

Climate warming delays and decreases seedling emergence in a Mediterranean ecosystem

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Temperature and moisture impact strongly on the early stages of a plant's life cycle. Global climate change is altering the environmental cues that seeds receive resulting in compromised seedling emergence and changes to seedling performance. Here, we investigate how temperature and moisture affect these early stages of plant development in four *Banksia* species collected from a longitudinal climate gradient in southwest Western Australia. A common garden was used to examine the between-species and among-population variation in seedling emergence, growth and leaf traits under two soil temperature regimes and three levels of precipitation. We predicted that reduced moisture and increased temperature would delay and reduce total seedling emergence and negatively affect seedling performance. Furthermore, we expected that within species there would be geographically structured variation in response to the treatments. Species differed significantly in all measured traits. Soil warming resulted in strong impacts on regenerative traits, significantly slowing seedling emergence in two species and reducing total seedling emergence in three species. In addition, warming altered seedling performance with significant reductions to the above-ground leaf biomass ratio of three species. In contrast, response to soil moisture manipulation was minimal across all species but possibly due to issues regarding implementation of an effective moisture treatment. The species that showed the greatest decline in emergence under warmed conditions (*B. quercifolia*) also showed the smallest vegetative shift; the species with the smallest decline in emergence (*B. coccinea*) showed a relatively large vegetative shift. Among-population differences were significant for many traits, however, trait differentiation was inconsistent across species and, contrary to our hypothesis, the variation we observed was not clearly associated with the climate gradient. As these among-population differences in traits are not easy to predict, we caution the use of simple rules for choosing seed populations for conservation and restoration.

Recruitment processes (seed germination and seedling emergence) are of major importance to the long term survival and persistence of sexually reproducing plant populations (Harper 1977, Silvertown and Charlesworth 2001). During these early life-cycle stages of a plant population performance is highly sensitive to environmental variation (Bond and van Wilgen 1996). Water is a major limiting factor for recruitment, particularly in seasonally dry climates, however, temperature is arguably the most influential climatic variable since it synchronises germination and emergence with environmental conditions optimal for subsequent seedling establishment. Temperature affects the number of seeds that germinate and the rate at which they germinate (Probert 2000), provided soil moisture is not limiting (Finch-Savage and Phelps 1993). In addition, temperature influences the productivity and growth of seedlings (Arnold 1974).

Many plant species have endured climatic fluctuations in the course of their evolutionary history, but recent anthropogenic climate warming could place increasing physiological constraints on recruitment and early growth, in particular

through their effects on seed and seedling traits (Walck et al. 2011). In this climate change context, increases in temperature and changes in timing and quantity of precipitation may result in significant ecological impacts on plant communities (Keeley and Fotheringham 2000, Lloret et al. 2005). Small changes in climatic conditions can result in large changes in seed responses: for instance, climatic changes in the maternal environment can alter a seed's dormancy status (Steadman et al. 2004, Hoyle et al. 2008), soil-stored seed longevity (Ooi et al. 2009) and seed mass (Hovenden et al. 2008); as the plant's maternal environment changes, seed characteristics and the seed's ability to decipher environmental cues can also change. This strong link between micro-climatic conditions during development of the seed and seed germination responses can potentially lead to the uncoupling of environmental conditions and specific seed germination cues resulting in lower fitness (Childs et al. 2010).

Species in the natural environment are distributed across heterogeneous landscapes that frequently encompass

significant climate gradients; as a consequence, among-population variation in environmental requirements and tolerances is common. Variation among populations may correspond to a combination of environmentally induced and genetically fixed differences between populations of a species and may be a sign that local adaptation and/or genetic drift have led to differentiation in trait means and plasticity across populations. Seed traits in particular provide good descriptors to differentiate species, and can often illustrate within-species variation across heterogeneous landscapes. Indeed, the increasing number of studies investigating the dynamics of seedling recruitment and early growth performance provide compelling evidence of this variation (Kunin et al. 2009, Vergeer and Kunin 2011). If populations of a given species are adapted to their habitats (local adaptation), then optimal conditions for germination, seedling emergence and establishment would be expected to vary according to local climate conditions across range distributions (Donohue et al. 2010). Populations locally adapted to current climatic conditions are expected to be at high risk of decline and / or extinction when environments change (Thomas et al. 2004). These risks may be ameliorated if individuals can exhibit plastic responses to changed conditions that improve fitness or minimise fitness losses. Genetic variation in both trait means and trait plasticity within and among populations may also buffer climate change impacts (Jump and Peñuelas 2005). The evolution of phenotypic plasticity has allowed species to cope with a wide range of climatic conditions (Pigliucci 2001), however, the presence of phenotypic plasticity may make it hard to predict the impact of changes in temperature and precipitation both at the species level and at the level of populations within species. Nonetheless, insight into plant responses to current and future environments will be key to understanding species' response to a rapidly changing climate.

