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The influence of collecting date, temperature and moisture regimes on the germination of epiphytic bromeliads

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Abstract

The understanding that many aspects of the spatial and temporal patterns of epiphyte communities may be explained by the comportment of early life stages has given rise to a considerable number of germination studies in recent years. Unfortunately, protocols frequently use unproven assumptions and arbitrary experimental conditions. To make future studies as ecologically meaningful as possible we address a number of potential pitfalls with a series of experiments with seeds from a total of 16 species. We show that it is safe to collect capsules for experiments before natural dehiscence – there is afterripening even in the case of very early collections. The application of fluctuating temperatures is not imperative, because there is no consistent difference in the germination response under constant versus fluctuating temperatures. The effects of different osmotic potentials and intermittent drought of varying intensity on germination are qualitatively, but not quantitatively, comparable. Due to the greater ecological realism, we encourage the use of the latter. However, care must be taken to use realistic temperatures – the impact of intermittent drought on germination is modulated by temperature. This highlights the need for data on the *in situ* temperature regimes during germination as an important prerequisite towards more realistic experiments in the field of germination ecology of vascular epiphytes.

Keywords: afterripening, Bromeliaceae, intermittent drought, methods, regeneration niche

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Introduction

Vascular epiphytes account for *c*. 27,000 species worldwide (Zotz, 2013b). Traditionally, physiological ecologists have studied larger individuals of this phylogenetically and ecologically diverse group of plants in an endeavour to understand species distributions in time and space (Zotz and Hietz, 2001), but more recently there has been increased interest in the earliest ontogenetic processes, namely germination and establishment (for field studies see, for example, Mondragón and Calvo-Irabien, 2006; Cascante-Marín *et al.*, 2008; Goode and Allen, 2009; and for laboratory studies, Fernandez *et al.*, 1989; Manzano and Briones, 2010; Tsutsumi *et al.*, 2011).

Germination is arguably the most vulnerable stage of the plant life cycle and thus a key element of plant life history strategy (Harper, 1977). A large proportion of published germination studies with plants in general, and epiphytes in particular, has been performed under controlled conditions in the laboratory. While allowing for strong inference by controlling relevant ambient factors, such studies may also lead to erroneous conclusions because of oversimplified conditions or arbitrary selection of treatment differences. One way to avoid such pitfalls is to inform experimental designs by field data (e.g. on *in situ* temperature regimes, Tsutsumi *et al.*, 2011), another is a general, critical revision of currently used methods (see also Baskin and Baskin, 2001).

For epiphytes, the majority of germination studies have been performed with members of a single family, the Bromeliaceae, and consequently our study also focuses on this group. We touch a number of issues, from the collection of samples and seed storage, to the appropriate design of experiments dealing with the influence of water supply and temperature on germination in epiphytic bromeliads.

Many authors give detailed and unambiguous information on the sampling procedure of the seeds used in experiments (e.g. Fernandez *et al.*, 1989;

Montes-Recinas *et al.*, 2012), in other studies there is room for interpretation (e.g. 'collected from natural populations', Bader *et al.*, 2009; or 'mature fruit', Pereira *et al.*, 2009). Since bromeliad capsules may look 'mature' weeks or even months before natural dehiscence, differences in germination success could easily stem partly, or entirely, from varying levels of maturity and not from difference among populations or treatment effects. For the proper interpretation of past and future studies, information on the potential of afterripening would be essential. To this end, we investigated whether collection time (from immediately after anthesis to close to dehiscence) affects the outcome of germination experiments in three species.

For soil-rooted terrestrial plants, there has been long-standing interest in the response of the germination process to different water potentials (Ψ), both in agriculture and in basic plant science, because germination is an important bottleneck for plant recruitment in natural systems (Evans and Etherington, 1990). A few studies with epiphytic bromeliads have also investigated this relationship (Pereira *et al.*, 2009), but it can be debated whether such experiments allow relevant conclusions for the situation in nature. In contrast to soils, which provide a relatively constant environment for germinating seeds over longer periods, conditions in the epiphytic habitat are extremely volatile, in particular in the case of bark epiphytes: their seeds are either wetted by rain or fog ($\Psi \approx 0$ MPa) or, with rapid transition, exposed to very dry conditions even in moist rainforest conditions. For example, a relative air humidity (rh) of 99% at 20°C already corresponds to a Ψ of -1.4 MPa, while rh of 90% represents a ten times lower Ψ (Nobel, 2005). For this reason, some researchers (e.g. Bader *et al.*, 2009) have begun to study the influence of water on the germination process in vascular epiphytes, not by varying Ψ, but by alternating periods of drought and wetness. Although this approach seems much closer to the real world with its irregular rainfall, the experimental details have not been analysed systematically.

Conceivably, the outcome of such studies may depend strongly on the relative lengths of wet and dry periods, or on a possible interaction between the moisture treatment and other factors, such as the temperature used. Consequently, we analysed how variation in the duration of dry/wet cycles affected the results of a germination experiment in four bromeliad species. In addition, for four other species we compared the outcome of an experiment with intermittent drought (Bader *et al.*, 2009) with the germination response to varying Ψ of the solutions in which the seeds were immersed.

