

## Differences in seedling water-stress response of two co-occurring *Banksia* species

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**Abstract.** In the South-west Australian Floristic Region, timing of rainfall is critical for successful seedling establishment, as is surviving the first year's summer drought for population persistence. Predictions of a warmer, drier future, therefore, threaten the persistence of obligate seeding species. Here, we investigate the drought tolerance of two co-occurring *Banksia* (Proteaceae) species by withholding water in pots to different extents of soil drying. Seed was collected from high- and low-rainfall populations, to test for niche differentiation in water-use strategies at the species level, as well as population differentiation. On the basis of a more negative leaf water potential at minimal levels of stomatal conductance and quantum yield, *B. coccinea* was considered to be more drought tolerant than *B. baxteri*. This was supported at the anatomical level according to xylem-vessel attributes, with a higher estimated collapse pressure suggesting that *B. coccinea* is less vulnerable to xylem cavitation. Population contrasts were observed mainly for *B. baxteri*, with a lower leaf-expansion increment rate in the low-rainfall population providing for drought avoidance, which was reflected in a higher rate of survival than with the high-rainfall population in which 87.5% of plants showed complete leaf senescence. The implications of species differences in water-use strategies are that community dynamics may start to shift as the climate changes. Importantly, this shift may be population dependent. A systematic understanding of adaptive capacity will help inform the choice of population for use in revegetation programs, which may lead to increased resilience and persistence in the face of environmental change. The results of the present study suggest that should declines in *B. baxteri* populations be noted, revegetating with seed collected from the low-rainfall population may help improve the chances of this species surviving into the future.

**Additional keywords:** *Banksia*, drought, niche differentiation, seedling, water use strategy.

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### Introduction

Recruitment processes such as 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). Success during these early life-cycle stages is especially dependent on environmental conditions (Bond and van Wilgen 1996), with water a major limitation in seasonally dry environments. Temperature is also very important because it can help synchronise germination and subsequent seedling emergence with environmental conditions optimal for seedling establishment. Many plant species have endured climatic fluctuations in the course of their evolutionary history; however, 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 the species-rich South-west Australian Floristic region (SWAFR; *sensu* Hopper and Gioia 2004), the genus *Banksia* L.f. (Proteaceae) is a major component of many vegetation communities. The climate is characterised by cool, wet winters and hot, dry summers, with fire being a recurring disturbance and driver of ecosystem dynamics (Cowling *et al.* 2005). Obligate-seeding *Banksia* is fire-killed and has an extended juvenile period, making them particularly vulnerable to frequent disturbance. Hence, successful seedling recruitment is crucial for persistence in fireprone landscapes. Timing of rainfall is also critical for successful seedling establishment in the SWAFR (Groom 2002), as is surviving the summer drought of the first year for population persistence (Enright and Lamont 1989; Enright and Lamont 1992). 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). Recent

investigation into the impact of warming on recruitment patterns in three *Banksia* species from SWAFR indicates that the mean time to emergence increases and final percentage emergence of seedlings decreases when seeds are exposed to above-average temperatures (Cochrane *et al.* 2015a). Thus, delayed emergence as a result of climate warming is likely to result in an increased risk of seedlings encountering water stress in their first year of establishment.

Seedling mortality rates may be ameliorated for *Banksia* species through their capacity to develop deep roots to avoid desiccation rather than tolerate low leaf water potentials. Plants that rely on access to groundwater are termed phreatophytes. Of the *Banksia* species so far investigated, *B. attenuata* and *B. menziesii* are categorised as facultative phreatophytes and *B. ilicifolia* is considered obligate (Zencich *et al.* 2002). Enright and Lamont (1992) showed that seedling survival of *B. attenuata* was greatest (34% survival) where seedlings were potentially accessing subsurface soil water in inter-dunal swales, compared with seedlings occurring on dune crests (4% survival). The vulnerability of phreatophytic *Banksia* species to changes in groundwater levels was also exemplified by Groom *et al.* (2000), who reported that up to 80% of all *Banksia* trees within the vicinity of the well field died as a consequence of rapid drawdown of the watertable because of increased summer abstraction. Thus, drought avoidance via deep roots is dependent on access to groundwater.

Reliance on groundwater availability is reflected in habitat preferences and water-use strategies of co-occurring *Banksia*, with contrasting avoidance and tolerance strategies (Groom 2002, 2004; Zencich *et al.* 2002), supporting niche differentiation (Chesson 2000; Silvertown 2004). In comparison, functionally similar species may instead rely on divergent life-history patterns for establishment opportunity (lottery model; Sale 1977; Chesson and Warner 1981), with resources similarly limiting mature stands (Lamont and Bergl 1991; Richardson *et al.* 1995).

*Banksia baxteri* R.Br. and *B. coccinea* R.Br. are two closely related *Banksia* shrubs that occur sympatrically across much of their range in the SWAFR. They differ in both their growth form and regeneration niche. *B. coccinea* has an erect growth pattern with little canopy spread, whereas *B. baxteri* is much broader with a higher degree of branching (Witkowski *et al.* 1991). In addition, *B. coccinea* is shorter-lived, matures faster, produces more and smaller seeds and is only weakly serotinous, (allowing for inter-fire recruitment; Lamont and Connell 1996) than *B. baxteri*.

The extent to which niche differences under current conditions affect the interactions among species under future conditions is unclear, as is the extent to which these interactions may vary across the ranges of such co-occurring species. Recent work investigated species and population divergence in traits underlying seed and seedling performance under contrasting temperature and moisture conditions (Cochrane *et al.* 2014,

2015a, 2015b). *Banksia coccinea* was shown to have a narrower temperature window for germination, with a steeper decline in percentage germination with increasing temperatures (Cochrane *et al.* 2014). However, at the seedling stage, *B. coccinea* was able to maintain growth to a greater extent than was *B. baxteri* (Cochrane *et al.* 2015a). Patterns of seedling growth were similarly mirrored under water-stress conditions, with *B. baxteri* suffering the greatest decline in biomass.