The genus *Banksia* (Proteaceae) is a major component of many vegetation communities in the species-rich South West Australian Floristic Region (SWAFR). *Banksia* are long-lived woody evergreens. Most species have a relatively long primary juvenile period, though flowering may occur within three to five years after germination. Pollination is mainly by nectar eating birds (honeyeaters of the family Meliphagidae), or insects, and sometimes small mammals such as honey possums *Tarsipes rostratus*, with seed set tending to be low (George 1984). The woody fruits are stored in the plant canopy as a persistent aerial seed bank (i.e. serotiny), with degree of canopy storage varying among and within species along climatic gradients (Cowling and Lamont 1985). Fire regimes largely determine the population dynamics of *Banksia* as many species require fire for seed release from woody fruits (Lamont et al. 2007, He et al. 2011). Seeds are non-dormant once released and recruitment occurs predominantly in the post-fire environment with the onset of rains and cooler temperatures in autumn and winter. Timing of rainfall is critical for successful seedling establishment (Groom 2002). Survival of the first year's summer drought is crucial for population persistence, yet typically less than 10% of *Banksia* seeds released after fire become seedlings, and less than 50% of these seedlings survive the first summer (Lamont and Groom 1998). These life history attributes make *Banksia* particularly sensitive to abiotic factors in the

landscape (Lamont et al. 2007), thus, along with many other Western Australian species, they are considered to be vulnerable to the predicted effects of climate change (Fitzpatrick et al. 2008, Yates et al. 2010).

At present the SWAFR has a typical Mediterranean climate consisting of cool wet winters and warm dry summers, with periodic drought and recurrent fires. Global climate model projections for the SWAFR forecast a 1–5.5°C increase in mean annual temperature and a 5–60% decline in mean rainfall during winter and spring by 2070, accompanied by a greater frequency of extreme drought and heat waves and an increase in fire frequency and intensity (Bates et al. 2008). Species in the SWAFR are projected to contract their ranges towards the cooler and wetter south west (Fitzpatrick et al. 2008). Much of the region's flora may already be at its physiological limits for survival with altered environmental conditions potentially impacting significantly on recruitment (Walck et al. 2011). The region therefore provides an ideal focal point for investigating the climate-sensitivity of plant traits.

To better understand the impact of changing environmental conditions on recruitment patterns, we assessed how temperature and moisture affect *Banksia* seed and seedling traits. We sowed seeds from six natural populations of four species from a longitudinal rainfall gradient under common garden conditions designed to simulate current and future climate regimes. We hypothesised that evolution and development under contrasting climates will have given rise to differentiation in seed and seedling traits and that populations from the hotter, drier end of the gradient would perform better in terms of emergence and growth with lower water availability or increased temperature than populations from the cooler, wetter end of the gradient. We addressed the following questions: 1) do species differ in mean trait values across the climate gradient? 2) Do populations within species exhibit differential patterns of response to temperature and moisture treatments? 3) Do population trait values and plasticity therein follow a geographic cline? We expected to see plastic responses to the treatments, with few negative impacts on regenerative traits and positive or no impacts on growth in seeds from the hot dry end of the gradient.

Material and methods

Study species and sites

Four non-sprouting, obligate seeding *Banksia* species endemic to the South West Australian Floristic Region (SWAFR) were chosen because they are locally common where they occur but are restricted to the southern coastal areas of the region (Supplementary material Appendix 1 Fig. A1, Table A1). *Banksia media* and *B. quercifolia* flower in the autumn–winter months, whereas *B. coccinea* flowers in winter, spring and summer and *B. baxteri* predominantly in summer. *Banksia baxteri* (ser. *Banksia*; subser. *Cratistylis*) and *B. coccinea* (ser. *Banksia*; subser. *Cratistylis*) co-occur across part of their distributional ranges, generally in tall shrubland in deep white sands on plains and dunes. *Banksia quercifolia* (ser. *Quercinae*) favours sandy depressions and is found on swamp margins, in shrubland–sedge or in low

woodland formations. *Banksia media* (ser. *Cyrtostylis*) grows in sand, loam and clay, sometimes over limestone or granite, in heath, shrub or open woodland and has a wider distribution than the other three species. The taxonomic arrangements in *Banksia* subgenus *Banksia* sensu George (1981) derive from Mast and Givnish (2002).

Taking into account the strong spatial and temporal heterogeneity in climate seen across the species' geographic distribution within the SWAFR, six discrete populations of each species were selected to represent a rainfall cline. The term 'population' is used to describe plants originating from a particular geographic and climatic location. Longitudinally, from west to east along the south coast, the seed source sites experience increasing autumn–winter temperatures (May–October), but the gradient in precipitation presents a declining mean to the east. There is a significant correlation between mean annual precipitation (MAP) and mean annual temperature (MAT) along the gradient ($r = -0.61$, $p < 0.05$, Supplementary material Appendix 1 Table A1). The difference between high rainfall (H) and low rainfall (L) populations of each species roughly coincided with a doubling of rainfall (Supplementary material Appendix 1 Fig. A1) but rainfall is more unpredictable where amounts are lowest. Medium rainfall sites (M) fell between the two extremes.

Within each population we sampled mature fruits from a minimum of 50 adult plants in order to obtain a broad range of genetic diversity. Fruits from each sampled individual were pooled, but populations were kept separate. Seeds were extracted from fruits within three months of collection by 1) burning the fruits with a gas torch until follicles split, 2) soaking them in water for 6–8 h and 3) drying them at 15°C and 15% RH for 1–2 weeks until seeds were released. Fruits found to be infested with seed eating larvae were discarded. All potentially viable seeds were then stored at 15°C and 15% relative humidity (RH) for up to six months prior to sowing in a common garden.

Seed viability was tested, within 3 months post-harvest, via germination in a constant temperature incubator (15°C) with a 12/12 h light/dark photoperiod. Under laboratory conditions, all seed populations were found to be highly viable ($86 \pm 2.5\%$ germination, data not shown).

Common garden design

A common garden was established in Albany on the south coast of Western Australia (34°98'64.9"S, E 117°90'91.3"E, 56 m a.s.l.), where the climate is Mediterranean with mild summers and cool, wet winters. Mean annual rainfall in Albany between 1971 and 2000 was 875 mm (<www.bom.gov.au>). The driest and hottest month is February with a long term mean of 23 mm rainfall and a mean maximum temperature of 22.9°C; July is the wettest and coldest month with 143.9 mm rainfall and a mean minimum of 8.1°C.

