



Seed number, germination and longevity in wild dry forest *Tillandsia* species of horticultural value



Alejandro Flores-Palacios^a, Angelita Belem Bustamante-Molina^a,
 Angélica María Corona-López^a, Susana Valencia-Díaz^{b,*}

^a Centro de Investigación en Biodiversidad y Conservación (CIByC), Universidad Autónoma del Estado de Morelos, Av. Universidad 1001, Col. Chamilpa, Cuernavaca Morelos C.P. 62209, Mexico

^b Centro de Investigación en Biotecnología (CEIB), Universidad Autónoma del Estado de Morelos, Av. Universidad 1001, Col. Chamilpa, Cuernavaca Morelos C.P. 62209, Mexico

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ABSTRACT

Life history traits like seed number, seed germination and seed longevity, are useful traits for the persistence, production and conservation of plants. We compared seed number per capsule, seed longevity and the effect of contrasting light conditions on seed germination in six wild *Tillandsia* species of tropical-dry-forest in central Mexico. For two species, we also tested the effect of storage temperature on seed longevity. Seed number per capsule differed among species and was positively associated with the time of fruit maturation. The seeds of all *Tillandsia* species were capable to germinate in light and dark conditions. Seed longevity in all species was at least 478 days after collection and improved under cool temperatures (7–11 °C). Seed germination followed a quadratic decline in *T. achyrostachys* and *T. schiedeana*, which become steeper toward the end of the rainy season. In the remaining species, seed germination followed a linear decrease. Our study suggests that *T. caput-medusae* is the most susceptible species to be produced for ornamental purposes because it presents numerous seeds with long longevity as well as high percentages of germination under both light and dark conditions. Moreover, these results could help to generate protocols of *ex situ* conservation for endemic species.

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1. Introduction

The Bromeliaceae is a Neotropical family that comprises 3160 species (Zotz, 2013a), many of which are of commercial value to the horticultural industry (as species, cultivars and sources for hybrids) and as non-timber forest products (Bennet, 2000; Pickens et al., 2003; Stringheta et al., 2005; Flores-Palacios and Valencia-Díaz, 2007; Haeckel, 2008; Toledo-Aceves and Wolf, 2008) and 56.0% of which are epiphytes (Zotz, 2013a). The genus *Tillandsia* represents 21% of species of Bromeliaceae and 94.6% of *Tillandsia* species are epiphytes (Zotz, 2013a).

In some forests, *Tillandsia* species are the most abundant or richest of the vascular epiphytes (Caldiz and Fernández, 1995; Merwin et al., 2003; Flores-Palacios and García-Franco, 2004; Vergara-Torres et al., 2010) and are extracted from their habitat for illegal trade (Flores-Palacios and Valencia-Díaz, 2007). For example; in several areas of Mexico and Guatemala, floral

arches are constructed for religious festivities, utilizing thousands of inflorescences of diverse *Tillandsia* species (Flores-Palacios and Valencia-Díaz, 2007; Haeckel, 2008; Toledo-Aceves et al., 2014a). Plant harvesting can thus represent an income for collectors (Toledo-Aceves et al., 2014a). Despite the abundance of some *Tillandsia* species, demographic projections have shown that intense collection may cause a decline in *Tillandsia* populations (Toledo-Aceves et al., 2014b). Understanding the characteristics of seed germination may enable the development of *ex situ* conservation and production protocols necessary to mitigate the effects of illegal exploitation of wild plant species (Pickens et al., 2003; Pereira et al., 2010; Toledo-Aceves and Wolf, 2008).

Several aspects of basic seed traits are still poorly understood for some epiphytic *Tillandsia* species, such as seed number per fruit, seed longevity and seed germination requirements. Knowledge of *Tillandsia* seed longevity (defined as the time that seeds remain alive, Ellis et al., 1982; Kochanek et al., 2010) may facilitate successful seed germination; however, data of seed longevity are available for just nine bromeliad species, including three *Tillandsia* species (Fernández et al., 1989; Goode and Allen, 2009; Zotz, 2013b) and it is known that storage at low temperatures increases longevity

* Corresponding author. Tel.: +52 777 3297000.