The current experiment continues the investigation of across-species and among-population comparisons of the response to environmental change in these species, to better understand how future climatic conditions may affect seedling performance and, ultimately, community composition. In this case, we examine whether seedling response to severe soil drying differs between the two co-occurring species, *B. baxteri* and *B. coccinea*, using seedlings grown from seed collected across the naturally occurring rainfall gradient in their distribution in SWAFR. We hypothesised that, at the species level, the higher growth rate of *B. baxteri* would result in a trade-off with drought tolerance and that, within each species, growth and survival would be most compromised in those plants grown from seed sourced from the high-rainfall site.

## Materials and methods

### Seed collection, germination and seedling transplantation

Two non-sprouting, obligate seeding *Banksia* species endemic to the SWAFR were chosen for their distribution along a longitudinal climate gradient, with annual rainfall roughly doubling from east to west (for a map of species distribution and sampling sites, refer to Fig. S1, available as Supplementary Material for this paper). At the high-rainfall (H) and low-rainfall (L) sites, *B. baxteri* and *B. coccinea* co-occur, generally in tall shrublands in deep white sands on plains and dunes (Table 1). The sandy soils were relatively homogenous within each site, but there were differences between sites. The substrate at the high-rainfall site was granitic, whereas limestone and quartzite constituted the base at the low-rainfall site. Individuals of the two species are interspersed in the habitat and show no indication of microsite differentiation. Seed were collected in the field from 10 maternal plants. For this experiment, we randomly selected four of these from each species at each site. For detailed information on seed collection and germination of the two species, refer to (Cochrane *et al.* 2015b).

When the seedlings were 6 months old, 128 healthy and similar-sized seedlings (64 seedlings of each species from two populations and four mother plants) were transplanted into 24.5-cm high × 9.5-cm-diameter PVC pots containing a low-phosphorous 2 : 1 mix of coarse white sand and low-phosphorous

**Table 1.** The geographic location and relevant biotic and abiotic data of seed-collection sites (WorldClim; Hijmans *et al.* 2005)

Site	Population name	Location	Altitude (m)	Annual mean precipitation (mm)	Precipitation of the warmest quarter (mm) <sup>A</sup>	Maximum temperature of the warmest period (°C) <sup>B</sup>	Total soil N (%)	Total soil P (mg <sup>-1</sup> )
High rainfall	'H'	118.422, -34.886	67	719	82	24	0.122	17.67
Low rainfall	'L'	119.516, -34.171	96	404	81	26.5	0.070	24.00

<sup>A</sup>The warmest quarter of the year occurs in summer (Dec.–Feb.), and the total precipitation over this period is calculated.

<sup>B</sup>The highest temperature of any weekly maximum temperature.

native potting mix (fertiliser rates included  $400 \text{ g cm}^{-3}$  superphosphate and  $1 \text{ kg cm}^{-3}$  of slow release premix fertiliser Osmoform 2 (Everris, Geldermalsen, Netherlands). The bottom of the pot was covered with shade cloth and absorbent cloth to prevent root escape and still allow for drainage. All pots were randomly arranged on a glasshouse bench in a metal-lattice framework, with squares of  $100 \text{ cm}^2$  to keep them upright. Planting density was one plant per  $200 \text{ cm}^2$ , with pots arranged diagonally from another pot to reduce shading effects. The plants were grown in a controlled-environment glasshouse with  $25^\circ\text{C}/15^\circ\text{C}$ , day/night temperature, under natural light conditions. Seedlings were well watered once every 2 days before treatment began 6 weeks after transplantation.

#### Watering treatments

Watering treatments included a well-watered control ('W';  $n = 64$  plants) and two water-stress treatment groups ('D1' water-withholding up to 10% water use, and 'D2' water-withholding for 2 weeks post-D1;  $n = 32$  plants per treatment). Forty-eight hours before the commencement of the drought treatments, all pots were watered to saturation to ensure that all plants started at a similar soil-water availability. The water-stress treatments were implemented by withholding water from pots. The first treatment (D1) involved withholding water until change in pot weight between days was 10% ( $\pm 5\%$ ) of the initial daily water use for each population-species combination. By this stage, it was assumed that stomata would be closed for the majority of the day because of water stress. Eight pots per population of each species were weighed at the same time every day to monitor water loss from pots. Because daily water use varied between population of each species, the time at which D1 was reached varied; 14 (*B. baxteri* H), 15 (*B. baxteri* L), 16 (*B. coccinea* H) and 18 (*B. coccinea* L) days after water was withheld. We chose to compare plant survival and recovery at a common functional point so as to test for differences in physiological drought tolerance and to accommodate for size effects on rate of water use. Once the target for D1 had been met, four plants per population of each species were assessed for physiological and morphological parameters. Another four plants were re-watered (R1) for a period of 4 weeks, before being similarly assessed. To test for drought tolerance after stomatal closure, we continued to withhold water for 2 weeks post-D1, on four individuals per species per population. Survival was assessed in terms of leaf senescence, with death defined as all leaves, especially tagged expanding leaves, having senesced at D2 and with no signs of recovery of existing tissue or regrowth 4 weeks after re-watering (R2). At each assessment point, four well-watered plants per population of each species were included for comparison. A schematic of the experimental design is given in Fig. S2 (available as Supplementary Material for this paper).

#### Physiological and morphological measurements

##### Growth rate

As a proxy of relative growth rate, leaf-length extension increment (the difference between the log of the length at Time 2 minus Time 1 divided by days;  $\text{mm day}^{-1}$ ) was measured on the three most recently initiated expanding leaves per plant. At the first measurement, the number of fully expanded

leaves was recorded and the youngest mature leaf of each plant was marked with a permanent marker pen. Immature leaves that were still curled within the bud were excluded. Leaf-expansion increments were measured non-destructively every 2 weeks during the experiment. Because growth rates of *Banksia* plants are inherently low and the duration of the experiment relatively short, growth rates were analysed only on well-watered plants to provide insight into differences in inherent biomass production between species and populations.

At 2 (D1) and 4 (D2) weeks, a cohort of four well-watered plants and four plants from the relevant drought treatment were harvested and plants were divided into three components, namely, leaf, stem and root, and dried at  $85^\circ\text{C}$  to a stable weight.