The use of constant versus fluctuating temperatures in experiments is a long-standing issue in germination biology ((Baskin and Baskin, 2001). Clearly, there are plant groups where fluctuating temperatures are *required* for germination, or are at least strongly stimulating, e.g. in many species of wetlands and flood plains (e.g. Mollard and Insausti, 2011; Carta *et al.*, 2013). In most studies with epiphytes, constant temperatures have been used, but this approach has apparently rarely been based on a critical evaluation of the effect of constant versus fluctuating temperatures on germination in this group. A few researchers did apply fluctuating temperatures, but only two studies compared the outcome of such a treatment with germination under the constant, mean temperature. The results were inconsistent. While germination of the two bromeliads studied by Pinheiro and Borghetti (2003) was slightly lower and slower under fluctuating conditions, germination in four other bromeliads was significantly enhanced by varying temperatures (Pereira *et al.*, 2009). Such inconsistent results are hard to interpret in an ecological context, because the maximum and minimum temperatures are rarely based on relevant measurements of thermal fluctuations in the field (but see, for example, Pinheiro and Borghetti, 2003). We compared germination under both conditions for a range of species. In addition, we studied the interactive effect of varying temperature and water supply on germination with another three species.

To conclude, laboratory experiments are simplifications and cannot capture the complexity of the natural situation, while field work (e.g. Cascante-Marín *et al.*, 2008) will hardly allow the unambiguous identification and quantification of the effect of individual factors, e.g. temperature, on germination. This contribution will hopefully assist in 'bridging the gap', by improving the quality of the results from controlled, *ex situ* experiments to help understand the complexities *in situ* for studies with vascular epiphytes.

Materials and methods

All experiments were conducted with material from epiphytic Bromeliaceae collected from natural populations in Panama (Table 1), which were brought to the Plant Functional Ecology Laboratory at the university in Oldenburg, Germany, where the experiments were carried out. Seeds were sown in disposable Petri dishes (100×15 mm) with filter paper (Machery-Nagel, 651 mm, Ø 83 mm), which were kept in climate cabinets (Economic Delux, Snijders Scientific, Tilburg, The Netherlands) with a light/dark period of 12/12 h (photon flux density *c.* 60 μmol m^{-2} s^{-1}). If not mentioned otherwise, temperature was set to 25°C. Before starting an experiment all seed comas were clipped off and the seeds sterilized, following Pickens *et al.* (2003). Coma removal can slow germination compared to intact seeds, as shown for *Catopsis sessiliflora*

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Table 1. Study species used in the five experiments (E1–E5). Collecting sites are lowland sites with wet (San Lorenzo), moist (BCI, Barro Colorado Island) and dry tropical vegetation (Azuero) and a lower montane site (Fortuna). Plant names follow The Plant List (2013)

		Experiments				
Species	Provenance	E1: collection time	E2: T constant versus fluctuating	E3: dry/wet cycles	E4: dry/wet cycles versus water potential	E5: E2 × E3
Guzmania monostachia	BCI	x	x	x		x
Tillandsia fasciculata	BCI	x	x	x		x
Vriesea sanguinolenta	BCI	x	x	x		x
Guzmania subcorymbosa	San Lorenzo		x			
Vriesea gladioliflora	San Lorenzo		x		x	
Catopsis sessiliflora	BCI		x			
Guzmania lingulata	BCI		x			
Vriesea heliconioides	BCI		x			
Tillandsia monadelpha	BCI		x			
Tillandsia bulbosa	BCI		x			
Tillandsia anceps	BCI		x			
Tillandsia subulifera	BCI		x			
Tillandsia flexuosa	Azuero			x		x
Werauhia lutheri	Fortuna				x	
Vriesea viridiflora	Fortuna				x	
Vriesea vittata	Fortuna				x	

(Wester and Zotz, 2011), but the final proportion of germinated seeds seems to be unaffected. In the experiments without special water treatments seeds were watered every other day to ensure continuously moist conditions. Germination (defined as breakage of the testa by the protruding, swollen hypocotyl) was recorded daily with a dissecting microscope (Zeiss 57 50 57, Jena, Germany). Before starting experiments with intermittent water supply it was necessary to determine the amount of water evaporating from Petri dishes with and without a lid. To this end, we added different amounts of water to the filter paper in open Petri dishes and weighed the dishes at 30-min intervals for 2 h. The same was done with closed Petri dishes, but at 6-h intervals over 24 h. This procedure was repeated in all chambers at all the temperatures used in the subsequent experiments. The results of these trials allowed us to vary the absolute amount of water applied to each Petri dish at the beginning of each wet period, so that the length of the wet and dry periods were as long as planned irrespective of temperature and chamber.

