



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

**INSTITUTO DE ECOLOGÍA
ECOLOGÍA**

**GERMINACIÓN DE ESPECIES
DE LA FAMILIA BROMELIACEAE:
ASPECTOS ECOFISIOLÓGICOS Y ANATÓMICOS**

TESIS

**QUE PARA OPTAR POR EL GRADO DE:
DOCTORA EN CIENCIAS**

PRESENTA:

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Presente

Me permito informar a usted que en la reunión ordinaria del Comité Académico del Posgrado en Ciencias Biológicas, celebrada el día 25 de febrero de 2013, se aprobó el siguiente jurado para el examen de grado de DOCTORA EN CIENCIAS de la alumna MONTES RECINAS SARAI con número de cuenta 92380633 con la tesis titulada: "GERMINACIÓN DE ESPECIES DE LA FAMILIA BROMELIACEAE. ASPECTOS ECOFISIOLÓGICOS Y ANATÓMICOS", realizada bajo la dirección de la DRA. ALMA DELFINA LUCIA OROZCO SEGOVIA:

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

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COORDINADORA DEL PROGRAMA

c.c.p. Expediente de la interesada.



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Resumen

Bromeliaceae es una familia Neotropical, cuyas especies habitan en ambientes méxicos, semi-méxicos o xéricos. El hábito de crecimiento de las bromelias puede ser terrestre, epifito o rupícola. La capacidad de las bromelias de colonizar diversos ambientes se atribuye a que éstas exhiben adaptaciones particulares a la aridez. Dentro de estas adaptaciones (descritas en estadios adultos) se encuentran el metabolismo ácido de las crasuláceas (CAM) y la presencia de escamas absorbentes de agua y nutrientes. La presente investigación abordó aspectos de la adaptación a la aridez en las fases tempranas del ciclo de vida de cuatro especies de bromelias. La pregunta general fue, ¿Qué adaptaciones estructurales y fisiológicas exhiben las semillas de bromelias para poder germinar en ambientes con poca o limitante disponibilidad de agua?

Los resultados de la investigación se presentan en tres capítulos (dos manuscritos y un artículo publicado).

El primer capítulo lo constituye un manuscrito enviado a la revista *Journal of Arid Environment* en el que se muestran los resultados del estudio de la biología de las semillas: fotoblastismo, respuesta al estrés hídrico y morfología-estructura (**The germination of *Hechtia confusa* seeds (Bromeliaceae): photoblastism, water stress and morpho-structure**). *Hechtia confusa* es una especie herbácea terrestre, dioica y endémica de México. Esta especie se distribuye en matorrales xerófilos de los estados de Puebla y Oaxaca; en donde, localmente exhibe una notoria dominancia o co-dominancia. Para llevar a cabo el presente trabajo, en primer término se identificó el requerimiento lumínico para la germinación de sus semillas. Posteriormente, el efecto de eventos de hidratación-deshidratación (en campo y bajo condiciones controladas) y de la exposición a alta temperatura (en semillas secas) sobre su germinación. Adicionalmente, para mejorar el entendimiento de las respuestas fisiológicas de las semillas de *H. confusa* estudiadas, y de observaciones de la dispersión de sus semillas (realizadas incidentalmente en campo), se describió la morfología y estructura de sus semillas. Los resultados más relevantes fueron: las semillas de *H. confusa* fueron quiescentes, fotoblásticas positivas y germinaron en luz blanca, roja y rojo-lejano. Las semillas de *H. confusa* también fueron tolerantes a eventos de hidratación-deshidratación (en campo y bajo condiciones controladas). Las semillas secas

fueron tolerantes a 42 °C, temperatura cercana a la más alta registrada en suelo sombreado de la zona de estudio. Las semillas de *H. confusa* presentaron un endospermo abundante y un embrión lateral pequeño. Una de capas de la cubierta seminal presentó alto contenido de taninos condensados. Los resultados anteriores, sumados a la persistencia de algunas de las semillas dentro de los frutos secos (observada en campo) y de la flotabilidad de las semillas en agua, sugieren que las semillas de *H. confusa*: tienen el potencial de formar bancos de semillas aéreas dentro de los frutos secos, podrían germinar principalmente en la superficie del suelo o a escasa profundidad y que pueden tener como un importante mecanismo de dispersión al agua.

El segundo capítulo lo constituye un artículo publicado en la revista *Plant Ecology* que describe los requerimientos de agua y temperatura para la germinación; así como el efecto de la hidratación discontinua sobre la germinación y supervivencia de las plántulas de *Tillandsia recurvata* (subfamilia Tillandsioideae, **Temperature and water requirements for germination and effects of discontinuous hydration on germinated seed survival in *Tillandsia recurvata* L.**). En este artículo se describen aspectos fisiológicos y estructurales de las semillas de *Tillandsia recurvata*; bromelia epífita con la distribución más amplia en América. Debido a que en esta especie no se presenta una emergencia conspicua del embrión a través de la cubierta seminal, se definieron dos estadios de germinación. El estadio-1 fue identificado por la imbibición de las semillas y la ruptura de la cubierta seminal en la región hypocotilar, y el estadio-2 por el cambio del embrión de no clorofílico a clorofílico. Las semillas de *T. recurvata* fueron colectadas en el matorral xerófilo del “Parque Ecológico de la Ciudad de México”. Los aspectos de la germinación evaluados fueron: 1) capacidad de las semillas de embeberse a partir del vapor de agua e iniciar el proceso de germinación, 2) temperatura requerida para la germinación del estadio-1 y los potenciales osmóticos ($\Psi_{\pi s}$) óptimo y crítico para la germinación en ambos estadios de germinación, 3) sobrevivencia de los embriones en estadio-2 de semillas incubadas en diferentes $\Psi_{\pi s}$ y expuestas a un evento de deshidratación, y 4) pérdida de tolerancia a la deshidratación. Adicionalmente, se obtuvieron imágenes de la estructura de la semilla de *T. recurvata*. Los resultados obtenidos mostraron que las semillas de *T. recurvata* requirieron estar en contacto con agua líquida para poder germinar. El estadio-1 de la germinación presentó un porcentaje de germinación similar a lo

largo del intervalo de temperatura utilizado (15–35 °C). El intervalo de Ψ_{π} que permite la germinación de las semillas de *T. recurvata* en diferentes porcentajes fue de 0 a –0.6 MPa. Por lo que, las semillas de *T. recurvata* requirieron de alta disponibilidad de agua para germinar, a pesar de ser una especie de amplia distribución en regiones semi-áridas. Bajos Ψ_{π} s afectaron la tolerancia a la deshidratación de los embriones a subsecuentes eventos de deshidratación. Finalmente, las semillas de *T. recurvata* exhibieron tolerancia a eventos de hidratación-deshidratación, pero esta tolerancia se redujo notoriamente una vez que el embrión presentó pigmentos fotosintéticos. Los resultados anteriores sugieren que, después de este momento, las lluvias esporádicas al inicio de la época de lluvias podrían afectar negativamente el potencial de reclutamiento de las poblaciones de *T. recurvata*.

En el tercer capítulo lo constituye un manuscrito que será enviado a la revista Journal of Arid Environment, en el que se comparó el efecto del estrés hídrico en la germinación de dos especies de bromelias que crecen en una región semiárida del centro de México (**Effect of water stress on the germination of two bromeliads from a semi-arid environment in Central México**). En éste se evaluó el requerimiento de disponibilidad de agua para la germinación de dos especies con diferente hábito de crecimiento que coexisten en el Valle de Zapotitlán de las Salinas, Puebla. Las especies estudiadas fueron *Hechtia tehuacana* y *Tillandsia circinnatioides*; con hábito terrestre y epifito, respectivamente. Se utilizaron diferentes Ψ_{π} s (0, –0.05, –0.1, –0.5, –1.0 and –1.5 MPa a 25 °C) y para ambas especies se registró la germinación en cada potencial osmótico. Como resultado se obtuvo que la germinación de *H. tehuacana* se redujo significativamente a los –0.05 MPa, pero su germinación todavía ocurrió a –1.0 MPa. Mientras que la germinación de *T. circinnatioides* ocurrió solo hasta los –0.05 MPa. Los resultados anteriores indican que ambas especies requieren de alta disponibilidad de agua para germinar y que, de las dos especies estudiadas, *T. circinnatioides* es la que requiere la mayor disponibilidad de agua. Los resultados obtenidos apoyan la propuesta de que en ambientes áridos, ciertas especies tienen como estrategia para incrementar el éxito en la germinación y en el establecimiento de las plántulas, el que su germinación ocurra solo cuando hay alta disponibilidad de agua. El mayor requerimiento de agua de la especie epifita *T. circinnatioides*, en comparación con la terrestre *H. tehuacana*,

podría tener relación con una disponibilidad de agua más efímera en la corteza de las ramas de los forofitos (árboles hospederos de epifitas), que en el suelo.

Los resultados obtenidos sugieren y/o corroboran que algunas de las adaptaciones de las semillas de las bromelias estudiadas, durante la germinación y el establecimiento de las plántulas en condiciones de aridez son, para el caso de las epifitas: a) una emergencia tardía del embrión, b) la delgadez y transparencia de la cubierta seminal, que permite que el embrión reciba luz, probablemente induciendo la síntesis de los pigmentos fotosintéticos, c) requerimiento de alta disponibilidad de agua para germinar, d) la tolerancia a eventos de hidratación-deshidratación y e) la rápida transformación de las semillas en plántula fotosintéticas. Las características para las especies terrestres son: f) el fotoblastismo positivo, g) la tolerancia a eventos de hidratación-deshidratación, y h) el requerimiento de alta disponibilidad de agua para germinar.

Abstract

Bromeliaceae is a Neotropical family, whose species inhabit mesic, semi-mesic or xeric environments. The grow habit of the bromeliads could be terrestrial, epiphyte or saxicolous. The diversity of habitats that the bromeliads may colonize is attributed to adaptations to arid conditions. Some of these adaptations (identified in adult stages) are the crassulacean acid metabolism (CAM) and modification of the epidermal trichomes in absorbent scales of water and nutrients. This research has focused on adaptive aspects of bromeliads to dry environments in the early stage of their life cycle; through the question, ¿Which structural and physiological adaptations show the seeds of epiphytic and terrestrial bromeliads to germinate in environments with few or intermittent water availability?

The results of this research are presented in three chapters (two manuscripts and an article published):

The first chapter is a manuscript sent to the Journal of Arid Environment. This chapter shows the study of biological aspects of *Hechtia confusa* L. B. Sm seeds (Bromeliaceae Family, Hechtioideae subfamily); as photoblastism, response to water stress and their structure-morphology (**The germination of *Hechtia confusa* seeds (Bromeliaceae): photoblastism, water stress and morpho-structure**). *Hechtia confusa* is a terrestrial herb, dioecious, and endemic species from México. This species is distributed in xeric shrubs of the Puebla and Oaxaca states; where this has a marked local dominance or co-dominance. To carry out this study, we first identified the light requirement for seed germination of *H. confusa* seeds. Then, we studied the effects of hydration-dehydration events (under field and controlled conditions) and exposure of dry seeds to high temperature on seeds germination. Additionally, to improve the understanding of physiological response and observations related with seeds dispersal (made in field) was described the morphology and anatomy of *H. confusa* seeds.

Hechtia confusa seeds were quiescent, positively photoblastic and germinated under white, red and far-red light. These seeds were also tolerant to hydration-dehydration events (under laboratory and field conditions) and to dry heat shock (42 °C, temperature close to highest temperature registered in shaded soil from study area). Within the most relevant results are:

Hechtia confusa seeds exhibited an abundant starchy endosperm and a small lateral embryo. One seed coat layer showed a high content of condensed tannins. The above results, the observation in the field that some of *H. confusa* seeds persist on dry fruits, in addition to their water buoyancy, suggest that these seeds have the potential to establish an aerial seed bank, its germination may occur on soil surface or in beneath depth soil and these may have like an important dispersal mechanism, the water.

The second chapter is an article published in the Plant Ecology journal. This article describes the water and temperature requirements of *Tillandsia recurvata* L. (subfamily Tillandsioideae) seeds to germinate. Further the effects of discontinuous hydration on *T. recurvata* seed germination and seedling survival. **Temperature and water requirements for germination and effects of discontinuous hydration on germinated seed survival in *Tillandsia recurvata* L.**, This article describes physiological and structural aspects of *T. recurvata* seeds. *Tillandsia recurvata* is the epiphytic bromeliad with the wider distribution in the Americas. The absence of radicle emergence in *T. recurvata* seeds resulted in establishment of two stages of germination. The stage-1 was identified by the seed coat broken in the hypocotylar area and the stage-2 by the change from non chlorophyllic embryo to chlorophyllic. The *T. recurvata* seeds were collected in the shrubland of “Parque Ecológico de la Ciudad de México”. We evaluated: 1) whether water vapour can provide a sufficient water source for germination, 2) the temperature required for germination stage-1 and the optimal and critical osmotic potentials for germination in both germination stages, 3) the effect of seed incubation at different osmotic potentials upon survival that undergo subsequent dehydration on survival in stage-2; and 4) the loss of dehydration tolerance during early post-seminal development. In addition, images of *T. recurvata* seed structure were obtained to illustrate its structures. The seeds required being in contact with liquid water for germinate. Germination stage-1 of *T. recurvata* seeds was rather similar across the tested temperature range (15–35 °C). The interval of osmotic potential facilitating both germination stages was from 0 to –0.6 MPa. So, *T. recurvata* seeds require high water availability to germinate, in spite that this specie is widely distributed in semi-arid regions. The osmotic potential during germination affected the tolerance of the chlorophyllic embryos to subsequent dehydration. Although germinated seeds displayed dehydration tolerance, this tolerance decreased in seeds with chlorophyllic embryos. These results suggest the sporadic rainfall events at the beginning

of the rainy season may negatively affect the recruitment of new individual to the *T. recurvata* populations.

The third chapter is a manuscript to be sent to the Journal of Arid Environment, in this study we compared the effect of water stress on seeds germination of two bromeliad species that growth in a semi-arid region in central Mexico (**Effect of water stress on the germination of two bromeliads from a semi-arid environment in Central México**). The species studied were the terrestrial *Hechtia tehuacana* and the epiphytic *Tillandsia circinnatioides* that coexist in the Zapotitlán Salinas Valley, México. The objective of this research was to assess the effect of the following osmotic potentials ($\Psi_{\pi s}$) on germination at 25 °C: 0, -0.05, -0.1, -0.5, -1.0 and -1.5 MPa. The germination of *H. tehuacana* was significantly reduced at -0.05 MPa, even though seed germination continued down to -1.0 MPa, whereas the germination of *T. circinnatioides* occurred only to -0.05 MPa. These results reveal that both species require high water availability to germinate and that *T. circinnatioides* showed the higher water requirements of the two studied species. The requirement of high water availability for germination in the studied species supports the proposal of this response as a strategy to survive in arid environments by improving the probability of achieving seed germination and seedling establishment. The higher water requirement of the epiphytic species, *T. circinnatioides*, may be related to the additional ephemeral water availability on tree branches than in the soil.

Results obtained lead to propose that some of the structural and physiological bromeliads seed characteristics as adaptations to germinate and seedlings establishment in conditions of water unavailability are, for epiphytic species: a) germination with scanty and late embryo emergency, b) a thin seed coat that allows the passage of light, c) the requirement of high water availability to germinate, d) tolerance to hydration-dehydration events, e) a fast transformation of embryo into chlorophyllic. The characteristics for terrestrial species are: f) positive photoblastism, g) tolerance to hydration-dehydration events, and also to high temperatures, and h) the requirement of high water availability to germinate.

Introducción general

Las bromelias

La familia Bromeliaceae (Juss.) forma parte de las angiospermas monocotiledóneas del orden Poales (APG, 2003). Es una familia con cerca de 56 géneros y aproximadamente 2920 especies (Luther, 2002); que se restringen a América (Baensch y Baensch, 1994), con excepción de *Pitcairnia feliciana* (A. Chev.) Harms & Mildbr., especie que habita en África (Porembski y Bartholott, 1999). Las bromelias se distribuyen en diferentes tipos de vegetación, desde el nivel del mar hasta los 4000 m snm (Smith y Downs, 1974; Benzing, 2000) y habitan en ambientes méxicos, semi-méxicos o xéricos (Varadarajan y Gilmartin, 1987).

Las bromelias son plantas generalmente herbáceas, acaules¹ y con hojas dispuestas a manera de rosetas; pueden ser terrestres, epifitas o rupícolas. Tradicionalmente, la familia se dividía en tres subfamilias (Martin, 1994): Pitcairnioideae con semillas aladas, Tillandsioideae con semillas con apéndices plumosos y Bromelioideae con frutos tipo bayas (Smith y Downs, 1974). Aunque por los estudios recientes, basados en secuencias nucleotídicas de cloroplasto (*ndhF*), en la actualidad para la familia Bromeliaceae se consideran ocho subfamilias: Pitcairnioideae, Puyoideae, Navioideae, Hechtioideae, Lindmanioideae, Brocchiniodeae, Bromelioideae y Tillandsioideae (Givnish *et al.*, 2007).

Al igual que las familias Orchidaceae y Araceae, la familia Bromeliaceae contiene gran número de especies con hábito epifito (Kress, 1986). Las bromelias epifitas colonizan especialmente las regiones de neblina y los bosques lluviosos. Aunque, también se les encuentra en regiones costeras secas y cálidas (*e.g. Tillandsia straminea, T. purpurea*). En desiertos crecen sobre cactus, en regiones pantanosas sobre mangles y en las alturas próximas al límite de las heladas sobre rocas desnudas. La mayor riqueza de especies se encuentra entre los 800 y los 2500 m snm (Baensch y Baensch, 1994).

¹ Acaule se refiere a las plantas con tallo tan reducido que parecen no tener tallo.

Aspectos fisiológicos de las bromelias

El hábito epifito de muchas de las bromelias, la diversidad de ambientes que colonizan (Benzing, 2000) y la importancia económica de algunas de sus especies, como la piña *Ananas comosus* (L.) Merr, han promovido el interés y estudio de sus adaptaciones a los distintos ambientes en los que habitan. De los hallazgos sobre aspectos morfo-fisiológicos propios de las bromelias se pueden mencionar los siguientes:

1) La presencia de escamas foliares (también conocidas como tricomas absorbentes) en la superficie de sus hojas (Varadarajan y Gilmartin, 1987). Las escamas foliares varían en complejidad y función entre las distintas subfamilias de Bromeliaceae (Benzing, 1977). En la subfamilia Pitcairnioideae las escamas foliares reducen la incidencia de luz y la evapotranspiración; mientras que en las especies epifitas de la subfamilia Tillandsioideae alcanzan una notable especialización en la captura de agua y nutrientes a partir de vapor de agua (Martin, 1994; Benzing, 2000).

2) Cuando las hojas de las especies con escamas foliares captadoras de agua y nutrientes (característica propia de *Tillandsia* spp.) se humedecen, se reduce su intercambio de CO₂ (Martin y Schmitt, 1989; Martin, 1994).

3) Muchas de las especies están adaptadas a sombra completa o parcial, aunque pueden crecer expuestas a luz directa (Martin, 1994).

4) Su fotosíntesis es óptima cuando la temperatura del día es cálida y la temperatura de la noche es fría (Martin, 1994).

5) Aproximadamente dos terceras partes de las bromelias estudiadas presentan el metabolismo ácido de las crasuláceas (CAM; Martin, 1994), ya sean especies terrestres (Skillman *et al.*, 1999) o epifitas (Martin y Schmitt, 1989; Martin, 1994), y pueden exhibir metabolismo CAM estricto o facultativo (Freschi *et al.*, 2010).

6) Pueden utilizar el CO₂ producto de la respiración, a través de su reciclamiento vía CAM (CAM_{idling}), especialmente en condiciones de estrés (Martin, 1994; Freschi *et al.*, 2010).

7) El potencial hídrico de los tejidos de las especies estudiadas raramente excede -1.0 MPa (Martin, 1994).

