| | 2015 | $F_{(3, 8)} = 20.88, P = 0.001$ | | |
| | | 0 | Control | 70.2 ± 2.29 a |
| | | 0 | NP | 55.7 ± 1.82 b |
| | | 30 | Control | 38.4 ± 4.21 c |
| | | 30 | NP | 54 ± 2.44 b |
| | 2016 | $F_{(5, 12)} = 63.81, P < 0.001$ | | |
| | | 0 | Control | 57.5 ± 0.91 a |
| | | 0 | NP | 54.2 ± 3.66 a |
| | | 30 | Control | 50.3 ± 1.05 a |
| | | 30 | NP | 41.6 ± 3.66 b |

| | | | | |
|---|---|---|---|---|
| | | 180 | Control | 19.6 ± 0.37 c |
| | | 180 | NP | 34.1 ± 1.23 b |
| *Damburneya coriacea** | 2015 | | | |
| | | 0 | Control | 64.9 ± 0.46 |
| | | 0 | NP | 64.9 ± 0.2 |
| | 2016 | $F_{(3, 8)} = 33.59$, $P < 0.001$ | | |
| | | 0 | Control | 57 ± 0.5 a |
| | | 0 | NP | 60.9 ± 1.7 a |
| | | 180 | Control | 30.6 ± 1.72 b |
| | | 180 | NP | 31.5 ± 5 b |
| *Magnolia perezfarrerae** | 2015 | $F_{(3, 8)} = 112.9$, $P < 0.001$ | | |
| | | 0 | Control | 24.6 ± 1.52 a |
| | | 0 | NP | 24.5 ± 1.38 a |
| | | 25 | Control | 8.2 ± 0.15 b |
| | | 25 | NP | 9.7 ± 0.29 b |
| | 2016 | $F_{(3, 8)} = 58.6$, $P < 0.001$ | | |
| | | 0 | Control | 19.9 ± 1.08 b |

| | | | | |
|---|---|---|---|---|
| | | 0 | NP | 30.4 ± 0.94 a |
| | | 25 | Control | 10.6 ± 0.16 c |
| | | 25 | NP | 18 ± 1.56 b |
| *Ternstroemia tepezapote** | 2016 | $F_{(3, 8)} = 4.513$, $P = 0.039$ | | |
| | | 0 | Control | 77.8 ± 4.15 ab |
| | | 0 | NP | 83.3 ± 6.1 a |
| | | 41 | Control | 59.3 ± 5.28 b |
| | | 41 | NP | 69.3 ± 3.83 ab |

Lowercase letters indicate significant differences (Tukey test, $P < 0.05$) between treatments for each year of seed collection for each species.

Table 3. Results of the two-way ANOVA to test the effects of seed treatment (natural priming, control), storage (stored, non-stored) and their interaction on germination parameters of *Chamaedorea glaucifolia* and *Cupania glabra* seeds. Storage times for *Chamaedorea glaucifolia* were 0 and 767 days, and for *Cupania glabra* were 0 and 15 days.

| | *Chamaedorea glaucifolia* | | | *Cupania glabra* | | |
|---|---|---|---|---|---|---|
| Source of variation | *F* | d. f. | *P* | *F* | d. f. | *P* |
| | Final germination (%) | | | | | |
| Treatment | 0.06 | 1,17 | > 0.05 | 5.34 | 1,17 | **0.04** |
| Storage | 28.13 | 2,17 | **< 0.001** | 65.62 | 2,17 | **< 0.001** |
| Treatment × Storage | 0.97 | 2,17 | > 0.05 | 2.17 | 2,17 | > 0.05 |
| | Maximum germination rate (% days$^{-1}$) | | | | | |
| Treatment | 4.74 | 1,17 | **0.05** | 90.11 | 1,17 | < **0.001** |
| Storage | 3.32 | 2,17 | > 0.05 | 202.71 | 2,17 | < **0.001** |
| Treatment × Storage | 3.48 | 2,17 | > 0.05 | 22.65 | 2,17 | < **0.001** |
| | Mean germination time (days) | | | | | |
| Treatment | 2.74 | 1,17 | > 0.05 | 8.49 | 1,17 | **0.01** |
| Storage | 176.91 | 2,17 | **< 0.001** | 1.21 | 2,17 | > 0.05 |
| Treatment × Storage | 1.37 | 2,17 | > 0.05 | 50.15 | 2,17 | < **0.001** |
| | Lag time (days) | | | | | |
| Treatment | 2.01 | 1,17 | > 0.05 | 23.2 | 1,17 | < **0.001** |
| Storage | 36.84 | 2,17 | **< 0.001** | 7.94 | 2,17 | **0.006** |
| Treatment × Storage | 3.56 | 2,17 | > 0.05 | 53.12 | 2,17 | < **0.001** |

Table 4. Results of the two-way ANOVA to test the effects of seed treatment (natural priming, control), storage (stored, non-stored) and their interaction on germination parameters of *Damburneya coriacea* and *Ternstroemia tepezapote* seeds. Storage times for *Damburneya coriacea* were 0 and 180 days, and for *Ternstroemia tepezapote* were 0 and 41 days.

| | *Damburneya coriacea* | | | *Ternstroemia tepezapote* | | |
|---|---|---|---|---|---|---|
| Source of variation | *F* | d. f. | *P* | *F* | d. f. | *P* |
| | Final germination (%) | | | | | |
| Treatment | 1.36 | 1,11 | > 0.05 | 0.62 | 1,11 | > 0.05 |
| Storage | 317.08 | 1,11 | **< 0.001** | 12.78 | 1,11 | **0.007** |
| Treatment × Storage | 0.02 | 1,11 | > 0.05 | 1.31 | 1,11 | > 0.05 |
| | Maximum germination rate (% days$^{-1}$) | | | | | |
| Treatment | 0.01 | 1,11 | > 0.05 | 7.44 | 1,11 | **0.02** |
| Storage | 5.88 | 1,11 | **0.04** | 0.53 | 1,11 | **> 0.05** |
| Treatment × Storage | 11,28 | 1,11 | **0.01** | 5.8 | 1,11 | **0.04** |
| | Mean germination time (days) | | | | | |
| Treatment | 159.51 | 1,11 | < **0.001** | 109.79 | 1,11 | < **0.001** |
| Storage | 131.12 | 1,11 | < **0.001** | 6.08 | 1,11 | **0.04** |
| Treatment × Storage | 3.43 | 1,11 | > 0.05 | 0.02 | 1,11 | > 0.05 |
| | Lag time (days) | | | | | |
| Treatment | 66.66 | 1,11 | < **0.001** | 53.26 | 1,11 | < **0.001** |
| Storage | 315.16 | 1,11 | < **0.001** | 3.28 | 1,11 | > 0.05 |
| Treatment × Storage | 54.93 | 1,11 | < **0.001** | 2.44 | 1,11 | > 0.05 |

