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Germination Responses to Water Potential in Neotropical Pioneers Suggest Large-seeded Species Take More Risks

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• *Background and Aims* In neotropical forests, very small-seeded pioneer species (<0.1 mg seed mass) recruit preferentially in small tree fall gaps and at gap edges, but large-seeded pioneers do not. Since water availability is related to gap size, these differences in microsite preference may reflect in part species-specific differences in germination at reduced water potentials.

• *Methods* For 14 neotropical pioneer species, the hypothesis is tested that small-seeded species, with shallow initial rooting depths, reduce the risks associated with desiccation by germinating more slowly and at higher water potentials than large-seeded species.

• *Key Results* Germination occurred both more quickly and at lower water potentials with increasing seed mass. For example, *Ochroma pyramidale* (seed mass 5.5 mg) had a time to 50 % germination (T_{50}) of 2.8 d and a median base potential for germination (ψ_{b50}) of -1.8 MPa while *Clidemia quinquenervia* (seed mass 0.017 mg) had a T_{50} of 17.6 d and ψ_{b50} of -1.1 MPa.

• *Conclusions* These data suggest that small-seeded species germinate only in comparatively moist microsites, such as small canopy gaps, which may reduce the risk of drought-induced mortality. Conversely, large-seeded species are able to germinate in the drier environment of large gaps, where they benefit by enhanced seedling growth in a high irradiance environment. The positive association of seed size and canopy gap size for optimal seedling establishment is maintained by differential germination responses to soil water availability coupled with the scaling of radicle growth rate and seed size, which collectively confer greater drought tolerance on large-seeded species.

Key words: Germination, seed size, Panamá, neotropical, pioneer, water potential.

INTRODUCTION

Seed size is a key variable for predicting patterns of plant regeneration. For example, in temperate herbaceous plant communities, seed persistence in the soil is associated with small seed size (Thompson, 1987; Thompson *et al.*, 1993), whereas in neotropical forests long-term persistence of pioneer species is associated with relatively large-seeded species (Dalling and Burslem, 2005; Dalling and Brown, unpubl. res.). Across a range of ecosystems small-seeded species are also more likely to require light as a cue to germinate in canopy openings or gaps in leaf litter (Milberg *et al.*, 2000; Pearson *et al.*, 2002; Jankowska-Blaszczuk and Daws, 2007). However, the importance of seed mass for other aspects of germination, such as the response to water potential, has received little attention and yet is a likely determinant of successful establishment.

Among tropical shrubs and trees, a functional group labelled 'pioneers' has been identified that consists of fast-growing early successional species that need canopy gaps for establishment (Swaine and Whitmore, 1988). In central Panamá, pioneers have seed sizes from approx. 0.02 mg to 100 mg (Daws *et al.*, 2007*a*) and occur in canopy gaps of differing size. Along the understorey–gap

continuum, the quantity of irradiance, and hence the amplitude of soil temperature fluctuations and the rate of soil surface drying, increase with increasing gap size (Denslow *et al.*,1998; Daws *et al.*, 2002, 2007*a*; Pearson *et al.*, 2002). For example, in large canopy gaps at the present study site in central Panamá (Barro Colorado Island), irregular rainfall during the wet-season can result in the soil matric potential at 10 mm depth falling to less than -1.5 MPa (the permanent wilting point) after just 6 d of drying (Engelbrecht *et al.*, 2006; Daws *et al.*, 2007*a*; Marthews *et al.*, 2008). In some small gaps, water extraction by the roots of the surrounding trees results in surface soil drying up to but not beyond the wilting point, while litter-free soil in the centre of large gaps continues to dry beyond the wilting point (Marthews *et al.*, 2008). This potential for rapid drying beyond the wilting point implies that the risk of mortality from desiccation for germinating seeds/establishing seedlings increases with gap size (Engelbrecht *et al.*, 2006; Daws *et al.*, 2007*a, b*).

Large-seeded neotropical pioneer species generally have a distribution that is biased towards the 'risky' environment of larger sizes of canopy gap (Brokaw, 1987; Pearson *et al.*, 2003*a*), although these studies did not invoke seed size to account for these differential distribution patterns (but see Dalling *et al.*, 2004). In addition, germination requirements

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of large-seeded pioneers typically appear to be 'cued' to large gap environments, i.e. they require either a high ratio of red : far red light (R : FR) or a large amplitude of diurnal temperature fluctuations for germination (Vázquez-Yanes and Orozco-Segovia, 1982; Daws *et al.*, 2002; Pearson *et al.*, 2002).