Twelve 5 × 5 m clear, polycarbonate-covered steel exclusion shelters were constructed on site to manipulate water availability and assess effects on seed germination, seedling emergence and seedling growth and survival. Two raised garden beds were placed below each shelter and filled with 2:1 compacted sand: native potting soil mix (pH 6). Water was collected from the roof of each shelter and passively irrigated

to the garden beds using weeper hose in each of three water treatments: reduced rainfall R (80% of total), natural rainfall N (100% of total rainfall), and increased rainfall I (140% of total). The hose delivered water directly to the soil and is designed for low pressure watering systems. Seven lines of hose were laid approximately 1 cm below the soil surface in each bed in a closed system.

Small polycarbonate open top chambers were used to warm the soil. Each shelter contained one garden bed fitted with 24 warming chambers (W) and one garden bed without warming chambers (C). Most heating in the chambers occurred in the daytime due to solar radiation, with a strong diurnal cycle, and day to day variation dependent on prevailing weather conditions. Chambers were used to increase mean soil temperature and provided variation in the micro-climate, a part of natural climate variability.

In each garden bed, seeds were sown approximately 1 cm below the soil surface in early winter (mid-June), prior to the onset of winter rain. Twenty seeds from each population were planted together in an area of approximately 20 cm diameter (hereafter termed 'experimental units'). Experimental units were arranged within each garden bed in a balanced array with three columns and eight rows, with seeds from all 24 populations sown in each garden bed (20 seeds × 6 treatments (3 water × 2 temperature) × 4 replicates = 480 seeds per population). Each row contained seeds from a single species. Each column in the array contained seed from the eight populations corresponding to a particular level of rainfall. Within these constraints the populations were divided into two sets of 12 so that the same 12 populations always occurred together in a set of four rows. The allocation of seeds to experimental units was fully randomised subject to the constraints of the design. This design was chosen partly for logistical reasons and partly to maximise the precision of estimation of the effects of interest.

Local air temperature and rainfall data were sourced from the Albany Meteorological Office (Station 0099500; 35°03'S, 117°88'E), located approximately 10 km from the study site. Soil moisture readings were taken in each experimental unit, after a significant rainfall and then repeatedly as the soils dried, using an moisture meter and hand held reader (<www.mea.com.au/>). These measures were used to describe the variation in water availability within each bed. We used restricted maximum likelihood (REML analysis) models for each set of moisture readings with water fitted as a fixed term; garden beds nested within shelters as a random term. The factor 'replicate' was included as a fixed effect to assess whether water treatment was homogeneous among replicate garden beds. Regression analysis on these moisture readings found no significant difference between water treatments for all but one mildly significant set of readings ($p = 0.045$). The general lack of significant differences between water treatments was likely due to unusually high spring rainfall, coupled with the passive nature of the irrigation system. Accordingly, we were not surprised to find that the effects of the water treatment were not significant except in a few cases, and in these we urge that water effects must be interpreted with caution.

Temperature loggers (<www.maxim-ic.com/>) recorded soil temperature every 30 min at 1 cm depth in warm and control experimental units (two buttons per garden bed). We

used a two-sample Students T-test on daily mean temperature values (control and warm garden beds, irrespective of water treatment), from a 12 month period, to assess temperature differences. Passive warming by chambers raised the mean daily soil temperature significantly ($F = 1.19$, $DF = 369$, $p < 0.001$) relative to the unwarmed controls (20.5 versus 19.5°C). The temperature range for control plots was 5.6 to 49.6°C; and 7.4 to 55.7°C for the warm plots, demonstrating the increase in temperature due to warming by the chambers.

Data collection

Seedling emergence (defined by visible cotyledon emergence from the soil), was recorded every 2–3 days from mid-July until mid-September, and on a monthly basis from mid-September until mid-December 2011 when no further germination had been recorded for two weeks. Emerged seedlings were marked with different coloured bamboo skewers to enable comparison of growth rates between different seedlings cohorts. When of sufficient size, three seedlings in each experimental unit were singled out for monthly measurement of height and every two months the number of leaf pairs per seedling was also recorded. In mid-November, remaining seedlings were thinned to the three measured seedlings per experimental unit to prevent overcrowding and competition for nutrients and water. In March 2012 above-ground plant biomass for each seedling was harvested; fully expanded leaves were counted, and the length of the stem and the longest leaf were recorded. Below-ground biomass was not sampled due to the potential for erroneous estimates when removing soil from roots (Poorter et al. 2012). In addition, the roots of plants were interwoven in the experimental units and the cluster roots that characterise *Banksia* species are difficult to sample under the best of conditions. Each component of biomass was bagged separately (leaves and stems) and dried at 60°C for 72 h prior to being weighed.