Table 5. Results of the three-way ANOVA to test the effects of seed treatment (natural priming, control), storage (stored, non-stored), year of seed collection (2012, 2015 and 2016 for *C. baillonii* and 2015 and 2016 for *M. perezfarrerae*) and their interactions on germination parameters of *Cymbopetalum baillonii* and *Magnolia perezfarrerae* seeds. Storage times for *Cymbopetalum baillonii* were 0 and 30 days, and for *Magnolia perezfarrerae* were 0 and 25 days.

| | *Cymbopetalum baillonii* | | | *Magnolia perezfarrerae* | | |
|---|---|---|---|---|---|---|
| Source of variation | *F* | d. f. | *P* | *F* | d. f. | *P* |
| | Final germination (%) | | | | | |
| Treatment | 0.83 | 1,35 | > 0.05 | 13.79 | 1,23 | **0.001** |
| Storage | 3.54 | 1,35 | > 0.05 | 23.29 | 1,23 | **< 0.001** |
| Year | 7.63 | 2,35 | **0.002** | 0.74 | 1,23 | > 0.05 |
| Treatment × Storage | 2.84 | 1,35 | > 0.05 | 10.62 | 1,23 | **0.005** |
| Treatment × Year | 0.14 | 2,35 | > 0.05 | 0.01 | 1,23 | > 0.05 |
| Storage × Year | 1.09 | 2,35 | > 0.05 | 2.69 | 1,23 | > 0.05 |
| Treatment × Storage × Year | 0.78 | 2,35 | > 0.05 | 0.01 | 1,23 | > 0.05 |
| | Maximum germination rate (% $days^{-1}$) | | | | | |
| Treatment | 16.78 | 1,35 | **< 0.001** | 16.08 | 1,23 | **< 0.001** |
| Storage | 5.06 | 1,35 | **0.03** | 0.23 | 1,23 | > 0.05 |
| Year | 34.65 | 2,35 | **< 0.001** | 0.01 | 1,23 | > 0.05 |
| Treatment × Storage | 1.54 | 1,35 | > 0.05 | 16.56 | 1,23 | **< 0.001** |
| Treatment × Year | 5.21 | 2,35 | **0.01** | 0.03 | 1,23 | > 0.05 |
| Storage × Year | 5.56 | 2,35 | **0.01** | 1.39 | 1,23 | > 0.05 |
| Treatment × Storage × Year | 3.64 | 2,35 | **0.04** | 0.15 | 1,23 | > 0.05 |
| | Mean germination time (days) | | | | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| Treatment | 391.58 | 1,35 | **< 0.001** | 225.12 | 1,23 | **< 0.001** |
| Storage | 2.22 | 1,35 | > 0.05 | 15.87 | 1,23 | **< 0.001** |
| Year | 88.46 | 2,35 | **< 0.001** | 0.07 | 1,23 | **0.01** |
| Treatment × Storage | 26.23 | 1,35 | **< 0.001** | 0.02 | 1,23 | > 0.05 |
| Treatment × Year | 0.77 | 2,35 | > 0.05 | 8.17 | 1,23 | > 0.05 |
| Storage × Year | 5.31 | 2,35 | **0.01** | 0.44 | 1,23 | > 0.05 |
| Treatment × Storage × Year | 4.08 | 2,35 | **0.03** | 0.01 | 1,23 | > 0.05 |
| | Lag time (days) | | | | | |
| Treatment | 229.46 | 1,35 | < **0.001** | 183.54 | 1,23 | **< 0.001** |
| Storage | 2.22 | 1,35 | > 0.05 | 10.07 | 1,23 | **0.006** |
| Year | 39.9 | 2,35 | < **0.001** | 0.03 | 1,23 | > 0.05 |
| Treatment × Storage | 19.78 | 1,35 | < **0.001** | 3.45 | 1,23 | > 0.05 |
| Treatment × Year | 18.03 | 2,35 | < **0.001** | 4.38 | 1,23 | > 0.05 |
| Storage × Year | 0.20 | 2,35 | > 0.05 | 15.62 | 1,23 | **0.001** |
| Treatment × Storage × Year | 1.20 | 2,35 | > 0.05 | 5.43 | 1,23 | **0.03** |

