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Evidence of population variation in drought tolerance during seed germination in four *Banksia* (Proteaceae) species from Western Australia

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Abstract. Given the predicted changes in rainfall patterns for many Mediterranean climate regions, identifying seed tolerance to moisture stress in the earliest phase of plant development is an important consideration for species conservation, management and restoration. Here, we used polyethylene glycol (PEG 8000) to induce plant water deficit similar to drought stress in a field situation. Seeds of four Western Australia *Banksia* R.Br. (Proteaceae) species were incubated at seven levels of moisture potential (0 to -1.5 MPa) and three constant temperatures (10°C , 15°C and 20°C). In the absence of moisture stress, germination was uniformly high, but increasing drought stress led to reduced and delayed germination in all species. Overall, the threshold moisture potential value for a significant decline, and delay, in germination was -0.25 MPa. Results suggested that one species (*B. coccinea*) is likely to be most vulnerable to germination failure under predicted changes in rainfall patterns, whereas another (*B. media*) is likely to be less vulnerable. There was significant variation in population response to drought stress. However, this variation could not be explained by rainfall across species distributions. We discuss the PEG approach for assessing seed sensitivity to moisture stress, particularly in the context of shifting rainfall under climate change.

Additional keywords: among-population variation, climate change, Mediterranean, temperature, water stress.

Received 17 June 2014, accepted 7 October 2014, published online 23 December 2014

Introduction

For sexually reproducing plants, regeneration by seed is a fundamental mechanism that influences the population and range dynamics of species (Harper 1977; Silvertown and Charlesworth 2001). Contemporary climate-driven changes in regeneration have the potential to cause significant declines in species abundance, changes in community composition and even local population extinction (Walck *et al.* 2011). Understanding how climatic variables influence early plant life-history phases helps in the prediction of the long-term viability of species under climate change. For example, seasonal timing of germination is regulated by temperature and moisture availability, with seedling emergence largely driven by the effect of moisture availability on seed imbibition and radicle growth that determines the minimum water potential (MPa or ψ) for germination (Finch-Savage and Phelps 1993). When MPa is zero, the soil is saturated, water is not limiting and germination is largely determined by temperature (Probert 2000).

However, as the soil dries out, MPa becomes increasingly more negative, making it harder for seeds to access moisture. Temperature can modify the range of soil MPa over which germination occurs affecting both total germination and its timing (Hegarty 1978; Gummerson 1986; Alvarado and Bradford 2002; Bradford 2002). Optimal temperatures for germination commonly coincide with the period of reliable rainfall, which favours seedling establishment and survival.

There are few options to simulate natural drought stress under controlled conditions, but the addition of solutes to water, such as the inert, water-binding polymer polyethylene glycol (PEG), will lower water potential. This induces a reduction in hydraulic conductivity at the seed–water membrane contact point, similar to drought stress in a field situation; the higher the concentration of solutes, the lower the ψ , and greater the moisture stress. PEG works as a simple yet informative indicator of drought stress that is harmless to seed viability. Such artificial methods have been reported to reduce,

delay or prevent germination completely, depending on the different PEG concentrations (Hegarty 1977; Cony and Trione 1998; Kaya *et al.* 2006; Perez-Fernandez *et al.* 2006; Van den Berg and Zeng 2006; Cavalcante *et al.* 2010; Sayar *et al.* 2010; Zeng *et al.* 2010). The role of moisture and temperature, and the relationship between delayed and reduced germination with increasing water stress, are well established (Sharma 1976; Hagon and Chan 1977; Bajpai 1997; Adams 1999; Tobe *et al.* 2001; Thomas *et al.* 2010; Boddy *et al.* 2012).

The application of PEG in seed-ecology studies is an underused approach for assessing drought response, and empirical data on seed response to drought stress exists for only a limited range of species, mostly of agricultural concern (but see Thomas *et al.* 2010). Few studies have investigated the response of seeds of long-lived species from Mediterranean climate ecosystems to impending drought stress as a result of projected climate change. Reductions in total rainfall are forecast for these regions and mean annual temperatures are expected to rise (IPCC 2007). These seasonal environments harbour a substantial proportion of the Earth's flora (Myers *et al.* 2000) and the warm, dry summers and cool, wet winters make these environments particularly vulnerable to a warming, drying climate (Sala *et al.* 2000; Klausmeyer and Shaw 2009). Water availability is already a major factor limiting seed germination in Mediterranean environments (Groom 2002; Lloret *et al.* 2005), with lack of soil moisture often a cause of seedling mortality (Lamont 1993). The few studies investigating the potential consequences of a warming and drying climate for plant population dynamics and community composition have suggested that a lack of soil moisture will have a stronger impact than does temperature on seed germination and seedling establishment in these ecosystems (Lloret *et al.* 2004, 2009). However, for many native species, the moisture requirements for germination, and their interaction with temperature, are currently unknown and more work is needed to understand how moisture availability and temperature act individually and interact to affect germination.