Los estudios ecofisiológicos sobre bromelias se ha realizado principalmente en *Ananas comosus* (Aragón *et al.*, 2012) y en las especies epifitas (*Tillandsia* spp., Castro-Hernández *et al.*, 1999). Los estudios de las especies epifitas se han centrado en identificar los factores que determinan: 1) su preferencia por forofitos¹ (ter Steege y Cornelissen 1989; Zimmerman, 1992; Callaway *et al.*, 2001; Callaway *et al.*, 2002; Muñoz *et al.*, 2003; Mehlreter *et al.*, 2005; Martínez-Meléndez *et al.*, 2008; Escutia, 2009; Vergara-Torres *et al.*, 2010) y 2) su distribución estratificada en el dosel (Graham y Andrade, 2004; Winkler *et al.*, 2005; Martínez-Meléndez *et al.*, 2008; Escutia, 2009).

Las semillas y la germinación de las bromelias

La biología reproductiva de las bromelias se consideraba un aspecto poco conocido para el grupo (Benzing, 2000). No obstante, recientemente se han realizado varios estudios sobre polinización y fenología floral, dentro de los que se pueden mencionar los realizados en *Werauhia gladioliflora* (Cascante-Marín *et al.*, 2005; 2009) y *Hechtia schottii* (Ramírez-Morillo *et al.*, 2008). En germinación, destacan los estudios realizados en *Dyckia encholirioides* (Pompelli *et al.*, 2006), *Tillandsia* sp, *Guzmania* sp, *Catopsis* sp, *Werauhia* sp (Cascante-Marín *et al.*, 2009), *Tillandsia eizii* (Toledo-Aceves y Wolf, 2008), *T. brachycaulos*, *T. elongata* (Graham y Andrade, 2004), *T. flexuosa* (Bader *et al.*, 2009), *Catopsis sessiliflora* (Wester y Zotz, 2011), *Alcantarea imperiales*, *Pitcairnia flammea*, *Vriesea heterostachys*, *Vriesea penduliflora* (Pereira *et al.*, 2009), *Pitcairnia albiflos* (Pereira *et al.*, 2010), *Tillandsia* spp. (Sosa-Luría, 2012).

Los estudios de la germinación de semillas de bromelias han permitido conocer que las especies epifitas *Catopsis sessiliflora* y *Tillandsia* spp. no presentan latencia² y no forman bancos de semillas³ (Winkler *et al.*, 2005). De la información contenida en la base de datos del

¹ Forofito es aquel árbol o arbusto hospedero de epifitas.

² Latencia es una o varias características morfológicas y/o fisiológicas de las semillas que impiden su propia germinación.

³ El banco de semillas (o retraso en la germinación) implica un grupo de semillas que no germinan y persisten en el suelo hasta por varios años. Los bancos de semillas se proponen como estrategias de supervivencia ante condiciones ambientales impredecibles y como manera de evitar altas densidades de plántulas.

Kew, Royal Botanical Garden (<http://www.kew.org>) se puede extraer que de las 103 especies reportadas, 17 son ortodoxas¹, 26 probables ortodoxas y tres intermedias. La longevidad de las semillas de bromelias es variada. Las semillas de *Billbergia elegans* -epífita de bosques tropicales de Brasil - son viables durante 18 meses, las de *Neoregelia concentrica* – terrestre o epífita de bosques tropicales de Brasil (con hojas dispuestas a manera de tanque para almacenamiento de agua) - durante 19 meses, las de *Puya* sp - taxón terrestre de Sudamérica - hasta por 30 meses (Benzing, 2000) y las de *Pseudananas sagenarius* - especie terrestres de pastizales y bosques tropicales de Brasil - hasta por 36 meses (Vieira y Silveira, 2010).

Los estudios realizados sobre la temperatura a la que germinan las semillas de las bromelias indican que éstas germinan a la temperatura que prevalece en su ambiente de origen. Las especies de regiones tropicales y subtropicales germinan entre los 20 y 30 °C, pero no germinan por debajo de los 15 °C. Mientras que la germinación de *Puya berteroniana*, especie de los Andes, declina a temperaturas superiores a los 15 °C (Benzing, 2000). Para las especies estudiadas en condiciones semi-controladas (a temperatura constante entre lo 20–25 °C y con luz blanca) reportadas en la base de datos del Kew, Royal Botanical Garden los porcentajes de germinación son de 75–100 %. Sin embargo, los estudios de germinación en condiciones naturales reportan bajos porcentajes de germinación de 0.83–30 % (Bernal *et al.*, 2005) y 4.7 % (Toledo-Aceves y Wolf, 2008).

La respuesta germinativa de las bromelias a la luz también es variada. Aunque resulta notoria la prevalencia de especies fotoblásticas positivas² (Benzing, 2000), como las epifitas *Dyckia sordida* y *D. ursine* (Tarré *et al.*, 2007), *Vriesea haematina*, *Nidularium fulgens*, *Aechmea coelestis*, *A. nudicaulis* (Pinheiro y Borghetti, 2003) y la saxícola *Pitcairnia albiflos* (Pereira *et al.*, 2010). Sin embargo, las mayoría de las especies epifitas estudiadas por Souza-Garcia y Gonçalves-Oliveira (2007), de los géneros *Aechmea*, *Neoregelia*, *Cryptanthus*, *Vriesea*, y *Tillandsia*; son indiferentes a la luz, es decir que germinan con o sin luz. Mientras

¹ Ortodoxas son aquellas semillas que pierden altos porcentajes de agua durante el secado de maduración y que pueden ser almacenadas por largos periodos sin que el embrión muera. Por el contrario, las semillas recalcitrantes pierden bajos porcentajes de agua durante su maduración y no pueden ser almacenadas por largos periodos. Intermedias son las semillas cuyos valores de porcentaje de pérdida de agua y de tiempo de almacenamiento se encuentran entre los extremos alcanzados por las semillas ortodoxas y por las recalcitrantes.

² Las semillas fotoblásticas positivas son aquellas que requieren luz para poder germinar, y en las semillas fotoblásticas negativas su germinación es inhibida por la luz.

que en otras especies, como en *Racinaea aeris-incola*, la respuesta fotoblástica depende de la temperatura (Souza-Garcia y Gonçalves-Oliveira, 2007).

El agua es otro factor esencial en la germinación. Dadas las condiciones de aridez y de estacionalidad de los matorrales xerófilos en los que se encuentra *Tillandsia recurvata* (Parque ecológico de la Ciudad de México), *Tillandsia circinnatioides*, *Hechtia confusa* y *H. tehuacana* (Valle de Tehuacán-Cuicatlán), uno de los ejes conductores del presente trabajo fue el efecto de la disponibilidad e intermitencia del agua sobre la germinación. Por lo anterior, cabe detallar aspectos relacionados con la dinámica de absorción de agua durante el proceso de germinación.

Cuando el agua se encuentra en óptima disponibilidad, las semillas se embeben y germinan siguiendo un comportamiento trifásico: imbibición, fase estacionaria y emergencia de la radícula (post-germinación; Bewley y Black, 1994). La segunda fase de la germinación ha recibido un particular interés debido a los numerosos cambios bioquímicos y funcionales que ocurren en ésta y que preparan a la semilla para la emergencia de la radícula y preparan al embrión para enfrentarse a su ambiente. Por ejemplo, la reparación de proteínas, ácidos nucleicos y organelos; así como la movilización de las reservas y la síntesis de proteínas y de ácidos nucleicos (Smith y Cobb, 1991; Khan, 1992; Ashraf y Bray, 1993).

Las semillas requieren un nivel de hidratación mínimo para poder concluir con el proceso de germinación, que se denomina nivel crítico de hidratación y es especie-específico (Hunter y Erickson, 1952; Hadas; 1970). Un método común, para determinar el nivel crítico de hidratación limitante para la germinación, es decir el potencial osmótico (Ψ_{π}) al cual las semillas ya no germinan; es mediante la exposición de las semillas a un gradiente decreciente de Ψ_{π} (Jordan y Nobel., 1979; Swagel *et al.*, 1997). Para especies de ambientes áridos, de manera general, se ha descrito un nivel crítico de hidratación de entre -1.0 y -1.5 MPa (Evans y Etherington, 1990).

El nivel crítico de hidratación para la germinación de las bromelias se conoce para: *Dyckia encholirioides* (Pitcairnioideae; entre $20-30$ °C, Pompelli *et al.*, 2006), *Aechmea nudicaulis*, *Neoregelia cruenta* (Bromelioideae) y *Vriesea neoglutinosa* (Tillandsioideae; 27 °C), cuya germinación ocurre hasta los -0.14 MPa (Mantovani e Iglesias, 2010).

Otra condición hídrica relevante para la germinación, es la continuidad o discontinuidad de la disponibilidad del agua. La interrupción de la hidratación de las semillas durante la germinación puede modificar algunos de los parámetros fisiológicos requeridos para este proceso, lo que a su vez repercute sobre el establecimiento de las plántulas (Baskin y Baskin, 1998). A pesar de que en algunas especies la exposición de sus semillas a eventos de hidratación-deshidratación (hidratación parcial) puede reducir la viabilidad o el porcentaje final de germinación (Thanos *et al.*, 1989), en otras especies la hidratación-deshidratación de las semillas puede incrementar la velocidad, el porcentaje y la uniformidad de la germinación; principalmente bajo condiciones desfavorables (Heydecker, 1974; Heydecker *et al.*, 1975; Nascimento, 2003). Estas fluctuaciones en el estado de hidratación también reducen el tiempo de emergencia de la radícula (Moradi *et al.*, 2008). Incluso en algunas especies los beneficios de dichos eventos pueden repercutir positivamente en el vigor de las plántulas (Parera y Cantliffe, 1992).

Con base en lo anterior, se han desarrollado diversos métodos de hidratación-deshidratación con diferentes acepciones: acondicionamiento, condicionamiento, tratamientos pregerminativos o *priming*¹. Tratamientos que tienen por objetivo mejorar las cosechas. Dentro de las especies en las que se han aplicado los tratamientos de hidratación-deshidratación, con impacto positivo en la germinación (*e.g.* incremento en el porcentaje de germinación), se encuentran: *Lycopersicon esculentum* (Solanaceae; Ozbingol *et al.*, 1999), *Cucumis melo* (Cucurbitaceae; Nascimento, 2003), *Capsicum annuum* (Solanaceae; Thanos *et al.*, 1989; Lanteri *et al.*, 2000), *Zea mays* (Poaceae; Moradi *et al.*, 2008), *Brassica oleraceae* (Brassicaceae; Gornik *et al.*, 1997), *Sorghum bicolor* (Poaceae; Foti *et al.*, 2002), *Triticum vulgare* (Poaceae; Idris y Aslam, 1975), entre otras (González-Zertuche *et al.*, 2000).

El efecto de la hidratación-deshidratación de las semillas también se ha estudiado en especies silvestres, como; *Larrea divacariata* (Zygophyllaceae; Barbour, 1968), *Callitris sp* (Cupressaceae; Adams, 1999), *Anastatica hierochuntica* (Brassicaceae; Friedman *et al.*, 1981) *Orquis palustris* (Orchidaceae; Esitken *et al.*, 2004), *Wigandia urens* (Hydrophyllaceae;

¹ Los tratamientos pregerminativos o de *priming* implican una hidratación parcial hasta alcanzar la segunda fase de la germinación con un posterior secado antes de la emergencia de la radícula.

González-Zertuche *et al.*, 2001), *Buddleja cordata* (Scrophulariaceae; González-Zertuche *et al.*, 2002), *Tillandsia flexuosa* (Bromeliaceae; Bader *et al.*, 2009).

Especies estudiadas de hábito terrestre

Hechtia es un género monotípico y xerofítico de Centroamérica y México que pertenece a la subfamilia Hechtioideae (Givnish *et al.*, 2007). Las especies de *Hechtia* son hierbas terrestres acaules de hojas suculentas fuertemente protegidas por espinas en sus márgenes y forman rosetas. Las hechtias presentan metabolismo CAM y en las localidades en donde se distribuyen son una especie dominante o co-dominante. Estas plantas son dioicas, tienen frutos secos (cápsulas septicidas) y sus semillas son aladas (Martínez-Correa, 2008). Cabe mencionar que *Hechtia* fue hasta hace poco tiempo, uno de los géneros menos atendidos por los botánicos mexicanos debido al breve periodo de floración de los individuos (menor a dos semanas), y por ende, a la dificultad de contar con ejemplares con inflorescencias de cada uno de los sexos (Espejo-Serna *et al.*, 2008; Ramírez-Morillo *et al.*, 2011), lo que ha limitado la claridad de sus *taxa* y el estudio de otros aspectos de su biología.

Hechtia confusa L. B. Sm. es una hierba acaule, rosetófila (**Fig. 1a**), con crecimiento cespitoso y presenta inflorescencias erectas (17–83 cm). Su floración puede ocurrir en los meses de mayo a diciembre y su fructificación de julio a diciembre. Sus semillas aladas miden de 2.2–3.6 mm de largo. *Hechtia confusa* es una especie endémica para los estados de Oaxaca y Puebla; y se distribuye entre los 1900 y 2600 m snm (Espejo-Serna *et al.*, 2004; Martínez-Correa, 2008).

Hechtia tehuacana B. L. Rob., es una hierba acaule, rosetófila (**Fig. 1b**), que presenta inflorescencias erectas (1.10–1.5 m). Su floración puede ocurrir de mayo a diciembre y los frutos maduros se pueden encontrar de marzo a abril o de agosto a septiembre. Sus semillas aladas miden de 3–3.8 mm de largo. *Hechtia tehuacana* se distribuye en los estados de Puebla y Oaxaca entre los 1550 y 2200 m snm (Martínez-Correa, 2008).

Especies estudiadas de hábito epifito y rupícola

El género ***Tillandsia*** pertenece a la subfamilia Tillandsioideae; y contiene hierbas acaules, cuyas hojas están cubiertas con escamas foliares absorbentes de agua y nutrientes a partir de

vapor de agua. Los frutos de *Tillandsia* son cápsulas que contienen semillas plumosas; con una coma, que consiste en numerosos tricomas que se extienden a uno o ambos extremos de la semilla (**Figs. 1c-d**). Los tricomas constituyen más de la mitad de la masa de las semillas, proveyéndoles de la capacidad de flotar en el aire y de un rápido engarce a sustratos (Benzing, 2000).

Tillandsia circinnatioides Matuda, tiene hojas de aproximadamente 12 cm de longitud que forman un pseudobulbo elíptico. Las hojas de esta especie presentan un tono plateado debido a lo abundante de las escamas foliares. Las hojas presentan numerosos haces vasculares longitudinales y se encuentran curvadas (**Fig. 1e**). Estas plantas crecen de manera cespitosa (de 6–7 rosetas) formando cúmulos sobre las ramas de árboles pequeños, cactáceas columnares o rocas. *Tillandsia circinnatioides* es una especie endémica para México; con distribución en los estados de Guerrero, Morelos, Puebla y Oaxaca y crece a 680–2200 m snm (Matuda, 1973; Espejo-Serna *et al.*, 2004).

Tillandsia recurvata (L.), tiene hojas lineares de 5–10 cm de largo (MacVaugh, 1992) cubiertas de tricomas absorbentes y presenta un sistema rudimentario de raíces. Esta especie crece en cúmulos que forman masas generalmente esféricas, las cuales frecuentemente rodean completamente las ramas sobre las que se desarrollan (**Fig. 1f**). El diámetro de las masas esféricas varía comúnmente entre los 4–15 cm y alcanzan los 23 cm cuando florecen. Su época de floración en el Ajusco Medio (Parque ecológico de la Ciudad de México) ocurre en verano y la dispersión de sus semillas ocurre de febrero a marzo. Los frutos contienen aproximadamente 40 semillas (Bernal *et al.*, 2005). *Tillandsia recurvata* se distribuye desde el sur de Estados Unidos hasta Argentina (Cáldiz *et al.*, 1993; Fernández *et al.*, 1989).

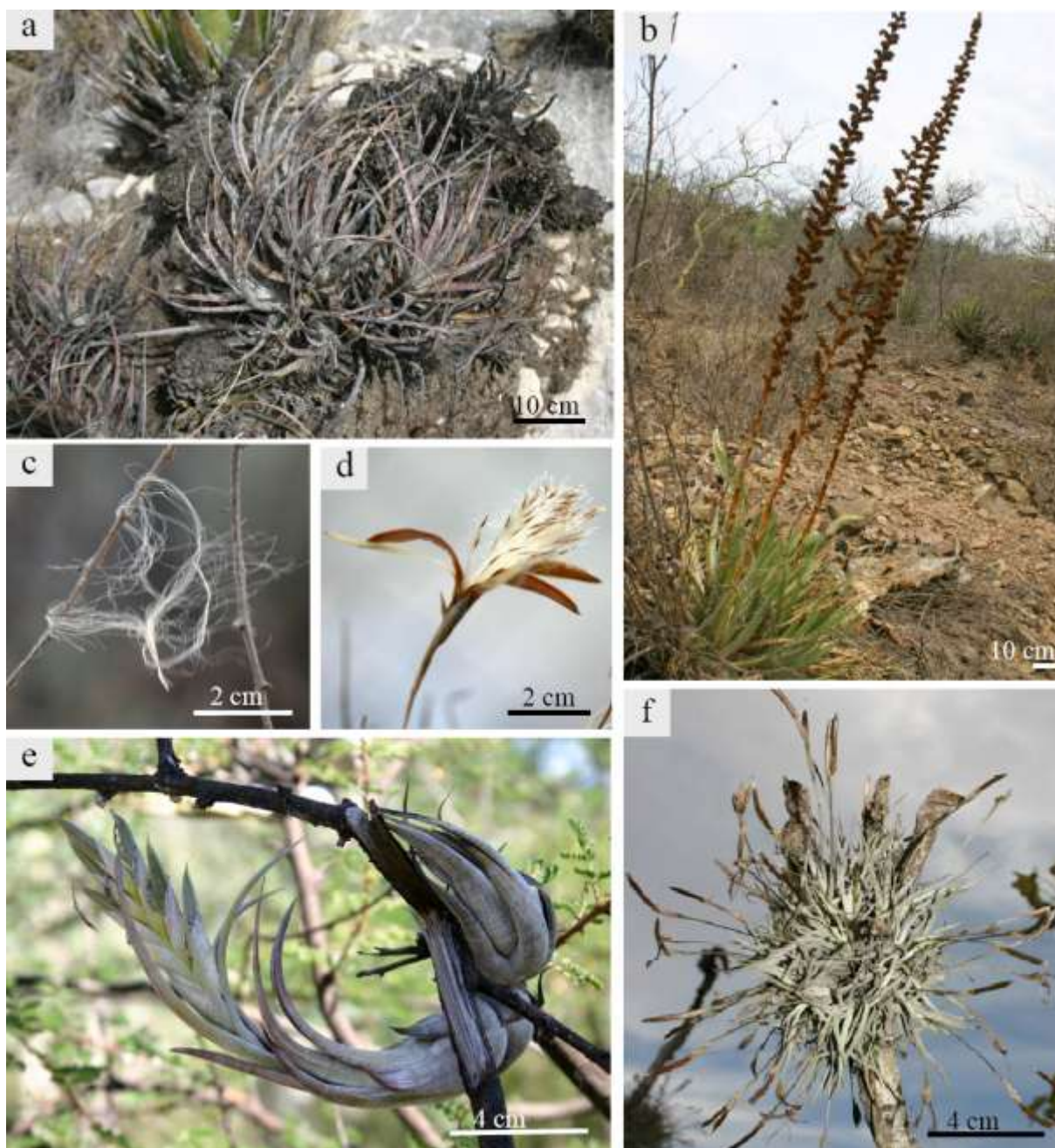


Figura 1. Especies de estudio. **a)** *Hechtia confusa*, **b)** *Hechtia tehuacana*, **c)** Semilla plumosa de *Tillandsia circinnatioides*, **d)** fruto con semillas de *Tillandsia recurvata*, **e)** *Tillandsia circinnatioides* con frutos, **f)** agregado de *Tillandsia recurvata* con frutos. Fotografías Saraí Montes.