## Figures

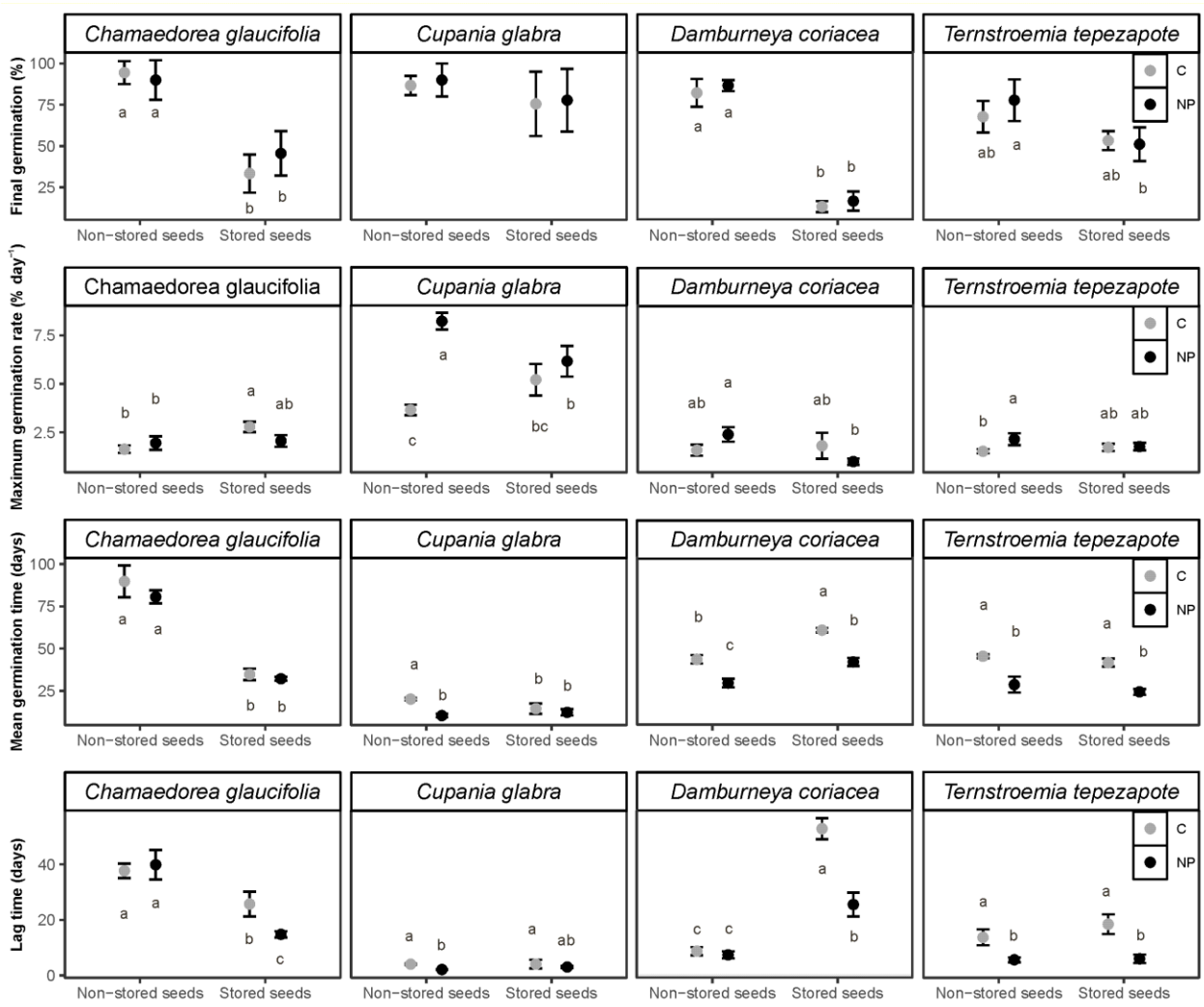


Figure 1. Mean values (±SE) of germination parameters of seeds exposed to natural priming (NP, black circle) and control seeds (C, gray circle), stored or non-stored, for the species indicated. Storage periods in closed glass jars at 15 °C were 767 days for *Chamaedorea glaucifolia*, 15 days for *Cupania glabra*, 180 days for *D. coriacea*, and 41 days for *T. tepezapote*. Lowercase letters indicate statistical differences within each germination parameter for each species (Tukey test, $P < 0.05$).