Final percentage emergence data were calculated as the percentage of seeds emerging over the course of the experiment. The mean time to emergence (MTE) was calculated using the formula:

$$\text{MTE} = \sum(n_i \times d_i) / n$$

Where n_i = number of seedlings that emerged in the i th scoring interval, d_i = time in days since seed was sown to the end of the interval, n = total number of seedlings that emerged. We also measured six above-ground plant traits commonly used in ecological investigations following standardised methods (Cornelissen et al. 2003): total above-ground biomass, leaf production rate, leaf number at harvest, leaf area of an individual leaf and specific leaf area (SLA). In addition we calculated the ratio of leaf biomass to total above-ground biomass (LMR_{AB} g). Leaves for SLA and leaf area (LA) assessment were taken from only three of the six populations (H, M and L) across the rainfall gradient due to time constraints. Leaf production rate (LPR) was calculated as the natural log of the difference between the number of leaves at harvest (March 2011) and the number of leaves at time period 1 (September 2010), divided by the number of days between the two time periods. We used a flatbed scanner to

take an image of a fresh leaf from each of the three marked plants, but only from three of the populations (H, M and L). Leaf area (mm^2) was then analysed using ImageJ (Rasband 1997–2012). Specific leaf area ($\text{cm}^2 \text{g}^{-1}$) was calculated by dividing the one-sided leaf area by leaf dry mass for each of the three marked seedlings.

Statistical analysis

A restricted maximum likelihood (REML analysis) model was used to fit mixed models for all measured traits (time to emergence and percent emergence, above-ground biomass, leaf above-ground biomass ratio, leaf number, leaf area, leaf production rate and specific leaf area). Species, populations nested within species, water and temperature were fitted as fixed terms (main and interacting effects); random terms were determined by the randomisation used in constructing the design. The factor 'replicate' was included as a fixed effect to assess whether treatment effects were homogeneous among replicate garden beds. Our analysis of the entire dataset showed substantial differences between species so we also applied the model at the individual species level to allow closer inspection of population differentiation for each species.

Prior to analyses each variable was examined for normality and transformed as needed to stabilize variance. We took the natural log of the mean time to emergence, leaf area, SLA and leaf number, and arc-sine transformed percentage emergence and above-ground biomass data prior to statistical analysis. Least significant differences (lsd) at $p < 0.05$ were used to determine significant differences between treatments, populations and species. Significant population main effects are likely to be indicative of genetic differentiation among populations. Differences in plasticity among species are inferred where the species by treatment interaction is significant; differences among populations in plasticity for a given species are inferred from significant population by treatment interaction terms. Where significant, the interactions are presented as norm-of-reaction diagrams with the environmental change on the x-axis and the mean trait value on the y-axis. The slope (reaction norm) represents phenotypic plasticity – the steeper the slope, the greater the plasticity. Predicted means were used in all figures.

To determine the relationship between mean population trait values and climate (MAP and mean MAT), at each seed source site, we used simple linear regression analysis with mean trait value for each population as the y-variate and climatic variables (MAP and MAT) as the x-factor. All analyses were performed using GenStat 15th edition (VSN Int.).

Results

Among species variation

There were significant differences among species for mean values of all eight plant traits (Fig. 1, Table 1). Of the four species, the minimum MTE was demonstrated by *Banksia quercifolia* and maximum by *B. baxteri*, with no difference in MTE between *B. coccinea* and *B. media* (Fig. 1a). Percentage emergence was greatest in *B. media* and least in *B. coccinea*; but did not differ between *B. baxteri* and *B. quercifolia*