E-mail address: susana.valencia@uaem.mx (S. Valencia-Díaz).

of *Tillandsia* seeds (Tompsett and Pritchard, 1993; Kochanek et al., 2010; Zotz, 2013b).

Along the vertical environment of the canopy, epiphyte seeds face heterogeneous conditions for germination (Winkler et al., 2005; Wagner et al., 2013); for example, they may be exposed to light, xeric areas in the upper layers or shaded, humid areas in the lower layers or crevices (Winkler et al., 2005; Wagner et al., 2013). Seed germination has been explored in 24 species of *Tillandsia*, with the atmospheric *T. recurvata* the subject of most study (Downs and Piringir, 1958; Benzing, 1978; Fernández et al., 1989; Pickens et al., 2003; Bernal et al., 2005; Stringheta et al., 2005; Winkler et al., 2005; Toledo-Aceves and Wolf, 2008; Bader et al., 2009; Valencia-Díaz et al., 2010, 2012; Montes-Recinas et al., 2012; Sosa-Luria et al., 2012; Toledo-Aceves et al., 2012; Zotz, 2013b).

Specifically for *Tillandsia* species of tropical dry forest, it is known that seed maturation and wind dispersal occur before the rainy season (Mondragón and Calvo-Irabién, 2006; Montes-Recinas et al., 2012), and germination can take place during the rainy season. However, due to the irregularity of precipitation in the tropical dry forest (Mondragón et al., 2004; Montes-Recinas et al., 2012), seeds along with higher longevity could “wait” the rainy season when it delays or is scarce and then germinate (Wagner et al., 2013).

In order to further the knowledge of seed number per fruit (capsule), germination light requirements and longevity in *Tillandsia* species, the specific objectives of this research were: (a) to report seed number per capsule in six *Tillandsia* species from a central Mexican tropical dry forest, (b) to evaluate germination under contrasting light requirements (light and dark) in six *Tillandsia* species, and (c) to measure seed longevity under room and cool temperatures in the species with highest seed availability: *Tillandsia recurvata* and *T. hubertiana*. We hypothesized that (a) *Tillandsia* seed germination would be indifferent to light or darkness conditions because canopy features different light conditions and (b) seed longevity would last until the end of the rainy season, declining afterwards, and would be longer under reduced storage temperatures.

2. Materials and methods

2.1. Collection site

All seeds were collected in the tropical dry forest of San Andres de la Cal, Tepoztlán, Morelos (18°57'22.2"W; 99°06'50.2"N, 1495 m asl), in central Mexico. Mean monthly temperatures range from 18 to 23 °C (Ruíz-Rivera, 2001). Highest temperatures (Fig. 1) occur during the seed dispersion period (April–May). Highest precipitation takes place after seed dispersion, during July–September (Fig. 1). Some of the dominant tree species in the study area are *Sapium macrocarpum* Müll. Arg. (Euphorbiaceae), *Bursera copallifera* (DC.) Bullock, *B. fagaroides* (Kunth) Engl., *B. glabrifolia* (Kunth) Engl. (Burseraceae), *Ipomoea murucoides* Roem. & Schult., *I. pauciflora* M. Martens & Galeotti (Convolvulaceae), *Conzattia multiflora* (B.L. Rob.) Standl. and *Lysiloma acapulcense* (Kunth) Benth (Fabaceae) (Vergara-Torres et al., 2010).

At the study site, the epiphytic community mainly consists of xerophytic or atmospheric members of the Bromeliaceae family, and includes *Catopsis nutans* Griseb., *Tillandsia achyrostachys* E. Morren ex Baker, *T. caput-medusae* E. Morren, *T. circinnatioides* Matuda, *T. cryptantha* Baker, *T. hubertiana* Matuda, *T. ionantha* Planch., *T. makoyana* Baker, *T. recurvata* (L.) L., *T. schiedeana* Steud., *T. lydiae* Ehlers and *Viridantha atroviridipetala* (Matuda) Espejo (Vergara-Torres et al., 2010). Of these, *T. achyrostachys*, *T. circinnatioides*, *T. cryptantha*, *T. hubertiana*, *T. makoyana*, and *T. lydiae* are endemic to Mexico (Espejo-Serna et al., 2004). Further details of the study area and regarding *Tillandsia* species