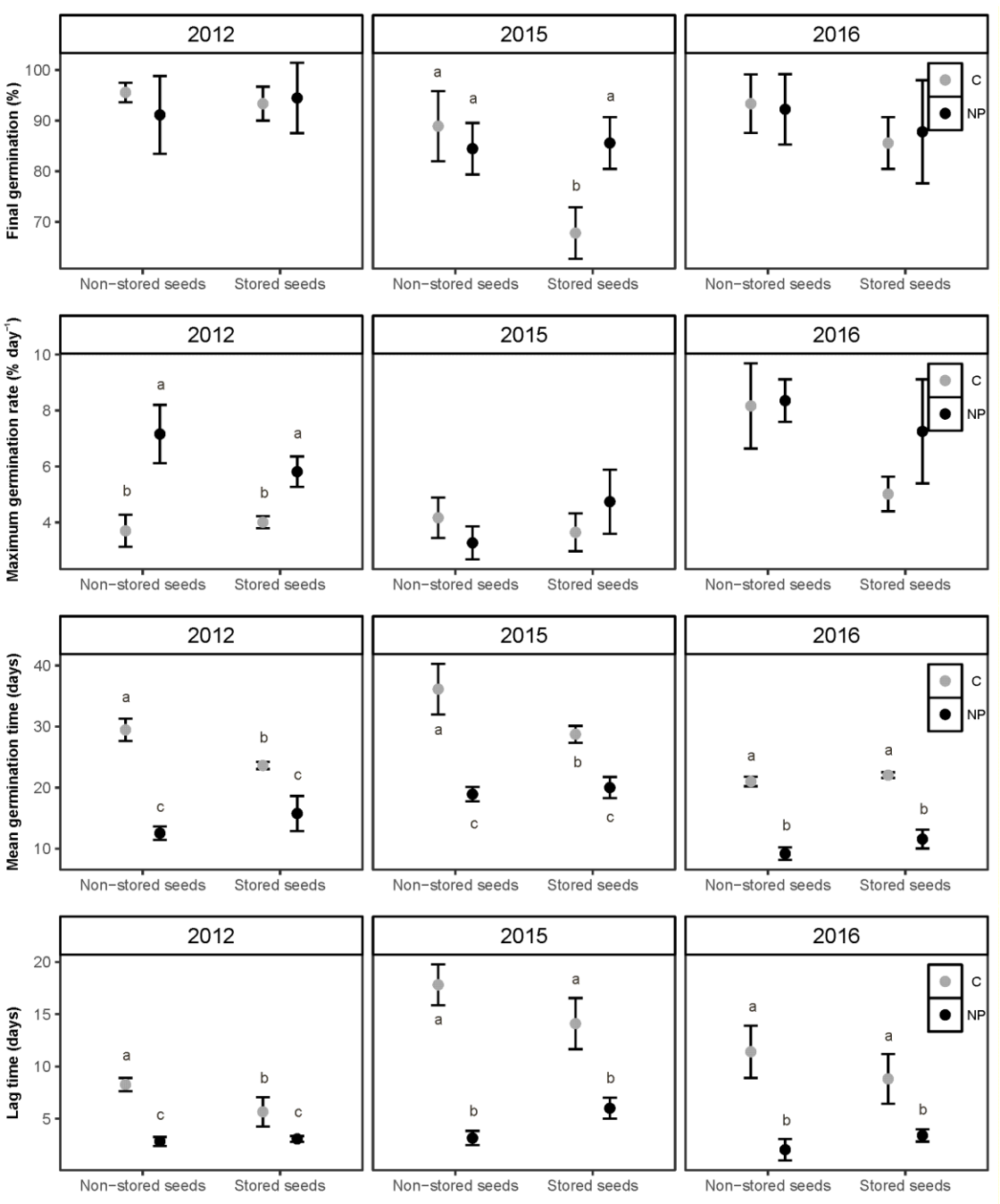


Figure 2. Mean values (±SE) of germination parameters of seeds exposed to natural priming (NP, black circle) and control seeds (C, gray circle), stored or non-stored, for *Cymbopetalum baillonii* collected in three years (2012, 2015, and 2016). The storage period in closed glass jars at 15 °C was 30 days. Lowercase letters indicate statistical differences within each germination parameter for each year (Tukey test, $P < 0.05$).

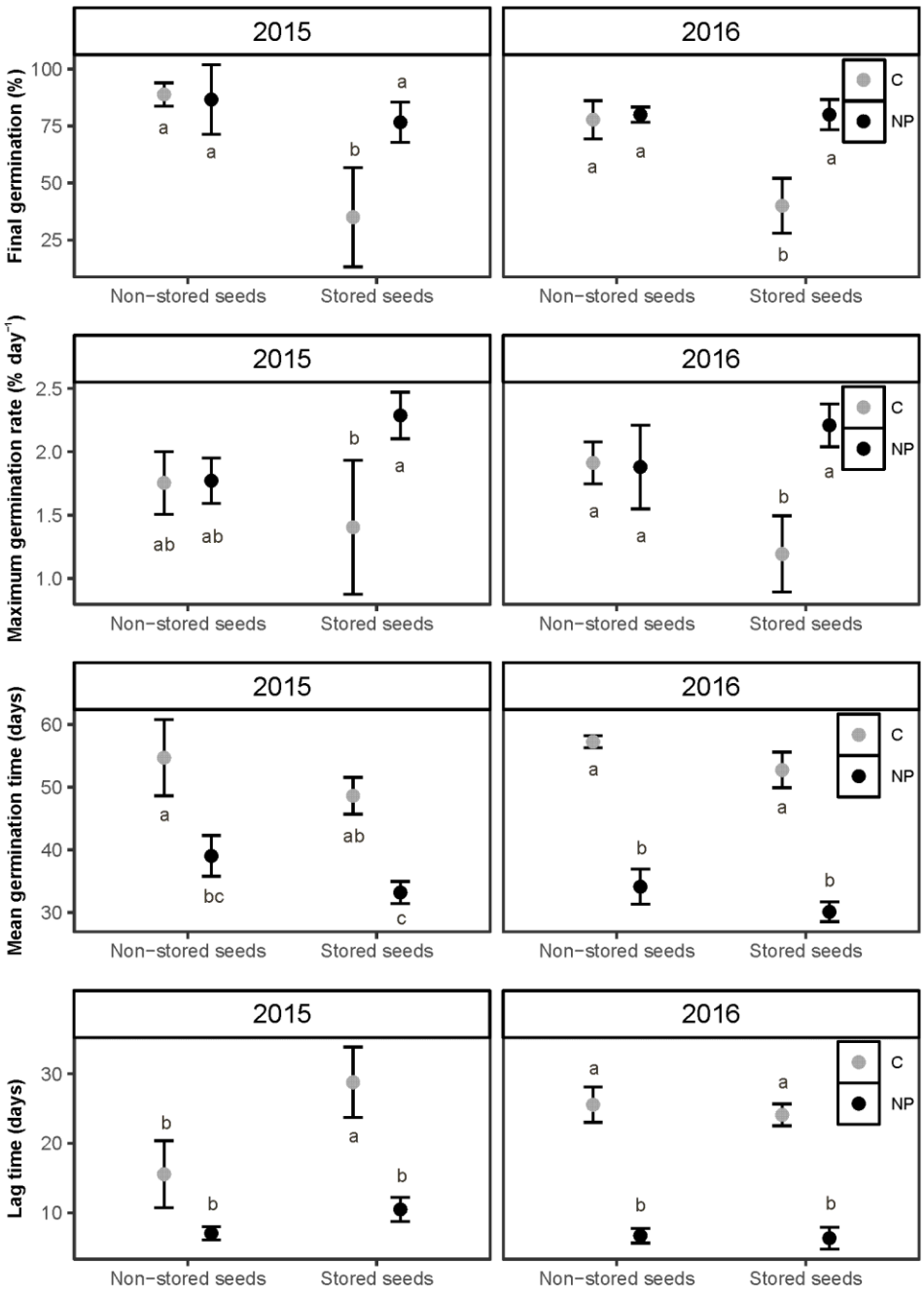


Figure 3. Mean values (±SE) of germination parameters of seeds exposed to natural priming (NP, black circle) and control seeds (C, gray circle), stored or non-stored, for *Magnolia perezfarrerae* collected in two years (2015 and 2016). The storage period in closed glass jars at 15 °C was 25 days. Lowercase letters indicate statistical differences within each germination parameter for each year (Tukey test, $P < 0.05$).