The risk of desiccation-induced mortality may be lower for seedlings from large seeds since such seedlings can emerge from greater soil depths (Bond *et al.*,1999; Pearson *et al.*, 2002) and have more rapid radicle growth rates (Daws *et al.*, 2007*a*) than seedlings from small seeds. Consequently, it is hypothesized that large-seeded species may be better able to 'cope' with the risk associated with large canopy gaps. The high light environment of large gaps is likely to be advantageous for seedlings that can establish successfully, by facilitating rapid post-establishment growth, as long as this occurs in advance of the frequent dry spells in the early wet season. For these potentially 'risk-taking' species, the risk of rapid emergence may be tempered by a rapid radicle growth and hence access to soil water at depth (Daws *et al.*, 2007*a*). However, for small-seeded species that can germinate only at shallow depths, where soil drying occurs rapidly, it is predicted that germination will occur more slowly. This response will limit germination either to large gaps in prolonged wet spells or to the less strongly drying environment of small gaps.

Soil water potential modulates seed germination, and species differ in the minimum water potential at which germination can occur (Kaufmann, 1969; Evans and Etherington, 1990; Choinski and Tuohy, 1991; Allen *et al.*, 2000; Daws *et al.*, 2002). For neotropical pioneer shrubs and trees, only four species from one genus have been characterized for their germination response to water availability (Daws *et al.*, 2002). These congeners had a limited range of seed mass values and showed only modest differences in the base water potential for germination (Daws *et al.*, 2002). Since large-seeded pioneers occur more frequently in large gap environments, it was predicted that they would germinate at lower matric potentials than small-seeded species. Such a response would potentially

complement rapid germination in enabling rapid establishment in the unpredictable large gap environment.

Consequently, in this paper, the predictions that for pioneer species (*a*) large-seeded species germinate more rapidly than small-seeded species and (*b*) that large-seeded species are able to germinate under lower water potentials (drier conditions) than small-seeded species are tested. The results are discussed in the context of risk-spreading in environments of unpredictable rainfall.

MATERIALS AND METHODS

Study site and species

Ripe fruits of the ten study species (Table 1) were collected between February and July 1999 from semi-deciduous forest on Barro Colorado Island (BCI; 9°09'N, 79°51'W), Republic of Panamá. Mean annual rainfall at BCI is approx. 2700 mm with about 93 % of the total occurring in the wet season between May and December (Croat, 1978). Fruits were collected from at least five individual plants per species and seeds extracted by mashing the fruits in water and decanting off the fruit pulp. Subsequently, seeds were air-dried in the dark, transported to Aberdeen, UK, and stored at room temperature prior to the start of germination experiments. In addition, seed mass and germination data for a further four pioneer species from BCI (*Piper dilatatum*, *P. hispidum*, *P. marginatum* and *P. peltatum*) were obtained from Daws *et al.* (2002).

Seed germination

Seeds were subjected to water potentials of approx. 0, −0·25, −0·5 and −1·0 MPa by placing three replicates of 25 seeds each on the surface of a wick connected to a reservoir of polyethylene glycol 8000 (PEG), at a concentration appropriate to the intended treatment (Michel and Kaufmann, 1973), following the method outlined by Daws *et al.* (2002). The PEG solution flowed over the wick, maintaining the seeds at a constant water potential.