In this study, we use solutions of PEG to examine the influence of drought stress on seed germination in long-lived woody *Banksia* species (Proteaceae) inhabiting the southern coast of the South West Australian Floristic Region (SWAFR). The SWAFR is one of the five Mediterranean climate regions of the world and has been recognised as a global biodiversity hotspot (Mittermeier *et al.* 2004). This region provides an ideal focal point for investigating the effects of moisture stress on recruitment in the context of climate change, because the region has undergone a 20% reduction in annual rainfall since the 1970s, with the greatest decline in rainfall occurring in the autumn–winter months (Bates *et al.* 2008, 2012). These changes are resulting in a shorter winter wet season, with a potentially smaller window of opportunity for germination. In Western Australia, *Banksia* species are generally confined by climate to the south-western corner of the region, with the limits for drought tolerance in the genus corresponding to 250 mm of rainfall per annum (Lamont and Connell 1996). As such, recent climate models have suggested that Western Australian *Banksia* species will be particularly vulnerable to the predicted effects of climate change, with sensitivity expected to be largely dependent on species tolerance to forecast

decreases in rainfall (Fitzpatrick *et al.* 2008; Yates *et al.* 2010). Members of this genus are an important component of heath and shrub vegetation in the SWAFR, providing wide structural and functional value to local fauna and surrounding vegetation (Lamont *et al.* 2007). Although *Banksia* species are foundation species in many ecosystems, there have been few empirical investigations into how these species will cope with the predicted changes (Witkowski and Lamont 2006; Yates *et al.* 2010), and, to our knowledge, no studies have been undertaken that explicitly considered moisture thresholds for germination in *Banksia* species, and across multiple populations.

Here, we examine the combined effects of water availability and incubation temperature on seed germination in four *Banksia* species endemic to the SWAFR. We test multiple populations of each species because predicting species response to changing moisture conditions requires an understanding of population differentiation, because populations located along a climate gradient are likely to experience a range of different environmental stresses, and subsequently respond in different ways (Cochrane *et al.* 2014). The four species (*Banksia baxteri*, *B. coccinea*, *B. media* and *B. quercifolia*) are fire-killed and rely wholly on seeds for regeneration. Fires are highly seasonal in this region, with wildfires tending to increase from west to east (Barrett *et al.* 2009). *Banksia* seeds will germinate readily post-fire, given appropriate environmental conditions. However, there is evidence to indicate recruitment failure in many such Proteaceae, owing to drought stress in the post-fire environment (Lamont *et al.* 1993; Groom 2002). The continued warming and drying in the region will affect fire regimes, with more extreme conditions potentially leading to reduced intervals between fires (Williams *et al.* 2009). The species are locally common where they occur, but largely restricted to the southern coastal areas of the SWAFR. Our overall hypothesis is that the germination response of the four *Banksia* species would be similar, but that the response of populations would differ in predictable ways (i.e. gradients in seed tolerance to drought would reflect rainfall gradients in the species' distributions). We hypothesised that seeds from the more xeric populations would be more tolerant of drought-stress treatments across all species.

Materials and methods

Seed material

To capture a broad range of environmental tolerances, seeds were sampled from three natural populations of each of the four species of *Banksia* (Table 1). The populations were located along a rainfall gradient that declined in an easterly direction. Mature, fruiting cones were collected from upwards of 50 individual maternal plants at each site. Seeds were extracted from fruits using the method of Cochrane *et al.* (2014). Seed mass was determined to the nearest μg by weighing 20 individual seeds from each population; however, preliminary analyses showed that seed mass varied as much within as between populations (data not shown) and was therefore not used as a covariate in subsequent analysis of germination response. Seeds from individuals collected from each population (high-, medium- and low-rainfall populations) were pooled