#### Gas exchange and leaf water status

Gas-exchange parameters, including assimilation rate ( $A$ ) and stomatal conductance ( $g_s$ ), were measured using a portable gas analyser (Li-6400; Li-Cor, Lincoln, NE, USA), with a light intensity of  $1000 \mu\text{mol quanta m}^{-2} \text{ s}^{-1}$  at a target air temperature of  $25^\circ\text{C}$  and vapour pressure deficit (VPD) of 1–1.2 KPa. The youngest fully expanded leaf per plant was sampled and the results adjusted for leaf area. Gas exchange and leaf water potential ( $\psi_{\text{leaf}}$ ) were measured between 1000 hours and 1300 hours. For the determination of  $\psi_{\text{leaf}}$ , a leaf, proximal to and of an equivalent developmental stage as the gas-exchange leaf, was removed and wrapped in damp paper towel and aluminium foil for immediate measurement with a Scholander pressure bomb (Model 1000, PMS Instrument Co., Albany, OR, USA).

#### Functional response curves to leaf water potential

After completion of the main water-withholding experiment, an additional set of five plants per population of each species that had been growing in the glasshouse were transferred to a growth chamber ( $25^\circ\text{C}/15^\circ\text{C}$ , day/night temperature, 50% humidity and 12 h daylight at  $\sim 800 \mu\text{mol quanta m}^{-2} \text{ s}^{-1}$  at the top of plant) to minimise fluctuations in growing conditions, particularly light levels. Water was withheld from plants and the youngest fully expanded leaf was sampled daily for measurements of stomatal and photosynthetic function (see below) between 1000 hours and 1200 hours. A maximum of five leaves were sampled on a minimum of three plants per species and population throughout the period of dry-down, to ensure that leaves sampled were of similar age.

Chlorophyll fluorescence of PSII was used to measure the sensitivity of photosynthesis to  $\psi_{\text{leaf}}$  during desiccation. The youngest fully expanded leaf was sampled on the plant and placed in a leaf clip (DLC-8) of a MiniPam (Walz, Effeltrich, Germany). The leaf segment was exposed to actinic light intensity of  $500 \mu\text{mol quanta m}^{-2} \text{ s}^{-1}$  for 90 s, and the quantum yield of PSII ( $\phi\text{PSII}$ ) was measured with a single saturating flash to the middle of the adaxial surface of the leaf.

Stomatal conductance was measured by steady-state diffusion using a SC-1 Porometer (Decagon, Pullman, WA, USA). Measurements were made immediately after the completion of  $\phi\text{PSII}$  measurements. The sample leaf was then excised and assessed for  $\psi_{\text{leaf}}$  with a pressure bomb, as previously described.

### Leaf anatomy

The vulnerability of xylem to declines in hydraulic conductivity when  $\psi_{\text{leaf}}$  drops (termed  $P_{50}$ ; Brodribb and Cochard 2009) has been related to plant survival under drought (Blackman *et al.* 2009; Brodribb and Cochard 2009) and climatic limits (Blackman *et al.* 2012; Choat *et al.* 2012). In turn,  $P_{50}$  is related to the theoretical capacity of the xylem to resist bending (Hacke *et al.* 2001) or collapsing (Blackman *et al.* 2010) under high tension, which is determined by conduit wall reinforcement  $(t/b)_h^2$ ; the thicker the double wall ( $t$ ) relative to its maximum span ( $b$ ), the greater the reinforcement against collapse (Hacke *et al.* 2001). We used  $(t/b)_h^2$  as a measure of drought tolerance and to see whether it may explain differences observed among populations or species in seedling mortality. The youngest fully expanded leaf from each of three well-watered plants per population of each species was collected and stored in formaldehyde–acetic acid–alcohol (FAA) until processing. Transverse sections of the petiole were sectioned on a freeze microtome and stained with toluidine blue and mounted on glass slides in phenol glycerine jelly. Lumen breadth ( $b$ ) and wall thickness ( $t$ ) were measured on all conduits of the largest vascular bundle, giving a total ~51 conduits per species. Images were taken using a Nikon DS-L1 digital camera (Nikon, Tokyo, Japan) connected to a light microscope at  $\times 100$  objective magnification and images were analysed in Image J (National Institutes of Health, Bethesda, MD, USA). Both  $b$  and  $t$  were measured by adjusting the microscope focus up and down to clearly define the thickest part of the cell wall;  $b$  was estimated as the side of the square with an area equal to the average conduit lumen, and hydraulically weighted according to hydraulic conductance (Mencuccini *et al.* 1997).

### Data analysis

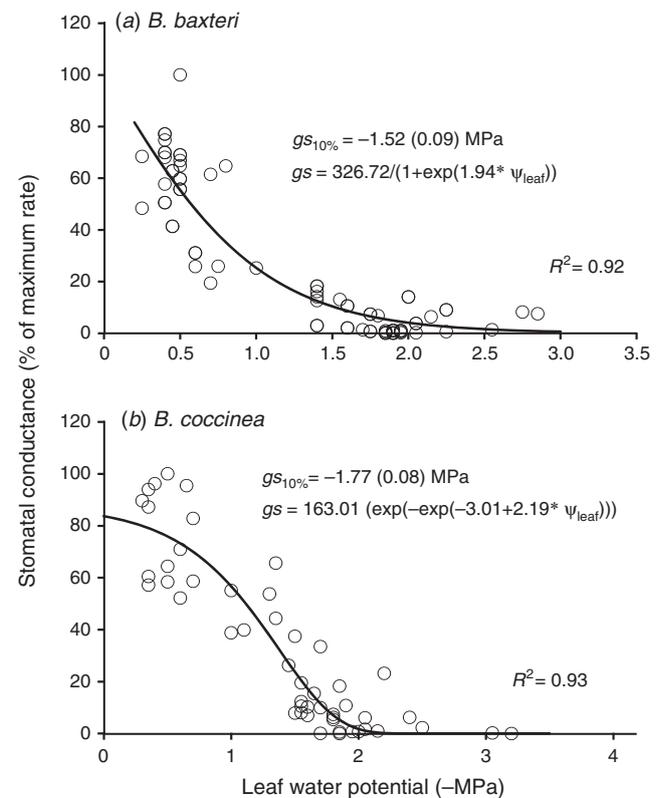
Gas exchange and harvest variables were analysed using general ANOVA in GENSTAT version 14, (VSN International, Hempstead, UK). We analysed the data in two separate stages, with the first stage considering innate species differences, looking only at the well-watered plants using data from all sampling dates, and the second delving into the treatment effects at particular stages in the dry-down experiment. For innate species differences in aboveground biomass and leaf-expansion increment, fixed terms in the model included species, population and measurement date (to incorporate the repeated nature of the design) and all interactions. Dry-down treatment effects were then assessed at the following individual time points: after 2 weeks of dry-down, 4 weeks of recovery, and 4 weeks of dry-down, using a model in which fixed terms were species, population and treatment (droughted vs well-watered). Maternal ID was used as a random factor in all models. Mortality following the extended dry-down (D2) was analysed using a replicated  $g$ -test across the four population-by-species combinations relative to an expectation of constant mortality rates across populations.