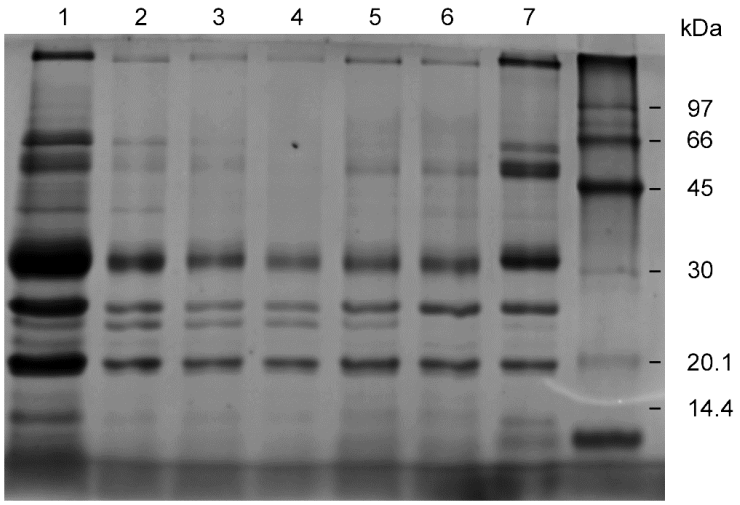


Figure 4. Total soluble protein profile of *Cymbopetalum baillonii* seeds, untreated (control) or treated with natural priming. For both cases, seed samples were obtained from 180-day-old stored seeds at 15 °C, and along with this initial stored seed sample, or P1, two additional samples were obtained from these stored seeds, but they were first placed in germination conditions, so we took seeds with a broken coat, or P2, and seeds with a protruding radicle, or P3. In addition, non-stored, non-treated seeds were evaluated. For each treatment, an equal amount of protein was loaded (15 µg) in each lane of a 12% SDS-PAGE gel. The molecular mass (kDa) is indicated next to the molecular marker lane (extreme right). Lanes: (1) stored control seeds at P1, (2) stored natural priming seeds at P1, (3) stored control seeds at P2, (4) stored natural priming seeds at P2, (5) stored control seeds at P3, (6) stored natural priming seeds at P3, and (7) non-stored control seeds.

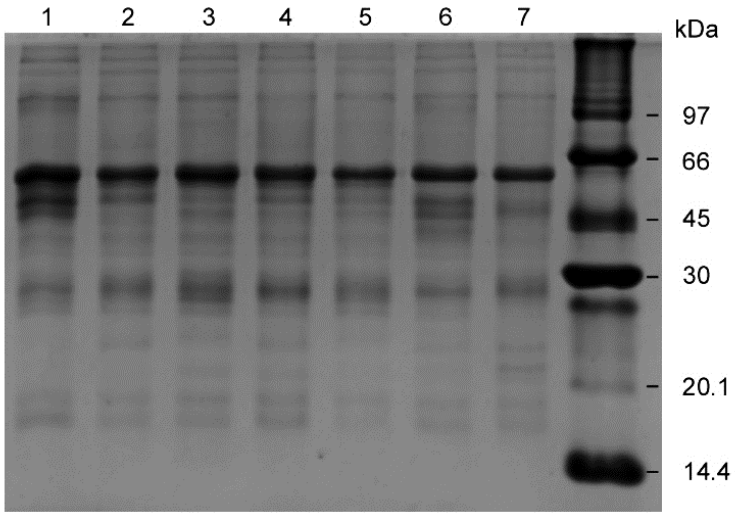


Figure 5. Total soluble protein profile of *Chamaedorea glaucifolia* seeds, untreated (control) or treated with natural priming. In both cases, stored seed samples were obtained after 426 days of storage. For each treatment, an equal amount of protein was loaded (15 µg) in each lane of a 12% SDS-PAGE gel. The molecular mass (kDa) is indicated next to the molecular marker lane (extreme right). Lanes: (1) non-stored control seeds, (2) non-stored natural priming / buried seeds during 18 days, (3) non-stored natural priming / buried seeds during 68 days, (4) stored control seeds at 23 °C, (5) stored natural priming seeds at 23°C, (6) stored control seeds at 15 °C, and (7) stored natural priming seeds at 15 °C.

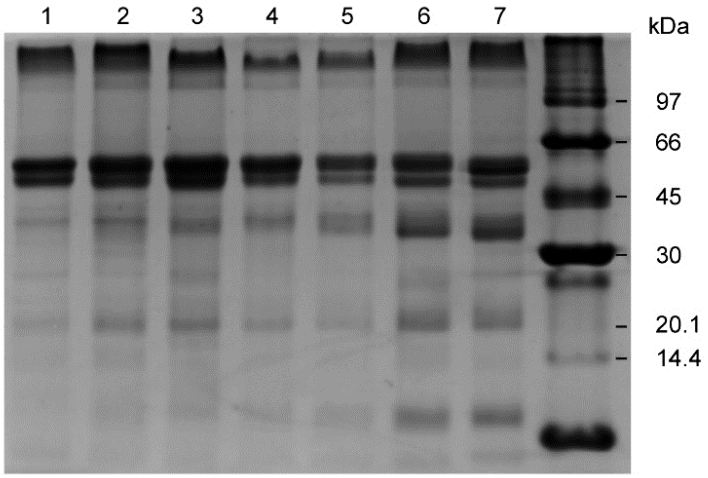


Figure 6. Total soluble protein profile of *Damburneya coriacea* seeds, untreated (control) or treated with natural priming either in 2015 or 2016. For 2016, stored seed samples were obtained after 180 days of storage. For each treatment, an equal amount of protein was loaded (15 µg) in each lane of a 12% SDS-PAGE gel. The molecular mass (kDa) is indicated next to the molecular marker lane (extreme right). Lanes: (1) 2015 non-stored control seeds, (2) 2015 non-stored natural priming seeds, (3) 2016 non-stored control seeds, (4) 2016 non-stored natural priming seeds, (5) stored control seeds at 15 °C, (6) stored control seeds at 5 °C, and (7) stored natural priming seeds at 15 °C.