Table 1. Ecogeographical data for populations for study species

Mean annual precipitation (MAP) and mean annual temperature (MAT) were derived from BioClim interpolation (Hijmans *et al.* 2005). Mean annual soil temperature and water content values were obtained from Hobo H21-002 micro weather stations recording soil parameters at ~5-cm depth at each site

Species	Site	Latitude	Longitude	MAP (mm)	MAT (°C)	Mean annual soil temperature (°C)	Mean annual soil water content (m ³ m ⁻³)	Seed mass ± s.e. (mg)
<i>Banksia Baxteri</i>	Waychincup National Park	34.89°S	118.38°E	726	15.0	17.45	0.0719	66.42 ± 2.83
	Stokes National Park	33.84°S	121.03°E	536	16.4	19.58	0.0217	46.23 ± 1.80
	Fitzgerald River National Park	34.17°S	118.52°E	404	16.3	18.54	-0.0171	46.07 ± 1.68
<i>Banksia coccinea</i>	Cheyne Beach	34.89°S	118.42°E	719	15.0	15.91	0.0590	18.84 ± 0.57
	Pfeiffer Road	34.64°S	118.21°E	598	14.7	16.18	0.0450	19.46 ± 0.55
	Stirling Range National Park	34.48°S	118.01°E	496	14.7	15.69	0.0693	16.74 ± 0.69
<i>Banksia media</i>	Alexander Bay	33.87°S	122.75°E	574	15.9	19.81	0.0196	45.69 ± 1.40
	Kundip Nature Reserve	33.71°S	120.21°E	438	16.2	18.45	0.0374	43.79 ± 1.71
	Peak Charles National Park	32.84°S	121.18°E	301	16.2	20.75	-0.0169	43.83 ± 3.19
<i>Banksia quercifolia</i>	Northcliffe	34.63°S	116.30°E	1139	14.7	16.48	0.1217	20.74 ± 0.66
	Torndirrup National Park	35.11°S	117.93°E	938	14.7	15.67	0.0652	16.23 ± 0.61
	Bakers Junction	34.92°S	117.94°E	800	15.2	15.09	0.0562	14.89 ± 0.58

and stored dry (15°C and 15% relative humidity) for 6 months until use.

Seed germination

Seven osmotic solutions of the inert, water-binding polymer polyethylene glycol (PEG as PEG 8000) between 0 (control) and -1.50 MPa were used to control water matric potential (MPa) during seed germination. Although it is unclear how these MPa classes precisely relate to actual field conditions, they were chosen to simulate a gradient of drought stress from unlimited water availability to a point akin to wilting point. The water potential of aqueous solutions of PEG is curvilinearly related to concentration (Michel and Kaufmann 1973) and, at a given concentration, ψ increases linearly with temperature. Therefore, solutions were made up to the appropriate strength with DI water, according to an empirical equation that accounted for temperature differences (Michel and Kaufmann 1973; Hardegree and Emmerich 1990). These osmotic potentials were verified using a Decagon WP4 Dewpoint PotentialMeter (Decagon Devices, Pullman, WA). The higher the concentration of solutes, the lower (more negative) was the osmotic potential and the greater the moisture stress. Seeds were sown in 90-mm-diameter Petri dishes on filter paper over 2-mm thick sponges, irrigated with 5 mL of the appropriate PEG solution (5 mL distilled water for 0 MPa control) before being placed in temperature- and light-controlled incubators using a 12-h photoperiod. Seeds were incubated at three constant temperatures (10°C, 15°C and 20°C) that were known to be adequate for eliciting germination in these species in the absence of moisture stress (J. A. Cochrane, unpubl. data). Four replicates of 10 seeds were used per MPa × temperature treatment.

To account for environmental gradients within the incubators, dishes were rotated in their placing each time germination was scored. Solutions of PEG may crystallise over time as seeds imbibe, resulting in a water potential more negative than expected. Therefore, to avoid PEG exclusion, ungerminated seeds were replated fortnightly and irrigated with fresh solution as previously described. Petri dishes were placed in

clear self-sealing plastic bags to further minimise changes in solution water potential owing to evaporation. Tests ran for 56 days and germination, defined as visible radicle emergence, was scored twice weekly, at which point seedlings were removed and discarded. Remaining seeds were subjected to a cut test. Seeds without an embryo were deducted from the total when calculating percentage germination.