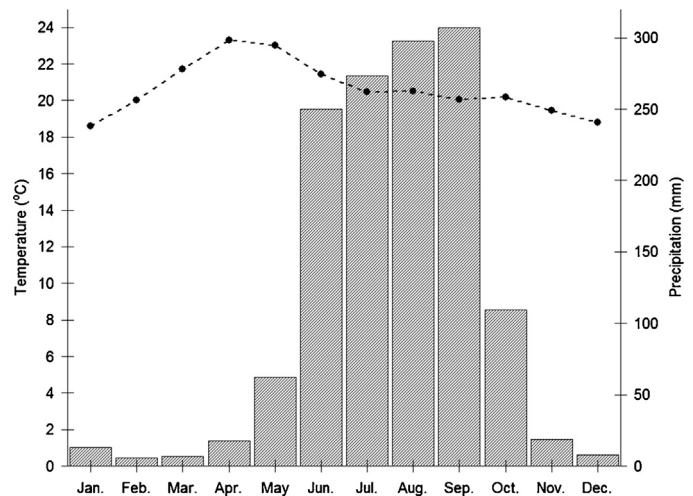


Fig. 1. Climograph of the lower area of Tepoztlán showing the monthly average temperature (line) and precipitation (bars). Data were obtained from the climatic monitoring station “Tepoztlán” (99°04'34"N, 18°59'10"O, 1703.22 m asl).

can be found in Ruíz-Rivera (2001), Vergara-Torres et al. (2010), Valencia-Díaz et al. (2007, 2010, 2014) and Orozco-Ibarrola et al. (2015).

In March 2009, infructescences from *T. achyrostachys* (Fig. 2a), *T. caput-medusae* (Fig. 2b), *T. circinnatioides* (Fig. 2c), *T. hubertiana* (Fig. 2d), *T. recurvata* (Fig. 2e) and *T. schiedeana* (Fig. 2f) were collected. We did not collect seeds from the other *Tillandsia* species because they were scarce, and because they were recorded at the site after our study (*C. nutans*, *T. cryptantha*), or no fruits were observed (*T. ionantha*) or because the suitable period for collection was unknown (*T. makoyana* and *V. atroviridipetala*).

2.2. Seed number

In order to avoid seeds mixture from different capsules and to allow the count of seed number per fruit, capsules were separated from their infructescences, labelled and stored in a paper bag at room temperature until maturation and dehiscence, a process that takes ca. 25 days. Once dehiscence took place, seeds were counted from all the capsules of *T. achyrostachys* (47 capsules from 11 plants), *T. circinnatioides* (17 capsules from 10 plants) and *T. schiedeana* (29 capsules from 19 plants). In the case of *T. caput-medusae* (32 capsules from 16 plants, 27 capsules were used), *T. hubertiana* (253 capsules from 8 plants, 25 capsules were used) and *T. recurvata* (575 capsules from >100 plants, 52 capsules were used), sample capsules and seeds for germination experiments were chosen at random from each batch.

2.3. Germination trials

Coma hairs were cut from seeds to facilitate manipulation and to reduce the risk of contamination during the experiments. In all the experiments, seeds were randomized; disinfected with sodium hypochlorite (0.25%) for 30 s and thoroughly rinsed with distilled water (following Valencia-Díaz et al., 2010). A mean number (\pm standard deviation) of 35 ± 2 seeds of *T. achyrostachys*, 62 ± 3 seeds of *T. caput-medusae*, 34 ± 1 seeds of *T. circinnatioides*, 32 ± 3 seeds of *T. hubertiana*, 10 ± 0 seeds of *T. recurvata*, and 34 ± 1 seeds of *T. schiedeana* were each placed in Petri dishes with Whatman No. 2 filter paper as a substrate. Three replicate Petri dishes were used for each experiment. The number of seeds per dish was constrained because we prepared homogeneous mixtures of seeds. In

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