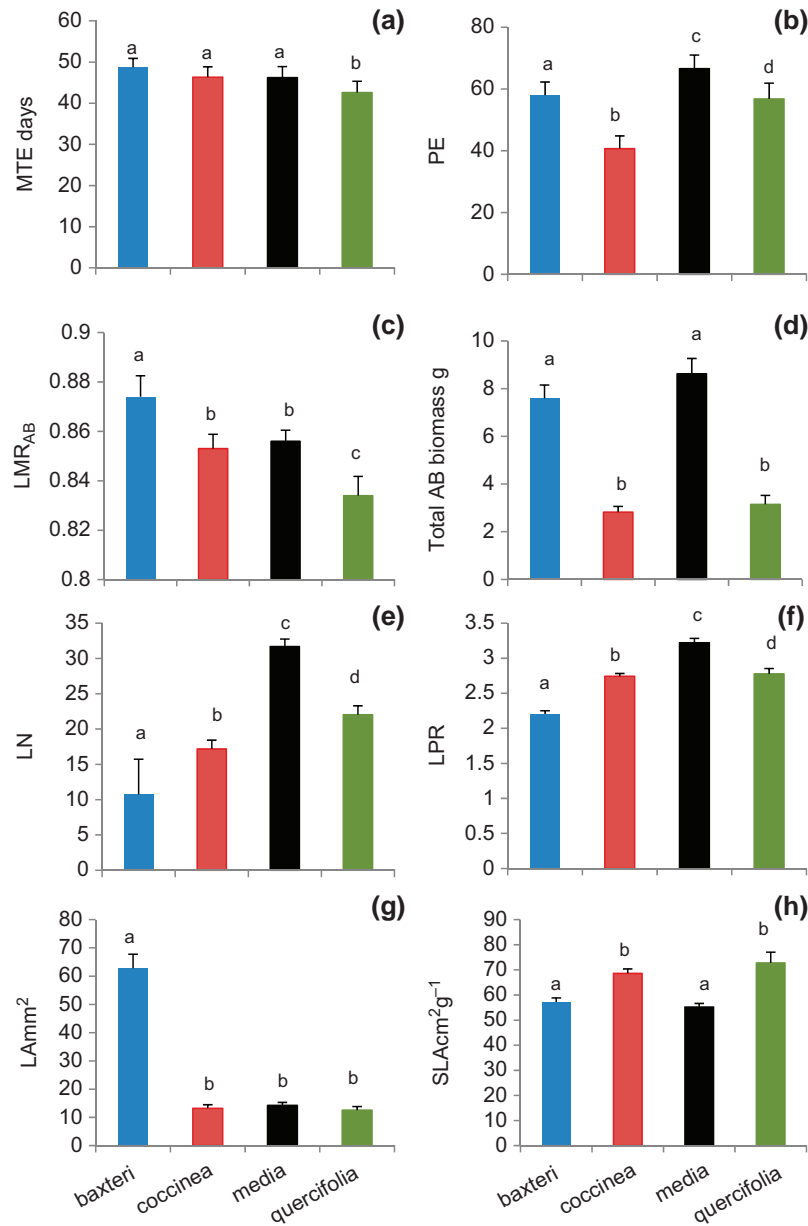


Figure 1. Mean species trait values \pm standard errors for eight measured plant functional traits: (a) mean time to emergence (MTE); (b) percentage emergence (PE); (c) above-ground leaf mass ratio (LMR_{AB}); (d) total above-ground biomass (total AB); (e) leaf number (LN); (f) leaf production rate (LPR); (g) leaf area (LA); and (h) specific leaf area (SLA). Within each graph, species that share the same letter/s are not significantly different from one another ($p < 0.05$).

(Fig. 1b). *Banksia baxteri* had higher LMR_{AB} (Fig. 1c) and leaf area values than all other species (Fig. 1g), though leaf number (Fig. 1e) and leaf production rate (Fig. 1f) were significantly lower in comparison with the other species. This result is not surprising as *B. baxteri* seedlings have relatively few leaves, but leaves are large. Values of above-ground biomass were larger for *B. baxteri* and *B. media* (Fig. 1d) and SLA was lower compared to either *B. coccinea* or *B. quercifolia* (Fig. 1h). *Banksia media* had greater leaf number and leaf production rate than any other species (Fig. 1e–f). Leaf production rate and leaf number did not differ between *B. coccinea* and *B. quercifolia*. Overall seedling survival was high (94%), but was significantly higher for

B. baxteri and *B. media*, compared to *B. coccinea* and *B. quercifolia* ($p < 0.001$) (data not shown).

Temperature had a significant main effect on MTE, percentage emergence and above-ground leaf biomass ratio (Table 1), however, species differed in their responses. In warmed soils all species exhibited delayed emergence compared to the control (Fig. 2a). *Banksia quercifolia* demonstrated the greatest delayed response and steepest reaction norm due in part to its initial faster emergence under control conditions. In contrast, *B. baxteri* showed least delay under warmed conditions but also had a longer emergence time under control conditions. Although species differed in MTE under control conditions, the species differences were not

Table 1. Results of cross-species REML analysis of the effects of temperature (temp) and water on mean time to emergence (MTE), percentage emergence (PE), above-ground leaf mass ratio (LMR_{AB}), total above-ground biomass (AB), leaf number (LN), leaf production rate (LPR), leaf area (LA) and specific leaf area (SLA). 'Pop' represents the different seed source sites (high, medium, low rainfall). Bold letters denote significance at $p < 0.05$.

Fixed term	DF	MTE	PE	LMR _{AB}	AB	LN	LPR	LA	SLA
Species	3	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Water	2	0.103	0.86	0.027	0.176	0.115	0.129	0.186	0.003
Temp	1	0.029	0.048	<0.001	0.234	0.201	0.18	0.625	0.421
Species.pop	20*	0.021	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.126
Species.water	6	0.849	0.1	0.143	0.397	0.209	0.261	0.803	0.088
Species.temp	3	0.001	<0.001	0.265	0.473	0.33	0.224	0.035	0.578
Water.temp	2	0.808	0.427	0.482	0.962	0.869	0.832	0.075	0.31
Species.pop.water	40*	0.236	0.283	0.043	0.182	0.796	0.77	0.499	0.468
Species.pop.temp	20*	0.19	0.544	0.834	0.081	0.328	0.147	0.806	0.64
Species.water.temp	6	0.284	0.826	0.877	0.181	0.556	0.63	0.945	0.133
Species.pop.water.temp	40*	0.685	0.721	0.431	0.163	0.678	0.738	0.899	0.489
Replicate	3	0.742	0.778	0.237	0.114	0.193	0.19	0.108	0.062

*DF for LA and SLA were eight for species.pop and species.pop.temp, 16 for species.pop.water and species.pop.water.temp.

significant under warmed conditions (Fig. 2a). Warming also reduced the total proportion of seedlings that emerged, with *B. quercifolia* demonstrating the most severe decline and

B. coccinea, the least (Fig. 2b). Emergence occurred over at least 12 weeks for all species, although more than 85% of total *B. quercifolia* seedlings emerged within the first six weeks after sowing, reduced to 70% under warmer soil conditions (data not shown). Aside from the two regenerative traits, the pattern of leaf allocation was the only other trait affected by warming: LMR_{AB} was significantly reduced for all species except *B. quercifolia* when soils were warmed (Fig. 2c). There was a mildly significant species by temperature interaction for leaf area, however, this was not apparent at the within-species-level (Table 2). Warming increased mortality slightly over the control, but not significantly (48 versus 40 seedlings died respectively). However, total emergence failure (i.e. no seedlings emerged in an experimental unit), was five times more common in warmed compared to control experimental units (26 versus 5 units, $r^2 = 14.226$, $DF = 1$, $p < 0.001$).

There was a lack of detectable moisture effect on most traits, however water was a significant main effect on SLA in *B. baxteri* and *B. media* (Table 2): SLA increased when water was both reduced or increased compared to the normal water treatment, a result hard to explain.

