SHORT COMMUNICATION

Germination and seedling survivorship of three *Tillandsia* species in the cloud-forest canopy

Tarin Toledo-Aceves[1], José G. García-Franco, Siunelly Landero Lozada, María Luisa León Mateos and Keith MacMillan

Red de Ecología Funcional, Instituto de Ecología, A.C., AP 63, 91070 Xalapa, Veracruz, Mexico

Low germination and seedling survival probabilities are reported in various species of epiphytic bromeliad (Benzing 1978, Hietz *et al.* 2011, Toledo-Aceves & Wolf 2008, Winkler *et al.* 2005; but see Cascante-Marín *et al.* 2008). If germination and seedling survival are limiting factors in the life cycle, differential germination and seedling survival between species should be reflected in the relative abundance of established plants (Cascante-Marín *et al.* 2006, 2008) and in their presence or absence in secondary vegetation (Hietz *et al.* 2011), while differential germination within the tree would be expected to contribute to a heterogeneous distribution of established plants within the canopy (Hietz *et al.* 2011, Zotz & Vollrath 2002). Many factors influence the performance and distribution of epiphytes, including forest condition, disturbance type, distance from seed source, tree size and species, microclimate, epiphyte population dynamics and physiology (Cascante-Marín *et al.* 2009, Hietz *et al.* 2011, Valencia-Diaz *et al.* 2010, Zotz & Hietz 2001). In this study, we tested whether germination and seedling survival rates differ between the epiphytic bromeliads *Tillandsia multicaulis* Steud., *T. punctulata* Schldl. & Cham. and *T. butzii* Mez, and whether species abundance reflects the ability to germinate and survive as seedlings within the cloud-forest canopy. We also explore how morphological and physiological traits of the studied species can influence their early establishment.

The three species are found in cloud forest, although not exclusively: *T. multicaulis* is an epiphytic tank bromeliad, with C3 metabolism, commonly found between 1150 and 1900 m asl. It produces one to five red inflorescences per plant. *Tillandsia punctulata* is of intermediate form between tank and atmospheric, with C3 metabolism, and is found between 1400 and 1800 m asl (Espejo-Serna *et al.* 2005). *Tillandsia butzii* is an atmospheric epiphyte, with CAM metabolism, found between 900 and 2100 m asl (Espejo-Serna *et al.* 2005, Hietz *et al.* 2002). The plumose seeds of all three species mature in about 11 mo and are wind-dispersed during the dry season (November–May; pers. obs.), with the rosettes dying after fruiting. The three species reproduce vegetatively; thus when a leading shoot dies, it is replaced by new offshoots (unpubl. data).

The study was carried out in tropical montane cloud forest (TMCF) fragments in the centre of Veracruz State, Mexico ($19°31'03''$N; $97°00'25''$W; 1660 m asl). Annual precipitation for the region is 1350–2200 mm and mean annual temperature is 18 °C. Mean annual precipitation reported in the region in 2010 and 2011 was 1567 and 1757 mm, respectively (National Water Commission). Microclimatic variables were measured in the forest canopy with four data loggers (HOBO) over a 4-d period in the rainy season of 2010. Photosynthetically active radiation (PAR) was 0.71 ± 0.11 mol m^{-2} s^{-1}, temperature was 16 °C (± 0.14 °C), and relative humidity 97% (± 0.61%). The forest fragments are secondary (tree diameter at 1.3 m height $= 27.4 \pm 0.81$ cm; unpubl. data) and immersed within a matrix of pasturelands and crops, mainly maize. The most dominant tree species in the study

area are *Quercus delgadoana* S. Valencia *et al.*, *Liquidambar styraciflua* L. and *Q. lancifolia* Schltdl. & Cham. (unpubl. data).

To determine the abundance of each species, single-rope techniques were employed to climb 24 trees and all plants ≥ 5 cm in longest leaf length recorded. Smaller plants were excluded as they could not be identified to species with confidence. Trees were divided into three zones (modified from Johansson 1974): T = trunk, I = canopy interior and E = canopy exterior. *Tillandsia multicaulis* was clearly the most abundant species (mean no. of plants per tree ± 1 SE; 209 ± 28), followed by *T. punctulata* (27 ± 6) and *T. butzii* (18 ± 4). Distribution among tree zones was compared with the χ^2 statistic (Quinn & Keough 2002). All species showed a preference for the trunk and exterior canopy (*T. multicaulis* $\chi^2 = 377.971$, *T. punctulata* $\chi^2 = 881.006$, and *T. butzii* $\chi^2 = 36.103$; df = 3, P = 0.001; Total no. of plants *T. multicaulis* T = 2070, I = 1204, E = 1753; *T. punctulata* T = 259, I = 132, E = 253, *T. butzii* T = 94, I = 16, E = 105).