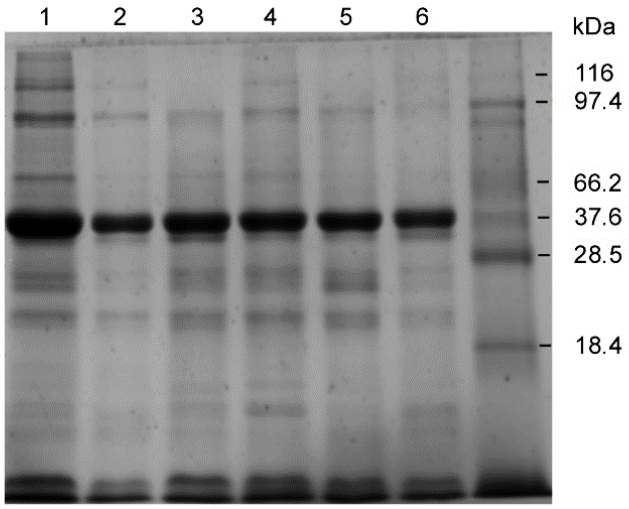


Figure 7. Total soluble protein profile of *Magnolia perezfarrerae* seeds, untreated (control) or treated with natural priming in 2016. In addition, samples were obtained from seeds that remain below litter at the same site and during the same period as natural priming seeds. Stored seed samples were obtained after 25 days of storage. For each treatment, an equal amount of protein was loaded (15 µg) in each lane of a 12% SDS-PAGE gel. The molecular mass (kDa) is indicated next to the molecular marker lane (extreme right). Lanes: (1) non-stored control seeds, (2) stored control seeds at 5 °C, (3) stored natural priming seeds at 5 °C, (4) non-stored natural priming seeds, (5) stored natural priming seeds at 15 °C, and (6) seeds from below litter.

## DISCUSIÓN GENERAL

Los esfuerzos hechos en la investigación sobre semillas recalcitrantes e intermedias, como el realizado en esta tesis, redundan en una mejor comprensión de su papel ecológico, lo que a su vez incentiva la conservación de estas especies. Recordemos que, al igual que las zonas de estudio de esta tesis, los remanentes forestales en el país mantienen una presión antropogénica constante que resulta en una amenaza inmediata más allá del potencial efecto del cambio climático. Al respecto, los resultados obtenidos aquí permiten 1) que se profundice en conocer la respuesta de las semillas de las especies estudiadas tanto al estrés hídrico como al acondicionamiento, y 2) que se establezca en primera instancia un esquema de manejo que mantenga a las semillas con niveles de viabilidad y vigor suficientes durante un mayor tiempo.

De manera general, pese a las diferencias encontradas, todas las especies evaluadas tuvieron semillas sensibles a la desecación y longevidad corta. Estas semillas presentaron características físicas (volumen de 150−400 $mm^3$ y peso de 0.16−0.62g), de contenido de humedad (20−75 % base seca) y de rapidez de germinación (tiempo medio de germinación de 20−60 días) que corresponden con características morfológicas y funcionales de especies de longevidad corta (Dickie y Pritchard, 2002). Además, los valores tanto de $WC_{50}$ (11−60%) como del tiempo en el que se alcanza este valor (9−150 días) para todas las especies salvo para *Chamaedorea glaucifolia*, corresponden al de semillas recalcitrantes. Estas características son adaptaciones a las condiciones climáticas de los ecosistemas tropicales (Dickie y Pritchard, 2002; Hamilton et al., 2013), razón por la que estos sitios albergan la mayor diversidad de especies con semillas recalcitrantes e intermedias. En el bosque tropical perennifolio, tipo de vegetación dominante de Los Tuxtlas, estas especies llegan a representar el 50% de la diversidad arbórea (Tweddle et al., 2003; Hamilton et al., 2013), Si bien esta diversidad se reduce conforme el clima del bosque se torna más seco (Dickie y Pritchard, 2002), la presencia de estas especies no deja de ser un punto en común en los bosques tropicales de México. Por ejemplo, cuatro de las cinco especies estudiadas habitan en la zona boscosa de la localidad Ocuilapa, sitio que, pese a ser más seco en términos de precipitación pluvial anual comparado con Los Tuxtlas (1100 mm y 4725 mm, respectivamente), resulta fundamental como zona de amortiguamiento del vecino bosque tropical perennifolio de El Ocote (Escobar-Ocampo y Ochoa-Gaona, 2007). En realidad, la vegetación de Ocuilapa es una transición entre la vegetación de El Ocote y el bosque tropical caducifolio de la Depresión Central de Chiapas.

Los métodos de evaluación del comportamiento en almacén de semillas con probable longevidad corta necesitan ser comprensiblemente rápidos y que a su vez brinden confiabilidad en los resultados. Al respecto, existen metodologías que hacen una estimación teórica de la sensibilidad a la desecación en las semillas, por ejemplo, con el cálculo del cociente entre la masa de la cubierta seminal con respecto a la masa total de la semilla (Daws et al., 2006; Gold y Hay, 2014). Este método resulta útil en especies de las que no se tiene información previa y que además se dispone de poco material biológico, tal y como ocurre en México. No obstante, una evaluación práctica como la hecha en este estudio, que además toma en cuenta la temperatura de almacenamiento entre otras variables, incrementa la confiabilidad en los resultados obtenidos. Con éstos, pueden realizarse otros análisis cuyos resultados coadyuven al conocimiento ecológico y a los esfuerzos de conservación de estas especies. Por ejemplo, aquí se analizó la relación de los datos morfológicos y funcionales de las semillas con la información sobre las características ambientales del hábitat de las especies. Los resultados obtenidos sugirieron que el clima local, junto con las características propias de la especie, influyen en la sensibilidad de la semilla a la desecación. Esta relación, bien documentada en especies de diferentes regiones en el planeta (Dussert et al., 2000; Oyerinde et al., 2023), enfatiza la vulnerabilidad del gremio funcional al cual pertenecen las especies bajo estudio ante los potenciales efectos perjudiciales del cambio climático. Finalmente, la consistencia de los resultados obtenidos, especialmente en las especies en las que se evaluaron años diferentes, sugiere que la metodología que se siguió en este estudio puede seguirse en nuevos estudios.