Among-population variation

Single species analysis revealed significant variation among populations for a number of the measured traits, with the pattern of among-population differences varying between species (Table 2). *Banksia baxteri* expressed significant population differentiation for all traits measured, with the exception of percentage emergence. Populations of *B. coccinea* differed in LMR_{AB}, leaf area, SLA and percentage emergence. For *B. media*, traits that differed among populations were LMR_{AB} and percentage emergence. For *B. quercifolia*, population differences occurred in leaf area and LMR_{AB}. In general, patterns of response to warming at the within-species level followed those at the cross-species level. Emergence was delayed and final percent emergence reduced by warming in all species, with the exception of *B. coccinea*.

We had expected that populations within species would differ in their responsiveness to temperature and water, and these differences would be evident as significant popula-

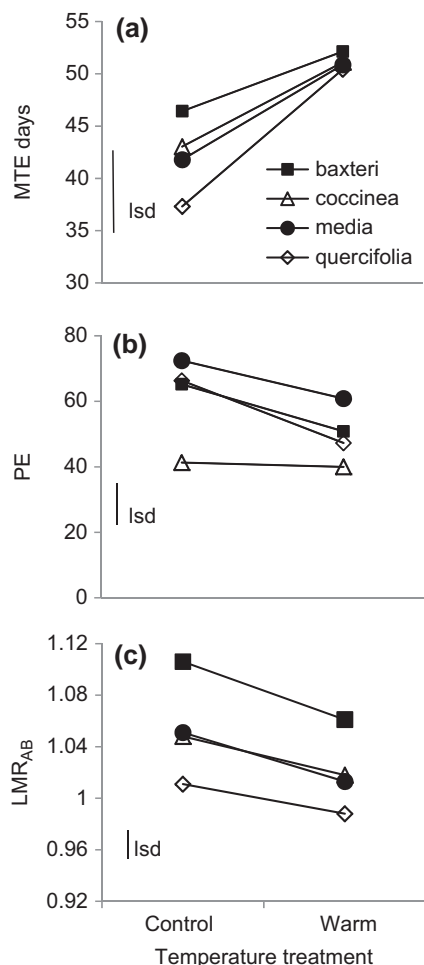


Figure 2. Predicted means \pm least significant differences (lSD) at $p < 0.05$ for significant species-level response to warming treatment (Table 1). (a) mean time to emergence (MTE) (lSD = 6.254); (b) percentage emergence (PE) (lSD = 9.058); and (c) above-ground leaf biomass ratio (LMR_{AB}) (lSD = 0.021).

Table 2. REML analysis results for temperature (temp) and water effects on mean time to emergence (MTE), percentage emergence (PE), above-ground leaf mass ratio (LMR_{AB}), total above-ground biomass (AB), leaf number (LN), leaf production rate (LPR), leaf area (LA) and specific leaf area (SLA) for the different species. 'Pop' represents the different seed source sites (high, medium, low rainfall). Bold letters denote significance at $p < 0.05$.

Fixed term	DF	MTE	PE	LMR _{AB}	AB	LN	LPR	LA	SLA
<i>B. baxteri</i>									
pop	5*	<0.001	0.14	<0.001	<0.001	<0.001	<0.001	0.027	0.007
water	2	0.137	0.653	0.057	0.459	0.433	0.435	0.584	0.04
temp	1	0.165	0.032	0.008	0.086	0.813	0.768	0.089	0.91
pop.water	10*	0.43	0.908	0.528	0.257	0.37	0.379	0.433	0.582
pop.temp	5*	0.293	0.344	0.736	0.806	0.358	0.405	0.399	0.393
water.temp	2	0.797	0.296	0.800	0.135	0.232	0.263	0.848	0.141
pop.water.temp	10*	0.68	0.822	0.256	0.268	0.157	0.201	0.237	0.438
replicate	3	0.743	0.816	0.549	0.357	0.89	0.881	0.409	0.6
<i>B. coccinea</i>									
pop	5*	0.102	<0.001	<0.001	0.124	0.594	0.630	0.032	0.029
water	2	0.175	0.982	0.185	0.238	0.279	0.234	0.292	0.668
temp	1	0.067	0.909	0.027	0.996	0.947	0.794	0.079	0.603
pop.water	10*	0.535	0.649	0.092	0.274	0.385	0.506	0.162	0.056
pop.temp	5*	0.761	0.783	0.342	0.246	0.463	0.534	0.292	0.192
water.temp	2	0.296	0.571	0.822	0.965	0.984	0.911	0.319	0.522
pop.water.temp	10*	0.949	0.992	0.824	0.214	0.208	0.170	0.589	0.123
replicate	3	0.386	0.920	0.218	0.134	0.237	0.192	0.070	0.192
<i>B. media</i>									
pop	5*	0.326	0.003	<0.001	0.443	0.470	0.445	0.294	0.104
water	2	0.173	0.814	0.219	0.266	0.134	0.142	0.088	0.017
temp	1	0.030	0.033	<0.001	0.159	0.097	0.095	0.509	0.101
pop.water	10*	0.718	0.044	0.373	0.814	0.919	0.904	0.274	0.312
pop.temp	5*	0.599	0.097	0.959	0.189	0.665	0.658	0.955	0.177
water.temp	2	0.794	0.626	0.087	0.209	0.276	0.307	0.659	0.193
pop.water.temp	10*	0.608	0.139	0.909	0.833	0.981	0.980	0.814	0.180
replicate	3	0.739	0.759	0.263	0.298	0.114	0.115	0.188	0.468
<i>B. quercifolia</i>									
pop	5*	0.802	0.067	<0.001	0.116	0.124	0.107	0.005	0.876
water	2	0.101	0.494	0.184	0.154	0.118	0.127	0.321	0.08
temp	1	0.012	0.022	0.175	0.257	0.215	0.161	0.494	0.963
pop.water	10*	0.523	0.264	0.030	0.225	0.678	0.608	0.917	0.537
pop.temp	5*	0.043	0.639	0.469	0.342	0.265	0.180	0.737	0.991
water.temp	2	0.662	0.453	0.605	0.806	0.911	0.938	0.379	0.53
pop.water.temp	10*	0.411	0.611	0.180	0.375	0.665	0.660	0.909	0.217
replicate	3	0.236	0.24	0.407	0.204	0.179	0.187	0.114	0.297