Sitios de estudio

El **Parque Ecológico de la Cd. de México** (PECM; 19°16′- 19°14′ N; 99°13′- 99°10′O; INEGI, 2000) representa una extensión de alrededor de 724 hectáreas, pertenecientes a la cordillera montañosa del Eje Neovolcánico Transversal. Esta zona presenta un suelo pobremente desarrollado, de origen volcánico. Tiene un clima, semifrío subhúmedo con lluvias en verano (Cb´(w2)(w)ig; INEGI, 2000). La zona dentro del parque conocida como Ajusco medio (2440–2850 m snm) tiene como tipo de vegetación un matorral xerófilo (**Fig. 2**).



Figura 2. Vegetación del Parque Ecológico de la Ciudad de México, durante la estación de lluvias. Fotografía Saraí Montes

El **Valle de Tehuacán-Cuicatlán** es un área de aproximadamente 10,000 km² que alberga de 10–11.4 % de diversidad de la flora mexicana. Este Valle está localizado al sur del estado de Puebla y al norte del estado de Oaxaca, entre 17° 39'–18° 53' N y 96° 55'–97° 44' O; a una altitud entre los 500 y 3200 m snm (Zavala-Hurtado y Díaz-Solis, 1995; Bernal *et al.*, 2005). El Valle de Tehuacán-Cuicatlán tiene un clima semi-árido con temperatura media anual de 14–26 °C y una precipitación media anual de 300–900 mm, que ocurre entre los meses de Mayo a Agosto (García, 1988). Dentro del Valle de Tehuacán-Cuicatlán se encuentra el **Zapotitlán de las Salinas (Fig. 3)**, un área montañosa en la porción sudeste del estado de Puebla, cerca de los límites con el estado de Oaxaca (18° 20' N, 97° 28' O) a una elevación de 1400–1600 m snm. En esta localidad la temperatura media anual es alrededor de los 20 °C la precipitación que fluctúa entre los 300–350 mm (Zavala-Hurtado 1982; García-Suárez *et al.*, 2003).



Figura 3. Valle de Zapotitlán de las Salinas, Puebla, México, durante la estación de lluvias. Fotografía Sarafí Montes

El presente trabajo tuvo como pregunta general, ¿qué aspectos estructurales y fisiológicos exhiben las semillas de las bromelias para poder germinan en ambientes con disponibilidad de agua escasa o intermitente?

Para contestar a dicha pregunta abordamos los siguientes objetivos particulares:

- 1) **Identificar los requerimientos hídricos para la germinación de las semillas de las bromelias estudiadas.** (Capítulos II y III).
- 2) **Identificar los requerimientos lumínicos para la germinación de las semillas de las bromelias estudiadas.** (Capítulo I).
- 3) **Evaluar el efecto de la temperatura sobre la germinación de las semillas de las especies estudiadas.** (Capítulo II).
- 4) **Evaluar la respuesta de las semillas (o plántulas) a la fluctuación o escasez de la disponibilidad de agua durante la germinación.** (Capítulos I y II).
- 5) **Describir las características morfológicas y/o estructurales de las semillas de las especies estudiadas y establecer posibles relaciones con los aspectos ecofisiológicos de su germinación.** (Capítulos I y II).

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Capítulo I

The germination of *Hechtia confusa* seeds (Bromeliaceae): photoblastism, water stress and morpho-structure

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The germination of *Hechtia confusa* seeds (Bromeliaceae): photoblastism, water stress and morpho-structure

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Abstract

Hechtia confusa L. B. Sm. (subfamily Hechtioideae) is an endemic dioecious herb from the Tehuacán-Cuicatlán Valley, Puebla, where it shows marked local dominance or co-dominance. In this study, different aspects of the seed biology of *H. confusa* were evaluated to gain an improved understanding on some of the physiological adaptations of this species to the marked seasonality of the Tehuacán-Cuicatlán Valley. We first determined the light quality required for germination. We then determined the effect on germination of hydration-dehydration events in the laboratory and in the field and the effect of the exposure of dry seeds to heat shock during the dry season in the field. Finally, to obtain an adequate description of the seed germination response, we studied the morphology and structure of the seeds. *Hechtia confusa* seeds are quiescent, positively photoblastic and can germinate in white, red and far-red light; they were also tolerant to hydration-dehydration events (in the laboratory and under natural conditions) and to dry heat shock. *Hechtia confusa* seeds have an abundant starchy endosperm and a small lateral embryo. One stratum of the seed coat has a high content of condensed tannins. The high resistance of the dry seeds to high temperatures, a seed coat that protects against herbivores and the persistence of the seeds on dry inflorescences observed in the field suggest that *H. confusa* seeds have the potential to establish an aerial seed bank.

Keywords: light; seed structure; hydration-dehydration; tannins; heat shock; natural priming

1. Introduction

Seed germination determines the potential of a plant for reproduction and persistence. Furthermore, water availability, temperature and light are abiotic factors to which the seeds respond. Several species inhabiting arid environments require light to germinate (positive photoblastism; e.g., *Mammillaria* spp., Benítez-Rodríguez et al., 2004). Nevertheless, light is not a limiting resource in arid environments. While in tropical forests photoblastism ensures that seeds germinate in places with sufficient light for seedling growth and development (Vázquez-Yanes and Orozco-Segovia, 1987). It has been hypothesised that positively photoblastic responses in arid environments represent a mechanism for detecting the appropriate depth of soil for germination (Rojas-Aréchiga et al., 1998; Gutterman, 1994).

The seeds of some species of Bromeliaceae are positively photoblastic (Benzing et al., 2000; Pinheiro and Borghetti, 2003; Tarré et al., 2007; Pereira et al., 2010), and others species are negatively photoblastic (germination is inhibited by light) at certain temperatures (Souza-Garcia and Gonçalves-Oliveira, 2007). Because different species of Bromeliaceae occupy distinct microhabitats in ecosystems, it is necessary to determine the type of photoblastism shown by previously unstudied species of bromeliads.

In arid environments, the availability of water is the principal constraint on seed germination. To achieve successful establishment, certain species in these environments disperse their seeds during the rainy season (Rojas-Aréchiga et al., 1997; Camargo-Ricalde et al., 2002). In these species, moisture availability may not be the principal constraint on seed germination (Camargo-Ricalde et al., 2002). In other species, however, seed dispersal does not coincide with the rainy season (Rojas-Aréchiga et al., 1997); in these species, the seeds will be exposed to temperature fluctuations and to hydration-dehydration (H-D) events in the days prior to the full onset of the rainy season. The seeds will also be exposed to biotic factors that may affect their ability to germinate successfully. Therefore, the germination of species from arid environments also depends on the ability of the seeds to tolerate H-D events.

Tolerance to dehydration allows the metabolic changes that occur in totally or partially hydrated seeds to be preserved after seed dehydration and storage (Bray, 1995). For many seeds of commercial and wild species (mainly orthodox seeds), the H-D events improve germination, allowing it to be more rapid, synchronic and/or increasing the germination

percentage (Bradford, 1986). For this reason, several types of H-D treatments have been developed, including hydropriming (hydration in water), osmopriming (hydration in osmotic solutions), matric priming (hydration in a moist solid matrix) and drum priming (hydration with water vapour). Additionally, wild species are exposed to H-D events in the soil (“natural priming”; González-Zertuche et al., 2001; 2002), and this exposure also improves seed germination.

Hechtia is a monotypic genus of the subfamily Hechtioideae (Bromeliaceae; Givnish et al., 2007) and shows high diversity and high endemism in the Tehuacán-Cuicatlán Valley, Puebla, Mexico (Dávila et al., 2002). The fruits of *H. confusa* develop from July to December (Martínez-Correa, 2008). Mature, partially open fruits have been observed in December–February (S. Montes-Recinas, personal observation). Consequently, some seeds of this species may remain exposed to high temperatures from March to May (35 °C for air, Rojas-Aréchiga et al., 1997), either inside the fruits or in the soil. Additionally, from April to the beginning of May, the seeds are exposed to the low and erratic precipitation occurring at the beginning of the rainy season. The purpose of this work was to evaluate and improve the understanding of some of the physiological adaptations of this species to the marked seasonality of the Tehuacán-Cuicatlán Valley. We first determined the light requirements for germination (darkness, white, red and far-red light). We then determined the effects of H-D events in the laboratory and under natural conditions (hydropriming and natural priming) and the effect of the exposure of dry seeds to heat shock during the dry season in the field. Finally, to obtain an adequate description of the seed germination response of the study’s species, we investigated the morphology and structure of the seeds.

2. Materials and Methods

2.1. Study area

The Tehuacán-Cuicatlán Valley is an area of approximately 10,000 km². An area of high biodiversity, home of 10–11.4% of the Mexican flora. The valley is located in the southern part of the state of Puebla and in northern Oaxaca, between 17° 39'–18° 53' N and 96° 55'–97° 44' W. The valley is a semi-arid region, with a mean annual temperature of 14–26 °C, a mean

annual precipitation of 300–900 mm (García, 1988) and an elevation ranging from 500 to 3200 m a.s.l. (Camargo-Ricalde et al., 2002).

2.2. *Study species*

Hechtia confusa L. B. Sm. is a dioecious and terrestrial herb endemic to the Tehuacán-Cuicatlán Valley, where it exhibits local dominance or co-dominance. This rosulate species grows in dense clumps and reaches 1.3 m in height. The inflorescences are erect panicles (Fig. 1A-C) that bloom from May to December and bear fruits from July to December. The fruits are brownish ovoid septicidal dry capsules 0.65–1 cm in length and 3–8 mm in diameter (Fig. 1 C-D, Martínez-Correa, 2008). The seeds are winged (the wing surrounding the seed) and brownish, 2.2–3.6 mm in length (Fig. 1E, Martínez-Correa, 2008). For this study, seeds were collected from more than five plants in December (2005) in Puebla state in the locality of Chapulco (18° 39' 20" N, 97° 24' 30" W, 2164 m a.s.l.). Voucher material was deposited at Herbario Metropolitano (UAMIZ), Universidad Autónoma Metropolitana, Iztapalapa (*N. Martínez-Correa, A. Espejo, A. R. López-Ferrari & S. Montes 13*). After collection, the seeds were stored in paper bags under laboratory conditions (23–25 °C and 30–40% relative humidity).

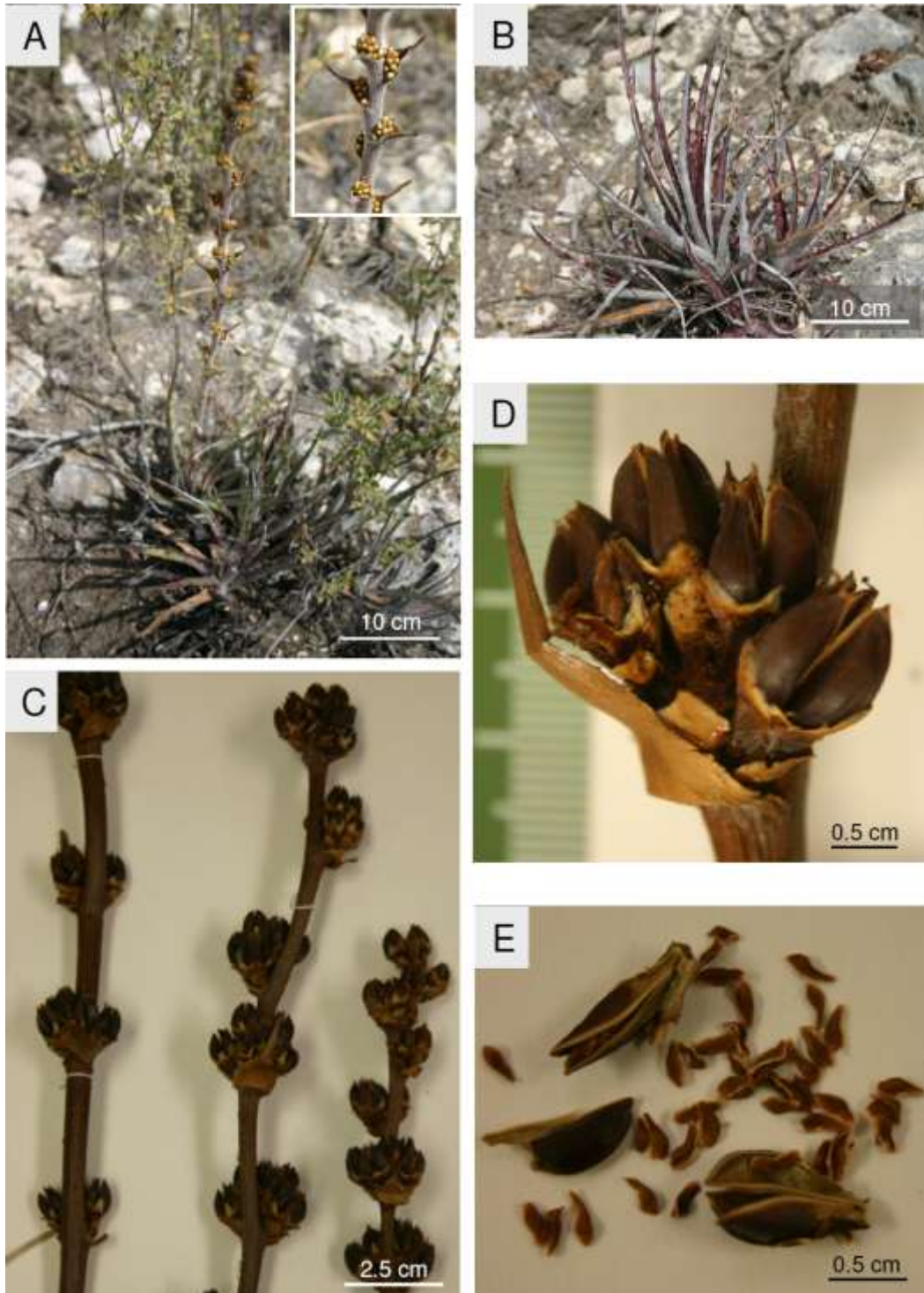


Figure 1. Some vegetative and reproductive characteristics of *Hechtia confusa* observed in the field and in an herbarium specimen. (A) Rosette with male inflorescence and detail of male floral buds, (B) rosette, (C) dry inflorescence with fruits, (D) detail of the dry dehiscent capsules and (E) open fruits with seeds.

2.3. *General procedures*

For all the experimental tests, the seeds were imbibed and germinated in Petri dishes (5 cm in diameter) on agar plates (1% in water; Bioxon, México). The dishes were placed inside germination chambers (Biotronette 844, Lab-Line Instruments, Inc. Melrose Park, IL, USA) at a constant temperature of 25 °C and under a 12-h photoperiod with a photosynthetic photon flux density of 30 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Germination was defined by the occurrence of 1 mm of radicle protrusion through the seed cover. Germination was recorded daily. The following three germination parameters were evaluated: 1) the lag time (at which the first seed germinated), 2) the germination rate and 3) the final germination percentage.

At the end of the germination tests, seedlings from each treatment were randomly selected and transplanted to 1 cm^3 black plastic pots. The pots were filled with ground limestone strained through a 0.8 mm^2 sieve and with peat moss (1:1) and were maintained under the same conditions used in the germination tests. The seedlings were watered every four days for two weeks, and then maintained for seven days without irrigation. After this period of water deprivation, the seedlings were watered every four days. At the end of 15 days under this watering regime, we quantified the seedling vigour (survival, number of leaves, plant cover, stem length and length of the longest root). The plant cover was calculated with the ellipse formula.

2.4. *Light requirements for germination*

To determine whether the seeds remained quiescent in the soil during burial treatments, we tested the germination response to four light conditions: white light (W), red light (R), far-red light (FR) and darkness (D). Fifteen seeds were sown on an agar plate in each of five Petri dishes and immediately exposed to different light conditions. For the red condition, the Petri dishes were placed inside a box (34 x 44 x 10 cm) made of red plexiglass (series 2424, Röhm & Hass, México DF; R:FR = 5.22, 9.297 $\mu\text{mol m}^{-2} \text{s}^{-1}$). A box constructed with one red and blue (Series 2423) plexiglass layer (R:FR = 0.05, 9.742 $\mu\text{mol m}^{-2} \text{s}^{-1}$) was used for the far-red condition. For the darkness condition, the Petri dishes were wrapped in three layers of

aluminium foil. All the Petri dishes were placed in a germination chamber, and the amount of germination was quantified after eight days.

2.5. *Effect of hydropriming and natural priming on germination and seedling survival*

To assess the effect of the sporadic and erratic precipitation at the beginning of the rainy season on *H. confusa* seeds present in the soil, we exposed the seeds to H-D cycles. The H-D treatments used were hydropriming (HP) and natural priming (NP). Under hydropriming, the seeds were hydrated in water at 25 °C. To ensure that each treatment included an adequate number of days, we determined the imbibition curve of *H. confusa*. Lots of 25 seeds were weighed ($n = 3$) and then sown on agar; daily, for six days, the seeds were blotted with a paper towel and then weighed (Fig. 2). The time to reach the three different germination phases was determined from this curve. Based on the resulting information, the germination length (phase III) was recorded after 5–6 days, whereas the HP treatment used an imbibition period of three days (seeds in phase II). The seeds were then dried for five days in the laboratory on paper towels. For natural priming, the seeds were buried in the soil of the “Helia Bravo” Botanical Garden in Zapotitlán (JBHB). Five grams of seeds were placed inside nylon bags (15 cm²; $n = 3$) and buried at a depth of 1 cm in the shadow of a *Prosopis laevigata* (Humb. & Bonpl. ex Willd.) M. C. Johnst. (Fabaceae) trees from May 6 to June 6, 2007. During this month, the temperature was recorded with dataloggers (HOBO U12-013 Onset Computer Corporation, Pocasset, MA, USA) capable of accepting 2 TMC6-HD thermocouple probes (Fig. 3). Two thermocouples were buried at a depth of 1 cm close to the seeds. At least one occurrence of rain (June 5) was recorded during the burial period. Because the seeds were damp after they were unearthed, they were air dried for 5 days in the laboratory on paper towels. Thirty seeds from the HP, NP treatments and control were rehydrated in Petri dishes ($n = 6$ each) under the conditions previously described, and the amount of germination was recorded each day for 14 days.

To evaluate the effects of HP and NP on seedling vigour, 25 seedlings from each treatment (HP, NP and control) were randomly selected, transplanted to plastic pots and exposed to the watering regime previously described. Sixty-two days after radicle protrusion, seedling

survival, the number of leaves, stem length, the length of the longest root and plant cover were determined.

2.6. Effects of dry heat shock on germination and seedling vigour

To investigate the effect on the seeds of the high temperatures occurring in the study area during the dry season, we recorded the temperatures from May to June. We selected a temperature (42 °C) near the highest temperature recorded (46.40 °C) in the shaded area in the JBHB for the heat shock treatment. Thirty seeds were mixed with ground limestone strained through a 0.8 mm² sieve (40 g) and placed in crystal Petri dishes (10 cm in diameter, n = 10). The Petri dishes were placed in an oven (model 136400, Boekel Scientific, USA) at 42 °C for three days. The dishes were then allowed to return to room temperature in the laboratory. The control seeds, kept at room temperature, and seeds treated were sown on agar and incubated inside germination chamber at conditions previously described, their germination was determined each day.

To evaluate the effect of the dry heat shock treatment on the vigour of the seedlings, 180 seedlings (for each condition) were randomly selected, transplanted to the plastic pots and exposed to the watering regime previously described. Fifty days after radicle protrusion, seedling survival, the number of leaves, stem length, the length of the longest root and plant cover were determined.

2.7. Morphology and structure of the seeds

To describe the micro-morphology of the *H. confusa* seeds, the seeds were dehydrated in a graded ethanol series (50, 70, 85, 98, and 100%) and dried with liquid CO₂ at the critical point (CPD 030; Balt-Tec AG, Balzers, Liechtenstein). The seeds were then mounted and covered with a layer of gold (200 nm; Desk II, Denton Vacuum Inc., Moorestown, NJ, USA), and images were obtained with a scanning electron microscope (JSM5310-LV, JEOL, USA).