Experiment 1: Seed maturity and germination response

One capsule per plant (Table 1, four individuals for each of three species) was collected at five different dates (November 2010–March 2011). The first collection was shortly after flowering (in October), the last immediately before natural dehiscence (in April). Seeds enclosed in the capsules were allowed to dry at room temperature (*c.* 22°C) and were kept in paper bags until the experiments were started a few weeks after the last collection date. We have shown recently that seeds of Tillandsioideae remain viable for at least 1 year under such conditions (Zotz, 2013a). For each species two replicates of every collecting date per individual with 20 seeds each were sown on filter paper in a Petri dish wetted with 2 ml of distilled water. The Petri dishes containing the seeds were sealed with Parafilm® in order to reduce evaporation and the germination success was monitored starting 3 d after sowing. Germination was followed for 19 d.

Experiment 2: Germination response to oscillating and constant temperatures

Germination with a fluctuating temperature regime (15–25°C) was compared with germination at the mean constant temperature of 20°C. In the case of *Guzmania monostachia*, *Tillandsia fasciculata* and *Vriesea sanguinolenta*, three replicates of 20 seeds were used; in the other nine species (Table 1) one batch of 20 seeds each were used. Germination was followed for 22 d.

Experiment 3: Germination response to wet–dry cycles of varying frequency

Seeds of four species (Table 1) were subjected to four different water treatments. Wet and dry periods made up consistently 50% of total time, but frequency of changes varied with cycles of 12/12 h, 24/24 h,

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36/36 h and 48/48 h (wet/dry). A control determined germination under continuous moisture. Each treatment was repeated in five Petri dishes containing 25 seeds each, except for *Tillandsia flexuosa* for which three replicates of 18 seeds were used, related to a scarcity of seeds of this species. The experiment was terminated when seeds in each of the treatments had experienced a total of 216 h (= 9 d) of wet conditions, which is enough for all controls to reach 100% germination.