*DF for LA and SLA were 2 for pop and pop.temp, and 4 for pop.water and pop.water.temp.

tion by treatment interactions. These were, however, rare. A significant difference in plasticity to temperature between populations would be reflected in the population by temperature interaction. Populations of *B. quercifolia* differed in the extent of their response for MTE to temperature, with some populations showing delayed emergence when soil was warmed ($F = 2.52$, $DF = 5$, $p = 0.043$) (Fig. 3, Table 2). Two out of six populations demonstrated significantly steeper reaction norm slopes than the other four populations. There were mildly significant population by water effects on LMR_{AB} in *B. quercifolia* and percentage emergence in *B. media* (Table 2), however the data must be viewed with caution given that measured soil moisture values did not differ significantly. The few examples of population by treatment interaction suggest that while populations differ in trait means there is little evidence of divergence in patterns of plasticity among populations.

Finally, we assessed whether there were significant relationships between the species trait means and climate of seed

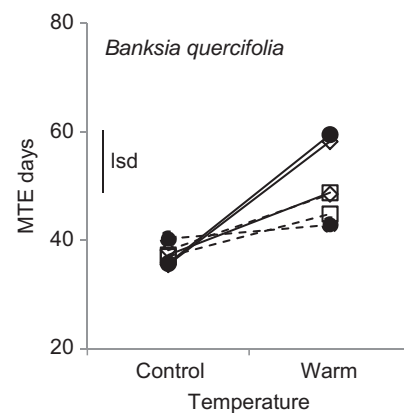


Figure 3. Predicted means \pm least significant differences (lsd = 11.874) at $p < 0.05$ for significant population-level response to warming for mean time to emergence (MTE) (Table 1). Each line represents a population of *Banksia quercifolia* sampled from high (2 × square), medium (2 × diamond) or low (2 × circle) rainfall zones.

origin. At the cross-species level, mean time to emergence was negatively correlated with MAP ($F = 8.17$, $DF = 23$, $p = 0.009$): as rainfall increased along the gradient, MTE decreased. Percentage emergence increased as the temperature rose along the gradient ($F = 4.5$, $DF = 23$, $p = 0.046$). Above-ground leaf mass ratio was negatively correlated with MAP ($F = 7.47$, $DF = 23$, $p = 0.012$) and positively correlated with MAT ($F = 13.69$, $DF = 11$, $p = 0.004$). In contrast, seedling SLA was greater when rainfall was higher ($F = 10.49$, $DF = 11$, $p = 0.009$), and temperature lower ($F = 13.69$, $DF = 11$, $p = 0.004$), along the gradient. However, despite among-population differences in trait means, there was very little evidence of differentiation in plasticity among populations due to treatment effects and so we did not examine the correlation between climate and plasticity per se.

Discussion

We examined the effect of temperature on seed and seedling traits in four woody *Banksia* species from the Mediterranean climate South Western Australia Floristic Region and found that warming was detrimental to both mean time to emergence and final percentage emergence and caused a reduction in leaf to stem biomass allocation in some species. Highly significant differences between species and between populations within species were seen along the climate gradient, but the direction and extent of the plastic response was generally not consistent for any given trait.

To our knowledge, this is the first example of decreased and delayed emergence of *Banksia* in response to warmer temperatures, and one of only a handful of reports from a Mediterranean ecosystem. Few studies have shown such a strong impact of warming on regenerative traits in woody species as we have in this study, and in a common garden designed to simulate current and future climates. We have found few reports of reductions and delays in seedling emergence with increasing soil temperatures (though see Chidumayo 2008). In contrast, soil warming has been reported to lead to earlier seedling emergence in *Pinus sylvestris* (Richter et al. 2012) and increased emergence in European understorey shrubs (De Frenne et al. 2012). In temperate grasslands, a reduction in seedling emergence with warming was almost solely explained by a decrease in soil moisture potential (Hovenden et al. 2008, Gao et al. 2012). Temperature has generally not been considered a direct controlling factor for seedling regeneration in Mediterranean climate ecosystems (Lloret et al. 2005). However, insight into the impact of warming soils on recruitment in *Banksia* may influence our understanding of recruitment in similar non-dormant / canopy-stored species in other fire-prone regions of the world that are also predicted to experience warmer, drier conditions in the future.

In seasonally dry ecosystems, like the Mediterranean climate ecosystem where *Banksia* occur, seedling emergence and subsequent growth and survival is highly dependent on rainfall, with increased access to water improving fitness and survival (Lloret et al. 2005, Padilla and Pugnaire 2007). Timing emergence to enable adequate seedling growth prior to the onset of summer drought is especially important in

resource-limited ecosystems (Moles and Westoby 2004, Castro 2006), as drought stress and desiccation in the early stages of seedling establishment are a common cause of seedling mortality (Lamont et al. 1991, Quintana et al. 2004, Gimenez-Benavides et al. 2007, Mustart et al. 2012). The faster seedlings can emerge in the cool winter months, the less likelihood of warmer spring temperatures compromising emergence. Early emergence is therefore a strong determinant of fitness and can substantially increase biomass and reproductive success, in particular when there is strong competition for resources (Verdú and Traveset 2005). Whilst we found differences between species in their mean time to emergence under unwarmed control conditions, these differences were lost after warming. This provides some evidence that warming will potentially change the separate germination niches that these species currently exploit.