To describe the structure of *H. confusa* seeds, the seeds were fixed in FAA: formalin (5%), acetic acid (4%), ethanol (48%) and water (43%) for two weeks. The seeds were then dehydrated in a graded ethanol series and embedded in regular paraplast (Sigma-Aldrich,

USA). Longitudinal sections 8 μm in thickness were obtained with a microtome (Leica RM 2135, Leica Microsystems Inc, Columbia, MD). To identify seed structures and the chemical composition of deposits, we applied three histochemical tests. Safranin-fast green stain was used for general staining of cytoplasm and cell walls. Lugol's iodine stain was used to identify starch, and vanillin stain was used to identify tannins. These histochemical procedures were performed according to López-Curto et al. (2005). The sections were observed with an Olympus photomicroscope (Provis AX-70; Olympus, Tokyo, Japan). Starch deposits were identified by the development of a dark purple colour in previously colourless tissue, and the presence of condensed tannins was identified by a change in the tissue colour from brown to reddish brown (Gardner, 1975; López-Curto et al., 2005).

2.8. *Statistics*

To obtain the germination rate (velocity) and the lag time, we fitted the accumulated germination percentage of each replicate to a sigmoid model with Table Curve 2D, v5.01.01 (AISN Software, Chicago, IL, USA). The germination rate was defined as the maximum value of the first derivative of the sigmoid curve. The germination percentages were arcsine transformed, and the plant cover data were square root transformed. If the data exhibited normality and homoscedasticity, statistical comparisons were performed with an analysis of variance (ANOVA). A Tukey test was used to perform the post hoc comparisons (Zar, 2009). If the normality or homoscedasticity assumptions were not satisfied, the data comparisons were performed nonparametrically with a Mann-Whitney *U*-test or a Kruskal-Wallis (*H*) test, and the post hoc comparisons were performed with a boxplot graph. The analyses were performed with the Statgraphics software package (Statistical Graphics Corporation, Englewood Cliffs, NJ, USA).

3. Results

3.1. Light requirements for germination

The seeds showed high percentages of germination (> 80%) under W, R and FR. The germination percentage observed under R was significantly highest ($F_{2,12} = 7.96$, $P < 0.01$; Fig. 2) than FR and D treatments. Seeds did not germinate in the darkness.

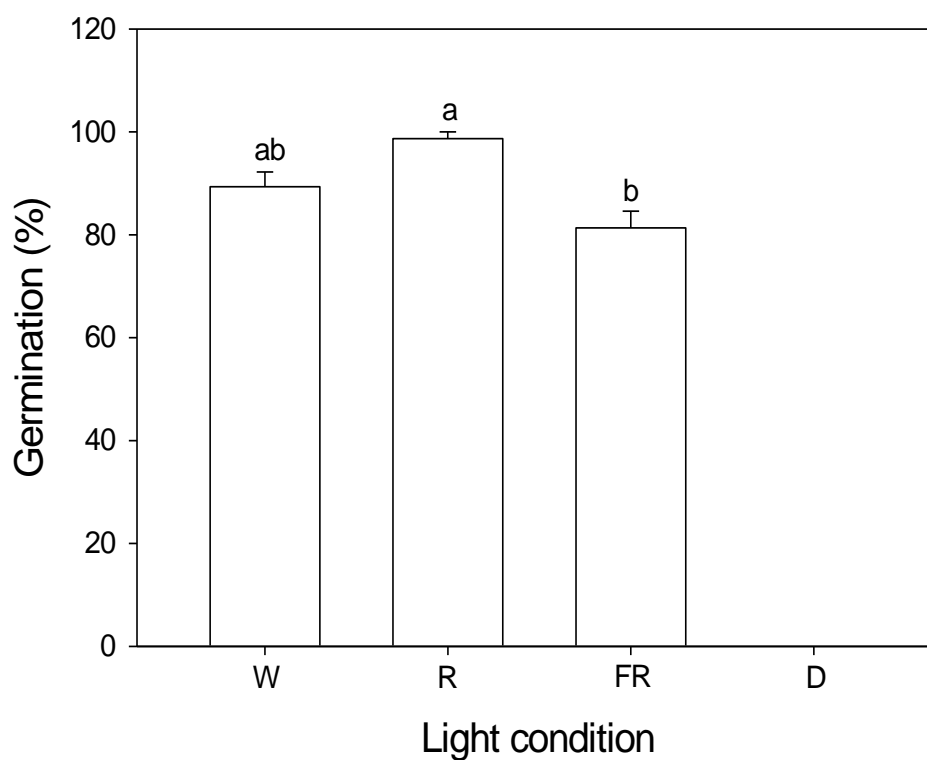


Figure 2. Germination of *Hechtia confusa* seeds under different light conditions: white light (W), red light (R), far-red light (FR) and darkness (D). Bars indicate standard error values, and different letters represent significant differences between treatments.

3.2. Effect of hydropriming and natural priming on germination and seedling survival

The imbibition curve of the *H. confusa* seeds at 25 °C showed that the imbibition phase of germination (phase I) lasted approximately 24 hours. Phase II (the lag phase) then began. The lag phase lasted approximately four days and ended with radicle protrusion (Fig. 3).

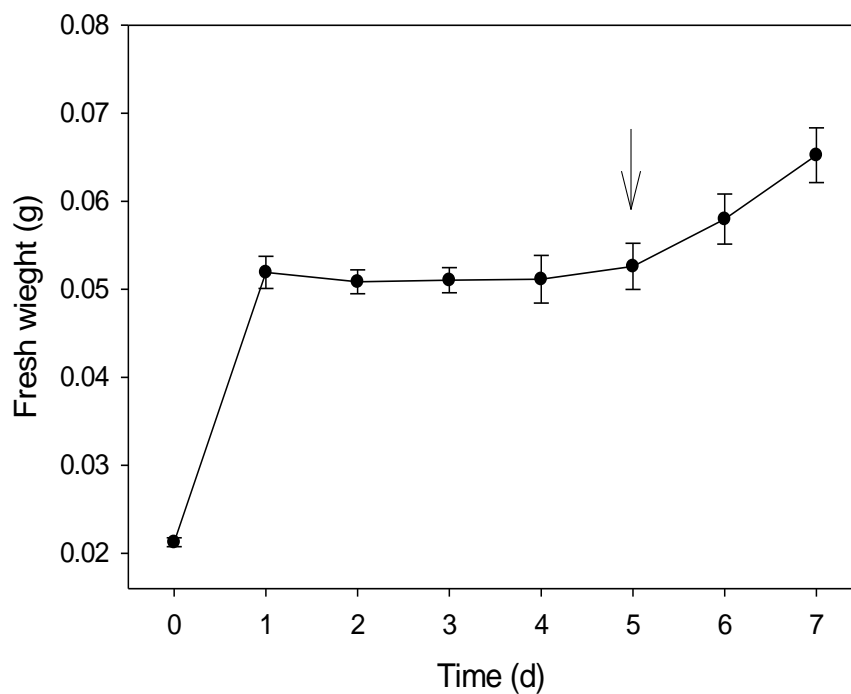


Figure 3. Imbibition curve of *Hechtia confusa*. The arrow indicates radicle protrusion. Error bars represent standard error values.

The soil temperature recorded from May 6 to June 6, 2007 in the Tehuacán-Cuicatlán Valley under the shadow of *P. laevigata* vegetation showed a mean temperature of 25.36 ± 0.27 °C, a minimum temperature of 14.00 °C and a maximum of 46.43 °C (Fig. 4).

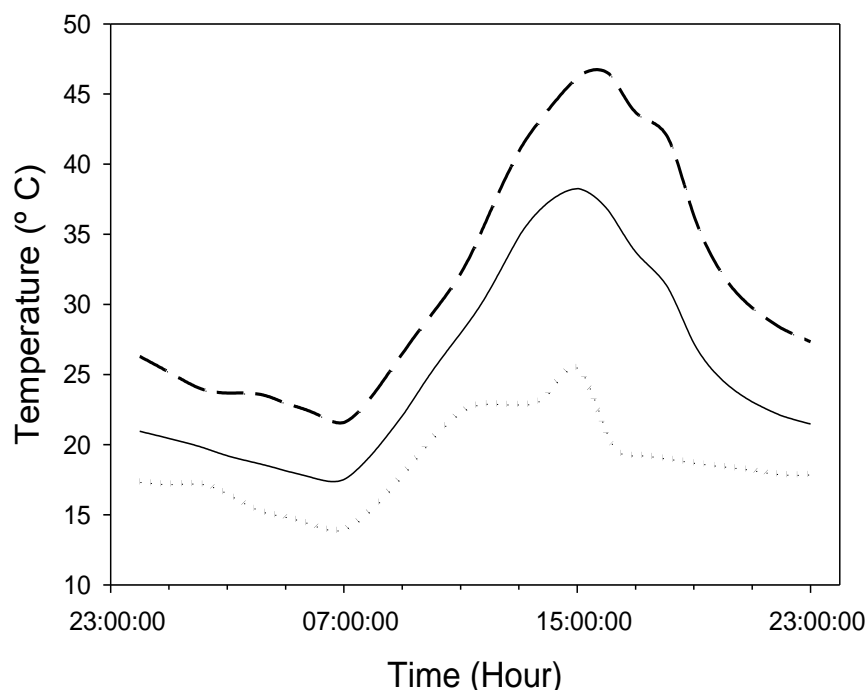


Figure 4. Mean (solid line), minimum (dotted line), and maximum (dashed line) soil temperatures at a depth of 1 cm in the shadow of *P. laevigata* vegetation in the “Helia Bravo” Botanical Garden, Zapotitlán de las Salinas, Puebla, May 6 to June 6, 2007.

The HP and NP treatments decreased the germination lag time of the *H. confusa* seeds relative to that of the control ($H = 10.48$, $P < 0.01$; Fig. 5). Neither the germination rate nor the final percentage of germination differed significantly from the corresponding values for the control ($H = 3.90$, $P = 0.14$; $H = 3.10$, $P = 0.21$, respectively; Fig. 5). At 62 days after radicle protrusion, the survival of the seedlings in the HP treatment was greater than that of the seedlings in the NP and control treatments ($H = 11.16$, $P < 0.01$; Fig. 6). All other parameters used to measure the vigour of the seedlings (the number of leaves stem length, the length of the longest root or plant cover) showed non-significant differences ($P > 0.065$).

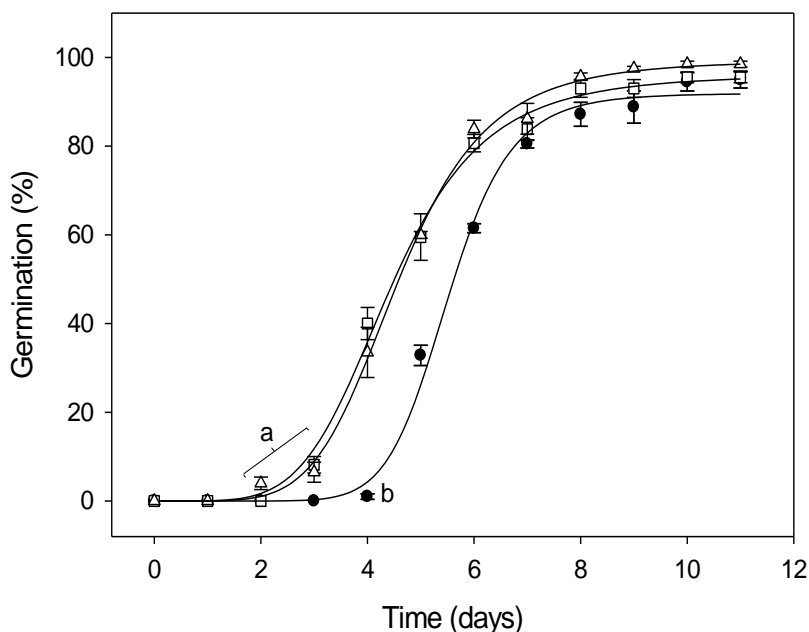


Figure 5. Cumulative germination curve for *Hechtia confusa*. Control (●), hydropriming (□) and natural priming (Δ). The bars represent the standard error. The different letters represent significant differences of lag time values between treatments ($H = 10.48$, $P < 0.01$). The germination rate and the final percentage of germination did not show significant differences between treatments.

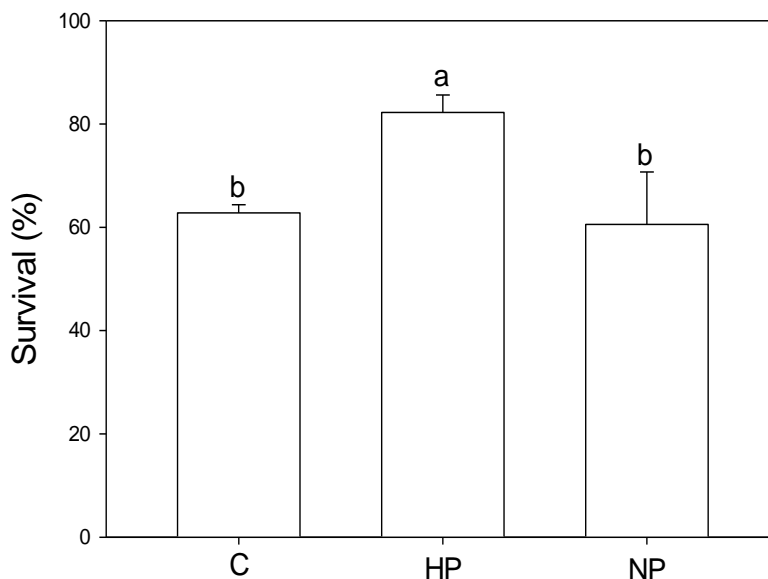


Figure 6. Seedlings of *Hechtia confusa* survival, after 62 days since radicle protrusion, and subsequent stress treatment (seven days without watering). (C) Control, (HP) hydropriming (NP) and natural priming. Bars represent the standard error and different letters represent significant differences between treatments ($H = 11.16$, $P < 0.01$).

3.3. Effects of dry heat shock on germination and seedling vigour

Heat shock (42 °C) did not produce significant effects on *H. confusa* seed germination ($P > 0.41$). The parameters of seedling vigour (the number of leaves, stem length, length of the longest root, plant cover and survival) showed no significant effects of heat shock ($P > 0.055$).

3.4. Morphology and structure of the seeds

Images obtained with scanning electron microscopy showed that the winged seeds have elliptic, planate or gyrose shapes (Fig. 7A-B). The hilum-micropylar region appeared as a wide area (Fig. 7A) with cells longer than the other cells of the seed surface (Fig. 7C). The surface of certain regions of the seeds, such as the areas near the hilum-micropyle, showed a reticular pattern (Fig. 7D).

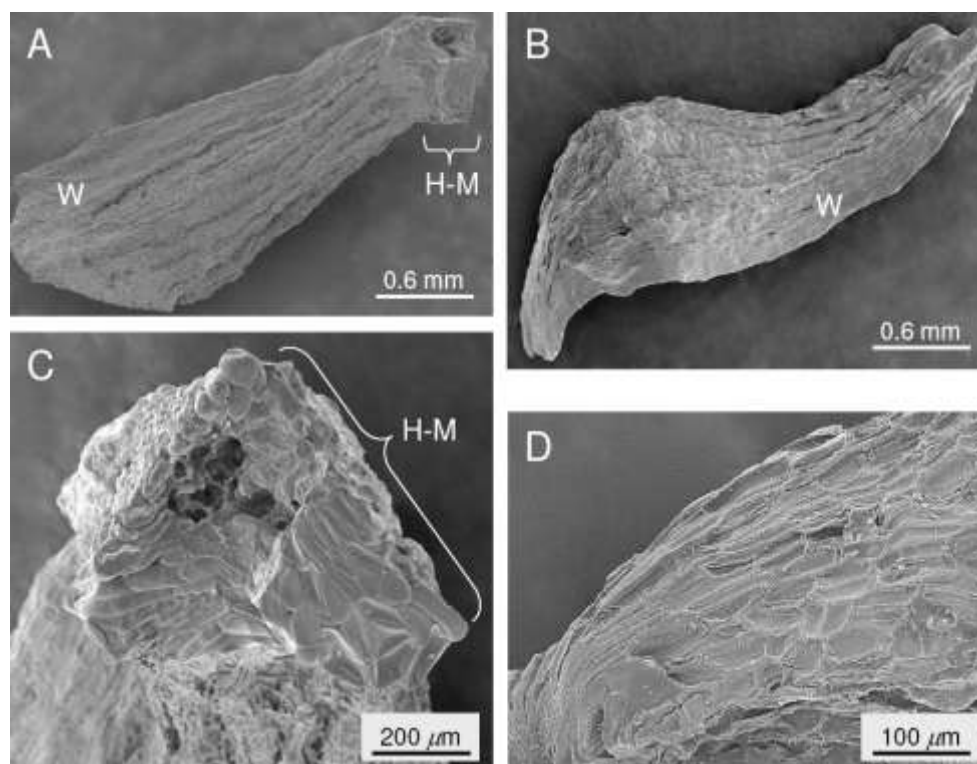


Figure 7. *Hechtia confusa* seeds morphology. Image obtained with scanning electron microscope. **A)** Seed shape and surfaces, (W) wing and (H-M) the hilum-micropylar region; **B)** Seed shape and (W) wing; **C)** Detail of the (H-M) hila-micropylar region; **D)** Detail of the reticular pattern of the seed surface (near the hilum-micropylar region).

Hechtia confusa seeds are endospermic and exhibit a lateral small embryo with an approximate 1:5 embryo:endosperm ratio. Their seed coat is formed by the wing and by a layer that contains dark inclusions (from one to several stratum). The wing is formed by dead cells filled with air (Fig. 8A). Below the layer with dark inclusions was the aleurone stratum (Fig. 8B). Aleurone stratum did not stain with Lugol's reagent (Fig. 8C) or with vanillin stain. The Lugol's histochemistry reagent showed that the endosperm of *H. confusa* contains starch (Fig. 8C). The vanillin stain revealed a layer with dark inclusion containing condensed tannins (Fig. 8D).

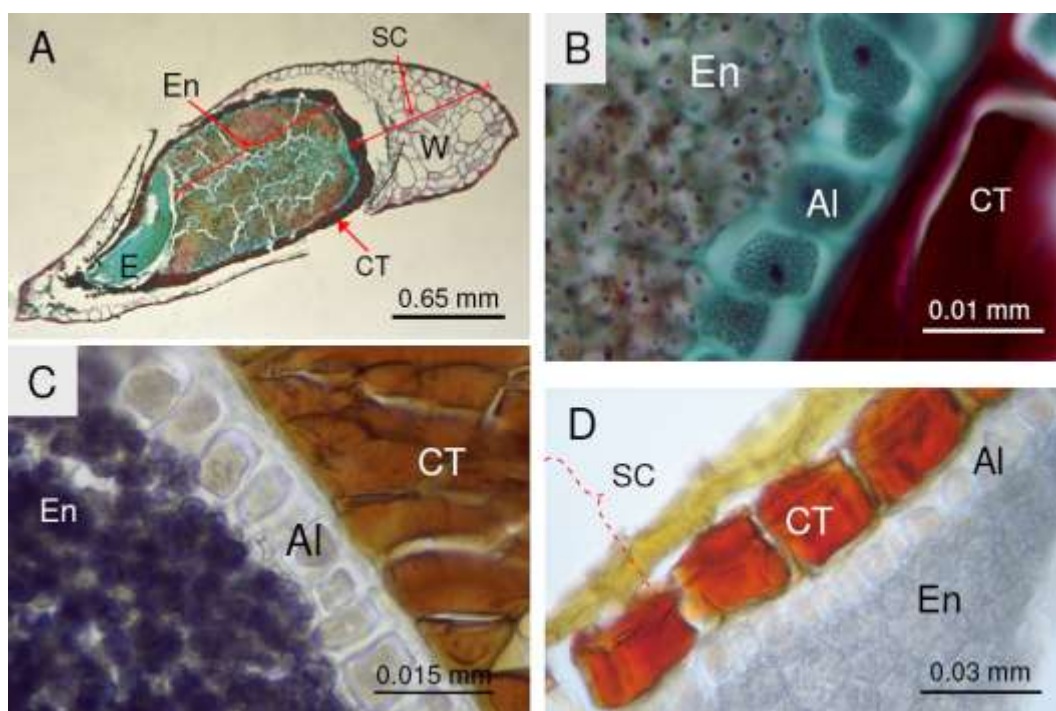


Figure 8. *Hechtia confusa* seeds structure. **A)** Longitudinal section stained with safranin and fast green; **B)** Detail of the endosperm, the aleurone cells and cells with condensed tannins; **C)** Detail of a seed section stained with Lugol's reagent; **D)** Detail of cells with condensed tannins stained with vanillin. (Al) Aleurone, (E) embryo, (En) endosperm, (CT) seeds coat cells with condensed tannins, (SC) seed coat and (W) wing of the seed.