De todas las especies, *Chamaedorea glaucifolia* fue la que presentó la menor sensibilidad a la desecación ($WC_{50}$ de 4.6 % y un tiempo para alcanzarlo de 426 días). Hemos discutido el papel de su estructura anatómica con respecto a su respuesta funcional. Muchas especies de palmas presentan latencia de tipo morfofisiológico (Orozco-Segovia et al., 2003; Baskin y Baskin, 2014). *Chamaedorea glaucifolia* tuvo un alto porcentaje de germinación después de la recolecta, al igual que las demás especies estudiadas, aunque el hecho de que su *lag time* (tiempo de inicio de la germinación) sea mayor que el de las semillas almacenadas sugiere una latencia marginal. Las semillas de longevidad corta usualmente no presentan latencia, si bien hay excepciones como en *Omphalea oleifera* (Sánchez-Coronado et al., 2007), especie que coexiste con *Cymbopetalum baillonii* en Los Tuxtlas. La presencia de latencia en *O. oleifera* depende del año de producción de las semillas. Por esta razón, esta característica funcional debe considerarse como un factor que

puede enmascarar el grado de tolerancia a la desecación de las semillas (Rodríguez et al., 2000; Sánchez-Coronado et al., 2007) y, como veremos más adelante, la respuesta de las semillas a los tratamientos de vigorización. La latencia es una característica funcional de la semilla que incrementa las probabilidades de germinación y de establecimiento de la plántula resultante (Baskin y Baskin, 2001; Fenner y Thompson, 2005). *Omphalea oleifera* permanece viable durante varios meses en el suelo, tanto por una resistencia anatómica de la semilla a la desecación, como por la presencia de latencia fisiológica en algunos años de producción (Sánchez-Coronado et al., 2007). De esta forma, resulta interesante evaluar a profundidad la relación entre el nivel de latencia de la semilla tanto con su longevidad como con su respuesta al acondicionamiento.

El acondicionamiento natural incrementó el vigor de las semillas de todas las especies. Un aumento en la rapidez de la germinación, i.e., *lag time* y tiempo medio de germinación más cortos respecto al control, fue el punto en común entre la mayoría de las especies estudiadas. Los cambios registrados en los perfiles de proteínas totales, si bien no son numerosos, podrían estar vinculados con el avance del proceso germinativo (memoria hídrica) en las semillas acondicionadas. Estos resultados sugieren que, por lo menos para las especies bajo estudio, el mecanismo por el cual opera el acondicionamiento a nivel fisiológico sería el mismo que el de las semillas de longevidad larga. Es decir, en el acondicionamiento natural, durante la permanencia de las semillas en el suelo, se inducen los procesos pregerminativos, pero dado que la hidratación se interrumpe antes de superar el *lag time* de cada especie, no ocurre la protrusión de la radícula, evento conocido como germinación (Orozco-Segovia y Sánchez-Coronado, 2009). Ya se ha propuesto que entre semillas tolerantes y no tolerantes a la desecación los procesos de desarrollo y de germinación son similares, es decir, los cambios estructurales, bioquímicos y moleculares mantienen un paralelismo entre sí; la diferencia entre ambas categorías estriba en el grado de desarrollo alcanzado por la semilla al momento de la dispersión (Obroucheva et al., 2017). De alguna manera, pese a su elevada actividad metabólica, la germinación tanto de semillas intermedias como recalcitrantes puede estar sujeta a un aporte hídrico extra que cubra su alta demanda de agua para una rápida germinación (Vázquez-Yanes y Orozco-Segovia, 1993; Pammenter et al., 1994). Cuando la disponibilidad hídrica se limita, como en la etapa final del acondicionamiento natural, la germinación de la semilla se evita, pero cuando las condiciones vuelven a ser óptimas la germinación no comienza desde cero, pues el avance conseguido durante la permanencia en el suelo se manifiesta en una mejora en el vigor germinativo a manera de memoria hídrica (Dubrovsky, 1996). Este efecto se presentó en las

semillas de todas las especies bajo estudio, pero no fue claro en *Chamaedorea glaucifolia*. En esta especie el efecto del acondicionamiento se manifestó solo después de 767 días en almacén, al disminuir el *lag time* respecto al control. El período en el suelo afecta no solo los procesos fisiológicos propios de la etapa de semilla, sino también la etapa de plántula, tal y como sucede en *Cymbopetalum baillonii* (Becerra-Vázquez et al., 2020).

Además de su acción favorecedora sobre el proceso germinativo de las semillas en las especies bajo estudio, el acondicionamiento tuvo el potencial adicional de inducir cambios que indujeron un incremento en su longevidad, como se observó en *Cymbopetalum baillonii* (2015) y *M. perezfarrerae* (2015 y 2016). El mantenimiento de un mayor vigor en las semillas acondicionadas respecto al control después del almacenamiento es un resultado destacable, dado que son semillas sensibles a la desecación. Además, se ha enfatizado que el acondicionamiento puede producir una disminución en la tolerancia a la desecación (Bruggink y van der Toorn, 1995; Chen y Arora, 2012). Lo anterior debido a que compuestos como dehidrinas y HSP (*heat-shock protein*), sintetizados durante la parte final del desarrollo de la semilla y que participan en la protección de membranas y proteínas, se degradan durante el proceso normal de germinación (Chen y Arora, 2012). Sin embargo, numerosos trabajos muestran que estas sustancias se sintetizan durante los tratamientos de acondicionamiento (Bruggink y van der Toorn, 1995; González-Zertuche et al., 2001; Gamboa de Buen et al., 2006). Esto se acentúa en los tratamientos de acondicionamiento que utilizan hormonas como el ácido abscísico (ABA), o bien sales como el cloruro de sodio (NaCl) o agentes osmóticos como el polietilenglicol (PEG). Por lo tanto, se ha planteado que el acondicionamiento *per se* conlleva un mecanismo de inducción de resistencia al estrés, pues las interrupciones tanto del proceso germinativo como de la protrusión de la radícula constituyen eventos que generan estrés hídrico (Chen y Arora, 2012). La permanencia de las semillas en el suelo también representa un período de estrés para ellas, debido a la variabilidad en la disponibilidad de agua en la matriz del suelo y al potencial mátrico de las semillas (Long et al., 2015). Finalmente, en los perfiles de proteínas se hallaron bandas con tamaños similares a dehidrinas, HSP o enzimas. Sin embargo, el análisis no establece con precisión la naturaleza de estas proteínas, lo que plantea la necesidad de realizar a futuro otro tipo de análisis bioquímicos (e.g., electroforesis en dos dimensiones) o bien estudios a nivel molecular (e.g., análisis proteómicos, transcriptómicos y genómicos) que revelen a detalle los cambios en las semillas acondicionadas.