The lack of detectable moisture effect on most measured traits suggests that the reduced water treatment was not sufficiently low to trigger responses, however, there is ample evidence in the literature pointing to the effects of water on plant traits. Indeed, higher than normal spring rainfall at the common garden site coupled with the passive nature of the water delivery undoubtedly contributed to both lack of water response and the high overall survival of seedlings despite our best intentions.

In addition to the general species-level patterns, many traits varied significantly among populations, within species. Notably, the species that showed the greatest decline in emergence (both MTE and PE) under warmed conditions (*B. quercifolia*), also showed the smallest vegetative shift (i.e. LMR_{AB}); the species with the smallest decline in emergence (*B. coccinea*) showed a relatively large vegetative shift. These species differences may reflect functional 'tradeoffs' between reproductive success and biomass allocation in response to soil warming and have repercussions for competitive interactions.

Whilst trait means varied among populations within species, direction and extent of the treatment responses did not generally vary significantly among the populations (i.e. there were few significant interactions between treatment and population). Thus, although we predicted that the populations from the hot, dry end of the gradient would be more robust to warming, they were not. The delays and reductions in emergence as demonstrated here suggest that impacts of warming at projected levels on recruitment of these species may be large, and may reflect the environmental or physiological limits of these species. What is of interest is the fact that we found some evidence of populations maintaining fitness homeostasis in response to stress. We assessed populations of all species from both central and range margins, and if patterns were to be found we would have expected to see them. However, if we were to investigate a larger number of populations we may find evidence of the potential for buffering against decline in emergence under future climate scenarios.

We had hypothesised that sites experiencing greater precipitation and cooler temperatures to show greatest effect of warming and drought and potentially greater variation in trait means, however, this was not the case. There appeared to be only a slight link between source climate and seed or seedling trait means. It is striking, given the large effect of

temperature on some measured traits, that measures of mean temperature were not correlated with trait values or their plasticity in these four *Banksia* species. Although there are many instances where such associations exist (for example (Hierro et al. 2009, Kim and Donohue 2011, De Frenne et al. 2012), there are occasions when few have been found (Schütz and Milberg 1997, Rustad et al. 2001, Richter et al. 2012, Vile et al. 2012).

Implications

When faced with environmental stress, species have often responded in a conservative manner, by migrating, rather than evolving (Bradshaw and McNeilly 1991). In the SWAFR, evidence suggests that species contracted to micro-refugia when adverse conditions were encountered (Keppel et al. 2012). However, in the current highly fragmented landscape of the SWAFR the potential for population migration is severely limited (Yates et al. 2010), and plants may need to tolerate or adapt to new conditions.

Our results support the view that *Banksia* may face an uncertain future as predicted by niche modelling (Fitzpatrick et al. 2008, Yates et al. 2010), and under a warmer climate, as predicted for the region, the continued persistence of these species in the broader landscape of the SWAFR may be constrained, primarily due to the sensitivity of seeds/seedlings to soil warming. The phenotypic responses of these four endemic *Banksia* species may not be sufficient to offset the negative effects of warming, with some populations potentially becoming disconnected from environmental germination cues in the future. If rapid adaptation is not possible, the risk of extinction rises (Dawson et al. 2011). In the wild, *Banksia* seedling emergence is not currently limited by low seed viability, however, only a small proportion of seeds become established seedlings (Lamont and Groom 1998). Our data suggest that even fewer seedlings will emerge under warmer conditions. Delayed emergence means that seedlings will be smaller when summer drought commences, such that the likelihood of desiccation is higher. A similar fate may also await long-lived woody species in other Mediterranean-climate regions of the world that share similar challenges (e.g. highly modified and populated regions that experience frequent disturbance by fire).

On the other hand, stabilising processes later in the life cycle could buffer the effects of warming and / or drought, especially relevant in Mediterranean environments (Lloret et al. 2012). These stabilising processes may include possible beneficial effects provided by warmer conditions to growth of seedlings, or a reduction in competition due to lower seedling density. Such stabilising processes that may compensate for losses during the recruitment phase also work at the population scale (Doak and Morris 2010,) and at even smaller spatial scales (Garcia-Camacho et al. 2012). Indeed, we found very little evidence of changes in seedling vegetative traits in response to climate manipulations with respect to above-ground biomass production suggesting that projected increases in temperatures will not have strong effects on these traits in seedlings of these species. These data highlight the need to consider responses to environmental change across a range of life history processes when predicting the effects of global warming.

The substantial population-specific differences in traits displayed by the species also indicate that using species mean trait values to describe species responses to environmental change is unsound, and should be replaced by population mean values or by some distribution of values as recommended by Albert et al. (2010). Although there were no fixed patterns of response to indicate local adaptation, the idiosyncratic variability demonstrated by these four *Banksia* species for the eight measured traits is worthy of further research, particularly considering the potential for identification of valuable among-population genetic variation, maternal or epigenetic effects. Among-population differences in traits are not easy to predict, and may be due to random genetic drift or founder effects. In the context of choosing the most appropriate seed material for restoration for future climates (i.e. seeds able to withstand warming and drying conditions), there may be no simple rules; hence, we argue the importance of assessing patterns of genetic variation within species before making decisions about restoration and conservation.

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Supplementary material (available online as Appendix oik.01359 at <www.oikosjournal.org/readers/appendix>). Appendix 1.