The approximate number of seeds produced per plant was: *T. multicaulis*: 1129 ± 102 (N = 7), *T. punctulata*: 571 ± 89 (N = 11) and *T. butzii*: 268 ± 107 (N = 11). To evaluate germination, seeds from at least 10 inflorescences of each species were collected. To determine germination *in vitro*, 1000 freshly collected seeds of each species were treated with fungicide (Daconil 3 g l^{-1}), placed in Petri dishes in a germination chamber (21°C : 12 h light) and frequently sprayed with distilled water for a period of 6 mo. To evaluate germination in situ, 1200 seeds of each species were placed on three different TMCF trees (nine trees in total) over January and February, 2010, when natural seed dispersal takes place. *Quercus sartorii* Liebm. was used for *T. multicaulis* and *T. butzii*, and *Q. lancifolia* for *T. punctulata*; tree species were selected based on abundance of established plants in the canopy. Due to the limiting nature of certain hosts for epiphyte establishment (Valencia-Díaz *et al.* 2010, Vergara-Torres *et al.* 2010), the germination results may not be representative of the entire forest. To evaluate the effect of position within the canopy on germination, 100 seeds were placed on the upper part of two branches in the canopy interior (I) and two sections of the main trunk of each tree (T) (100 seeds × 4 positions (two on branches and two on trunk) × 3 trees = 1200 seeds). The exterior canopy was not evaluated because it could not be accessed safely. Seed appendages were intertwined in a thin natural fibre string, which was then attached to the tree. The seeds were thereby held in place while remaining in contact with the tree bark. Seed germination and seedling survival and growth were recorded every 30 d for 12 mo and every 3 mo thereafter, up to 18 mo. Germination among species was compared with a GLM: epiphyte species was included as a fixed factor and individual tree as a random factor

(Quinn & Keough 2002). Percentage germination was square root-transformed prior to analysis. The Bonferroni test was used to compare between means. Differences in the number of germinated seeds between two positions were analysed for each species with the chi-square test (χ^2). Minitab 16 (Minitab Inc., State College, PA, USA) was used for statistical analysis.

Maximum germination (total number of germinated seeds) was reached after 80 d in the germination chamber for all species: *T. multicaulis* = 91.3%, *T. butzii* = 84% and *T. punctulata* = 49.8%; however, most seeds germinated between 14 and 20 d after sowing (*T. multicaulis* reached 81% of germination, *T. butzii* 75% and *T. punctulata* 42%). In the canopy, maximum germination was attained after 210 to 240 d. The effect of individual tree was not significant (P = 0.27), but species had a significant effect (F = 17.0, df = 2; P < 0.001): *Tillandsia punctulata* germination in the field was higher (31.8% ± 2.4%) than that of *T. multicaulis* (13.7% ± 2.9%) and *T. butzii* (10.0% ± 2.7%). Germination values found in the field are similar to those of previous studies of epiphytic bromeliads in Mexican TMCF (Hietz *et al.* 2011, Toledo-Aceves & Wolf 2008, Winkler *et al.* 2005). Winkler *et al.* (2005) report 0.2% germination in *T. multicaulis*, which could be due to the use of non-viable seeds. Considering that relative to the other species, *T. punctulata* had the lowest percentage germination *in vitro*, it is remarkable that this species displayed the highest germination in the forest canopy. Conditions in the germination chamber, such as high humidity, may have been less favourable for this species than field conditions.

In all three species, percentage germination on the trunk was significantly higher than on the inner canopy branches (*T. multicaulis* $\chi^2 = 199$, *T. punctulata* $\chi^2 = 15.7$, *T. butzii* $\chi^2 = 322$; df = 5; P = 0.01; *T. multicaulis*: T = 19.5% ± 4.4% and I = 8.0% ± 2%; *T. punctulata* T = 34.7% ± 4.1% and I = 29.0% ± 2.5%; *T. butzii* T = 14.7% ± 4.1% and I = 5.3% ± 2.5%). This could be the result of the thicker trunk bark, which can have a higher water-retention capacity thus favouring epiphyte germination (Mehltreter *et al.* 2005). Hietz *et al.* (2011) found that germination of *Tillandsia* species was largely controlled by microclimate for tanks and tank-atmospheric intermediate species, but that substrate had a larger effect on *T. juncea*, an atmospheric CAM-species. Interestingly, the atmospheric CAM-species *T. butzii* (Hietz *et al.* 2002) displayed a percentage germination almost three times higher on the trunk than on the branches. While the role of canopy position shows contrasting results in terms of effect on germination probability, humidity has repeatedly been considered a determinant factor in this process (Hietz *et al.* 2011, Toledo-Aceves & Wolf 2008, Winkler *et al.* 2005). A similar pattern was observed in terms of abundance of established plants within the phorophyte: the tree trunks supported the

highest number of plants. Because asexual reproduction is common among these plants, vegetative growth via resprouts may thus augment the observed distribution patterns by contributing to the prevalence of zonation within the tree. Nonetheless, these data do not prove a causal relationship and experiments involving the complete life cycle of plants are required in order to accurately determine the mechanisms involved (Zotz & Hietz 2001). For instance, germination success did not seem to reflect the relative abundance of a species; *T. multicaulis* is the most dominant species in the study area, but *T. punctulata* presented an almost three times higher percentage of germination. Similarly, *T. butzii* is the least abundant, yet displayed similar germination percentage to *T. multicaulis*. Cascante-Marín *et al.* (2008, 2009) have suggested that other factors, such as dispersal and seed availability as well as chance and historic events related to seed dispersal, must be considered as influential on community assembly. *Tillandsia butzii*, for example, produces 75% and 50% fewer seeds than are produced by adult plants of *T. multicaulis* and *T. punctulata*, respectively: such reduced production of propagules per individual in *T. butzii* may contribute to the lower abundance of this species.