Functional response of gas exchange to  $\psi_{\text{leaf}}$  was analysed using non-linear regressions fitted to the data by using `proc nlmixed` procedures in SAS, version 9.1 (SAS Institute Inc., Cary, NC, USA). A log-likelihood ratio chi-square test was used to compare nested models, for the purpose of selecting the most parsimonious model. The  $P$ -value for model selection was derived from the difference in the  $-2$  log-likelihood statistics

between the two models, assuming chi-square distribution with degrees of freedom given by the difference in the number of parameters between models. Where there was no significant difference, the reduced model was selected. Akaike's information criterion (AIC; Akaike 1974) and biological reasonableness were used to distinguish between non-nested models. The same logic was applied to testing differences in response curves between populations, whereby the full model fitted two curves and the reduced model fitted one curve to the combined population data. The coefficient of determination ( $R^2$ ) was used to indicate the level of variance explained by the model and was expressed as  $R^2 = 1 - (SS_{\text{error}}/SS_{\text{total}})$ , where  $SS_{\text{error}}$  = residual sum of squares and  $SS_{\text{total}}$  = uncorrected total sum of squares for non-linear models without an intercept. Parameter estimates including  $gs_{10\%}$  and  $yield_{10\%}$  were derived using SAS procedures, with corresponding standard errors approximated using the delta method (Billingsley 1986). Species and population differences for  $(t/b)_h^2$  were analysed using ANOVA within the SAS procedure 'proc glm' (version 9.4, SAS Institute Inc., Cary, NC, USA).

### Results

Withholding water from pots resulted in a rapid decline in the rate of water loss, with plants reaching 10% of maximum water-loss rates between 14 and 18 days after withholding water. *Banksia Baxteri* closed stomata rapidly with the onset of decline in



**Fig. 1.** The relationship between stomatal conductance ( $gs$ ) and leaf water potential ( $\psi_{\text{leaf}}$ ) is represented by a half sigmoidal curve in (a) *Banksia baxteri* and a sigmoidal curve in (b) *B. coccinea*. The  $\psi_{\text{leaf}}$  at 90% loss of  $gs$  is given with standard error in parentheses, as well as coefficient of determination.

$\psi_{\text{leaf}}$ , closing at  $-1.5$  MPa, which is a higher (less negative, more hydrated) water potential than with *B. coccinea* (Fig. 1). *Banksia coccinea* was insensitive to  $\psi_{\text{leaf}}$  until  $-1$  MPa, after which stomata steadily closed reaching  $gs_{10\%}$  by  $-1.77$  MPa. Although plants were the same age, *B. baxteri* plants were generally larger at the start of the experiment, reflecting higher growth rates. This may explain why *B. baxteri* reached  $gs_{10\%}$  at a faster rate, despite more conservative stomatal regulation. Accordingly, aboveground biomass was greater for *B. baxteri* than for *B. coccinea* (Fig. 2a). Within each species, the high-rainfall population had a greater biomass than did the low-rainfall population (Fig. 2b). This result was similarly reflected in leaf-expansion interval of well-watered plants across the experimental period. A significant interaction between species and population was observed with leaf-expansion rate of well-watered individuals; it was higher in the high-rainfall population than in the low-rainfall population of *B. baxteri* (Fig. 2c). No significant difference between populations of *B. coccinea* was detected (Table 2).

Instantaneous measures of function, including assimilation rate and  $\psi_{\text{leaf}}$ , were used to assess the effect of water-withholding. At the end of D1, assimilation rate had fallen to 14% of the well-watered rate (Fig. 3a) and there was a concurrent significant decline in  $\psi_{\text{leaf}}$  to an average of  $-1.5$  MPa (Fig. 4a) in all experimental plants regardless of species or population. The

additional 2 weeks of water withholding in the D2 treatment resulted in near-zero assimilation rates of water-stressed plants (Fig. 3b), with leaves dehydrated further to  $-2.4$  MPa (Fig. 4b), resulting in widespread leaf senescence.

The water-stress effects were reversible at D1, with re-watered plants regaining well-watered photosynthetic rates (Fig. 3c) and  $\psi_{\text{leaf}}$  (Fig. 4c) when assessed 4 weeks later. Despite there being a significant species and population effect on  $\psi_{\text{leaf}}$  at R1, the values were biologically comparable to those observed for well-watered plants at D1 and D2. Similarly, there was a significant species by population interaction observed for assimilation rate at R1; all values were elevated compared with well-watered plants at D1 and D2; however, whether this result reflects simple variation among individuals or some form of overcompensation is difficult to say. Drought did lead to stimulation of root growth such that the leaf to root ratio increased by 21% across species and populations ( $P=0.01$ ).