4. Discussion

4.1. Light requirements for germination

The seeds of *H. confusa* are quiescent. For this reason, their positive photoblastism is necessary to ensure an adequate place and time for germination. Because the light photon flux and the R:FR ratio (Smith, 1982) decrease over a scale of millimetres as soil depth increases (Bliss and Smith, 1985), the positive photoblastism of *H. confusa* seeds may limit their germination to a specific range of soil depths and may ensure the germination of the seeds at the correct soil depth (Gutterman, 1994). This depth may be at the soil surface or a few millimetres beneath the surface, a pattern that is commonly found in the germination of the Poales and of the monocotyledons (Tillich, 2007). In deserts, most seeds are situated on the soil surface or in the uppermost 0–2.5 cm after dispersal (Gutterman, 1994), and a previous study has shown that most seedlings emerged from depths of 2 to 4 cm (Gulshan and Dasti, 2012). *Hechtia confusa* seeds might also germinate under far-red light, which penetrates deeper in the soil than white or red light (Bliss and Smith, 1985). For this reason, the depth limitations on *H. confusa* seed germination may be less restrictive than those affecting species that can only germinate in white or red light. The germination response of *H. confusa* seeds to far-red light might also favour access to moisture for a longer period, beneath the surface, as has been proposed for other species inhabiting arid environments (Benítez-Rodríguez et al., 2004).

The photoblastic responses depend of the phytochromes receptors. In positively photoblastic species, phytochrome B is present from the time of seed dispersal and requires 1–1000 $\mu\text{mol m}^{-2}\text{s}^{-1}$ to induce germination. In contrast, only a few milliseconds of light exposure are enough for germination to occur (Smith 2000) due to phytochrome A (Botto et al., 1996), which induces germination at 0.0001–0.05 $\mu\text{mol m}^{-2}\text{s}^{-1}$. Phytochrome A is synthesised when the seeds are imbibed in darkness after the first rains and could induce germination even if the seeds are buried and remain in near-darkness.

Photoblastism shows a clear tendency in certain plant families. Fabaceae and Poaceae tend to be dark germinators, whereas others (e.g., Asteraceae) require light for germination. For this reason, photoblastism has been considered to include a phylogenetic component (Fenner

and Thompson, 2005). Positive photoblastism is more frequent in the previously studied saxicolous and terrestrial species (Benzing et al., 2000; Tarré et al., 2007; Pereira et al., 2009; 2010). The positive photoblastism exhibits by *H. confusa* coincides with the light response tendency of the terrestrial bromeliads. The fact that the epiphytic species of Bromeliaceae studied to date do not show a clear trend of light seed responses (these species can be positive photoblastic negative photoblastic or indifferent (Souza-Garcia and Gonçalves-Oliveira, 2007) may be result of an loss or physiological modification from and original condition of positive photoblastism in early bromeliads (terrestrial from sunny areas in rainforest, Givnish et al., 2007) that changed its light germination responses in relation with the micro-environment condition in the canopies, further studies are needed to clarify this aspect.

4.2. *Effect of hydropriming and natural priming on germination and seedling survival*

Despite the importance of light for germination and seedling emergence from the soil, water is the primary limiting factor affecting all stages of the life cycle in desert plants (quantity and continuity in availability in time, Gutterman, 1994). The tolerance to H-D events demonstrated by the response of *H. confusa* seeds to the HP and NP treatments indicates that most of the seeds may survive until the erratic rains occur at the beginning of the rainy season. Moreover, the tolerance of *H. confusa* to H-D events also may allow seeds to advance the germination process during sporadic hydration events and retain these advances during subsequent dehydration events. For *H. confusa* seeds, these metabolic advances resulted in the early emergence of seedlings in both the HP and NP treatments. Rapid germination may occur under field conditions when the rainy season is well established, as in sharply seasonal environments in the Mediterranean (Thanos et al., 1995). This physiological advance may have strong positive effects on the fitness of the plant (e.g., for growth and fecundity; Verdú and Traveset, 2005).

Nevertheless, the rapid emergence of seedlings observed in *H. confusa* after the NP and HP treatments in the field may also induce a high risk of mortality due to desiccation because tolerance to dehydration is acquired at the end of seed development and is lost as germination advances (Vertucci and Farrant, 1995). Additionally, the seedlings have an unfavourable surface:volume ratio for water conservation (Jordan and Nobel, 1979). However, after 62 days

followed by a week without watering, the seedlings of *H. confusa* showed survival rates greater than or equal to 60% depending on the type of pre-treatment. One week without watering was sufficient to cause the substrate to become completely dry. Hydropriming produced 80% seedling survival. This outcome is known to occur for seeds of economic value that are regularly exposed to HP prior to storage and sale (Halmer, 2004). The seedlings appeared wilted after the non-watering treatment. However, they recovered fully after hydration, indicating that the permanent wilting point was not reached for the surviving seedlings. The failure of natural priming to improve seedling survival could be a result of insufficient exposure of the seeds to hydration before they were unearthed. However, the amount of exposure to hydration was sufficient to improve the germination of these seeds. It is necessary to explore the effects of repetitive cycles of H-D and the combination of H-D events with other environmental conditions (biotic and abiotic) on *H. confusa* germination and seedling survival in field conditions.

Seeds of *H. confusa* can survive for at least six years in suboptimal storage (in paper bags and in laboratory conditions) (S. Montes-Recinas, personal observation). Because the seeds also survived the H-D treatments, these findings suggest that the seeds may form a seed bank. Moreover, the seeds survived 72 h of exposure at 42 °C. Indeed, higher temperatures may occur in open areas under field conditions. The effect of soil temperatures on seed survival and during H-D should be explored. Exposure of the seeds to a temperature of 42 °C did not affect the viability or several of the germination parameters. The findings for the germination parameters suggest that the effect on the seed coat of exposure to a temperature of 42 °C may not be necessary for the germination of a quiescent, vigorous and mature embryo. As a result, the fitness responses of germination, seedling establishment and survival depend on the germination and seedling requirements: light, moisture and, most likely, temperature. However, the effect of temperature on imbibed seeds might differ from the effect on dry seeds. In hydrated seeds, temperatures as high as 42 °C may induce germination inhibition, secondary dormancy or seed death (Hill and van Staden, 2003). Even seedling tolerance to stress also depends on the temperature during HP (Bray, 1995).

4.3. Morphology and structure of the seeds

The characteristics of *H. confusa* seeds, including small size (2.2–3.6 mm in length; Martínez-Correa, 2008), a reticulate surface and the air-filled dead cells of the seed coat (of the wing; Fig. 8A), suggest wind dispersal. However, these same characteristics may explain why *H. confusa* seeds float on water (S. Montes-Recinas, personal observation). This morphology is also related to hydrochory in other species (Werker, 1997). Hydrochory has also been hypothesised for other species of bromeliads (*Pepinia aphelandriflora* (Lem.) André and *P. punicea* (Scheidw.) Brongn. ex André) with spongy-winged seeds (Benzing et al., 2000). Additionally, the water buoyancy of *H. confusa* seeds may help to prevent the burial of the seeds at soil depths where the amount of light is insufficient for germination.

Additionally, it was necessary to shake the dry inflorescences to obtain the seeds because the fruits did not open completely and the seeds could remain trapped inside the fruits. Hence, *H. confusa* seeds may remain inside the fruits and be dispersed from the fruits until the rainy season, as described for other *Hechtia* species (e.g., *H. schottii*; Ramírez-Morillo et al., 2007). Therefore, *H. confusa* may establish a temporary aerial seed bank. However, more field data on the maturation of *H. confusa* fruits are required to confirm this hypothesis.

The brown cells with condensed tannins observed in this study may correspond to the inner integument described in other bromeliads such as *Ananas comosus* (L.) Merr. (Rao and Wee, 1979). Specifically, these cells may correspond to the endotegmen, as suggested for *Tillandsia* spp. (Magalhães and Mariath, 2012). However, embryological studies of the genus *Hechtia* are needed to confirm this hypothesis. Tannins are commonly responsible for the brown coloration of seeds (Werker, 1997), have the property of precipitate proteins and make plant tissues unpalatable. For these reasons, tannins are considered one of the most important plant defences against herbivores and omnivores (Moilanen and Salminen, 2008).

Leaching of the tannins was observed during the germination experiments with the *H. confusa* seeds (S. Montes-Recinas, personal observation). The leaching of the water-soluble fraction of the tannins during hydration events may decrease the mechanical strength of the seed coat and facilitate the protrusion of the radicle. Additionally, the loss of tannins may reduce the opacity of the seed coat. Moreover, the leaching of the tannins during hydration events (inside the fruits or in the soil) may protect the seed against pathogens.

An aleurone layer has been described in the seeds of *Tillandsia* spp. In *H. confusa* seeds there is a layer (that did not stain with Lugol's reagent) that occupied the same position as the aleurone layer in *Tillandsia* spp. seeds and it might also mediate the mobilisation of reserves by secreting enzymes to break down the starch and proteins of the endosperm, as previously proposed for *Tillandsia* spp. (Magalhães and Mariath, 2012).

Hechtia confusa seeds are albuminous, as are the seeds of other bromeliad species such as those of the genus *Tillandsia* (Montes-Recinas et al., 2012; Magalhães and Mariath, 2012). However, the endosperm proportion of *H. confusa* seeds is higher than that found in the seeds of *Tillandsia* spp. A small embryo characterises seeds with a well-developed endosperm (Werker, 1997), as is the case in *H. confusa*. The abundant starch in the endosperm of *H. confusa* seeds (Fig. 8A-D) may participate in the dynamics of water flow into the seeds, reducing their hydric potential (Bewley and Black, 1994) and helping to retain the water within the seeds, as previously described in cereals (Allen et al., 2000) and tobacco seeds (Manz et al., 2005). However, structural and biochemical studies are needed to confirm this hypothesis.

5. Conclusions

Hechtia confusa seeds are quiescent, orthodox and show positive photoblastism; they can germinate in white, red and far-red light. This response to light may be an adaptation to germinate under favourable conditions of temperature and moisture in the field to facilitate seedling establishment. Dry seeds of *H. confusa* tolerate high temperatures, and imbibed seeds tolerate H-D events. Hydration-dehydration events reduce the lag time for seed germination, but the ecological implications of this effect have not yet been investigated. The partial seed retention observed in the fruits suggests that *H. confusa* may establish a temporal aerial seed bank, by the seeds water buoyancy, is suggested also hydrochory in addition to anemochory as dispersal mechanism.

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Capítulo II

Temperature and water requirements for germination and effects of discontinuous hydration on germinated seed survival in *Tillandsia recurvata* L.

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Temperature and water requirements for germination and effects of discontinuous hydration on germinated seed survival in *Tillandsia recurvata* L.

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Abstract *Tillandsia recurvata* is an epiphytic bromeliad with a wide distribution in the Americas; however, little is known about the development of its post-seminal adaptations for survival in epiphytic environments. The purpose of this study was to define the temperature and water requirements for the germination of *T. recurvata* seeds. The absence of radicle emergence in *T. recurvata* seeds resulted in 2 stages of germination: swollen with broken seed coat (stage-1) and chlorophyllic embryos (stage-2). The effects of partial or discontinuous hydration on germinated seed survival were also assessed. The seeds were collected in a semi-arid shrubland of Mexico City. We explored: (1) whether water vapour can provide a sufficient water source for germination; (2) the temperature required for germination stage-1 and the optimal and critical osmotic potentials for germination in both germination stages; (3) the effect of seed incubation at different osmotic potentials that undergo subsequent dehydration on their survival in stage-2; and (4) the loss of dehydration tolerance

during early post-seminal development. In addition, an image of *T. recurvata* seed anatomy was obtained to illustrate its structures. Germination stage-1 of *T. recurvata* seeds is rather similar across the tested temperature range. The seeds required to be in contact with liquid water to germinate. The interval of osmotic potential facilitating both germination stages was from 0 to -0.6 MPa. Although germinated seeds displayed dehydration tolerance, this tolerance decreased in germination stage-2. The osmotic potential during germination affected the tolerance of the chlorophyllic embryos (stage-2) to subsequent dehydration.

Keywords Germination · Epiphyte · Osmotic potential · Regulated hydration · Anatomy

Introduction

Tillandsia (Bromeliaceae Juss.) is a widely distributed American epiphytic genus that includes some of the most drought-tolerant epiphytic bromeliads (Smith 1989; Benzing 1990). *Tillandsia* species have several notable adaptations that allow them to colonise microsites with little or no soil and limited and patchy availability of nutrients and water, including tree bark, rocks (Bennet 1991) and electric wires. One such adaptation is leaves covered with absorptive trichomes (scales) that reflect solar radiation (reducing heat loads and photo damage; Lüttge et al. 1986) and participate in collecting water from fog (Guevara-Escobar et al.

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2010). Therefore, the roots in *Tillandsia* species are used only as holdfasts. Based on these features, the *Tillandsia* species have been assigned to ecophysiological type IV, ‘atmospheric-absorbing trichomes’, where the additional water reservoir known as phytotelmata or ‘tank’ (formed from overlapping leaf bases, evident in the category of ‘tank-absorbing trichomes’) is reduced or absent (Smith 1989). Other important adaptations include crassulacean acid metabolism (CAM) and the ability of some species (e.g., *Tillandsia recurvata*) to tolerate large amounts of water loss (Benzing et al. 2000). However, some of these strategies are not present in seeds during germination or in the early post-seminal development of the *Tillandsia* species (Bader et al. 2009). Survival strategies of the *Tillandsia* species during early developmental stages are relatively unknown, and the underlying factors explaining the high mortality of seedlings and juveniles of *Tillandsia* species under field conditions remain unclear (Castro-Hernández et al. 1999; Bernal et al. 2005; Winkler et al. 2005).

Tillandsia is a vascular epiphytic genus that is common in tropical dry forests and semi-arid shrublands (Mondragón et al. 2004). These environments are sharply seasonal; however, before the true start of the rainy season, rains often occur erratically. Thus, seeds in these environments are exposed to a partial imbibition or periodic hydration and subsequent dehydration without radicle protrusion. These variations in seed hydration result in water stress and/or desiccation events for seeds; water stress might result in delayed and reduced germination or prevent germination completely (Hegarty 1977; Bradford 1995). Nevertheless, exposure to discontinuous hydration is not necessarily detrimental to seed germination. For many cultivated species, partially controlled seed imbibition with subsequent dehydration is used to improve and homogenise germination parameters (e.g., lag time, rate, and final percentage of germination) and to increase the seedlings’ desiccation tolerance (Bruggink and van de Toor 1995). These effects represent advantages to seedling establishment and survival (Bray 1995). The effects of ‘cycling hydration’ have been described in some wild species from arid environments with resulting improvements in seed germination (hydric memory, Dubrovsky 1996, 1998) and/or seedling vigour (Lobato et al. 2008). These germination responses can also be obtained through the exposure of the seeds to field conditions

that include several precipitation events (González-Zertuche et al. 2001; Gamboa de Buen et al. 2006).

The effect of discontinuous hydration events and the availability of water (osmotic potential, Ψ_{π}) on seed germination have been extensively studied in crop species (Bray 1995) and some wild species (Evans and Etherington 1990; Swagel et al. 1997). However, despite the unique water relations exhibited by adult epiphytes—including their adaptation to a shallow substrate and, for most *Tillandsia* species, a short period of water availability—little is known about their water relations during the early stages of development (Benzing et al. 2000).

Therefore, the purpose of this study was to define the temperature and water requirements for the germination of *T. recurvata* seeds and characterise the effects of discontinuous hydration on the embryos survival. We explored the following questions: (1) whether water vapour can provide a sufficient water source for germination; (2) the temperature required for germination as well as the optimal and critical Ψ_{π} for germination; (3) the effect of seed incubation at different Ψ_{π} on the survival of embryos under subsequent dehydration and (4) the loss of dehydration tolerance during early post-seminal development.

Methods

Study species

We focused on *T. recurvata* L. (Bromeliaceae: Tillandsioideae), a small atmospheric bromeliad with CAM (Benzing et al. 2000) that inhabits the canopies of shrubs and trees in Neotropical semi-arid ecosystems (Montaña et al. 1997). *Tillandsia recurvata* flowers in the autumn (Bernal et al. 2005), and the capsules mature in winter (February), when they release small plumose seeds (2–3 mm in length; Fernández et al. 1989). The seeds are fusiform (Fig. 1a) and have a plumose coma (flight apparatus) at the apical extreme consisting of long trichomes derived from cells of the external layer of the testa (external integument; Benzing et al. 2000; Wester and Zotz 2011). This coma facilitates effective wind-dispersal and promotes seed adhesion to specific substrates (Benzing et al. 2000).

Germination of *T. recurvata* does not occur until the rainy season (June–September). In many

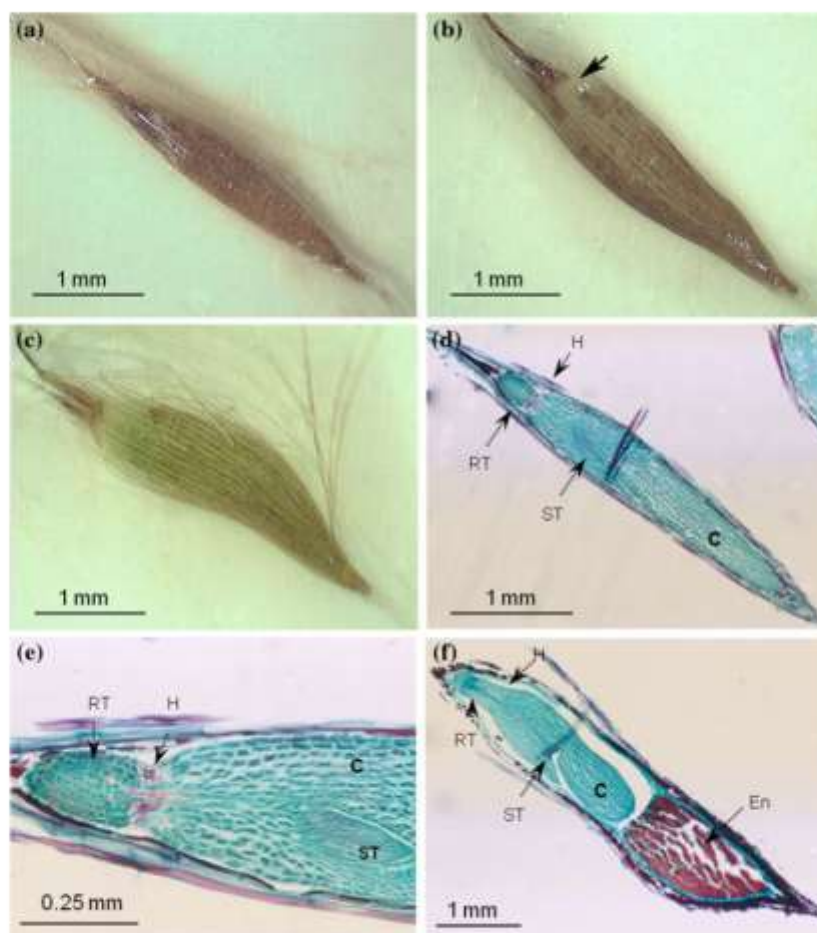


Fig. 1 Morphology of germination stages (S1, S2) of *Tillandsia recurvata* and the anatomy of *T. recurvata* and *Tillandsia prodigiosa* seeds. *T. recurvata*: **a** Dry mature seed with seed coat. **b** Germination stage-1 at 4 h of incubation; the seed is swollen and the seed coat broken in the hypocotilar region (arrow). **c** Germination stage-2; chlorophyllic embryos after 5 days of incubation. **d** Longitudinal section of a dry mature

seed. *H* hypocotyl-root axis, *RT* root tip, *ST* shoot tip, plumule, *C* cotyledon. **e** *H* narrow hypocotyl-root axis region with dead cells, *RT* root tip, *ST* shoot tip. **f** Longitudinal section of a dry mature *Tillandsia prodigiosa* seed with endosperm, which is missing in *Tillandsia recurvata* endosperm (*En*), a hypocotyl-root axis longer than in *T. recurvata* with living cells in the hypocotilar region (*H*), *RT* root tip and *ST* shoot tip

angiosperms, germination is evidenced by the emergence of the radicle (Bewley 1997). In the Tillandsioideae, seed germination occurs through an intercalary growth near the base of the hypocotyl, which pushes part of this organ and the adjacent radicle through the seed coat (Benzing et al. 2000). However, for *T. recurvata* seeds, the hypocotyl and radicle emergence do not occur; the seed coat is broken a few hours after imbibition, and subsequently, the embryo turns green upon chlorophyll synthesis (personal observation). After 1 month (30–40 days),

the first true leaf emerges across the seed coat and the root appears several months later (Benzing 1980). This type of germination has been reported in other monocots, e.g., *Cocos nucifera*, where the first structure that emerges is the plumule, and several months later, the root emerges (Orozco-Segovia et al. 2003). The morphological changes of *T. recurvata* seeds (deprived of a seed coat) from germination to seedling development have been described as a continuum (Fernández et al. 1989). However, when the seed coat is present, this process occurs in delimited steps, and

few studies (as in Bader et al. 2009) report the germination criterion used.