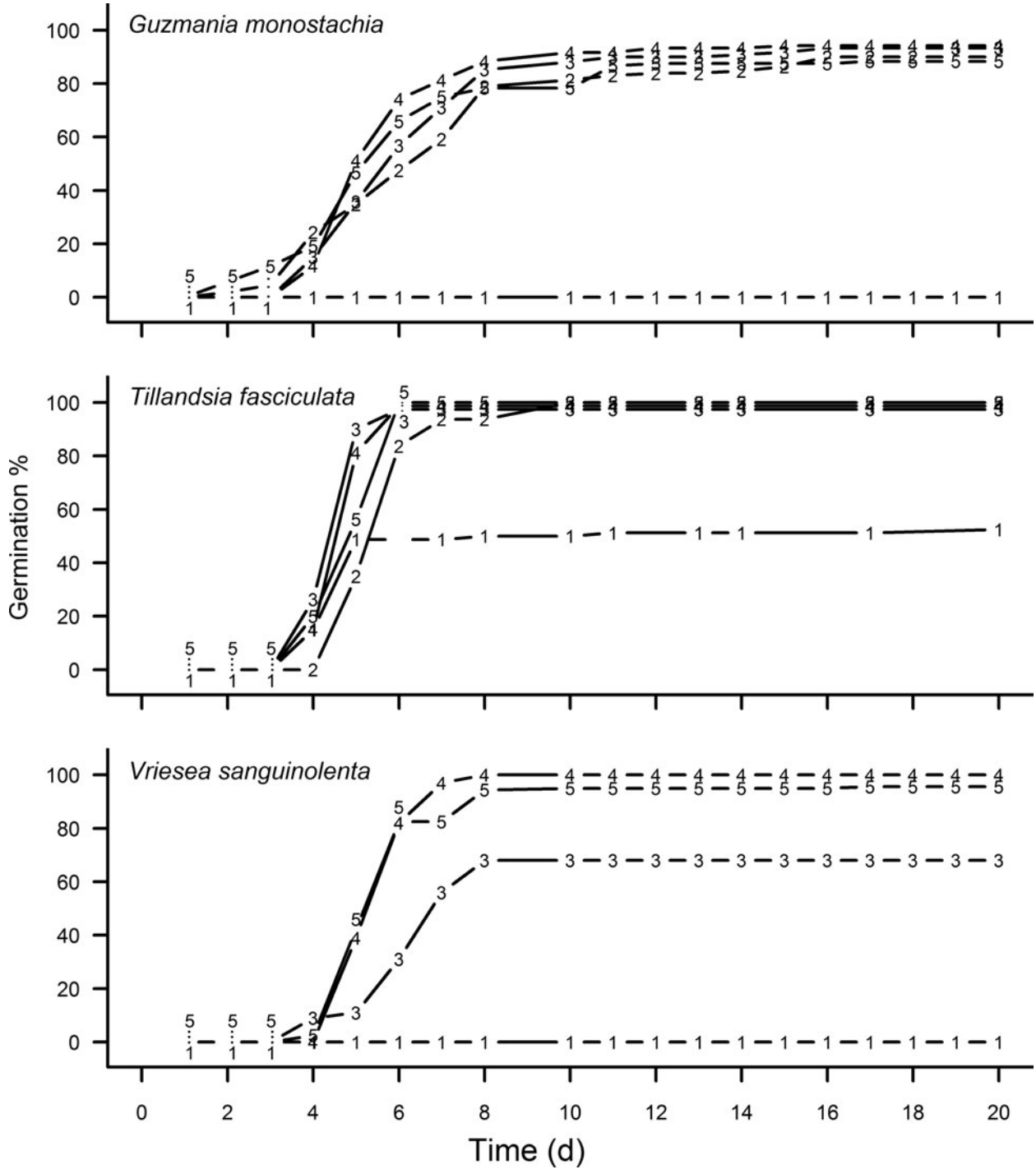

Figure 1. Cumulative germination of seeds collected at different stages of maturity in three bromeliad species. Data points are means of four individuals. Numbers (1–5) indicate the different collection dates: 1, immediately after anthesis (4 November 2010); 2, *c*. 2 months after anthesis (8 January 2011); 3, *c*. 3 months after anthesis (2 February 2011); 4, *c*. 4 months after anthesis (23 February 2011); 5, *c*. 5 months after anthesis (17 March 2011). For the species *Vriesea sanguinolenta*, data for the second collecting time are missing because all seed batches were attacked by a fungus.

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Experiment 4: Germination response to varying water potentials

Germination in seven solutions with varying osmotic potential was studied over 35 d with four species (Table 1). Solutions with osmotic potentials of −0.10, −0.25, −0.50, −1.0, −1.50 and −2.0 MPa were produced by varying the concentrations of mannitol; a control used distilled water (0 MPa). Treatments and control were replicated three times with 20 seeds each per species. Using the same seed batches, we also studied germination with different moisture regimes following the protocol described in Bader *et al.* (2009):

D0: continuous moisture in closed Petri dishes, adding 1 ml distilled water every other day to compensate for evaporation;
D1: mild repeated drought with 2 h dryness per day;
D2: moderate repeated drought with 6 h dryness per day;
D3: severe repeated drought with 22 h dryness in 2 d.

Each treatment was replicated three times with 20 seeds each. Germination was followed for 35 d.

Experiment 5: Simultaneous variation in the water and temperature regime and germination

Seeds of four different species (five Petri dishes with 20 seeds each, Table 1) were subjected to three different water treatments (D0, D1 and D2 at four different temperatures (15°C, 20°C, 25°C and 32.5°C). Consistent durations of dry periods were achieved by applying different amounts of water at the beginning of each wet period and removal of the lid about 2 h before the planned beginning of the subsequent dry period. Germination was followed for 35 d.

Data analysis

For statistical analysis we used R 3.0.3. (R Development Core Team, 2014). There is a range of response variables that can be used to analyse germination experiments (Scott *et al.*, 1984). In most cases we used the germination index (GI), which is defined as:

$$\mathrm{GI} = \frac{(\sum T_i N_i)}{S},$$

where T_i is the number of days after sowing, N_i is the number of seeds germinated on day i, and S is the total number of seeds planted. This index reflects both the speed of germination and the final germination success. Usually, we standardized GI by setting the maximum observed value in each experiment and species to unity, which facilitates comparison across experiments. Since final germination percentages under favourable conditions were close to 100% in individual seed batches in all experiments, we interpreted lower germination success as indicative of a treatment effect and not as an indicator of non-viable material, although we did not specifically test for viability after termination of an experiment. The responses of germination to varying wet/dry periods were analysed with one-way analyses of variance (ANOVAs) for each species separately, using 'lm'. Within-group differences were explored with Tukey's honest significance difference (HSD) tests. The effects of the combined drought and temperature treatments were studied with a three-way ANOVA. The response to constant and fluctuating temperature was analysed with a *t*-test.

Table 2. Germination success under constant and fluctuating temperature regimes in 12 epiphytic bromeliads (Experiment 2). Results are final germination in % after 22 d or the germination index. Sample size was 1 or 3 times 20 seeds, in the latter case the data are averages

	Final germination (%)		Germination index		
Species	Fluctuating	Constant	Fluctuating	Constant	*n*
Guzmania monostachia	90	97	1833	2344	3 × 20
Tillandsia fasciculata	97	97	1707	2072	3 × 20
Vriesea sanguinolenta	94	98	1890	2264	3 × 20
Guzmania subcorymbosa	85	89	1493	1792	1 × 20
Vriesea gladioliflora	100	100	1095	1694	1 × 20
Catopsis sessiliflora	100	100	1905	1630	1 × 20
Guzmania lingulata	100	93	1558	1293	1 × 20
Vriesea heliconioides	95	79	1708	1164	1 × 20
Tillandsia monadelpha	95	100	1588	1750	1 × 20
Tillandsia bulbosa	90	90	1485	1405	1 × 20
Tillandsia anceps	90	100	1615	2340	1 × 20
Tillandsia subulifera	90	90	1845	2365	1 × 20

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Results

Experiment 1: Seed maturity and germination response

With the exception of the first collection date, germination success was invariably close to 100% (Fig. 1), and we found no significant effect of collection date on final germination percentage for any of the three species (one-way ANOVA, $P > 0.05$). Seeds from capsules collected shortly after anthesis in November 2010 did not germinate in the case of *G. monostachia* and *V. sanguinolenta*. Remarkably, those of *T. fasciculata* showed about 50% germination, although capsules were still greenish when collected – about 5 months before natural dehiscence and seed dispersal.