Data analysis

Germination was expressed as the cumulative percentage of the number of seeds that had germinated at the final recording. The mean time to germination (MTG, described as the length of the lag period from the start of imbibition to radicle protrusion for each treatment) was calculated per treatment as: $MTG = \sum(n_i \times d_i) / N$, where n_i = number of seedlings germinated in each scoring interval, d_i = time in days since seed was sown to end of the interval and N = total number of seedlings germinated.

Germination data (final percentage germination and mean time to germination) for each species were analysed using linear models (GENSTAT 16th edition, VSN International, Hemel Hempstead, UK), with populations, temperature and moisture potential as treatment factors. Both linear and non-linear effects were considered in the analyses with main and interacting treatment effects considered significant at $P < 0.05$. Percentage-germination data were arcsine transformed and mean-time-to-germination data log-natural transformed before analyses. This was performed to stabilise the variance and to achieve approximate normality. Predicted means were used in all figures.

Results

Effect of PEG concentration and temperature

When seeds were incubated in the presence of water only (i.e. without PEG), germination was uniformly high (over 80% under all temperature conditions) for three of the four species. The exception was *B. coccinea*, and this species was most affected by the different temperature regimes (Fig. 1). The optimal germination temperature (T_{opt} – temperature at which the

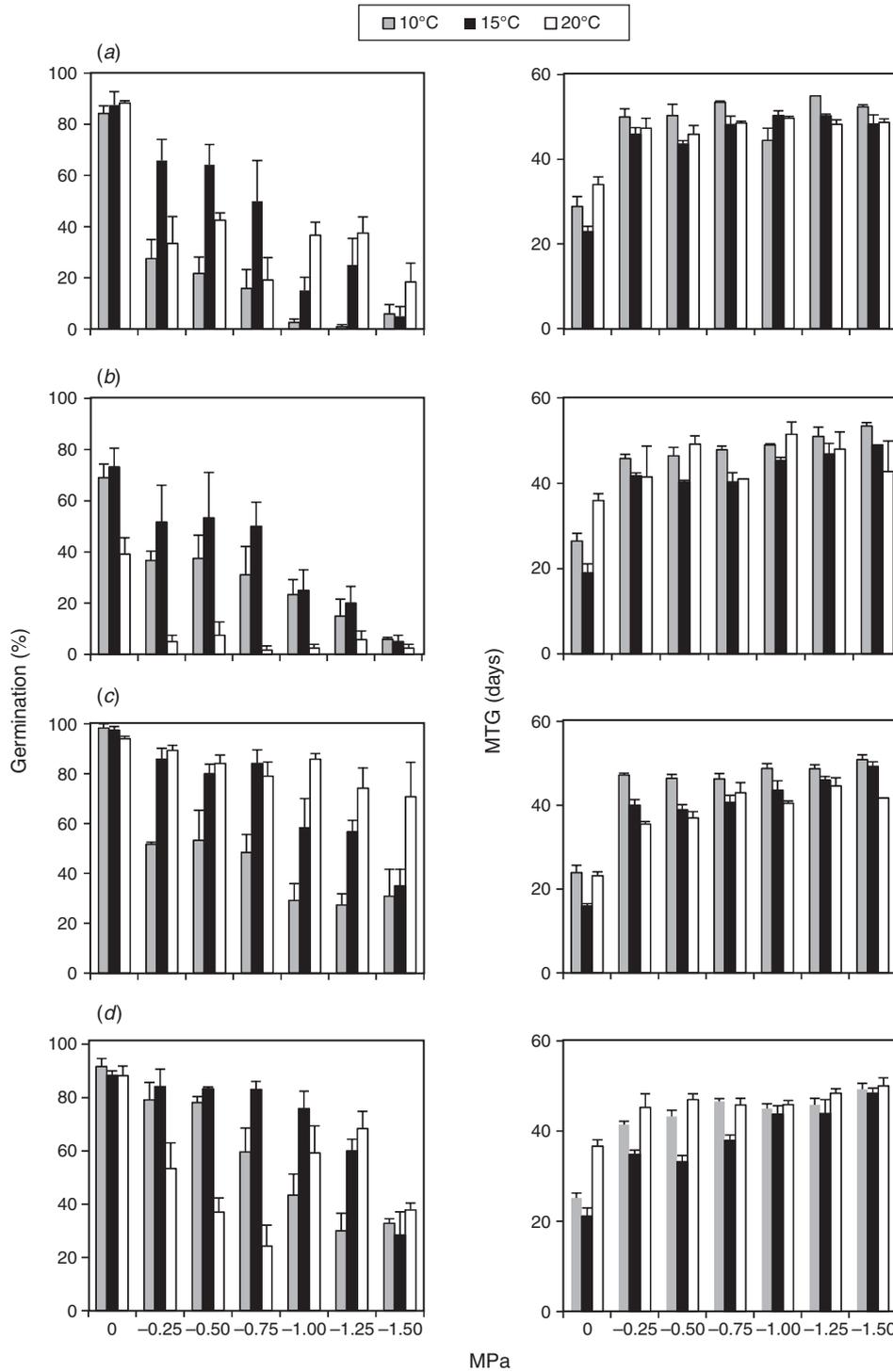


Fig. 1. The response of *Banksia* seeds to a gradient of moisture stress at three constant temperatures. Data points represent mean species values (pooled data from all populations of each species) at each moisture level for final percentage germination (left panels) and mean time to germination (right panels). (a) *B. baxteri*, (b) *B. coccinea*, (c) *B. media* and (d) *B. quercifolia*.

highest germination was achieved in the shortest time) for all species was generally 15°C. Moisture availability and temperature had a significance impact of germination traits (Table 2: $P < 0.001$ for all species). When seeds were