Recovery after D2 was minimal with plant death significantly greater in the high-rainfall population of *B. baxteri* (7 of 8 plants died) than in either of the other treatments (Fig. 5). In both the low-rainfall *B. baxteri* population and high-rainfall *B. coccinea* population, only two of the eight plants died, and in the low-rainfall population of *B. coccinea*, only one of the eight plants died. This pattern of mortality resulted in a significant g-test for

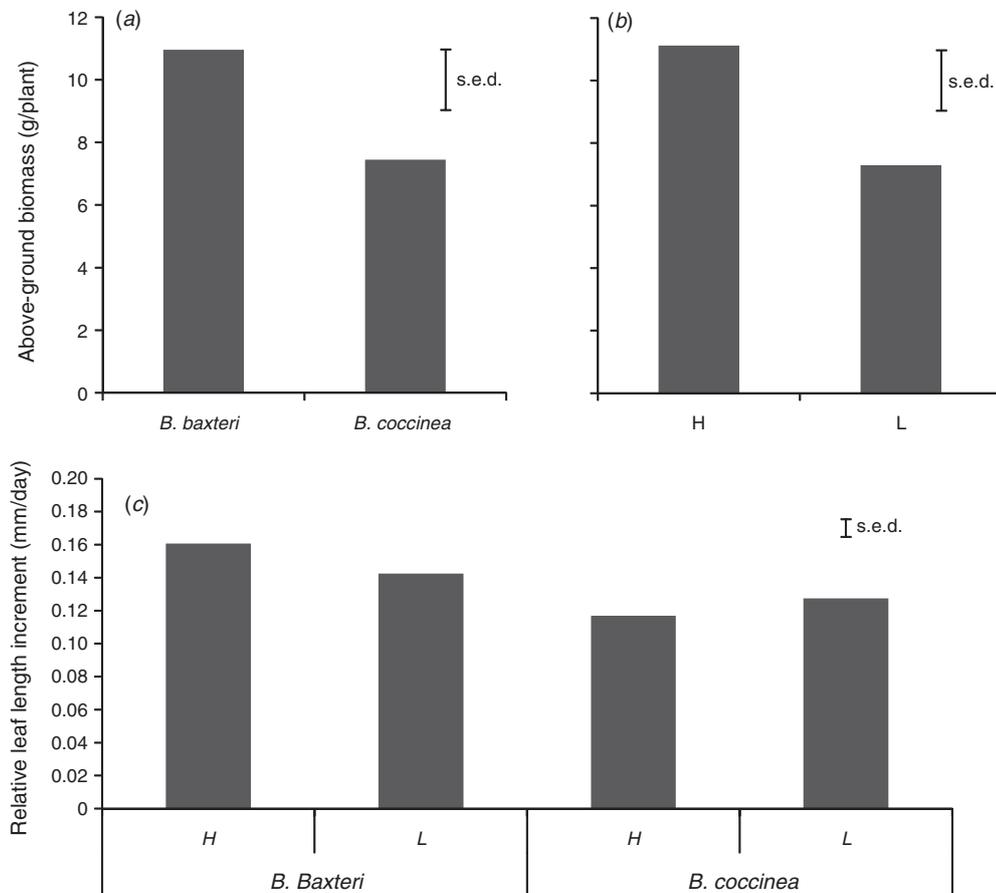
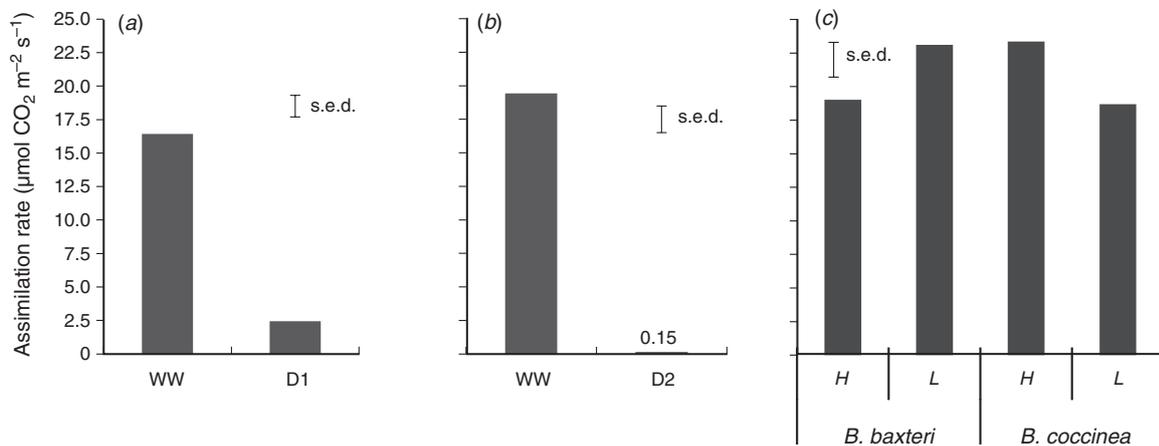


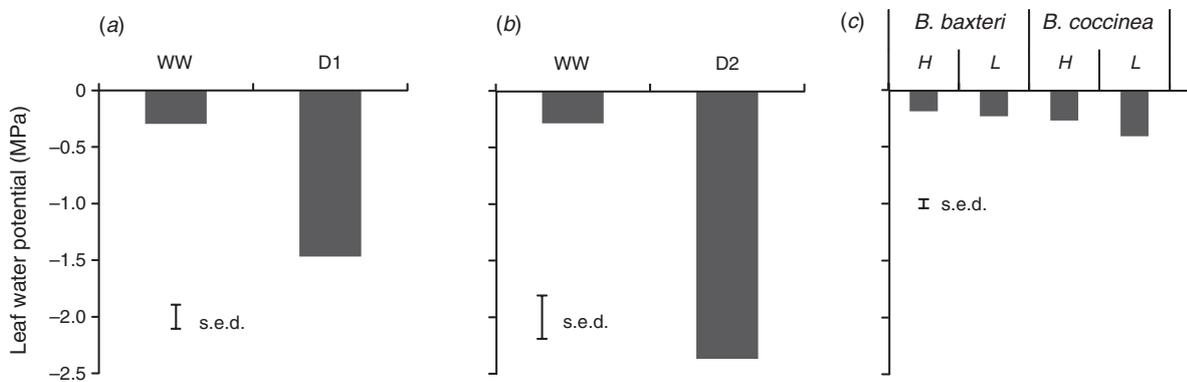
Fig. 2. Biomass and growth-rate patterns between *Banksia* species and populations of well-watered plants; (a) species and (b) population differences in aboveground biomass, and (c) species by population interaction in relative leaf-expansion increment.