Study area and seed collection and preparation

Seeds of *T. recurvata* were collected from open capsules in February 2010 from 50 individuals growing in xeric shrub vegetation inside the protected area 'Parque Ecológico de la Ciudad de México' (PECM; 19°10'–19°14'N, 99°16'–99°13'W; 2,360–2,860 m elevation). The PECM is in the southwest of the Valley of México, with a seasonal climate (dry during October–May and rainy during June–September). The average temperatures of the warmest and the coldest months are 14 and 9 °C, respectively, with a mean thermal oscillation of 5 °C. The average monthly rainfall ranges from 9–230 mm (González-Hidalgo et al. 2001). The seeds were obtained from the mature capsules of *T. recurvata* plants growing on branches of *Dodonaea viscosa* (L.) Jacq. (Sapindaceae), which is a common phorophyte for *T. recurvata* in the PECM.

For experiments on optimal germination temperatures, the *T. recurvata* seeds were also collected in February 2010 from the valley of Zapotitlán de las Salinas, Puebla, México (18°20'N, 97°28'W; 1,400–1,600 m elevation). The average annual temperature is approximately 20 °C, and the mean annual precipitation is approximately 300–350 mm (Zavala-Hurtado 1982).

After collection, the seeds were stored in paper bags under laboratory conditions (23–25 °C and 30–40 % relative humidity, RH) until use. The laboratory experiments and germination tests were conducted under a 12-h photoperiod with a photosynthetic photon flux density (PPFD) of 30 $\mu\text{mol m}^{-2} \text{s}^{-1}$ at 25 °C in germination chambers (Biotronette 844, Lab-Line Instruments, Inc. Melrose Park, IL, USA). Before laboratory germination, the distal part of the coma appendage (plume) was cut from each seed (to facilitate seed manipulation), and the seed surface was sterilised with 1 % Na-hypochlorite for 2 min.

Due to the absence of radicle emergence and the inconspicuous emergence of the hypocotyl, the completion of *T. recurvata* germination is not easy to determine. Therefore, we used 2 morphological changes to recognise the post-seminal development of the embryo. Germination was defined by 2 post-seminal development stages: seed swollen with the seed coat broken (0.25 mm wide) on the base of the

hypocotyl area (stage-1, S1; Fig. 1b) and green chlorophyllous embryos (stage-2, S2; Fig. 1c). These morphological changes were observed using a stereomicroscope (Stereo Star Zoom, American Optical Spencer, USA).

Seed anatomy

To describe the structure of the *T. recurvata* seed and relate it to their physiological response, the seeds were fixed in formalin (5 %), acetic acid (4 %), ethyl alcohol (48 %) and water (43 %; FAA) over 3 weeks, dehydrated using an alcohol series (50, 70 85 98 and 100 %), and subsequently placed in xylene and embedded in Paraplast® Regular. The seeds were cut longitudinally into 8- μm sections using a Microtome Leica RM 2135 (Leica Microsystems Inc, Columbia, MD). The sections were stained with Safranin (overnight) and Fast Green (2s) and observed using a photomicroscope (Olympus Provis AX-70; Olympus, Tokyo, Japan). Because the atmospheric (ecophysiological type IV) *T. recurvata* endosperm is missing, we compared the structure of this species with the seed anatomy of *Tillandsia prodigiosa* (Lem.) Baker, a tank-absorbing trichomes species (ecophysiological type III). Seeds of this species were collected in 'Las Joyas' Research Station (ECLJ; 19°34'–19°37'N, 104°14'–104°18'W). In this area, the climate is markedly seasonal, and dry in summer; the average annual temperature is 15 °C, and the average annual precipitation is 1,800 mm (Cuevas and Peñáz 2004).

Determination of the sufficiency of water vapour for germination

The different relative humidities and water potentials were established with saturated salt solutions (Table 1; Stokes and Robinson 1949; Greenspan 1977) and calculated using the following formula (Lambers et al. 2008):

$$\psi = -RT/V_w^0 \ln(\% \text{RH}/100)$$

where R is the gas constant ($\text{J mol}^{-1} \text{K}^{-1}$), T is the temperature in K and V_w^0 is the partial molar volume of pure water ($\text{m}^3 \text{mol}^{-1}$).

The salts were added to distilled water at approximately 40 °C with stirring until the salt was completely dissolved. Additional salt was added to ensure an excess of the saturating salt (Greenspan 1977). The

Table 1 Relative humidity and water potential established with saturated salt solutions, at constant 20 °C

Salt	Relative humidity (RH %)	Water potential $-\Psi$ (MPa)
Mg (NO ₃) ₂	50	93.80
NaCl	75	38.93
KCl	85	21.99
K ₂ SO ₄	99	1.36

T. recurvata seeds were attached by the hairs of the coma to glass sticks, which were suspended at 3 mm above the surface of different saturated salt solutions (10 mL) in 25-mL assay tubes sealed with parafilm in accordance with the Wuest (2007) technique. The assay tubes were incubated under a 12-h photoperiod with a PPFD of 30 $\mu\text{mol m}^{-2} \text{s}^{-1}$ at 20 °C in a germination chamber. Five seeds were used per assay tube, with 3 replicates per treatment, and daily observations were made over 3 weeks.

Determination of optimal temperature and optimal and critical Ψ_x for germination

The optimal temperature for germination S1 was compared between 2 populations of *T. recurvata* (from PECM and 'Zapotitlán de las Salinas'). The seeds were placed in Petri dishes containing 1 % agar (Bioxon, México) and incubated at different constant temperatures (15, 20, 25, 30 and 35 °C). Thirty seeds per dish with five replicates per treatment were used. The final germination S1 percentages were recorded after 2 days of incubation.

The seeds were placed on nylon mesh (to avoid oxygen deprivation) in Petri dishes and incubated at 25 °C with different solutions (10 mL): 0, -0.04, -0.1, -0.6, -0.75, and -0.86 MPa. These potentials were generated with polyethylene glycol 8000 (PEG 8000, J.T. Baker, USA). The PEG solutions were prepared according to the solute potential, using molar-molal-g solute/g water interconversion software (SPPM, Michel and Ratcliffe 1995). The Petri dishes were weighed and sealed with parafilm to reduce water loss. The dishes were weighed every other day, and the water lost through evaporation was replaced. Thirty seeds per dish with six replicates per treatment were used. The germination stages (S1 and S2) were recorded during the 4-h incubation period

and then daily until there were no more changes in germination (13 days).

Effect of seed incubation at different Ψ_x on germinated seed survival under subsequent dehydration

The seeds were placed in Petri dishes on nylon mesh and were incubated with 10 mL of 1 of several solutions at -0.04, -0.1 and -0.6 MPa. The Petri dishes were sealed with parafilm, and the water lost through evaporation was replaced every other day. Thirty seeds per dish with six replicates per treatment were used. After 15 days of incubation, the chlorophyllic germinated seeds (S2) were washed with tap water to eliminate PEG, blotted on a paper towel and transferred to Petri dishes containing filter paper (Whatman USA, No. 1). Hydration occurred at 25 °C, and dehydration occurred at laboratory conditions (23–25 °C and 30–40 % RH). The germinated seeds (S2) were deprived of water for 5 days (dehydration treatment) and rehydrated on wet filter paper using tap water for 3 days. This dehydration–rehydration (5–3 days) cycle was applied twice to the same S2. Fifteen germinated seeds (S2) per dish with ten replicates per treatment were used, and survival was quantified at 15 days after the end of the second dehydration–hydration cycle.

Loss of hydration tolerance during early post-seminal development

The seeds were incubated for different time periods (1, 2, 3, 5, 7, 9 and 10 days). For each treatment, 30 seeds per dish with six replicates were shown. At the end of each incubation period, the germinated seeds (S1 and/or S2) were deprived of water for 1 day and rehydrated on wet filter paper for 10 days. Survival was evaluated at the end of this rehydration period.

Data analysis

Because the data did not comply with the assumptions of homogeneity of variances, the data were compared using the Kruskal–Wallis test (*H*; Zar 1984) and visually compared using box-and-whiskers plots (Tukey 1977; Statistical Graphics Corporation software, Englewood Cliffs, NJ, USA).

Stages germination percentages (arcsine transformed) obtained in the Ψ_{π} treatments were fitted to a sigmoid model using Table Curve 2D, v3 (AISN software, Chicago IL, USA).

$$y = a/[1 + b^{(-cx)}]$$

Two germination parameters were obtained from the sigmoid fittings: (1) the time at which the first seed germinated (lag time) and (2) the rate of germination (velocity, first derivate maximum of the sigmoid curve, González-Zertuche et al. 2002). The data were also analysed using the Kruskal–Wallis test. The survival percentages of stage-2 seeds obtained in the dehydration tolerance loss test were also fitted to a sigmoid curve using Table Curve 2D.

Results

Seed anatomy

The following observations were noted from the histological observation of a longitudinal section of the *T. recurvata* seed (Fig. 1d): a thin layer of trichomes in the external integument, an inner layer formed by one stratum of cells containing pigments, a lineal embryo filled the seed, the cotyledon was surrounding the shoot tip and no endosperm was present. Further, the hypocotyl–root axis was a narrow area of dead cells (Fig. 1d, e).

The longitudinal section of the *T. prodigiosa* seed (Fig. 1f) showed the trichome layer, the inner layer of cells with pigments, which were thicker than in *T. recurvata*, and the lineal embryo, which accompanied the endosperm. In contrast with *T. recurvata*, in *T. prodigiosa* the hypocotyl–root axis is longer and their cells are not dead.

Seed exposure to water vapour

During the 3 weeks of exposure to water vapour at different relative humidities (50, 75, 85 and 99 %), some cases of water condensation were observed, but the condensation never reached the seeds. During this period, *Tillandsia recurvata* seeds did not show any of the post-seminal morphological changes considered to be evidence of germination.

Optimal temperature and optimal and critical Ψ_{π} for germination

The seeds of both localities germinated (S1) at all the temperatures tested (15–35 °C). The final germination percentages were significantly higher in seeds from Zapotitlán de las Salinas than in seeds from PECM ($H = 20$, $P < 0.001$; Fig. 2).

From 0 to –0.6 MPa, the lag time for germination S1 was between 4–24 h, and at –0.75 MPa, germination was retarded significantly ($H = 14$, $P < 0.05$; Fig. 3a). At –0.9 MPa, germination did not occur. The germination rate to reach S1 showed a significant reduction at –0.75 MPa with respect to Ψ_{π} values less negatives ($H = 16$, $P < 0.05$; Fig. 3a). The final percentage of germination (S1) was at least 60 % from 0 to –0.6 MPa but showed a significant reduction at –0.75 MPa ($H = 20$, $P < 0.001$; Fig. 3a).

Significant differences were recorded in the lag time for germination S2 from 0 to –0.6 MPa versus –0.75 MPa ($H = 26$, $P < 0.001$; Fig. 3b). The rate at which the embryos became chlorophyllic showed significant differences between different Ψ_{π} from 0 to –0.6 MPa versus –0.75 MPa ($H = 20$, $P < 0.001$; Fig. 3b). The final percentage germination S2 was higher in solutions with higher Ψ_{π} (0 to –0.6 MPa) versus –0.75 MPa ($H = 21$, $P < 0.001$; Fig. 3b). Germination was not observed at –0.9 MPa (Fig. 3a).

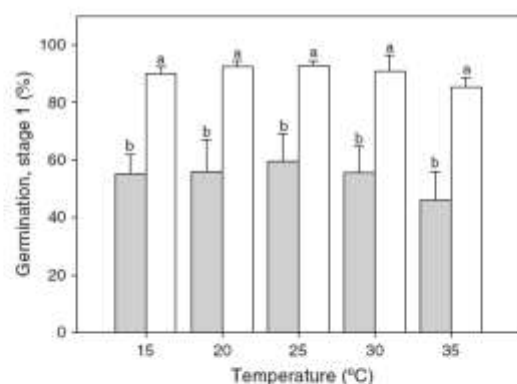


Fig. 2 Germination of *Tillandsia recurvata* a in temperature gradient. Final percentages of stage-1 germination in Zapotitlán de las Salinas (white) and PECM (grey) seeds along a temperature gradient (x-axis). The bars indicate the standard error

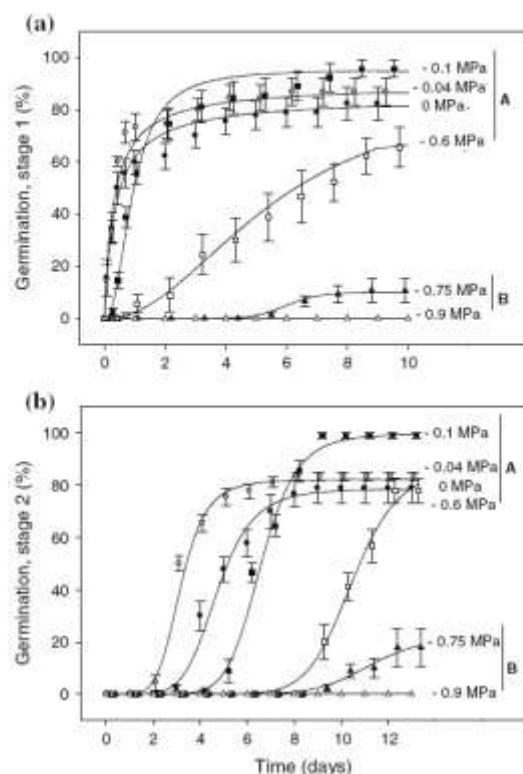


Fig. 3 Germination of *Tillandsia recurvata* in an osmotic potential gradient. Cumulative curves of the germination of *Tillandsia recurvata* seeds incubated in PEG solutions with different osmotic potentials; **a** germination stage-1, **b** germination stage-2. Capital letters in each panel indicate significant differences. The bars indicate the standard error

Effect of seed incubation at different Ψ_{π} on the dehydration tolerance of germinate seeds (S2)

There was evidence that the Ψ_{π} at which the incubated seeds germinated affected their survival when the germinated S2 seeds were exposed to two dehydration–hydration (5–3 days) cycles. The germinated S2 seeds displayed the highest survival when incubated at -0.04 and -0.1 MPa and the lowest survival when incubated at 0 and -0.6 MPa ($H = 11$, $P < 0.05$; Fig. 4).

Dehydration tolerance loss of germinated seeds

The survival of the germinated seeds was reduced as the incubation period increased, following a negative

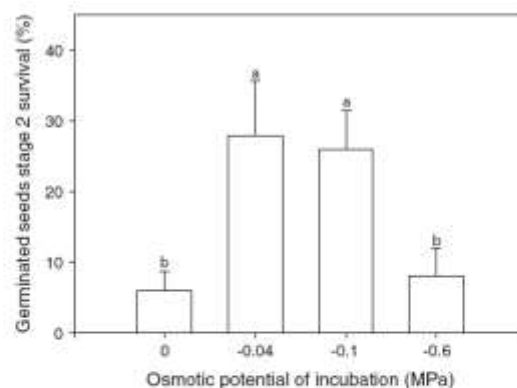


Fig. 4 Effect of osmotic potential on germinated seed (stage-2) survival. Survival percentage embryos exposed to 2 dehydration–hydration (5–3 days) cycles. The Ψ_{π} in which the seeds were incubated is indicated on the x -axis. The bars indicate the standard error

sigmoid pattern ($R^2 = 0.64$; Fig. 5). This finding was indicated through lower survival percentages after 7 days of incubation. ($H = 21$, <0.05 ; Fig. 5). Although the reduction of the germinated seeds survival, caused by subsequent dehydration, was not significant after five incubation days, it did show a strong tendency to reduction. Moreover, this time period coincides with the change from S1 to S2 when embryos become chlorophyllous (Fig. 5).

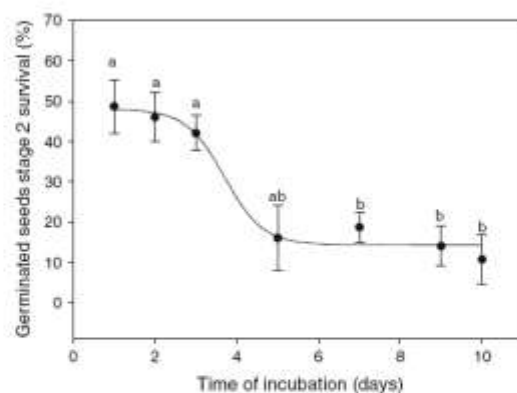


Fig. 5 The loss of dehydration tolerance during the germination of *Tillandsia recurvata*. Survival percentage of germinated seeds (S2) incubated at different time points (x -axis) and exposed to a single dehydration day and subsequent hydration for 10 days. The bars indicate the standard error

Discussion

A typical physiological strategy of seedlings and adult stages of *Tillandsia* species is the ability of their leaves to obtain water from vapour (Guevara-Escobar et al. 2010). Even some seeds of terrestrial species, such as wheat, can use water vapour to germinate (Wuest 2007). However, *T. recurvata* seeds required contact with liquid water to initiate imbibition. Direct contact with liquid water could represent a signal that the water is sufficient to complete germination and offer a more successful strategy to seasonal environments instead of germination with water vapour or in a highly negative Ψ_{π} . However, the water vapour source might be important once germination has begun (especially the fast germination of *T. recurvata*) because the liquid and vapour sources might occur simultaneously (Wuest 2002) or consecutively, when environmental conditions favour high values of RH.

Liquid water upon the surface of the bark or rocks is usually only available for a short time following rain events. The Ψ_{π} would be expected to decrease rapidly when these surfaces are exposed to radiation or wind. In laboratory conditions of 25 °C and 40 % RH, *D. viscosa* branches were immersed in water for 5 min and retained in water for 4 h (S. Montes Recinas, unpublished data). Hence, *Tillandsia recurvata* might quickly germinate before the Ψ_{π} value becomes lower than the critical Ψ_{π} for germination, as is known to occur in other species (Allen and Meyer 1998).

The small seeds and seedlings of *T. recurvata* might result from a large surface-to-volume ratio that makes them more susceptible to rapid hydration (Baskin and Baskin 1998) and, consequently, dehydration. However, *T. recurvata* seeds showed a thin covering of hygroscopic trichomes that most likely favour fast water absorption and might also reduce water loss while the germinated seeds are inside the seminal coat, as demonstrated in the bromeliad *Catopsis sessiliflora* (Wester and Zotz 2011). Once the scales appear on the embryo, at night, these would uptake a provision of water, as in *Tillandsia usneoides* (Martin and Schmitt 1989). This provision of water may reduce the duration of the dehydration events.