Experiment 2: Germination response to oscillating and constant temperatures

The 12 tested bromeliad species showed no significant difference in germination response under constant and fluctuating temperature (Table 2, *t*-test, $P = 0.42$ for final germination percentage and $P = 0.17$ for GI). In three of the species, these trials were replicated three times, and again no treatment-related differences were found [*G. monostachia* (*t*-test, $P = 0.08$), *T. fasciculata* (*t*-test, $P = 0.51$) and *V. sanguinolenta* (*t*-test, $P = 0.63$)].

Experiment 3: Germination response to wet–dry cycles of varying frequency

Germination was invariably fastest in the controls and slowest in the treatment with the most frequent dry periods (12 h/12 h), with intermediate responses of the other drought-treated samples (see supplementary Figure S1). A different picture emerged when relating the GI to the periods of moisture, i.e. hydrotime (Fig. 2). Although the 12 h/12 h treatment continued to impose a significant effect on germination (ANOVAs, HSD tests $P < 0.05$; with one exception, *Guzmania monostachia*), the effects of the other treatments were basically indistiguishable statistically, both between each other and compared to the control (ANOVAs, HSD tests $P > 0.05$).

Experiment 4: Germination response to varying water potentials

The GI followed a logistic relationship with varying water potential (Fig. 3). Remarkably, two of the four species still germinated, if at low percentage, at water potentials < -1.5 MPa. Aligning these response curves with the GIs of the four drought treatments (D0-D3) yielded an inconsistent quantitative pattern. For example, the germination response under the driest wet/dry treatment in *Vriesea gladioliflora* was comparable to that with a water potential of -1.7 MPa,

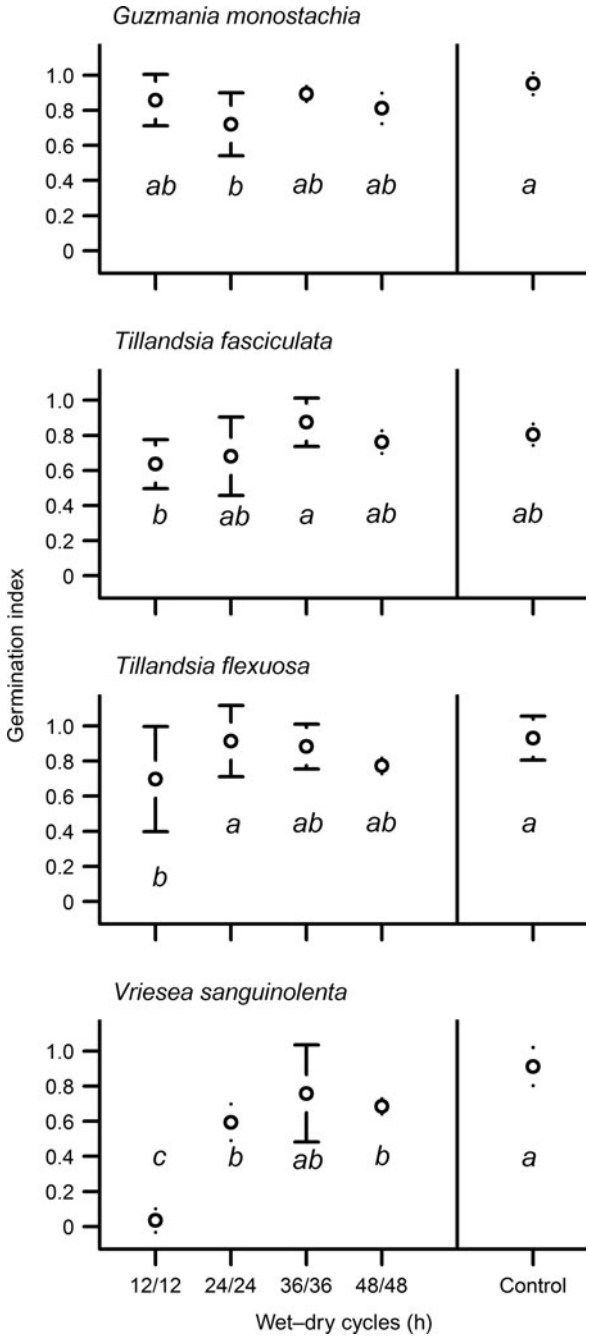

Figure 2. Standardized germination indices as a function of different wet–dry periods in four bromeliad species (left panels) and a control (constantly wet, right panels). Data are means ± SD; sample size was 5 × 25 seeds, except for *Tillandsia flexuosa* with 3 × 18 seeds. Significant differences (ANOVA, HSD, $P < 0.05$) are indicated by different letters. Detailed germination kinetics are shown in supplementary Figure S1.