TABLE 1. *Summary information of seeds used in this study*

Species	Family*	Habitat information	Seed mass (mg)	T_{50} (d)
Apeiba tiborbou	Malvaceae−Tiliaceae	Abandoned farms, road edges, canopy gaps[†]	5·6	7·1
Cecropia insignis	Moraceae	Canopy gaps[†]	0·68	5·6
Cecropia longipes	Moraceae	Canopy gaps[‡], open areas[§]	1·2	6·3
Cecropia obtusifolia	Moraceae	Tree fall areas[§]	0·58	5·9
Cecropia peltata	Moraceae	Tree fall areas[§]	0·59	14·0
Clidemia octona	Melastomataceae	Forest edge[§]	0·015	21·4
Clidemia quinquenervia	Melastomataceae	Edge of clearings[§]	0·017	17·6
Luehea seemannii	Malvaceae−Tiliaceae	Along roads, canopy gaps[†]	1·7	4·1
Miconia argentea	Melastomataceae	Along roads, canopy gaps[†]	0·076	11·3
Ochroma pyramidale	Malvaceae−Bombacaceae	Large clearings[†] Disturbed areas[§]	5·5	2·8

* From APG II (2003).
[†] From CTFS (2004).
[‡] From Würth *et al.* (1998).
[§] From Croat (1978).

Sub-samples of the wicks were removed after the experiment had been running for 20 d, and their actual water potential determined using an SC-10A Richards thermocouple psychrometer (Decagon Devices, Pullman, WA, USA). Measured rather than nominal values of water potential were used in all analyses; measured values were all within 20 % of the target values and had standard deviations <15 % of the mean value.

Seeds of *Ochroma pyramidale* and *Apeiba tibourbou* require a dormancy-breaking treatment. Consequently, to ensure germination in the water potential treatments, seeds of *A. tibourbou* and *O. pyramidale* were treated with hot water at 80 °C and 90 °C, respectively, for 2 min (Acuña and Garwood, 1987; Daws *et al.*, 2006).

The germination system was housed in transparent polyethylene boxes and placed in a growth chamber at 26 °C, with a 12-h photoperiod. Although for some species base potential has been shown to be dependent on temperature (Dahal *et al.*, 1993; Kebreab and Murdoch, 1999), a single temperature (26 °C) was used. Even though soil surface temperatures in large gaps are elevated close to mid-day, time-courses of temperature at 1-cm depth suggest that values for both small and large gaps are within 1 °C of 26 °C for a mean of 87·5 % and 67·4 %, respectively, each day (Daws *et al.*, 2002). Irradiance inside the transparent boxes averaged 5 μmol m^{-2} s^{-1}, the R : FR was approx. 2·0. Seeds were scored daily for germination for up to 50 d with germination defined as visible radicle emergence.

Data analysis

Time to 50 % of maximum germination (T_{50}) at 0 MPa (water) was determined for each species from plots of germination against time.

The effect of water potential on time to germination has been described by the hydrotime model of germination (Bradford, 1990). In this model seeds germinate only when they have accumulated sufficient hydrotime (θ_H). At and below a base water potential (ψ_b) seeds do not start to germinate. Above this potential, seeds accumulate hydrotime. Within seed lots a normal distribution of base potentials is typically observed (Bradford, 1990; Dahal and Bradford, 1990; Gummerson, 1996) while the hydrotime for germination is constant for all individual seeds (Bradford, 1990; Dahal and Bradford, 1990). Thus, the fastest seeds to germinate in a population have the lowest base potentials and are able to accumulate hydrotime at lower potentials than slower-germinating seeds. Thus germination can be described as:

$$\theta_{Hg} = (\psi_w - \psi_{bg})t_g \qquad (1)$$

where θ_{Hg} is the hydrotime (in MPa h^{-1}) required for germination of proportion g of the seed lot, ψ_w is the actual water potential (MPa), ψ_{bg} is the base potential of proportion g of the seed lot and t_g is the length of time since the start of imbibition.

By rearranging eqn (2) in terms of base potential:

$$\psi_{bg} = \psi_w - (\theta_H/t_g) \qquad (2)$$

it is possible to determine ψ_{b50} and the standard deviation of base potential ($\sigma_{\psi b}$) by plotting experimentally determined germination progress curves, on a probit scale (for the experimentally studied range of water potentials), against base potential [$\psi_w - (\theta_H/t_g)$]. Thus, the unknown values of θ_H can be estimated iteratively (Bradford, 1990). If the value of θ_H is repeatedly changed until the best fit (i.e. the value with minimal residual variation) is obtained when a single least squares regression line is fitted to the data for the range of water potentials, then ψ_{b50} corresponds to the base potential when germination is 50 % of the maximum observed germination level and the reciprocal of the slope of the regression line is the standard deviation of the base potential ($\sigma_{\psi b}$).