incubated with PEG solutions of -0.25 MPa and below, germination was dramatically reduced in almost all cases (Fig. 1), with the decline in germination greatest between 0 and -0.25 MPa. Temperature moderated the strength of the

Table 2. Results of linear model analyses for arcsine-transformed percentage germination and mean time to germination for each of the four study speciesd.f. = degrees of freedom. Bold values denote significant differences at $P=0.05$. pop = source population; MPa = moisture potential; temp = temperature

Source of variation	d.f.	<i>B. baxteri</i>		<i>B. coccinea</i>		<i>B. media</i>		<i>B. quercifolia</i>	
		<i>F</i> -statistic	<i>P</i> -value	<i>F</i> -statistic	<i>P</i> -value	<i>F</i> -statistic	<i>P</i> -value	<i>F</i> -statistic	<i>P</i> -value
Percentage germination									
pop	2	18.98	<0.001	21.93	<0.001	7.12	0.001	9.75	<0.001
MPa	6	138.41	<0.001	58.28	<0.001	64.33	<0.001	45.88	<0.001
temp	2	47.66	<0.001	101.71	<0.001	96.25	<0.001	30	<0.001
pop.MPa	12	0.54	0.884	4.62	<0.001	3.68	<0.001	1.84	0.044
pop.temp	4	9.12	<0.001	8.06	<0.001	1.06	0.376	1.63	0.168
MPa.temp	12	8.46	<0.001	5.14	<0.001	7.38	<0.001	12.05	<0.001
pop.MPa.temp	24	2.02	0.005	1.69	0.028	1.72	0.024	1.28	0.181
Mean time to germination									
pop	2	15.03	<0.001	15.3	<0.001	2.31	0.103	14.25	<0.001
MPa	6	292.37	<0.001	110.67	<0.001	446.34	<0.001	212.45	<0.001
temp	2	24.98	<0.001	43.08	<0.001	85.65	<0.001	139.75	<0.001
pop.MPa	12	3.78	<0.001	4.85	<0.001	3.45	<0.001	3.24	<0.001
pop.temp	4	4.49	0.002	3.64	0.008	9.8	<0.001	1.69	0.153
MPa.temp	12	17.79	<0.001	11.65	<0.001	19.46	<0.001	19.3	<0.001
pop.MPa.temp	19	2.34	0.003	3.43	<0.001	2.93	<0.001	2.55	<0.001

decline, and the shape of that decline was often non-linear. The impact of increasing moisture stress on seeds of *B. baxteri* and *B. coccinea* was greatest at 10°C and 20°C, and least at 15°C. For *B. media*, the combination of moisture stress at the lower temperature of 10°C caused the steepest decline in germination relative to the control; for *B. quercifolia*, germination under moisture stress at 20°C resulted in least germination. *B. coccinea* was most affected by increasing drought stress and at -1.5 MPa, <10% of seeds germinated regardless of temperature. *B. media* was least affected by declining water availability; at 20°C the negative effect of high PEG concentrations was ameliorated and germination remained high (70%).