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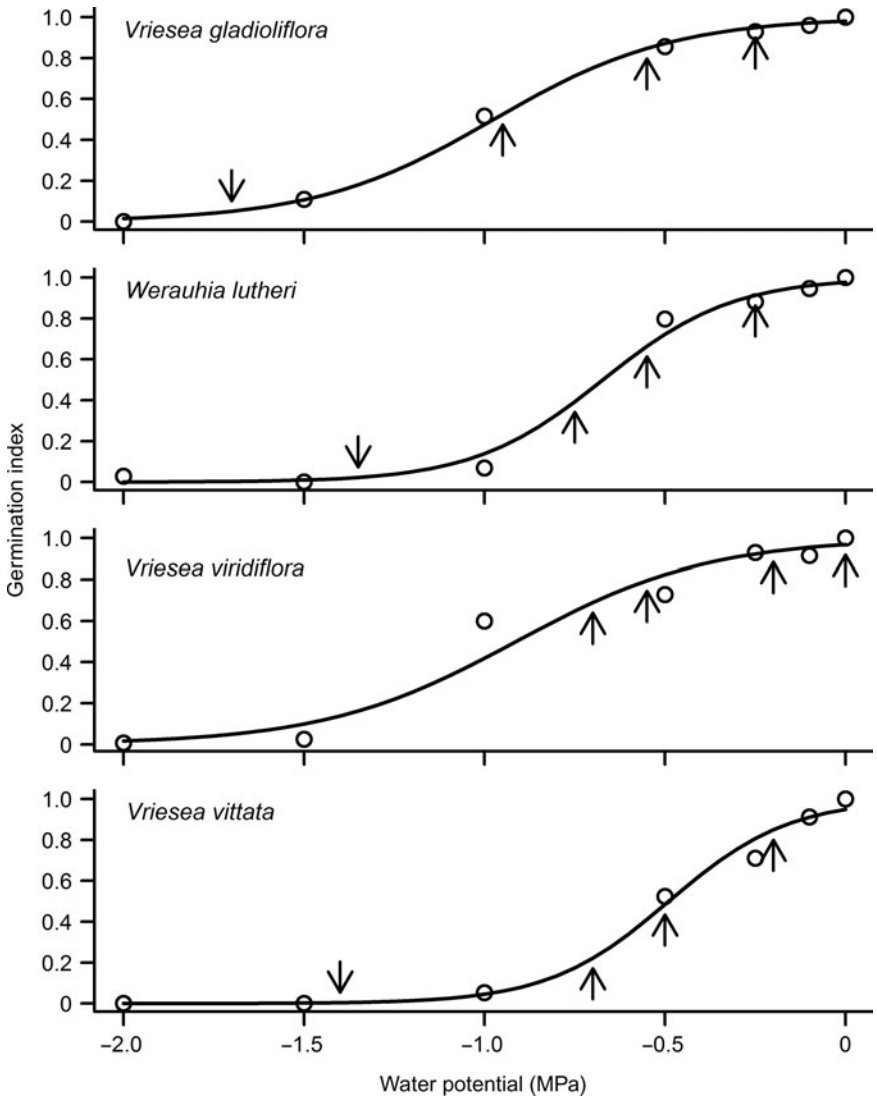

Figure 3. Germination responses to different water potentials in four epiphytic bromeliads. Open symbols are the mean germination indices at different water potentials (0 to -2.0 MPa), lines show logistic regressions fitted to these data, and arrows indicate the mean germination indices of the four different treatments with intermittent drought (D0–D3). Detailed germination kinetics are shown in supplementary Figure S2.

while the effect of the same treatment on *Vriesea viridiflora* was equivalent to that of an osmoticum of only −0.8 MPa.

Experiment 5: Simultaneous variation in the water and temperature regime and germination

Temperature, water regime and species affected germination (Table 3, Fig. 4). Germination success of all four studied lowland species varied consistently with temperature – it increased from 15°C to 25°C with no further increase, or even a decrease, at 32.5°C. Much less consistent was the response to intermittent drought. A negative effect of longer drought on germination was strongest at low temperatures (−63% to −100%), intermediate at 20 and 25°C (−24% to −81%), while at 32.5°C a reduction was only found in *V. sanguinolenta* (−80%, Fig. 4).