**Table 2.** Probabilities from REML analysis of aboveground biomass of well-watered plants pooled across time points, relative leaf-length increment during the period of D1 and R1, and assimilation rate and leaf water potential at three time points comparing droughted and well-watered plants  
Values significant at  $P=0.05$  are shown in bold. n.a., not available

Parameter	Aboveground biomass (g)	Relative leaf-length increment (mm day <sup>-1</sup> )	Assimilation rate (μmol CO <sub>2</sub> m <sup>-2</sup> s <sup>-1</sup> )			Leaf water potential (-MPa)		
	Well-watered	Well-watered	D1	D2	R1	D1	D2	R1
Species	0.329	<b>&lt;0.001</b>	0.690	0.632	0.965	0.203	0.633	<b>0.007</b>
Population	0.402	0.309	0.939	0.857	0.764	0.371	0.649	<b>0.042</b>
Treatment	n.a.	n.a.	<b>&lt;0.001</b>	<b>&lt;0.001</b>	0.269	<b>&lt;0.001</b>	<b>&lt;0.001</b>	0.545
Species × population	<b>0.002</b>	<b>0.002</b>	0.372	0.507	<b>&lt;0.001</b>	0.402	0.793	0.261
Species × treatment	n.a.	n.a.	0.525	0.659	0.909	0.111	0.793	0.716
Species × population × treatment	n.a.	n.a.	0.061	0.754	0.083	0.669	0.599	0.402



**Fig. 3.** A significant effect of dry-down on assimilation rate was observed at the end of each drought period (a) D1 and (b) D2 compared with well-watered (WW) plants. After D1 plants were re-watered for 4 weeks, the effect of water-withholding was no longer apparent and (c) a species by population interaction was detected.



**Fig. 4.** A significant effect of dry-down on leaf water potential at the end of each drought period (a) D1 and (b) D2 compared with well-watered (WW) plants. After droughted plants were re-watered for 4 weeks post-D1, (c) the effect of water-withholding was no longer apparent. Although a species by population interaction was indicated, the water potentials were below or within the error of the well-watered plants at D1 and D2.

*B. baxteri* individually, relative to an expectation of constant mortality rates among populations and significant heterogeneity among populations ( $g=8.64$ , d.f. = 1,  $P<0.01$  for *B. baxteri*,  $g=12.29$ , d.f. = 3,  $P<0.05$  for heterogeneity).

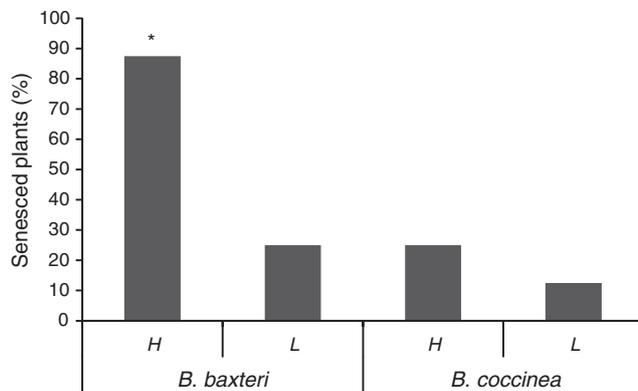
Anatomical assessment of the theoretical collapse pressure of xylem conduits in the leaf petiole  $(t/b)_h^2$  was used as a surrogate measure of drought tolerance, along with the  $\psi_{leaf}$  at which

stomatal closure and minimum levels of quantum yield were observed. Species differences were found, with higher conduit reinforcement in *B. coccinea* than in *B. baxteri*, suggesting that xylem conduits could be exposed to lower water potentials (or higher pressures) before vessel collapse would occur and lead to reduced water transport capacity ( $P=0.0076$ ; Fig. 6). The results suggested that *B. coccinea* could also maintain open stomata

and Photosystem II function at lower  $\psi_{\text{leaf}}$ . However, unlike the response of stomata to  $\psi_{\text{leaf}}$  (Fig. 1), quantum yield was much more sensitive to declining  $\psi_{\text{leaf}}$  in *B. coccinea* than in *B. baxteri* (Fig. 7). A single model could describe the response of quantum yield to  $\psi_{\text{leaf}}$  for all species and populations, but parameter coefficients differed among *B. baxteri* populations. The low-rainfall population demonstrated a much shallower decline in quantum yield, with  $\psi_{\text{leaf}}$  reaching a minimum at  $-2.89$  MPa, compared with  $-2.65$  MPa for the high-rainfall population. There was no significant difference in model parameters for populations of *B. coccinea*.

## Discussion

We examined drought tolerance in seedlings from two geographically distinct populations in a co-occurring, congeneric species pair. *Banksia baxteri* and *B. coccinea* grow sympatrically across much of their geographic range. The species share several similarities (fire-killed, obligate seeding, canopy seed storage) but differ in the degree of serotiny each exhibit (George 1984) and in other recruitment-related traits including seed size, time to germination and percentage germination (Cochrane *et al.* 2014, 2015a). In response to heat and mild drought stress, the species show phenotypic divergence,

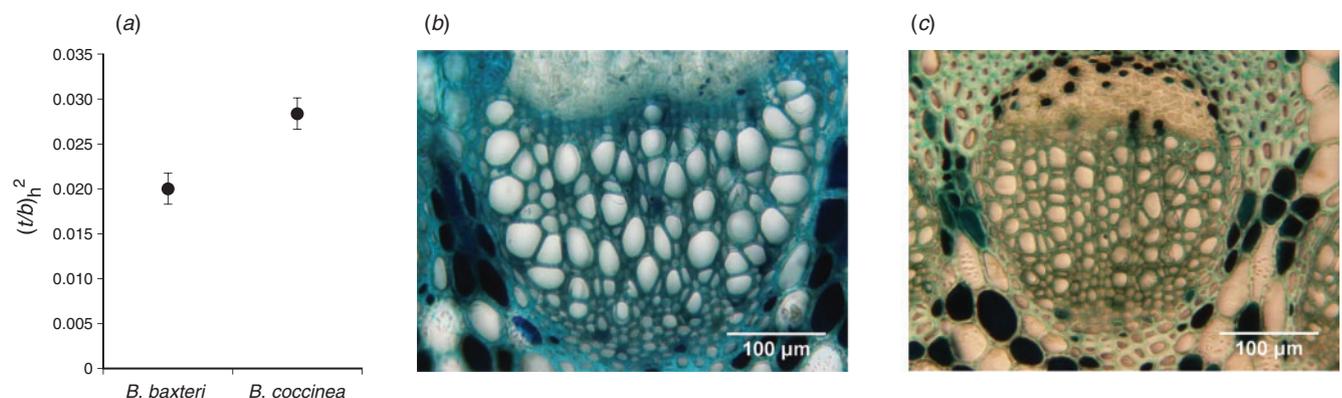


**Fig. 5.** Percentage of plants ( $n=8$ ) that had complete leaf senescence at the end of D2. The asterisks indicate that *Banksia baxteri* (H) was significantly ( $P < 0.05$ ) different from the other groups.