RESULTS

Effect of water potential on germination

For all ten species, germination percentage and rate of germination decreased as water potential became more negative. Thus, germination was most rapid and reached the highest percentage at 0 MPa (water) and was lowest at 1·0 MPa. In addition, the negative effect of -0.25 and -0.5 MPa was relatively minor for most species, with a greater impact of -1.0 MPa (data not shown).

Plotting germination (in probits) against base potential indicated that the median base water potential (the base potential when germination in probits is 5; ψ_{b50}) ranged from -1.07 to -2.06 MPa (*Clidemia quinquenervia* and *Apeiba tibourbou*, respectively; Fig. 1). On Fig. 1 each point represents an observation from the four germination progress curves with values of base potential determined after iteration of the hydrotime constant, θ_H, in the expression [$\psi_w - (\theta_H/t_g)$] to maximize the fit of the linear regression. Therefore species that take longer to germinate have more observations per germination progress curve, which is reflected in a greater number of points on the corresponding graphs.

For the ten study species and four additional species from Daws *et al.* (2002), there was a significant negative relationship between the median base water potential and seed mass (linear regression, $R^2 = 0.457$, d.f. $= 12$, $P = 0.008$): small-seeded species were less able to germinate at low water potentials (Fig. 2A).

Time to 50 % germination and seed mass

Time to 50 % germination (T_{50}) in the 0-MPa (water) treatment for the 14 species was significantly negatively related to seed mass (linear regression, $R^2 = 0.684$, d.f. $= 12$, $P < 0.001$; Fig. 2B). For example, *Ochroma pyramidale* (seed mass $= 5.5$ mg) reached 50 % of maximum germination in 2·8 d while *Clidemia octona* (seed mass $= 0.015$ mg) took 21·4 d. In addition, T_{50} and ψ_{b50} were significantly correlated, i.e. the faster-germinating species
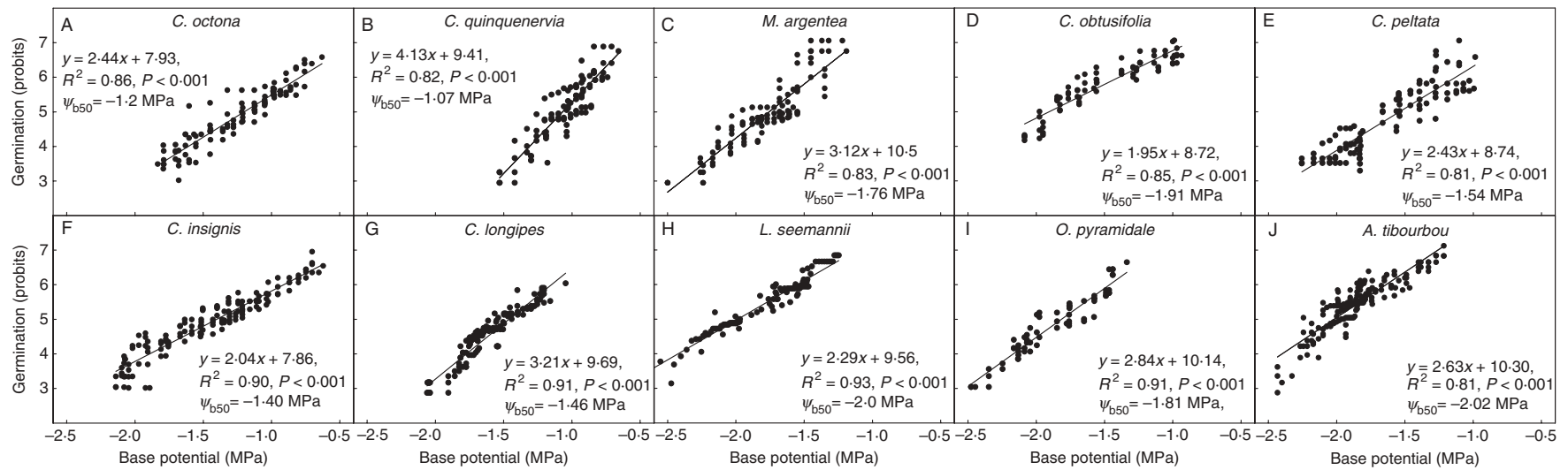
FIG. 1. Plots of germination, on a probit scale (a probit value of 5 corresponds to 50 % germination), against the median base water potential for germination (ψ_{b50}) for the ten study species. Species (A–J) are ranked in order of increasing seed mass.
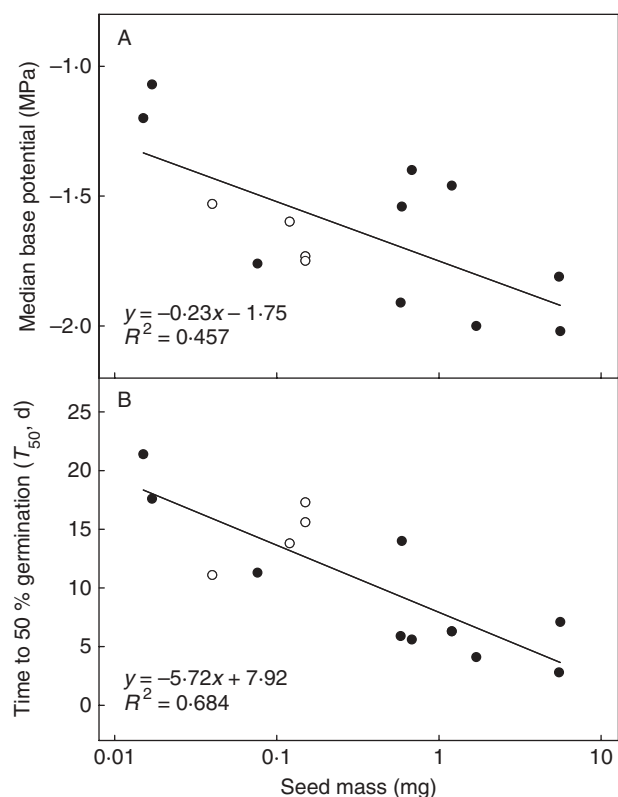
FIG. 2. The relationship between seed mass and (A) the median base water potential for germination (ψ_{b50}) and (B) time to 50 % germination (T_{50}) at 0 MPa (water) for 14 pioneer species. Closed symbols refer to data from this current study, open symbols to data for *Piper dilatatum*, *P. hispidum*, *P. marginatum* and *P. peltatum* from Daws *et al.* (2002).