Discussion

Only a decade ago Baskin and Baskin (2001) concluded in a general review that 'not much is known about the germination ecophysiology of epiphyte seeds'. This situation has improved considerably by now, with a number of recent publications on the subject (e.g. Cota-Sanchez and Abreu, 2007; Toledo-Aceves and Wolf, 2008; Bader *et al.*, 2009; Goode and Allen, 2009; Manzano and Briones, 2010; Valencia-Díaz *et al.*, 2010; Montes-Recinas *et al.*, 2012; Sosa-Luría

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Table 3. Results of a three-way ANOVA on the effects of water, temperature and species on the standardized germination index for the species *Guzmania monostachia*, *Tillandsia fasciculata*, *Tillandsia flexuosa* and *Vriesea sanguinolenta*

Factor	df	*F*	*P* value
Temperature (T)	3	367.5	<0.001
Water (W)	2	158.8	<0.001
Species (S)	3	81.4	<0.001
T × W	6	11.1	<0.001
T × S	9	15.8	<0.001
W × S	6	6.9	<0.001
T × W × S	18	3.5	<0.001
Error	191		

et al., 2012). Overall, however, our understanding of this aspect of epiphyte ecology is still rather sketchy and highly biased – the majority of studies have focused on bromeliads. This contribution addresses methodological issues in an effort to ensure ecologically meaningful germination studies with bromeliads and epiphytes in general.

A first consideration in any germination study is to be sure about the level of maturity of the material used (Baskin and Baskin, 2001). Unless seeds are collected from naturally opened fruit, 'maturity' is not unambiguously defined. In our experience, fruit may look 'mature' long before natural opening. Information on afterripening in epiphytes is scarce (Fernandez *et al.*, 1989, Schwallier *et al.*, 2011). In *Tillandsia recurvata*, immature seeds germinated at a relatively high percentage, but lost viability much faster than mature seeds (Fernandez *et al.*, 1989). The advantage of collecting capsules before dehiscence are obvious: (1) during collecting trips material from species of different phenologies and conspecifics of varying developmental state can be collected together; and (2) as discussed by Ruiz *et al.* (2008) seeds inside unopened capsules are in sterile conditions, which makes sterilization procedures unnecessary. The results of our germination trials with immature seeds (Fig. 1) suggest that it is safe to collect material before natural dehiscence and obtain meaningful results, although it is certainly preferable to collect mature seeds whenever possible (Baskin and Baskin, 2001). The resulting question why these epiphytes postpone apparently possible dispersal for months may have a simple answer. Dispersal in the late wet season or early dry season would obviously offer no advantage for establishment, while seeds are clearly better protected in capsules until released immediately before the wet season. If this logic is true, afterripening should be less pronounced or absent in species with natural dehiscence during the wet season.

In nature, fluctuating rather than constant temperatures are the rule. Although some authorities therefore advocate the invariable use of alternating temperature regimes in germination studies (Baskin and Baskin, 2001), it is far from clear that *arbitrarily* set temperature variation yields results that allow better, i.e. less ambiguous, interpretation in an ecological context. Undoubtedly, experimental designs informed by data of field conditions are preferable, but in the absence of such data we see no reason to promote fluctuating temperatures in studies with epiphytic bromeliads. This statement is based on the inconsistent results in published studies and our own results. For instance, some studies have shown that bromeliad species have elevated germination percentages at alternating temperatures (Pereira *et al.*, 2009), while others showed the opposite pattern (Pinheiro and Borghetti, 2003). The bromeliad *Pitcairnia albiflos* even failed to germinate in an alternating temperature regime (Pereira *et al.*, 2010). Our results with 12 species of epiphytic bromeliads (Table 2) are in line with these previous observations. There was no consistent difference between germination behaviour in constant and fluctuating temperature conditions.

Two recent papers have used wet/dry cycles to study the effect of intermittent water supply on germination in epiphytes (Bader *et al.*, 2009, Wagner *et al.*, 2013). The ecological realism of this new approach is certainly much higher than the use of different osmotica, but considering the frequent use of the latter in germination studies (Baskin and Baskin, 2001) a comparison of the effects of both approaches is desirable. Although there was a consistent, and unsurprising, reduction in germination response with increasing drought (Fig. 3), either produced by shorter periods of wetness or lower water potential, a quantitative comparison revealed substantial interspecific variation in that relationship: different osmotica are clearly not a simple proxy for the impact of intermittent droughts of differing severity.

There are many other conceivable variations of wet/dry cycles in nature. In another experiment, we explored how the length of wet and dry periods affected gemination, while keeping the total duration of wet and dry periods constant, with 216 h each in *c.* 18 d. Compared to the controls, the delay in germination was inversely related to the length of the wet/dry cycles (see supplementary Figure S1). However, calculating the GI for periods of hydration only (i.e. for hydrotime; Black *et al.*, 2006), revealed very similar kinetics in most treatments (Fig. 2). Apparently, the germination process is only temporarily arrested during dry periods, and continues immediately after remoistening. A substantial reduction in the GI by the most rapid fluctuations of wet and dry periods (12 h/12 h) was only observed in one species, *V. sanguinolenta*. This is remarkable, because this tank bromeliad tends to occupy more exposed, and hence drier, microsites than two of the