The mean time for germination was relatively quick (MTG < 23 days) under optimal conditions (0 MPa and 15°C; Fig. 1). As water became less available (i.e. declining MPa), germination underwent significant delays; although beyond -0.25 MPa, the effect of drought stress on MTG steadily weakened.

Population response

Response to drought stress varied significantly among populations (Table 2; $P < 0.001$ for all species for both percentage germination and mean time to germination, with the exception of mean time to germination for *B. media*); the extent of this variation frequently depended on incubation temperature (pop.MPa.temp). Population performance was generally similar across the species: there was commonly a steep decline in germination at low to medium moisture stress and a tailing off at higher stress levels to low (*B. media* and *B. quercifolia*) or no germination (*B. coccinea*). As germination declined most rapidly between 0 and -0.25 MPa, we illustrate this by graphing the relative decline in percentage germination for each population that occurred between these moisture-stress levels (Fig. 2a-d). These graphs clearly demonstrate the interaction between temperature and moisture for each of the

three populations at the point where decline is sharpest. Germination temperature is optimal where the percentage decline is smallest. At 15°C, seeds of the high-rainfall population of *B. coccinea* outperformed those of medium- and low-rainfall populations; at 10°C and 20°C, the populations did not differ significantly in their response. Low- and medium-rainfall populations of *B. baxteri* at 15°C and *B. quercifolia* at 10°C outperformed other populations at most moisture levels; at 15°C, all *B. quercifolia* populations germinated well (>80%), even at -1 MPa. The most notable result was the ability of more arid-sourced seeds of *B. media* to maintain fitness at the highest temperature and under intense water stress, with almost 100% final germination at -1.5 MPa. There was also a significant interaction between moisture, temperature and population for all species (Table 2) that affected delays in germination; the least delay in mean time to germination generally occurred at 20°C, and invariably in low-rainfall populations of all species (Fig. 2e-h).

Discussion

We examined germination traits (final percentage germination and mean time to germination) under conditions of increasing moisture stress in multiple populations of four endemic Western Australian *Banksia* species. The results demonstrated that moisture stress, including its interaction with temperature, can have considerable impacts on germination of all species. The species most constrained by low water availability were *B. coccinea* and *B. baxteri*. However, these two species showed a divergence in their germination strategies, with *B. coccinea* expressing lower germination under the higher temperature, and *B. baxteri* lower germination under the lower temperature. In the SWAFR, moisture stress commonly occurs in late spring-summer when temperatures rise and rainfall declines, typical of Mediterranean-climate ecosystems. It is possible that recruitment in *B. coccinea* is constrained to winter when soils are cool and moist, whereas in *B. baxteri*