To estimate seedling survival rates, we used the Kaplan–Meier model at 4, 5, 6, 9 and 13 mo after sowing (Crawley 2002). Survival curves were obtained for each species; however, due to the reduced number of surviving seedlings as the experiment progressed, the effect of position was not evaluated and only the factor of species was tested. We compared survival rates between species with the Log-rank statistic, assuming a chi-square distribution. Seedling survivorship probability differed among species (Log-rank $\chi^2 = 44.4$, df $= 2$, P < 0.0001; Figure 1). Percentage of surviving seedlings 13 mo after sowing was higher in *T. punctulata* (35.6%) than in *T. multicaulis* (11.5%) and *T. butzii* (8.3%). Longest leaf length at 13 mo after sowing was similar among species: *T. multicaulis* $= 6.2 \pm 0.8$ mm; *T. punctulata* $= 6.5 \pm 0.6$ mm and *T. butzii* $= 6.0 \pm 0$ mm (P > 0.05). Final seedling size is within the range ($\sim$ 1 cm) reported for related species (Mondragon *et al.* 2004, Toledo-Aceves & Wolf 2008, Winkler *et al.* 2007) but the high rates of survival found are remarkable for such relatively small seedlings given the reported positive correlation between seedling size and survival (Toledo-Aceves & Wolf 2008). Since epiphytic bromeliad germination and seedling survival have proved to be vulnerable to dry conditions (Benzing 1978, Mondragón *et al.* 2004, Toledo-Aceves & Wolf 2008), the survival rates found are surprisingly high given the relatively low levels of precipitation during the experimental period. The attachment method (natural fibre string) could explain the success compared to the synthetic materials used in previous approaches (glue and segments of carpet): it is possible that these natural fibres
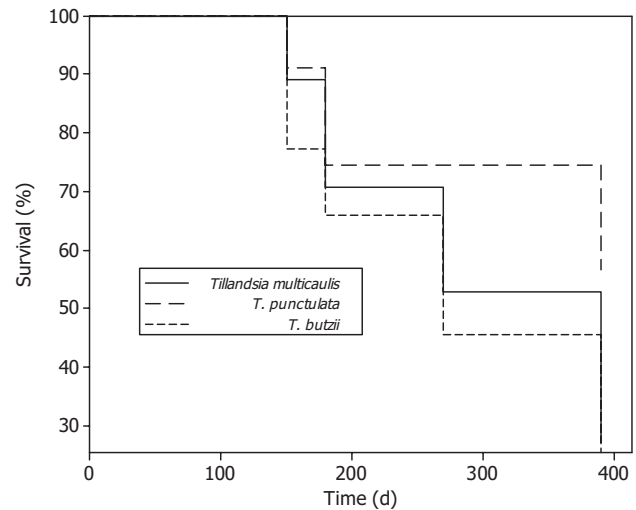

Figure 1. Seedling survival (%) based on Kaplan–Meier estimates in the epiphytic bromeliads *Tillandsia multicaulis*, *T. punctulata* and *T. butzii*, in the canopy of *Quercus* spp. in tropical montane cloud forest in Veracruz, Mexico. Survival curves are significantly different (log-rank test P < 0.001).

retain more humidity than is possible with the synthetic methods.

Approximately 1 y after sowing, seedling survival in *T. punctulata* was considerably higher than reported in previous studies for this and other related species (Cascante-Marín *et al.* 2008, Toledo-Aceves & Wolf 2008, Winkler *et al.* 2005). In comparison to *T. multicaulis*, which is almost restricted to TMCF, *T. punctulata* distribution suggests a higher drought tolerance: it is common in drier and disturbed forests of the region (Hietz & Hietz-Seifert 1995, Hietz *et al.* 2006), coffee plantations (Toledo-Aceves *et al.* 2012) and isolated trees that are exposed to higher radiation and evaporative demand, relative to the TMCF interior (Flores-Palacios & García-Franco 2004). Thus, the disturbed conditions of the studied forest may favour the establishment of bromeliad species such as *T. punctulata*, with lower atmospheric humidity requirements than more shade-tolerant species (Hietz *et al.* 2006). Even though the relative contribution of germination and seedling survival to the population growth of epiphytic bromeliads is considered minor based on demographic models (Mondragon *et al.* 2004, Winkler *et al.* 2007, Zotz 2005, Zotz *et al.* 2005), successful recruitment is a key process in long-term population viability (Silvertown & Lovett-Doust 1993). Changes in establishment patterns as a result of forest disturbance can affect the composition of the epiphyte community. An evaluation of the early establishment of a more complete group of closely related species, covering a gradient of morphological and physiological attributes over a disturbance gradient, is necessary in order to further our knowledge of these processes.

ACKNOWLEDGEMENTS

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