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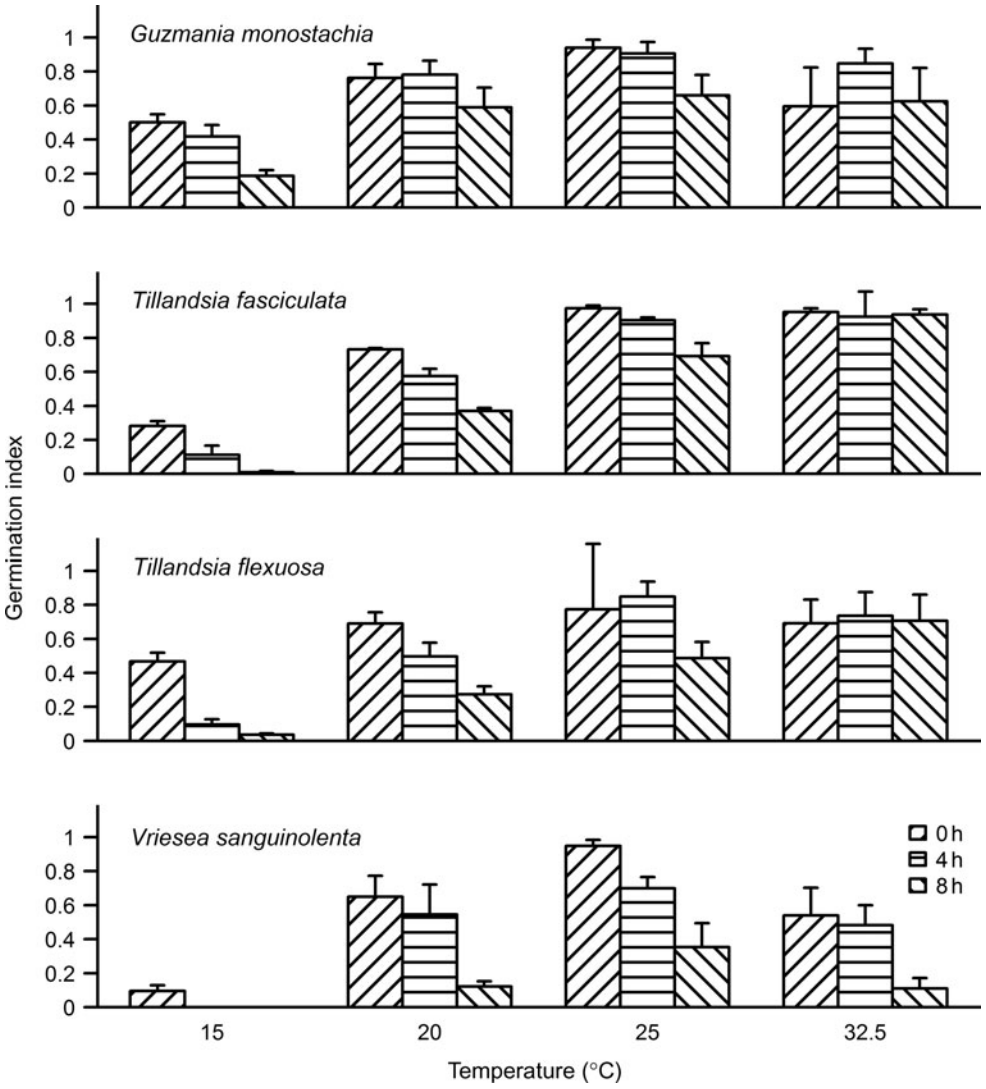

Figure 4. Germination indices in response to different water and temperature treatments for four epiphytic bromeliads. Water treatments: continuous moisture, 4-h dry period and 8-h dry period. Temperatures: 15°C, 20°C, 25°C and 32.5°C. Data are means ± SE. Detailed germination kinetics are shown in supplentary Figure S3.

other studied species (*G. monostachia* and *T. fasciculata*; Zotz, 1997), which were hardly affected.

The response to intermittent water supply depends on temperature as well (Fig. 4). Although the expected reduction in GI with increasing duration of intermittent drought was found at most temperatures, GI did not show this trend in three of four species at the highest temperature. Moreover, the proportional reduction varied substantially among lower temperatures. Hence, results of such experiments may be quantitatively, and even qualitatively, misleading when experimental conditions are not based on the typical situation in the field. Unfortunately, we are largely ignorant of the temperatures that germinating seeds of epiphytic plants experience in nature. Records from climate stations may be used as a first approximation, but conditions, particularly on exposed branches, are likely to deviate considerably from such standardized measurements.

To conclude, we present the results of a series of germination experiments with epiphytic bromeliads. We show that it is safe to collect capsules for experiments before natural dehiscence. The application of fluctuating temperatures is not imperative. The effects of different water potentials and intermittent drought on germination are not quantitatively comparable among species, and we advocate the use of the latter because of the greater ecological realism. However, the impact of intermittent drought on germination depends on temperature. Hence, data on *in situ* temperatures during germination are needed to design experiments in such a way as to ascertain unambiguous interpretation of the results in an ecological context.

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Supplementary material

To view supplementary material for this article, please visit http://dx.doi.org/10.1017/S0960258514000312.

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Conflicts of interest

None.

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