Another adaptation of *T. recurvata* seeds to ephemeral water availability might be the absence of endosperm reserves (Fig. 1d) that is present in other *Tillandsia* species, such as *Tillandsia usneoides* (Benzing et al. 2000) and *Tillandsia prodigiosa* (Fig. 1f).

Such an absence suggests that the embryo absorbs the reserves during its development, as occurs in many monocots (Bewley and Black 1994; Orozco-Segovia et al. 2003). The fast germination of *T. recurvata* (Fig. 3) and its endosperm absence supports the observation that the species with storage cotyledons and little endosperm germinate faster and require a shorter time to seedling establishment than species with a small embryo and copious endosperm (Vivrette 1995).

The critical Ψ_{π} at which *T. recurvata* can still germinate (−0.75 MPa) is not as low as critical values observed for some other species of arid environments. For example, *Kochia prostrata* (Chenopodiaceae) germinates at Ψ_{π} of −1.85 MPa (Allen et al. 2000), while the hemiepiphyte *Ficus aurea* (Moraceae) germinates at Ψ_{π} of −1 MPa (Swagel et al. 1997). The critical Ψ_{π} value of −0.75 MPa required for the germination of *T. recurvata* seeds is similar to that required for the epiphyte *Rhipsalis baccifera* (Cactaceae −0.77 MPa; De la Rosa and Briones 2010). It is relevant to consider that these results were obtained under constant Ψ_{π} conditions, and therefore, they might differ from those prevailing in field conditions (Hadas 2004).

The germination of *T. recurvata* seeds begins with a slightly swollen seed followed by the growth of the cotyledon near the hypocotyl area, which is accompanied by the rupture of the seed coat (germination S1; Fig. 1b). The absence of development and growth of the primary root might be explained by the narrow hypocotylar area of dead cells (before the root tip; Fig. 1c), which might interrupt the flow of water to the radicle during all germination steps. In addition to the slow-growing embryo, the embryo remains inside the seed coat for several weeks and is protected even after the development of scales on the embryo surface (S. Montes-Recinas, unpublished data).

The thin seed coat (Fig. 1d) might not represent an obstacle to light transmittance, thus favouring early photomorphogenesis and enabling photosynthetic activity in the embryo after 4–9 days incubation at −0.1 MPa. The early *T. recurvata* embryo became chlorophyllous (and the possible subsequent photosynthetic activity), which might be related to the correlation between *T. recurvata* distribution and photosynthetic active radiation of the *D. viscosa* branches (Escutia-Sánchez 2009).

The germination response of *T. recurvata* seeds matches the type 2 response defined by Evans and Etherington (1990) for species growing in dry and

well-drained habitats (e.g. *Reseda luteola* (Reseda-ceae) and *Teucrium scorodonia* (Lamiaceae)). This type of response is characterised by high germination levels maintained within a certain Ψ_{π} range. If this limit is exceeded, there is a sharp reduction in germination (Fig. 2). In this study, the upper limit for *T. recurvata* was estimated at -0.6 MPa. However, the species considered by Evans and Etherington (1990) germinate in a wider Ψ_{π} interval from 0 to -1 and -1.5 MPa. The shorter Ψ_{π} interval in which *T. recurvata* germinates might be related to ephemeral liquid water availability on the phorophyte's bark, representing a safer signal for germination.

The type 2 germination response is considered a possible advantage for establishment under water stress conditions (Evans and Etherington 1990). Contrary to the short Ψ_{π} interval where *T. recurvata* seeds germinate, this species had a wide temperature range where germination S1 occurred, which might be related to the wide distribution of *T. recurvata*.

The Ψ_{π} for S2 germination was higher at <0 , though close to it (-0.04 and -0.1 MPa), compared to 0 MPa. Slow water uptake determined by low Ψ_{π} could protect embryonic cells from injury caused by a high rate of water uptake in the seeds (imbibitional injury; Leprince and Golovina 2002). However, high survival at these Ψ_{π} might also be related to the tolerance to dehydration acquired in the early germination phases during incubation in PEG (Bruggink and van de Toor 1995).

A gradual decrease in desiccation tolerance can be expected as germination proceeds (Farrant et al. 1988). Our results show that *T. recurvata* embryos (S1 and S2) are desiccation-tolerant under laboratory conditions. However, this tolerance began to decrease when the embryos turned chlorophyllic, and only 10 % of the germinated S2 seeds survived day 1 of desiccation, which was imposed after 10 incubation days (Fig. 5). This finding is consistent with the proposal that relatively high rates of metabolism are related to an increase in desiccation sensitivity (Farrant et al. 1997). This low survival percentage might have an important ecological value for maintaining *T. recurvata* populations in the field.

The loss of dehydration tolerance might be due to water condensation on the branches, even during the dry season, which could induce germination (S1 and S2), thus increasing desiccation sensitivity and consequently causing the death of chlorophyllic embryos. This

outcome was suggested by the observations of the dead embryos under a stereomicroscope. In addition, other environmental factors in the field, such as wind and increased temperature, might cause different kinetics of water uptake and bark water loss. The low field survival of *Tillandsia* species observed by several authors (Castro-Hernández et al. 1999; Bernal et al. 2005; Winkler et al. 2005) can be related to the occurrence of germination and the transformation into chlorophyllic embryos before the establishment of rainy season. Other limiting factors for establishment are high temperature, pathogens (Zotz and Hietz 2001) and chemicals present in the bark (Valencia-Díaz et al. 2010).

In dry environments, the strategy of fast germination under high Ψ_{π} can ensure sufficient water availability to complete germination and achieve establishment. For seeds, remaining as resistant structures versus germination and dehydration tolerance loss (metabolism reactivation) is a trade-off. The rapid germination of *T. recurvata* might be related to different species that *T. recurvata* can use as phorophytes, which in turn is related to the wide distribution of this species in the Americas. This physiological aspect of *T. recurvata* supports the idea of rapid germination as an advantage for plant fitness (Allen et al. 2000; Verdú and Traveset 2005). However, the germinated seeds are particularly sensitive to dehydration from the time they become chlorophyllic (S2), presumably until the appearance of absorbent scales. The negative effect of intermittent dry periods upon germination has also been reported for other atmospheric bromeliads, e.g. *Tillandsia flexuosa* and *Tillandsia fasciculata* (Bader et al. 2009). Therefore, the first erratic rains at the beginning of the rainy season might be an important constraint for the establishment of the *Tillandsia* species, with its high mortality being compensated through copious seed production.

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Capítulo III

Effect of water stress on the germination of two bromeliads from a semi-arid environment in Central México

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Effect of water stress on the germination of two bromeliads from a semi-arid environment in Central México

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Abstract

Terrestrial *Hechtia tehuacana* and epiphytic *Tillandsia circinnatioides* coexist in the Zapotitlán Salinas Valley, México. The objective of this research was to assess the effect of the following osmotic potentials on germination at 25 °C: 0, -0.05, -0.1, -0.5, -1.0 and -1.5 MPa. The germination of *H. tehuacana* was significantly reduced at -0.05 MPa, even though seed germination continued to -1.0 MPa, whereas the germination of *T. circinnatioides* occurred only to -0.05 MPa. These results reveal that both species require high water availability to germinate and that *T. circinnatioides* requires the higher level of the two studied species. The requirement of high water availability for germination in the studied species supports the proposal of this response as a strategy to survive in arid environments by improving the probability of achieving seed germination and seedling establishment. The higher water requirement of the epiphytic species, *T. circinnatioides*, may be related to the additional ephemeral water availability on tree branches than in the soil.

Keywords: Osmotic potential; *Hechtia tehuacana*; *Tillandsia circinnatioides*; Epiphyte; Zapotitlán; Tehuacán-Cuicatlán

1. Introduction

Seeds need to imbibe water and reach a species' specific critical water content for germination (Hunter and Erickson, 1952). A common method used to determine the critical seed hydration level for germination is to hydrate the seeds in a series of solutions with a decreasing gradient of osmotic potential (Ψ_{π} ; Swagel et al., 1997). The Ψ_{π} values of solutions can be established with different substances, such as sodium chloride, mannitol, glycerol or polyethylene glycols of 4000–20,000 (nominal molecular weights). In general, seed imbibition rates and the germination percentage decrease as the Ψ_{π} value of the incubation medium decreases (Hunter and Erickson, 1952; Evans and Etherington, 1990). Most of the species studied to date germinate from 0 to -3.0 MPa under controlled conditions (Swagel et al., 1997; Allen et al., 2000).

According to Evans and Etherington (1990), the germination responses to water availability (water stress) of species from a range of contrasting habitats can be categorised as four different types. Response **type 1**, germination is virtually unaffected by changes in the soil osmotic potential (Ψ_{soil}) and is typical of ruderal species. Response **type 2**, characterised by high percentages of germination in soil as dry as -1.0 MPa, but germination does not occur at lower Ψ_{soil} , is typical of species in arid or well-drained habits. Response **type 3**, the species exhibit a germination that progressively decreases as Ψ_{soil} decreases, until -1.5 MPa, most species from a range of habitats exhibited a similar response (*e.g. Typha latifolia*). Response **type 4**, germination substantially decreases in soils drier than -0.05 MPa, thus the seeds require moist soil conditions to germinate; it is typical of wetland species.

Arid and semi-arid environments are distinguished by a low amount and unpredictable distribution of rains (Gutterman, 1994). Hence, water availability in these environments is a major limiting factor for plant establishment (Adams, 1999). The species that coexist in arid and semi-arid environments may exhibit different strategies for survival (Flores and Briones, 2001; Kos and Poschlod, 2008). Some species of arid environments germinate at a very negative Ψ_{π} value (*e.g., Caragana korshinskii*, 15.0% at -2.1 MPa; Zeng et al., 2010) in similar way as the response type 3.

Other strategy that is associated with species from environments with unpredictable rains is the requirement of high water availability for seed germination. This requirement improves

the probability of successful germination and seedling establishment by reducing the risk of germination in a rapidly drying seed zone (Gutterman, 1994; Allen et al., 2000). Accordingly, a high sensitivity to water availability for germination is proposed as an adaptive mechanism for native xerophytic species (*e.g.*, those inhabiting arid dunes in China; Zeng et al., 2010). The requirement of high water availability corresponds to the germination response type 4 of Evans and Etherington (1990).

The purpose of this study was to identify the sensitivity to water stress for germination of two coexisting bromeliad species from the “Zapotitlán de las Salinas” Valley, a semi-arid region of central Mexico, to determinate the Ψ_{π} interval at which seed germination can occur. In addition, we sought to compare the water requirements between two species with different growth habits: terrestrial *Hechtia tehuacana* and epiphytic *Tillandsia circinnatioides*.

2. Materials and Methods

2.1. Study area

Seeds were collected in the Zapotitlán de las Salinas Valley in the Mexican state of Puebla (18°20' N, 97°28' W). This valley forms a subsystem within the Tehuacán Valley, which is well known for its cactus diversity and its overall floristic endemism (Zavala-Hurtado and Díaz-Solís, 1995). The climate is dry with summer rains. Annual mean temperature is ca. 20 °C and precipitation fluctuates between 300–350 mm. The soils are shallow, stony and halomorphic. The vegetation has been classified as xerophyllous scrub (Zavala-Hurtado et al., 1996).

2.2. Study species

Hechtia tehuacana B. L. Rob. (Bromeliaceae: Hechtioideae; Givnish et al., 2007) is a dioecious and terrestrial species that grows in xeric scrubs and is locally dominant or co-dominant. The succulent leaves form a rosette and its inflorescence attains 1.10 to 1.5 m in height. The inflorescences form erect panicles that bloom from May to December and bear fruits (capsules) with winged seeds (3–3.8 mm) from March to April, from August to

September and in December. *Hechtia tehuacana* is endemic to México and is distributed in the Puebla and Oaxaca states at 1550–2200 m asl (Martínez-Correa, 2008).

Tillandsia circinnatioides Matuda (Bromeliaceae: Tillandsioideae) is an epiphytic species; its curved leaves (12 cm in length) forming an elliptical pseudobulb, are densely covered with silvery scales. The inflorescence develops on the short terminal part of the scape, and the fruits are dehiscent capsules with plumose coma seeds (approx. 3.5 mm without the coma). This species grows in a caespitose manner, with 6–7 rosettes, on rocks, column cacti or shrubs (Matuda, 1973). In the Zapotitlán Salinas Valley, *T. circinnatioides* grows on the phorophyte *Prosopis laevigata* (Humb. & Bonpl. ex Willd.) M. C. Johnst. and on *Cercidium praecox* (Ruiz et Pavón) Harms. *Tillandsia circinnatioides* is endemic to México and is distributed in the Guerrero, Morelos, Puebla and Oaxaca states at 700–1500 m a.s.l. (Pulido-Esparza et al., 2004).

2.3. General procedures

The seeds of *H. tehuacana* and *T. circinnatioides* were collected in February (2007) from the mature fruits of different individuals (5 and 17, respectively) found inside of the Botanical Garden “Helia-Bravo Hollis” in the Zapotitlán Salinas Valley. After collection, seeds were stored in paper bags under laboratory conditions (23–25 °C and 30–40% relative humidity, RH) until their use one month later.

To avoid oxygen deprivation, the seeds of both species were placed on a nylon mesh fixed in a Petri dish, just in contact with solutions of different osmotic potential (10 mL): distilled water (0 MPa) and –0.05, –0.01, –0.5 –1.0 and –1.5 MPa. These potentials were generated using polyethylene glycol 8000 (PEG 8000, J.T. Baker Research Products, Phillipsburg, USA), and the PEG solutions were prepared using the water inter-conversion software SPPM (Michel and Ratcliffe, 1995). The Petri dishes were incubated in germination chambers (Biotronette 844, Lab-Line Instruments, Inc. Melrose Park, IL, USA), under a 12-h photoperiod with $30 \mu\text{mol m}^{-2} \text{s}^{-1}$ of photosynthetic photon flux density at 25° C. For *T. circinnatioides*, the seed plumose coma was cut prior to sowing to facilitate seed manipulation. The Petri dishes were sealed with Parafilm to reduce water loss. The dishes

with seeds were weighed every other day, and the water lost through evaporation was replaced. Thirty seeds per dish, with four replicates per treatment, were used.

The germination of *H. tehuacana* was evaluated by radicle emergence from the seed coat. However, because the *T. circinnatioides* radicle does not protrude, germination was registered when the embryo turned green (this was possible due to the partial transparency of the seed coat, as in other *Tillandsia* spp. (Montes-Recinas et al., 2012). Germination was registered by 30 days for *H. tehuacana* and by 10 days for *T. circinnatioides*. Because the ungerminated *T. circinnatioides* seeds had rotted after 10 days, the seeds were dissected, and lack of development or scanty embryo development was noted.

2.4. Data analyses

The cumulative germination percentages (arcsine-transformed) obtained at the different $\Psi\pi$ values were fitted to sigmoid or exponential sigmoid models using Table Curve 2D, v5.01.01 (AISN software, Chicago IL, USA).

$$y = a / [1 + b^{(-cx)}]$$

and

$$y = a / [1 + b(\exp(-cx))]$$

Where “y” = dependent variable which represents the germination percentage measured at the time “x”; “a” = parameter which represents the highest germination percentage reached, it is determined when the curve becomes asymptotic; “b” = parameter which denotes the point of inflexion; “c” = rate to which the logarithmic function of germination percentage changes per unit of time (the slope of the curve); “x” = independent variable which represents the time in days.

The two germination parameters were obtained from the fittings, as follows: 1) the time at which the first seed germinated (lag time) and 2) the rate of germination (velocity, first maximum derivate of the sigmoid curve; González-Zertuche et al., 2001). The above parameters, together with the final germination percentages, were analysed using the Kruskal-Wallis test and visually compared using box-and-whiskers plots (Statistical Graphics Corporation software, Englewood Cliffs, NJ, USA).

3. Results

3.1. For both species we obtained low germination percentages, 44.6 ± 5.2 for *H. tehuacana* and 12.2 ± 1.9 for *T. circinnatioides*. The highest germination values for both species were obtained at -0.1 MPa. The *H. tehuacana* seeds germinated in a Ψ_{π} interval from 0 to -1.0 MPa. The optimal Ψ_{π} interval for *H. tehuacana* seed germination was from 0 to -0.5 MPa. The lag time increased significantly at -0.5 and at -1.0 MPa ($H = 15$; $P < 0.01$). Additionally, the germination rate for this species was significantly reduced at -1.0 MPa ($H = 12$; $P < 0.05$), as was the final germination percentage ($H = 10$; $P < 0.05$) (Table 1). The *T. circinnatioides* seeds germinated in a Ψ_{π} interval from 0 to -0.5 MPa, with the lag time and velocity both reduced at -0.5 MPa; the final germination percentage was the highest at -0.1 MPa. However, the differences observed were not statistically significant (Table 1). The water requirement for germination and the germination velocity were higher in *T. circinnatioides* than in *H. tehuacana*.

Table 1. Germination parameters evaluated on *Hechtia tehuacana* and *Tillandsia circinnatioides* (lag time, velocity and final germination percentage) at different values of osmotic potential, as established with polyethylene glycol (8000) at constant 25° C. The different letters represent significant differences between treatments $P < 0.05$.

	Osmotic potential condition	Lag time (days)	Velocity (germination %/day ⁻¹)	Final germination (%)
<i>Hechtia tehuacana</i>	Distilled water	2.3 ± 0.1 ^a	14.5 ± 2.1 ^a	39.2 ± 6.1 ^a
	-0.05 Mpa	2.0 ± 0.3 ^a	12.9 ± 2.4 ^a	42.5 ± 8.6 ^a
	-0.1 Mpa	2.8 ± 0.3 ^a	16.1 ± 2.3 ^a	44.6 ± 5.2 ^a
	-0.5 Mpa	4.8 ± 0.5 ^b	10.4 ± 1.6 ^a	41.7 ± 6.7 ^a
	-1.0 Mpa	9.4 ± 0.4 ^c	3.1 ± 0.3 ^b	15.0 ± 3.2 ^b
	-1.5 Mpa	0	0	0
<i>Tillandsia circinnatioides</i>	Distilled water	2.3 ± 0.0	27.2 ± 4.0	7.8 ± 2.5
	-0.05 Mpa	2.6 ± 0.2	33.2 ± 1.4	6.7 ± 1.4
	-0.1 Mpa	2.3 ± 0.1	17.8 ± 2.9	12.2 ± 1.9
	-0.5 Mpa	4.0 ± 0.0	23.3 ± 1.7	8.3 ± 1.2
	-1.0 Mpa	0	0	0
	-1.5 Mpa	0	0	0

4. Discussion

In this study, we confirmed that *H. tehuacana* seeds are orthodox (in agreement with the seed databases of Kew, Royal Botanical Garden; <http://www.kew.org>) and quiescent. Because *H. tehuacana* seeds are dispersed over an extended period, *i.e.*, March–April, August–September and December (Martínez-Correa, 2008), distinct seed batches may be exposed to different environmental conditions, potentially increasing the chance of successful establishment. The germination response to water stress exhibited by the *H. tehuacana* seeds corresponds to response type 4, as proposed by Evans and Etherington (1990). This response indicates that germination is associated with high soil moisture and supports the hypothesis of a high water requirement as one of the strategies followed by species of arid environments (Gutterman, 1994; Allen et al., 2000).

However, high moisture in the soil of Zapotitlán Salinas is present only during the rainy season and sufficient water availability for *H. tehuacana* seed germination may occur only during years with high levels of precipitation. In contrast, the germination of epiphytic *T. circinnatioides* is more sensitive to water availability than terrestrial *H. tehuacana*, which may be related to its epiphytic habitat, which has lower water availability than soil.