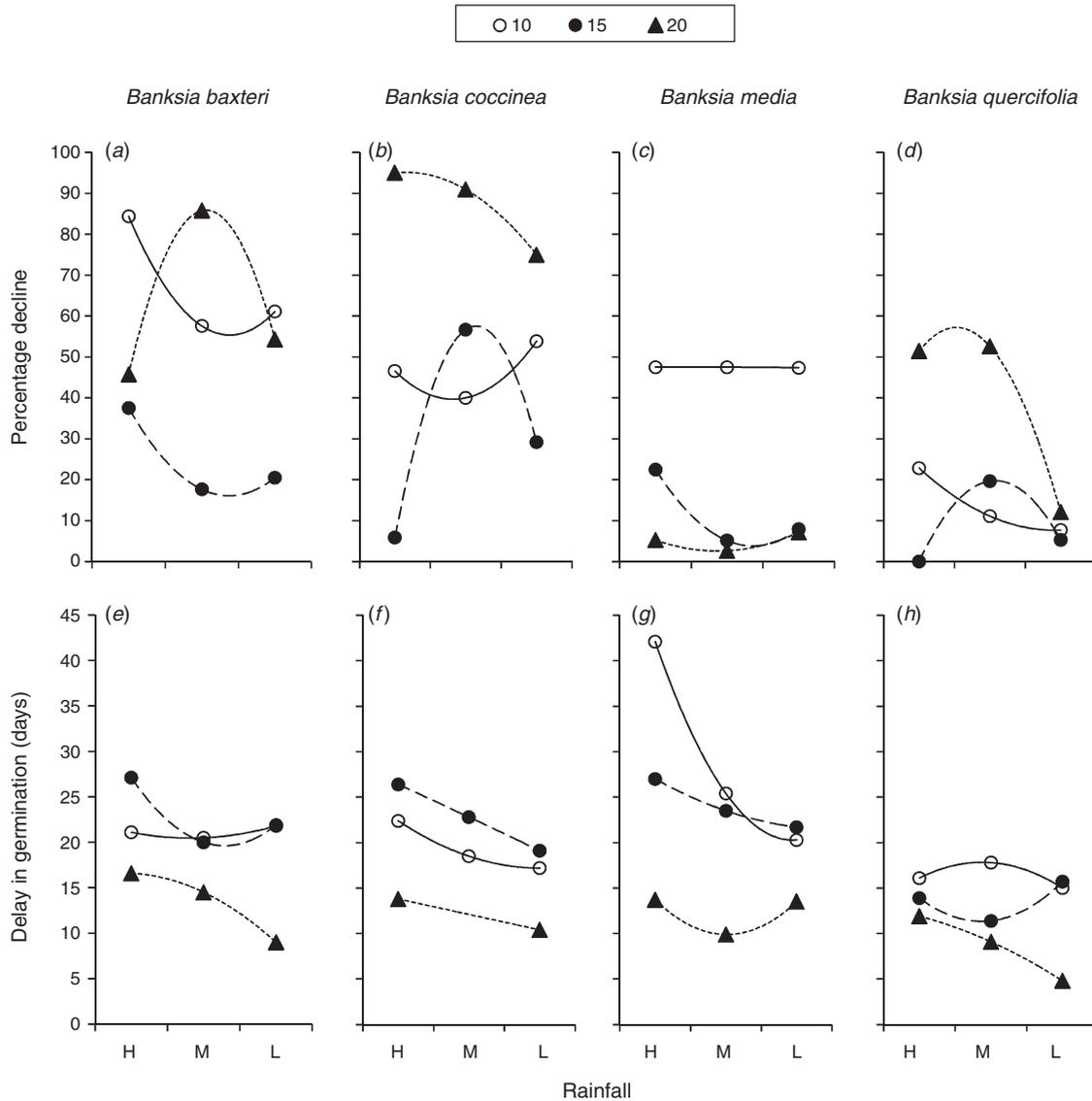


Fig. 2. Comparison of (a–d) relative decline in total germination and (e–h) relative delay in mean time to germination for three populations located along a rainfall gradient (H = high rainfall, M = medium rainfall, L = low rainfall) in response to changes in water availability (0 to –0.25 MPa) at 10 (○), 15 (●) and 20°C (▲). (a, e) *B. baxteri*, (b, f) *B. coccinea*, (c, g) *B. media* and (d, h) *B. quercifolia*. Second-order polynomial curves were fitted to the data points.

recruitment may be possible in both early autumn and spring when soils are still warm, but soil moisture is not limiting. *B. baxteri* and *B. coccinea* frequently co-occur and the divergent germination strategies may help permit coexistence.

In contrast, *B. media* and *B. quercifolia* seeds appear more drought and temperature tolerant. In particular, *B. media* seeds show stronger germination capacity under water limitation and relatively high temperature. Edaphic factors may be important determinants of germination behaviour under moisture stress and the heavier loamy soils supporting this species may maintain higher available water for longer than the free-draining deep white sands associated with the other species. Being located at the xeric end of the gradient may also contribute to tolerance to moisture stress by *B. media*; annual precipitation across this species range is considerably lower

(300–600 mm year⁻¹), and more variable, than for the other species. When germinated at optimal temperatures and above, *B. quercifolia* also exhibited some moisture-stress tolerance and the loamy soils associated with this species potentially provide some moisture buffering for seeds during the critical process of germination. Living at the edges of ephemeral swamps, *B. quercifolia* can experience pronounced differences in hydraulic conditions across seasons, potentially leading to evolution of adaptation to environmental stress, in particular warmer temperatures coupled with low moisture conditions that would occur as soil dries towards late spring and early summer.