could germinate at more negative water potentials (Pearson's correlation coefficient, $r = 0.573$, d.f. $= 12$, $P < 0.05$).

DISCUSSION

There were substantial differences in the germination responses of BCI pioneer species in both T_{50} and ψ_{b50} that were related to seed size. The low base potential and rapid germination (low T_{50}) of large-seeded species suggests a 'risky' germination strategy that has the potential to enable germination in large gaps and result in microsite pre-emption in advance of slower-germinating species. However, smaller-seeded species are more 'conservative', germinating slowly and at less-negative water potentials, which may reduce the likelihood of seedling mortality by ensuring germination occurs primarily in either small gaps or large gaps during prolonged wet periods. For the 14 species examined here and by Daws *et al.* (2002) seed size differences are confounded by phylogenetic contrasts at the family level, and there were no relationships between T_{50} or ψ_{b50} and seed size within any of the four families (Fig. 2). Consequently, phylogenetic effects independent of seed size may also be important: data from a larger sample of species and families would help to clarify this issue.

Base water potential and germination microsite

The 14 species exhibited differences of >1 MPa in ψ_{b50}. Species with more negative values of ψ_{b50} (e.g. *Ochroma pyramidale*, -1.8 MPa) can progress towards germination under drier conditions, which may facilitate germination in large (drier) gaps. In contrast, a higher ψ_{b50} will limit germination to small gaps and potentially also to large gaps during extended wet periods. A wide range of ψ_{b50} values has also been reported for other species. For example, Allen *et al.* (2000) report values for 24 desert species ranging from -5.92 to -0.07 MPa, although most values were clustered in the range -1.8 to -0.07 MPa. Similarly, Evans and Etherington (1990) found that among 15 British plant species there was a wide range of responses to water potential with differences between species partly corresponding to habitat type. Thus, the three species with germination most sensitive to decreasing water potentials were all wet-land species (e.g. *Juncus articulatus*). However, among the remaining species there was little clear pattern in sensitivity to water potential in relation to habitat type.