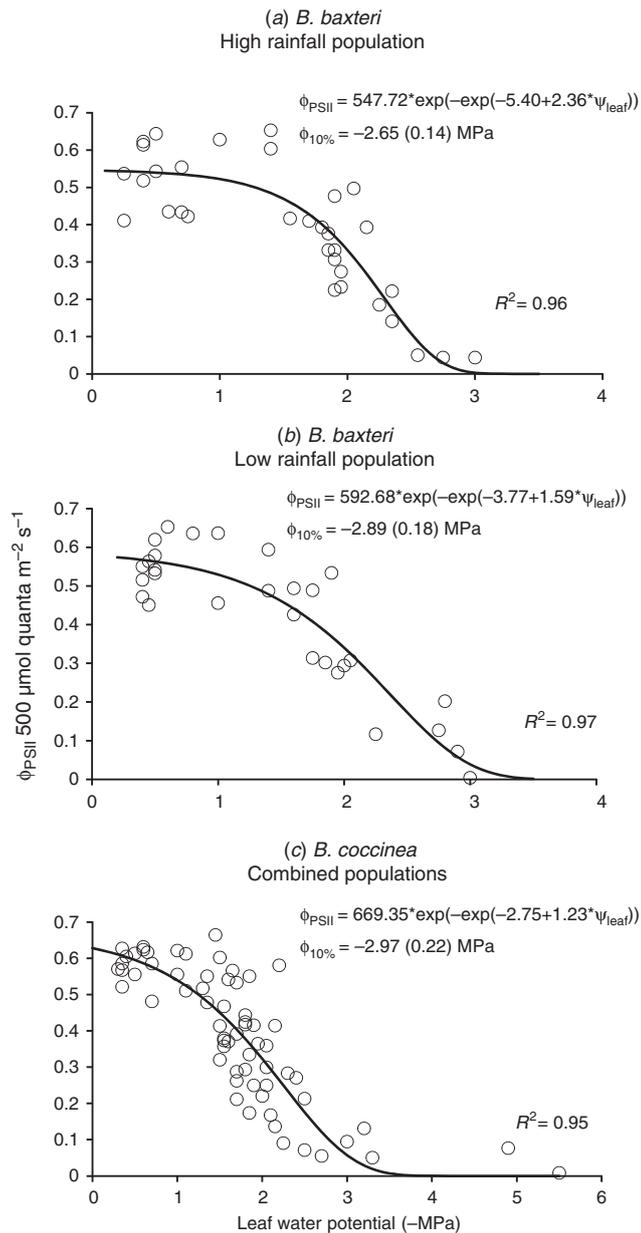
supporting the idea of niche differentiation (Cochrane *et al.* 2015b). As such, our main interest was to determine whether the two species also differed in their ability to tolerate severe soil water deficits, given that future climate projections suggest warmer, drier conditions for the region (CSIRO 2007; Bates *et al.* 2008, 2012), which may have an impact on groundwater availability (Hughes *et al.* 2012) and delay seedling emergence (Cochrane *et al.* 2015a), increasing the risk of exposure to drought stress before seedlings have established.

Drought resistance was tested by withholding water in pots to a similar functional state (D1) and recording plant survival in terms of complete senescence of existing leaves 2 weeks beyond minimal stomatal conductance (D2). The more negative  $\psi_{\text{leaf}}$  at plant functional limits (10% stomatal conductance and quantum yield) suggests that *B. coccinea* is more drought tolerant (Figs 1, 7). Anatomical differentiation between the species provided further support, with the petiole xylem vessels of *B. coccinea* found to have a higher estimated collapse pressure (Fig. 6), and they are therefore likely to be less vulnerable to xylem cavitation. Maintaining water transport capacity has been shown to be imperative to plant survival, with leaf death often observed once the  $\psi_{\text{leaf}}$  reaches 50% loss of hydraulic conductivity (Brodribb and Cochard 2009). This is especially true of woody plants and has been shown to be related to a species' drought tolerance (Brodribb and Hill 1999; Pockman and Sperry 2000; Blackman *et al.* 2009; Maherali *et al.* 2004) and distributional range (Blackman *et al.* 2012). Accordingly, the greatest plant mortality was observed for *B. baxteri* and this result was population dependent, with only 12.5% of plants from the high-rainfall population surviving the extended water-withholding period. In contrast, 75% of plants in the low-rainfall *B. baxteri* population and 75% and 87.5% of plants in the high- and low-rainfall populations of *B. coccinea*, survived respectively (Fig. 5).

Despite closing stomata at a high  $\psi_{\text{leaf}}$  to minimise further water use, the higher growth rate of *B. baxteri* under well-watered conditions resulted in greater aboveground biomass before commencement of the water-stress treatment and, therefore, greater total water demand for maintenance of leaf hydration (Fig. 2). This was especially the case in the high-rainfall population, which had the highest leaf-extension increment and



**Fig. 6.** The theoretical capacity of xylem conduits in the petiole to resist bending  $(t/b)_h^2$ , (a) with a significant difference found at the species level, with examples of xylem anatomy observed in (b) *Banksia baxteri* and (c) *B. coccinea*. Photos taken at  $\times 20$  objective magnification.