The average time to germination was also affected by temperature and moisture conditions with least delays in germination occurring at 15°C, which is a temperature

experienced in the SWAFR during autumn–winter and spring when moisture is generally freely available. Speed of germination is key for coping with declining water availability; the quicker a seed can germinate, the greater its chance of exploiting resources for establishment, growth and survival, particularly in seasonal climates (Verdú and Traveset 2005). At this time, seedlings use available water to establish root systems that can facilitate survival. An important aspect of regional climate change is alteration in availability of soil moisture. Declining osmotic potentials slow down the uptake of water by seeds, thereby inhibiting imbibition and limiting germination. Rapid drying of the soil surface would initiate this osmotic stress. In a future warmer world, low soil-moisture conditions may well coincide with what is currently the germination window in the post-fire environment. It may be that the steep declines, and delays, in germination that occur at small negative water potentials reflect adaptations that preclude germination under conditions of transient, or low, moisture availability (Romo *et al.* 1991) and may act as a survival strategy in more xeric environments (Zeng *et al.* 2010).

We also observed wide variation in among-population tolerances to the treatment conditions. This was not surprising, considering seeds originated from along a climate gradient. However, there was little indication of local adaptation, because seed sensitivity to treatments could not be adequately explained by rainfall or temperature conditions at sites of seed origin. Instead, there were some unpredictable and locally specific responses, indicating that populations can differ in their response to the same stressors. This raises the question as to whether the lack of responses in the direction expected may be related to either genetic or historical constraints in these particular species. Although there was some support for our hypothesis that low-rainfall populations would be more tolerant of environmental stress, we cannot assume that seeds from the xeric end of a species range will be best adapted for future drier conditions. Most noteworthy was that the low-rainfall *B. coccinea* population may already be close to its optimal temperature and moisture conditions; further shifts in climatic conditions, as forecast for the region, may result in local population decline.

Here we have assessed the moisture response of non-dormant seeds the germination timing of which is dictated by seed release from the canopy seed bank. Fire and declining rainfall are co-drivers of population dynamics (Lamont and Groom 1998), and the timing and severity of fire will strongly affect recruitment (Cowling and Lamont 1987). Although adults are sufficiently long-lived to buffer the short-term effects of reduced seedling recruitment, in the long term, if adult mortality exceeds recruitment, then populations will trend toward decline. This will most likely occur following small fires at short intervals in climates of unreliable rainfall. Differences in fire response among populations (Lamont and Markey 1995) may have a major effect on reproductive biology (Lamont and Groom 1998), so cannot be ignored.

Banksia species, however, represent only one type of germination behaviour (i.e. non-dormant) and many native species have complex barriers to germination (i.e. dormancy mechanisms). Conditions that impose or overcome dormancy may be differently affected by environmental stress. In addition,

it is probable that environmental thresholds for seedling emergence and survival may be very different from those for germination, with the transition from germinated seed to emerged seedling a major bottleneck in the recruitment process (James *et al.* 2011). The ability to germinate under water limitation may co-evolve with traits that affect seedling drought tolerance (Kos and Poschlod 2008); however, there are indications that germination may be a more sensitive life stage to climate warming than is seedling root growth for some species (Cochrane *et al.* 2011).

Benefits of PEG

Mimicking moisture stress with PEG allowed us to demonstrate the divergence in stress tolerance between and within species. The approach is relatively underused for native species, but a highly convenient and effective means for assessing species vulnerability to forecast change, which is especially important, given the predicted changes in precipitation patterns for much of the world, including the Mediterranean-climate SWAFR. Whether it is a valid method for assessing the *in situ* effect of declining rainfall on plant regeneration by seed needs to be proven with field experiments, as controlled-environment studies cannot fully reflect the complexities of natural abiotic and biotic conditions. In addition, how the moisture-potential values used in the present study relate to predicted climate-change scenarios is uncertain, and how often species are likely to be faced with conditions equivalent to this water stress is unknown. Nevertheless, identifying species and population variation in drought tolerance is vital for effective restoration in a warming, drying world. Tolerance to water stress in the germination phase is a highly desirable trait for population persistence. Such tolerance may enable maintenance of current geographic range despite changing environmental conditions, and the capacity to establish in areas where less drought-tolerant species cannot. Winters in the SWAFR are predicted to get warmer, rains will be postponed and reduced, and evaporation will increase. These changes will alter germination timing and ultimately regeneration success. Populations known to be least vulnerable to the negative effects of drought (regardless of their origin along a climate gradient) should be utilised in restoration for more successful outcomes.

Acknowledgements

This work was supported by the Western Australian Department of Parks and Wildlife and the Australian National University. We thank Anne Monaghan and Simone Dudley for technical assistance and two anonymous reviewers for constructive comments on a previous draft.

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