Role of seed size in germination

Brokaw (1987) inferred gap partitioning among the pioneer species *Miconia argentea*, *Cecropia insignis* and *Trema micrantha*, based on the observation that plants of these species grow to maturity in gaps larger than 102, 215 and 376 m^2, respectively, on BCI. While these differences have been related to a trade-off between growth-rate in high light and survival in the shade (Brokaw, 1987), seed size also increases in the same order in these three species: *Miconia argentea* < *Cecropia insignis* < *Trema micrantha*. Similarly, large-seeded species such as *Ochroma pyramidale* and *Cecropia obtusifolia* also occur preferentially in larger gap sizes (Croat, 1978; Pearson *et al.*, 2003*a*). On the other hand, the small-seeded *Clidemia octona* and *Clidemia quinquenervia* occur preferentially in small gaps or the edges of large gaps (Croat, 1978). A role for seed mass in contributing to observed gap size preferences is supported by the correlation between ψ_{b50} and seed mass: small-seeded species germinate less well at low water potentials and hence are less able to germinate in the drier environment of large gaps.

Successful establishment in large gaps may necessitate rapid germination to enable emergence in advance of potentially rapid soil drying (at least close to the soil surface), which is supported by the rapid germination of the larger-seeded species (Fig. 1B). Large seed size may also facilitate germination and establishment in large gaps by enabling emergence from greater soil depths (Bond *et al.*, 1999; Pearson *et al.*, 2002) where soil drying will be less rapid. However, even for shallowly buried large seeds, the risk of seedling mortality may be lower than for small-seeded species since the rate of radicle extension is allometrically related to seed mass: radicle growth of seedlings from large seeds may progress faster than the soil drying front in the event of a post-germination dry spell (Daws *et al.*, 2007*a*). Indeed, several studies have reported that seedlings

from small seeds have higher mortality under water stress than those from large seeds (Buckley, 1982; Leishman and Westoby, 1994). Thus, for these pioneer species it is proposed that for small-seeded species, a combination of rapid germination and germination at low water potentials is unlikely since it would result in extremely high seedling mortality in the event of a post-germination dry spell. Similarly, Doussi and Thanos (2002) suggested that slow germination of Mediterranean species was an adaptation to unpredictable water availability since this strategy ensures that germination only occurs with the onset of reliable winter rainfall (i.e. it prevents germination occurring following sporadic early autumn/winter rainfall).

However, the mechanism(s) resulting in slow germination in the small-seeded species is unclear. Small seeds have a faster water absorption capacity than large seeds resulting from a larger surface area to mass ratio (Kikuzawa and Koyama, 1999), suggesting that the opposite pattern might be expected. Alternatively, the mechanical restraint provided by the seed coat might contribute to delaying germination. However, Daws *et al.* (2007*a*) found no relationship between seed mass and the proportion of seed mass that constituted seed coat, which suggests that this may not contribute to the differences in T_{50}. Consequently, further studies are needed to understand the physiological basis of these differences in germination timing.

The present data for neo-tropical pioneers suggest a continuum between large-seeded 'risk-takers', with rapid germination and a low ψ_{b50}, and small-seeded 'risk-averse' species with slow germination and a high ψ_{b50}. This trend is further supported by the pattern of response in germination observed in relation to seed mass and R : FR for pioneer taxa on BCI. Thus, Pearson *et al.* (2003*b*) reported that among species with photoblastic germination, large-seeded species require a higher R : FR to trigger germination than small seeds. In this scenario, large-seeded species only germinate in the higher risk environment of large gaps, while the small-seeded species can germinate in all gap sizes. However, these kinds of patterns are not evident in the data of Allen *et al.* (2000) for 24 desert species where no relationship between ψ_{b50} and germination speed was observed. One explanation for this discrepancy is that the analysis of Allen *et al.* (2000) was potentially confounded by the inclusion of species belonging to various functional groupings (halophytes, psammophytes and generalists) while the present study is based on species from one functional group collected from the same location. Clearly, further studies are needed to test the generality of seed mass, germination rate and ψ_{b50} relationships using co-occurring species from additional habitat types that span a range of seed masses and that belong to the same functional group.

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