The sensitivity of germination to water availability has also been described for other bromeliad species that can colonise epiphytic habitats, such as *Vriesea heterostachys* (Baker) L. B. Sm. and *Vriesea penduliflora* L. B. Sm. from a rain and cloud forest, respectively, with the germination of these species decreasing below -0.75 MPa (Pereira et al., 2009). The germination of *Tillandsia recurvata* L. seeds from a population of a seasonal and temperate sub-humid climate, decreases below -0.6 MPa (Montes-Recinas et al. 2012), whereas the germination of *T. circinnatioides*, a species from a semi-arid environment, did not occur below -0.5 MPa. The high water sensitivity for germination observed in *T. circinnatioides* may be related with lower water availability in the arid environment where it lives, representing a prudent strategy to germinate.

By low viability of the *T. circinnatioides* seeds was not possible establish a concordance with the germination response types proposed by Evans and Etherington (1990). The low germination percentage of both of the studied species may be related to drought stress during seed development. In the two years prior to the year of seed collection (*i.e.*, 2007),

precipitation was reduced by 16% with respect to the two ensuing years (2008 and 2009; <http://smn.conagua.gob.mx>). In addition, the seeds of both species showed germination percentages above 80% when they were collected (S. Montes-Recinas, personal observations). The rapid germination exhibited by *T. circinnatioides* and also observed in *Tillandsia recurvata* (Montes-Recinas et al., 2012) may be a relevant adaptation for an epiphytic habitat.

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Discusión general

Las características morfológicas, estructurales y fisiológicas de las semillas de las especies estudiadas (*Hechtia confusa*, *H. tehuacana*, *Tillandsia recurvata* y *T. circinnatioides*) revelan algunas de sus estrategias adaptativas a la escasa disponibilidad de agua, que caracteriza a los ambientes estacionales en los que habitan.

Semillas de *Hechtia confusa* y *H. tehuacana*

La dispersión de las semillas puede jugar un papel importante en la composición de las comunidades vegetales (Turnbull *et al.*, 2000). El tamaño pequeño de las semillas de *H. confusa* (2.2–3.6 mm de longitud, Martínez-Correa, 2008) y de otras especies del género *Hechtia*; así como su forma alada, podrían sugerir como su principal mecanismo de dispersión a la anemocoria¹. Sin embargo, parte de los resultados de la presente investigación, como son: el patrón reticulado de la parte central de la superficie de las semillas de *H. confusa*, la flotabilidad de sus semillas en agua y la persistencia de las éstas en los frutos secos (observada en campo); sugieren que la hidrocoria² también podría jugar un papel relevante en la dispersión de las semillas de *H. confusa*.

La dispersión de las semillas de *H. tehuacana* y *H. confusa* en el Valle de Tehuacán-Cuicatlán se observó restringida al entorno inmediato de la planta madre. Observación también señalada para *Hechtia schottii* Baker; una bromelia terrestre de la selva baja caducifolia de Calcehtok, Yucatán; y que condujo a proponer para esta especie un radio de dispersión reducido, a menos de que sus semillas sean removidas por herbívoros o por la lluvia (Ramírez-Morillo *et al.*, 2008).

Las semillas de *H. confusa* presentan un endospermo con almidón en una relación aproximada de 5:1, con respecto al embrión. Estas reservas, si bien confieren energía para la germinación y establecimiento de la plántula, también representan una valiosa fuente de alimento para los granívoros. Sin embargo, en las observaciones de campo en Zapotitlán de las Salinas se registró la permanencia de las semillas de individuos de *Hechtia* spp. por más de un mes en el suelo próximo a la planta madre, sin una aparente remoción por granívoros. Lo

¹ Anemocoria es la dispersión de las semillas u otras diásporas, como son los frutos, por el viento.

² Hidrocoria es la dispersión de las semillas u otras diásporas por el agua.

anterior, podría ser consecuencia de la unpalatabilidad que los taninos confieren a los tejidos vegetales. La falta de remoción de semillas con acumulación de metabolitos secundarios en la cubierta seminal también ha sido reportada para especies de otros hábitats; como *Omphalea oleífera* (Euphorbiaceae) especie de la selva alta perennifolia que comparte con las hechtias el patrón de distribución agregado, y cuyas semillas son dispersadas por barocoria¹ combinada con la fuerte escorrentía en la época de lluvias (Dirzo y Mota-Bravo, 1997; Sánchez-Coronado *et al.*, 2007).

Con base en lo anterior, proponemos dos aspectos de la dispersión de las semillas de *H. tehuacana* y *H. confusa*:

1) La anemocoria puede ser un medio de dispersión poco eficiente para *H. tehuacana* y *H. confusa*, debido al tamaño pequeño del ala de sus semillas (Figura 7, Capítulo 1) y a la acumulación abundante de taninos en la cubierta seminal (que podría implicar un mayor peso de éstas). Mientras que la dispersión hidrócora podría transportar a las semillas de *Hechtia* spp., a través de las corrientes de agua que se forman tras las lluvias intensas que ocurren en el Valle de Tehuacán-Cuicatlán (Castillo y Valiente-Banuet, 2010). La hidrocoria podría ser un medio de dispersión primario para las semillas que aún permanecen dentro de los frutos secos y secundario cuando éstas han caído al suelo o han sido previamente dispersadas por viento (Vander Wall, 2002). Sobre las semillas de *Hechtia* spp. se requieren futuros estudios en campo del alcance de la dispersión de sus semillas, de la existencia de dispersores y/o granívoros y del potencial de reclutamiento de nuevos individuos a la poblaciones naturales (dados sus altos porcentajes de germinación bajo condiciones controladas); así como de los factores que determinan el patrón de distribución agregada y dominancia de cierta especie en cada uno de los numerosos sub-valles que conforman el valle de Tehuacán-Cuicatlán.

2) La retención de una proporción de semillas de *H. confusa* en los frutos secos (registrada en campo), aún después de la dehiscencia de éstos, implica la posibilidad de la formación de bancos de semillas aéreos y temporales dentro los frutos secos.

La retención de un porcentaje de las semillas maduras en los frutos se ha considerado como un tipo de serotinia. En un inicio, el término serotinia se acuñó para la vegetación susceptible

¹ Barocoria es la dispersión de las semillas u otras diásporas por acción de la fuerza de gravedad.

a incendios, en donde las semillas se retenían en los frutos hasta que el fuego las liberaba, algo común para especies leñosas, *e.g. Pinus contorta* (Heinselmann, 1981). Aunque el uso del término serotinia se ha extendido a plantas anuales y a suculentas de ambientes áridos (*e.g. Mammillaria hernandezii*, Santini y Martorell, 2013). La serotinia brinda a las semillas dispersadas a distinto tiempo, diferentes oportunidades de establecimiento. Además, las semillas dentro de los frutos secos quedan protegidas de altas temperatura en el suelo y de los granívoros. La serotinia se ha propuesto como una latencia establecida por las condiciones ambientales (Lamont y Enright, 2000), que propicia la dispersión de las semillas hidrócoras en periodos más favorables para la germinación y el establecimiento de las plántulas.

Recientemente, se ha descrito que las raíces de individuos adultos de *Hechtia* spp. presentan asociaciones micorrízicas (Camargo-Ricalde *et al.*, 2002; asociaciones simbióticas de raíces de las plantas con hongos). Por lo que, otro aspecto por estudiar de la biología de *Hechtia* spp. es el papel que podrían jugar las micorrizas durante las primeras fases del establecimiento de sus plántulas.

Fotoblastismo

El fotoblastismo positivo ha sido propuesto como una estrategia que previene la germinación en momentos y lugares no favorables para el establecimiento de las plántulas (Fenner y Thomson, 2005). El fotoblastismo es un mecanismo que permite percibir de manera indirecta la profundidad a la que las semillas se encuentran enterradas en el suelo, a través de la cantidad y calidad de luz que éstas reciben (Bliss y Smith, 1985).

De acuerdo con Gutterman (1994), la germinación de las especies de ambientes áridos ocurre generalmente en los primeros 5 mm de profundidad. El fotoblastismo positivo que presentan las semillas de *Hechtia confusa* sugiere que su germinación ocurre en la superficie del suelo o en profundidades muy cercanas a ésta. Además, apoyando lo anterior, se encuentra el crecimiento escaso del tallo de las plántulas de *H. confusa*; que impediría su emergencia a la superficie, en caso de que la germinación ocurriese a profundidades mayores a los 3-5 mm (altura aproximada de las plántulas recién emergidas).

El fotoblastismo positivo identificado en las semillas de *H. confusa*, coincide con la tendencia de las bromelias terrestres y rupícolas a tener esta respuesta (Benzing *et al.*, 2000; Tarré *et al.*, 2007; *Pitcairnia albiflos*, Pereira *et al.*, 2010). Sobre este tema, se requieren estudios comparativos que incluyan el hábito de las especies y su tipo de fotoblastismo. Lo cual permitiría identificar en qué grado participan las relaciones filogenéticas en las adaptaciones que las semillas de las bromelias presentan a las condiciones lumínicas de los hábitats en los que se distribuyen.

Semillas de Tillandsia

La longitud de las semillas de *Tillandsia recurvata* y *T. circinnatioides* (3–3.8 y 3.9–4.9 mm, respectivamente) es similar a la longitud de las semillas del género *Hechtia* (2.2–3.6 mm). Sin embargo, las semillas de *Tillandsia* presentan menor proporción de endospermo (relación 1:1 endospermo-embrión) o incluso ausencia del mismo (Montes-Recinas *et al.*, 2012; Magalhães y Mariath, 2012). La escasa cantidad de reservas en las semillas de *Tillandsia* puede ser una adaptación a la colonización del ambiente epifito; mediante la reducción del peso de las semillas, lo que facilita la dispersión por viento. Adicionalmente, las semillas de las especies de *Tillandsia* estudiadas presentan menor cantidad de taninos condensados en la cubierta seminal (Capítulo 2) con respecto a las semillas de *Hechtia confusa* (Capítulo 1). La menor cantidad de taninos en la cubierta seminal de las semillas de las especies de *Tillandsia* spp. (Montes-Recinas *et al.*, 2012; Magalhães y Mariath, 2012) puede haber sido resultado de la reducción de la selección de defensas contra granívoros, ya que los predadores son menos abundantes en el ambiente epifito (Winkler *et al.*, 2005).

Un caracter morfológico notorio de las semillas de *Tillandsia* spp. son los tricomas que constituyen su cubierta seminal. Los tricomas facilitan la dispersión de las semillas (particularmente durante la época de secas¹, cuando los frutos abren) y la adhesión de las mismas a los sustratos. Además, los tricomas constituyen una cubierta seminal delgada e higroscópica, como se ha reportado para semillas de *Catopsis*² spp. (Wester y Zotz, 2011). Una cubierta seminal delgada también reduce el gasto energético del embrión para emerger a

¹ Cuando ocurren las lluvias los tricomas se aglutinan, lo que reduce la flotabilidad de las semillas, y por ende su radio de dispersión.

² *Catopsis* es otro género de la familia Bromeliaceae, cuyas semillas también presentan tricomas.

través de ésta. Además, la delgadez de la cubierta seminal de las semillas de *Tillandsia* spp. permite que la luz llegue al embrión (como ocurre para la orquídea *Dichromanthus aurantiacus*; Suárez-Quijada, 2010) y permita un inicio de la actividad fotosintética.

Las características particulares de la cubierta seminal de *Tillandsia* spp., junto con la atrofia de la raíz primaria, observada en *Tillandsia recurvata* (Montes-Recinas *et al.*, 2012) y en otras *Tillandsia* spp. (Magalhães y Mariath, 2012) permiten que el embrión incremente su tiempo de permanencia dentro de la cubierta seminal, protegiéndole de la deshidratación (Wester y Zotz, 2011). Por lo que, la cubierta seminal junto con la atrofia de la raíz primaria pueden ser consideradas de las adaptaciones morfológicas más importantes de los primeros estadios del desarrollo de las bromelias epifitas. Posteriormente, para enfrentar la baja disponibilidad de agua, el desarrollo del embrión incluirá la aparición de las escamas foliares absorbentes.

Pérdida de tolerancia a la deshidratación

Dentro de las principales causas de mortalidad de las bromelias epifitas se encuentra la sequía y la caída de los adultos de las ramas de los forofitos que las sostienen. La mayor mortalidad de individuos se presenta en el primer año de vida de las plántulas y se ve reducida conforme aumenta el tamaño de la planta (Benzing *et al.*, 2000). Por los resultados obtenidos con la epífita *Tillandsia recurvata* la tolerancia a eventos de hidratación-deshidratación disminuye una vez que el embrión ha iniciado su actividad fotosintética (Montes-Recinas *et al.*, 2012). Dicha sensibilidad a la deshidratación explica los bajos porcentajes de germinación obtenidos en condiciones naturales para *Tillandsia* spp., a pesar de los porcentajes de germinación, cercanos al 100 % obtenidos bajo condiciones controladas (Castro-Hernández *et al.*, 1999; Bernal *et al.*, 2005; Mondragón y Calvo-Irabien, 2006). Este aspecto puede ser compensado por la alta producción de semillas y por la capacidad de las plántulas de absorber la humedad atmosférica (Bader *et al.*, 2009) una vez que se han desarrollado los tricomas absorbentes; así como por la reproducción vegetativa. El inicio de la fotosíntesis representa el estadio de mayor vulnerabilidad a la sequía. Por lo que, las lluvias erráticas e intermitentes que ocurren previamente al establecimiento de la época de lluvias pueden tener efectos negativos en la supervivencia de las plántulas de *Tillandsia recurvata*; y posiblemente en otras especies de *Tillandsia* de ambientes áridos.

Respuesta de las semillas a la hidratación-deshidratación

La fase de plántula temprana es considerada el estadio más vulnerable en el ciclo de vida de las plantas (Harper, 1997). Los resultados obtenidos para las especies estudiadas y en particular para *Tillandsia recurvata*; aunados a los reportados para *Tillandsia flexuosa* (Bader *et al.*, 2009) coinciden con esta propuesta. En las especies estudiadas la fase tardía de la germinación (en *T. recurvata*, germinación fase-2) y el periodo inmediato a la conclusión de ésta son más susceptibles a eventos de hidratación-deshidratación.

Los eventos de hidratación-deshidratación de las semillas de *Hechtia* y *Tillandsia* estudiadas, bajo condiciones controladas (*hidropriming*), redujeron el tiempo de inicio de la germinación o incrementaron la velocidad de la misma. Sin embargo, la exposición de las semillas a eventos de hidratación-deshidratación bajo condiciones naturales redujo la supervivencia de las plántulas. Por lo que, si bien las semillas de las especies estudiadas presentan mecanismos de tolerancia a la hidratación-deshidratación, bajo condiciones naturales factores biótico o abióticos pueden comprometer dicha tolerancia.

Una vez que las plántulas de las especies de *Hechtia* estudiadas emergieron por completo a través de su cubierta seminal o las semillas de *Tillandsia* desarrollaron las escamas absorbentes, las plántulas fueron notablemente resistentes a la deshidratación (observaciones personales, Sarafí Montes). Aún desconocemos con precisión el grado de tolerancia a la deshidratación y los cambios metabólicos que subyacen a dicha adquisición o pérdida. Podemos considerar que la estrategia de las especies estudiadas es, desde estadios tempranos de plántula, de tolerantes a baja disponibilidad de agua. Aunque son particularmente susceptibles cuando *T. recurvata*, durante su germinación, inicia la síntesis de pigmentos fotosintéticos.

Dentro de los mecanismos que pueden estar relacionados con la tolerancia a la deshidratación o a la poca disponibilidad de agua se pueden mencionar: la síntesis de osmolitos compatibles (Hussain *et al.*, 2011) y/o de proteínas relacionadas con respuestas a estrés hídrico, como son las proteínas de choque térmico (Sun *et al.*, 2001) y las proteínas LEA (late embryogenesis abundant, Saijo *et al.*, 2001; Hussain *et al.*, 2011).

Por otra parte, la dispersión de las semillas ha sido propuesta como el factor más importante en la definición de la distribución de las bromelias epifitas (Cascante-Marín *et al.*, 2009). Sin embargo, nuestros resultados sugieren que la respuesta de las semillas a las fluctuaciones de humedad (que ocurren principalmente en los periodos previos al establecimiento de la época de lluvias) podría representar un factor limitante; tanto para las tillandsias, como para las hechtias estudiadas. Además, como ocurre para otras especies de zonas áridas (Gutterman, 2001), las bromelias estudiadas podrían germinar solo en los años de mayor precipitación, y por ende más propicios para su germinación y establecimiento (debido a su requerimiento de alta disponibilidad de agua para germinar). Se requieren estudios comparativos entre la respuesta de especies de bromelias de ambientes méxicos y xéricos para conocer si su germinación y establecimiento obedecen a los mismos factores limitantes.

Se ha propuesto que la fisiología de las semillas tiene un importante componente ambiental (Dubrovsky, 1996) y posiblemente filogenético (Thanos *et al.*, 1989). La respuesta de las semillas de las especies estudiadas de los géneros de *Hechtia* y *Tillandsia*, a los tratamientos de *hidropriming* y *natural priming* sugieren que en las bromelias; a pesar de sus diferentes formas de vida y morfo-anatomía de sus semillas, exhiben similitud en la respuesta a eventos de hidratación-deshidratación. Lo anterior, se podría explicar por la condición de aridez compartida en los ambientes terrestres y epifitos en los que se distribuyen las especies estudiadas.

Ambientes áridos, altos requerimientos de agua para germinar

El intervalo de Ψ_{π} al que germinaron las semillas de *Hechtia tehuacana* (0 a -1.0 MPa) y las semillas de las epifitas (*Tillandsia recurvata* y *T. circinnatioides*; 0 a -0.6 y 0 a -0.5 MPa, respectivamente) indican un alto requerimiento en la disponibilidad de agua para germinar. Esta respuesta se ha considerado como una estrategia que propicia que la germinación ocurra cuando la disponibilidad de agua es suficiente para concluir con la germinación y el establecimiento de las plántulas (Gutterman, 1994; Allen *et al.*, 2000). El alto requerimiento de agua para germinar también lo exhiben otras especies del Valle de Tehuacán, como son: *Cercidium praecox*, *Prosopis laevigata*, *Neobuxbaumia tetetzo*, *Pachycereus hollianus*, *Beaucarnea gracilis* y *Yucca periculosa* (Flores y Briones, 2001).

Los resultados obtenidos indican que las bromelias epifitas (*Tillandsia recurvata* y *T. circinnatioides*) requieren mayor disponibilidad de agua para germinar, con respecto a *H. tehuacana*. Este mayor requerimiento podría ser una respuesta adaptativa a la menor disponibilidad de agua en el ambiente epifito. Aunque la capacidad de las semillas de *Hechtia* estudiadas, de germinar a Ψ_{π} s más negativos que las epifitas, también podría tener relación con la alta salinidad del suelo de Zapotitlán de las Salinas, Puebla (Cruz *et al.*, 1995). Pero esta última explicación la contradice el alto requerimiento de disponibilidad de agua para germinar que también exhiben otras especies terrestres del Valle de Tehuacán, mencionadas en el párrafo anterior.

La fisiología de la germinación de las bromelias requiere de estudios comparativos que incluyan un mayor número de taxa de diferentes ambientes y que permitan definir si la estrategia de germinación de las bromelias a Ψ_{π} s poco negativos es una generalidad.

Por otra parte, dada la importancia de la temperatura en la movilización de solutos, también se requieren estudios que incluyan gradientes de temperatura y temperatura fluctuante; y en caso de ser requerida la implementación de tratamientos pregerminativos en semillas de la familia Bromeliaceae se puede partir de un Ψ_{π} óptimo para la germinación de las especies estudiadas, -0.1 MPa.

La discontinuidad en la disponibilidad de los recursos influencia procesos ecológicos como la competencia, la coexistencia, el mantenimiento de la diversidad y la invasión (Lundholm y Larson, 2004). En relación a las bromelias, particularmente para las subfamilias Bromelioideae y Tillandsioideae, se propone su origen a partir de ancestros de hábitats abiertos y secos (Givnish *et al.*, 2007). Por lo que, para estos taxa, la discontinuidad en la disponibilidad de agua pudo ser una presión importante para su surgimiento y posterior diversificación.

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