Como se ha señalado, el grado de madurez de la semilla varía en función de ciertos procesos bioquímicos y moleculares que ocurren en ella durante la etapa de maduración y secado. En las semillas recalcitrantes e intermedias, pese a su variabilidad intra e interespecífica, en general los cambios relacionados con la maduración son pocos en comparación con los que ocurren en las semillas ortodoxas (Kermode y Finch-Savage, 2002). Tanto las semillas ortodoxas como las recalcitrantes e intermedias tienen altos niveles metabólicos y respiratorios mientras permanecen en la planta madre. En las semillas ortodoxas ocurre un control genético hormonal que produce la disminución en el nivel metabólico y respiratorio, así como una reducción drástica en su contenido de humedad (hasta alrededor de10%; Kermode y Finch-Savage, 2002; Bewley et al., 2013). Estos cambios evitan que estas semillas germinen, pese a que el embrión es germinable al final de la etapa de histodiferenciación, e inducen una serie de modificaciones relacionadas con la resistencia a la desecación y con la longevidad. En contraste, en las semillas recalcitrantes e intermedias la dispersión ocurre cuando su nivel metabólico y respiratorio es elevado, razón por la que el tiempo hacia su germinación es corto comparado con las ortodoxas (Vázquez-Yanes y Orozco-Segovia, 1993; Berjak y Pammenter, 2013). Sin embargo, el acelerado metabolismo de estas semillas causa que continúen incrementando su biomasa aun y cuando no reciban el aporte hídrico externo necesario para la culminación de la germinación (Pammenter et al., 1994; Finch-Savage y Blake, 1994). Este hecho junto con la variabilidad microambiental en la humedad, temperatura, entre otros factores, posibilitarían que durante el período de enterramiento ocurra un cierto grado de maduración adicional en la semilla. Este efecto probablemente haya ocurrido en las semillas acondicionadas de todas las especies. El período de enterramiento hizo que las semillas acondicionadas se sembraran después que el control tanto antes como después del almacenamiento. En el control la germinación se evaluó alrededor de dos días después de haberse realizado la recolecta. Pese a esto, las semillas acondicionadas manifestaron igual o mayor vigor que el control al inicio y/o posterior al almacenamiento. Solo las semillas acondicionadas de *Cymbopetalum baillonii* de la recolecta 2015 tuvieron un contenido de humedad menor que el control, es decir, se deshidrataron después del período de enterramiento. En esta especie se ha reportado un incremento en el porcentaje de germinación después de una ligera deshidratación de las semillas (Rodríguez et al., 2000). Esto se explica por el hecho de que la deshidratación *per se* es una señal que acelera la germinación de las semillas, permitiendo que la radícula-raíz acceda a otras fuentes de agua (Berjak y Pammenter, 2013). No obstante, este efecto no fue observado en las semillas de *Cymbopetalum*

*baillonii* de la recolecta 2015. Por otra parte, durante la maduración de las semillas, cuando el nivel de deshidratación se aproxima al contenido crítico de humedad de la semilla, ésta disminuye su tasa metabólica y respiratoria, lo cual conlleva a un mayor tiempo para la expresión de compuestos relacionados con la reparación de daños celulares (Kranner et al., 2010). Por ejemplo, se han observado modificaciones en el sistema antioxidante en semillas recalcitrantes que permanecieron enterradas temporalmente (Marques et al., 2017). Estos reportes plantean posibles enfoques para estudios futuros en las especies abordadas aquí.

## CONCLUSIONES

- Pese a las variaciones encontradas todas las semillas de las especies estudiadas, a través de su respuesta a la deshidratación durante el almacenamiento, tuvieron valores de $WC_{50}$ y de T $WC_{50}$ que corresponden a semillas con sensibilidad a la desecación.
- Todas las especies manifestaron longevidad corta en sus semillas. Cada una de ellas respondió diferencialmente al período de almacén dependiendo de la temperatura y/o el año de recolecta.
- Si bien las semillas de *Chamaedorea glaucifolia* se mantuvieron viables después de 767 días, tanto su latencia inicial como sus características anatómicas influyeron en dicha longevidad (además de influir en su respuesta al acondicionamiento).
- Se concluye que las semillas de *Chamaedorea glaucifolia* son de tipo intermedio y para el resto de las especies estudiadas son de tipo recalcitrante.
- Existe una relación entre la longevidad de la semilla con sus características germinativas, morfológicas y anatómicas, con la época en la que son dispersadas y con el clima de su hábitat. Esta relación debe tomarse en cuenta tanto para el manejo de las semillas con fines de conservación-restauración, como para enfatizar la connotación ecológica en la historia de vida de estas plantas.
- El acondicionamiento natural mejoró el vigor germinativo de las semillas de las especies estudiadas. Se concluye que el efecto fisiológico del tratamiento es similar al que presentan las semillas de tipo ortodoxo.

- El perfil de proteínas totales en las semillas acondicionadas de las especies estudiadas correspondería principalmente a cambios pregerminativos, como los observados en semillas ortodoxas. Se necesitan análisis futuros a nivel bioquímico y molecular.
- Dado el mayor vigor de las semillas acondicionadas después de un período de almacén, es posible que en ellas hayan ocurrido, además de los cambios relativos a la germinación, procesos que implican un aumento de la madurez, o bien, la expresión de compuestos relacionados con la resistencia al estrés hídrico.

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