**Fig. 7.** The relationship between quantum yield of Photosystem II ( $\phi_{\text{PSII}}$ ) and leaf water potential ( $\psi_{\text{leaf}}$ ) in the (a) high-rainfall population of *Banksia baxteri*, (b) low-rainfall population of *B. baxteri* and (c) combined populations of *B. coccinea*. The  $\psi_{\text{leaf}}$  at 90% loss of  $\phi_{\text{PSII}}$  is given with standard error in parentheses, as well as coefficient of determination.

aboveground biomass of the two populations. Similar growth patterns have been observed for these same species in common-garden and glasshouse experiments (Cochrane *et al.* 2015a, 2015b), as well as in the field (Lamont and Witkowski 1995). However, when exposed to mild, extended water stress, growth of *B. baxteri* declined more sharply (Cochrane *et al.* 2015b). Thus, although *B. baxteri* may be more vulnerable to drought-induced cavitation, the consequences of xylem attributes in terms of drought tolerance seem to be moderated by drought-avoidance traits. These include a more conservative growth strategy

(Fig. 2c), which was identified in the low-rainfall *B. baxteri* population and aided by conservative stomatal regulation in this species (Fig. 1a). The  $\psi_{\text{leaf}}$  at which minimal Photosystem II function was observed was also more negative in the low-rainfall population of *B. baxteri* than in the high-rainfall population (Fig. 7b vs Fig. 7a), suggesting greater resistance of the photosynthetic apparatus to desiccation. Importantly, across both species and both populations, stomata closure occurred at water potentials less negative than those that caused a significant depression in Photosystem II function, supporting the idea that stomatal closure occurs as a protective mechanism against permanent functional damage (e.g. Tyree and Sperry 1989; Brodribb and Holbrook 2003).

Species differences in water-use strategies observed in the current study contrast those described for *B. menziesii* and *B. littoralis* (Groom 2002). The less conservative stomatal regulation of *B. littoralis* is considered an opportunistic ‘water-spender’ strategy, reflecting its constrained distribution to low-lying seasonally wet areas. Furthermore, the conservative stomatal regulation of *B. menziesii* was also associated with lower maximum rates of stomatal conductance. In contrast, in our study, the higher growth rate of *B. baxteri* reflected a trade-off with the capacity to tolerate low  $\psi_{\text{leaf}}$ . Importantly, different combinations of water-use traits may have different survival outcomes depending on accessibility to water in the landscape. Unlike *B. menziesii* and *B. littoralis*, neither *B. baxteri* nor *B. coccinea* have yet to be classified as being phreatophytes, and, on the basis of position in the landscape, we see no evidence that they divide the habitat spatially according to microsite conditions, as might be expected if they differed markedly in root architecture and access to ground water. That said, it is interesting to note that seedling root growth was stimulated even during rapid exposure to water stress, with the root-mass ratio increasing by 21% across both species and populations. Given that *B. coccinea* was able to maintain stomata open at lower  $\psi_{\text{leaf}}$  (Fig. 1b), this may result in a growth advantage in the field, with continued carbon uptake able to support further root growth.

In terms of niche differentiation, our results suggest that *B. baxteri* may be more dependent on achieving a deep root system during the early years of establishment through higher growth rates, compared with *B. coccinea*, which may be able to tolerate changes in water accessibility to a greater degree. For *B. coccinea*, drought tolerance may in fact be necessary given the short life span and greater investment in seed over biomass production (Witkowski *et al.* 1991; Lamont and Witkowski 1995). Furthermore, *B. coccinea* displays inter-fire recruitment (Lamont and Connell 1996), which, consequently, exposes seedlings to more hostile establishment conditions, and has smaller seeds and, therefore, reduced reserves for rapid establishment to ensure access to water (Witkowski *et al.* 1991). Such differences in both aboveground phenology and temporal exploration of the soil profile may help facilitate co-existence (Parrish and Bazzaz 1976; Davis and Mooney 1986). However, as suggested by Lamont and Bergl (1991), belowground niche separation may not necessarily be required for co-existence to be achieved. This is based on having observed little differences in patterns of water use in either space or time in three co-occurring *Banksia* species, although in that case, they all had a similar growth form.

Field measurements to explore spatial and temporal patterns in water use are clearly needed to confirm the implications of leaf-level strategies within the context of soil water profiles (Lamont and Bergl 1991; Dodd and Bell 1993; Zencich *et al.* 2002; Groom 2004). Poot and Veneklaas (2012) used such an approach to examine species distribution and local dominance of four *Eucalyptus* species in south-western Australia. They identified the two species typically found growing under wetter conditions, *Eucalyptus marginata* and *Corymbia calophylla*, as vulnerable to crown decline under reduced precipitation on the basis of their greater susceptibility to xylem cavitation and reduced ability to draw water at low soil water potentials to maintain function. However, consistent geographic patterning of plant traits within species is not always apparent. In the study by Cochrane *et al.* (2015b), morphological responses of seedlings to warming and/or drying did not consistently support the hypothesis that *Banksia* populations at the warmer and drier end of their distribution would show lower plasticity, and neither was there a population that displayed superior performance under all conditions. Acclimation that requires plasticity in functional traits can be an important strategy to mitigate the impacts of soil drying. This has been observed in populations of *B. attenuata* and *B. menziesii* in terms of xylem vulnerability to cavitation (Canham *et al.* 2009), and deserves greater consideration for understanding and predicting the adaptive capacity of species and populations.

The implications of species differences in water-use strategies coupled with life-history patterns suggest that some species may be more vulnerable to climate change than others, with consequent shifts in community dynamics (Fitzpatrick *et al.* 2008; Yates *et al.* 2010). The results of the present study suggest that with continued declines in groundwater levels (Hughes *et al.* 2012), *B. baxteri* may lose out during seedling establishment relative to *B. coccinea*. Conservation efforts will be aided by better understanding population differences (Cochrane *et al.* 2015c), in particular the evolutionary potential for adaptation to changing environmental conditions, which is considered imperative for long-term persistence (Weeks *et al.* 2011). A systematic understanding of adaptive capacity will help evaluate species vulnerability and, where deemed appropriate, inform the choice of population for use in revegetation programs so that managers can improve outcomes in the face of environmental change (Hewitt *et al.* 2011). In light of the continued warming and drying climate forecast for the region, the results of the present study suggest that if *B. baxteri* populations from the wetter end of the species range begin to show decline, then revegetating with seed collected from the low-rainfall end of the range may improve the chances of this species persisting across its range into the future.

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