Hydrological and thermal responses of seeds from four co-occurring tree species from southwest Western Australia

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Seed germination is a critical stage in the life cycle of most plants and is defined by specific tolerance thresholds beyond which rates and success of germination rapidly decline. Previous studies have demonstrated that widespread plant species commonly germinate over a broad range of temperatures and water stress levels, whereas range-restricted species often exhibit a narrower germination window in terms of temperature and moisture. We investigated the relationships of the key germination traits of maximum germination ($G_{max}$) and time to 50% germination ($t_{50}$) in response to temperature (5–35°C) and water stress (−1.5–0 MPa) in four co-occurring Western Australian native Eucalyptus species with widely varying biogeography. Eucalyptus caesia subsp. caesia and E. ornata exhibit a highly localized distribution and a narrow geographical range, being restricted either to granite outcrops or the upper slopes and tops of lateritic rises, respectively. These two species were compared with the two widespread and dominant congeners E. salmonophloia and E. salubris. There was a distinctive hump-shaped response of $t_{50}$ to temperature and an exponential response to water stress, characteristic of rate- and threshold-limited processes, but no consistent pattern in the response of $G_{max}$. The four species were significantly different in their thermal performance of $t_{50}$, with E. caesia and E. ornata displaying narrower thermal tolerance ranges than the two widespread species. In terms of mean final germination percentage, the two range-restricted endemic taxa exhibited higher lability in their response to thermal stress and drought stress compared to the two broadly distributed congeners. These findings indicate a link between distributional extent, temperature and water stress tolerance and may have implications for identifying ecological filters of rarity and endemism.

Keywords: Conservation, drought stress, performance model, seed germination, thermal tolerance, threatened flora

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Introduction

High levels of biodiversity and endemism are often harboured in range-restricted niche habitats on rocky outcrops such as banded ironstone formations (BIFs) and granite outcrops (Porembski and Barthlott, 2000; Jacobi et al., 2008; Gibson et al., 2010). Opportunities for evolution in these habitats largely result from edaphic isolation from the surrounding vegetation matrices and the unique and highly localized environmental conditions commonly found in these niche landscapes (Porembski and Barthlott, 2000; Withers, 2000; Jacobi et al., 2008; Gibson et al., 2010). Consequently, the plant communities of rock outcrop habitats are often unique and comprise combinations of taxa that are regionally widely distributed as well as range-restricted ecological specialists that are highly adapted to various local microhabitats (Gibson et al., 2010; Porembski and Barthlott, 2012; Do Carmo and Jacobi, 2016). The result is that rock outcrop communities are generally speciose compared to adjacent vegetation on deeper soils (Main, 1997; Mares, 1997; Withers, 2000; Yates et al., 2003; Schut et al., 2014) and contribute significantly to regional biodiversity (Hopper and Gioia, 2004; Safford et al., 2005; Jacobi and Fonseca do Carmo, 2008). For example, the granite outcrops of Western Australia host 17% of the flora native to the South Western Australian Floristic Region (SWAFR; Hopper and Gioia, 2004), including many range-restricted plant taxa that are threatened, yet granite outcrops occupy less than 1% of the land area in the SWAFR (Byrne and Hopper, 2008; Wege et al., 2015).

Previous studies have highlighted topographic factors, edaphic isolation and climatic variables as major factors determining the distributional extent of narrow-range endemics (Yates et al., 2004; Carta et al., 2013; Tapper et al., 2014a; Cross et al., 2015a). For example, recent studies on the germination ecology of ephemeral taxa have revealed that hydrology regimes and hydroperiod are major ecological filters that determine species distributional range in temporary wetland habitats (Cross et al., 2015a; Cross et al., 2015b; Carta et al., 2013; Cross et al., 2018). Widely distributed species in Mediterranean climatic regions commonly germinate over a relatively wide range of temperatures and water stress levels (Cochrane, 2017; Cochrane, 2018), whereas the germination response of range-restricted taxa has been shown in several species to be limited to a narrower window (Luna et al., 2012; Turner et al., 2018). Edaphic isolation and local topographic elements have been identified as driving forces of the patterns of plant diversity observed in rocky outcrop habitats such as BIFs and inselbergs (Jacobi et al., 2007; Gibson et al., 2010; Porembski and Barthlott, 2012; Do Carmo and Jacobi, 2016). Granite outcrops (and their immediate surroundings) represent a fine-scale mosaic of habitats, and where the ecophysiology of different elements of the floristic community might vary substantially (Withers, 2000; Byrne and Hopper, 2008; Tapper et al., 2014b). Microhabitats in granite outcrop environments often harbour range-restricted and highly specialized species, as well as taxa that are widespread across different parts of the landscape (Hopper et al., 1997; Withers, 2000). Exposed granite surfaces are characterized by high temperatures (particularly during summer) and low moisture availability due to high water runoff and limited capacity for moisture to soak into the subsurface environment (Withers, 2000; Porembski and Barthlott, 2012). However, following rainfall events, weathering of granite produces various highly localized, shaded, mesic microhabitats that retain water for periods of time including rock pools, crevices, gullies, talus and exfoliating sheets of granite where water collects and losses via evaporation and soil percolation are reduced (Wyatt, 1997; Withers, 2000; Liu et al., 2007). The ecological filters underlying patterns of plant diversity in outcrop habitats are yet to be clearly identified and understood (Byrne and Hopper, 2008). However, the substantial proportion of range-restricted plant species endemic to rock outcrops suggests that the traits enabling these plant taxa to persist and flourish in their rocky niche may consequently reduce their competitiveness in other environments (Byrne and Hopper, 2008; Anacker et al., 2011; Tapper et al., 2014b), and these warrant further investigation.

The transition from seed to seedling represents one of the most critical stages of the plant life cycle (Lloret et al., 2004; James et al., 2013). Seeds are therefore highly adapted to their habitat in order to maximize recruitment success, as essentially seeds have only one attempt at successfully transitioning from a seed to a viable and healthy seedling (Walck et al., 1997; Tweddle et al., 2003; Luna et al., 2012). Consequently, the environmental requirements for dormancy alleviation and seed germination are usually definable, highly nuanced and species-specific (Turner et al., 2018). Seed germination occurs in response to specific combinations of environmental cues above critical thresholds with two of the most important being temperature and soil moisture (Bell, 1994; Bell et al., 1995; Merritt et al., 2007). It is reasonable to expect that range-restricted species, and particularly species occurring only in specific microhabitats such as rocky outcrops, may have narrow germination niches as these habitats provide environments that are likely to differ markedly from other parts of the landscape (Turner et al., 2018; Elliott et al., 2019). Consequently, investigation of the germination ecology of seeds from range-restricted and ecologically specialized flora should be a principle area of research to better understand their demographic limitations which may assist with their ongoing conservation and management (Luna et al., 2012; Clemente et al., 2017). Furthermore, identifying some unifying theoretical constraints to seed germination is essential for constructing a priori, mechanistic hypotheses underpinning these demographic limitations.

There have been efforts made to develop models of seed germination in relation to temperature and water stress (Bradford, 2002), but these have been heavily data-referential, and have not been consistent with the theoretical underpinnings of the wider thermal performance literature (Angilletta Jr
et al., 2009). As such, the statistical fitting is potentially over-simplified, and the resulting parameters may be inaccurate and difficult to place in a broad theoretical context. According to the collision theory of chemical kinetics, reaction rates increase exponentially with increasing temperature (Gates, 2016). However, metabolic reactions are catalyzed by enzymes that have a specific thermal threshold beyond which they denature (Peterson et al., 2007). The interaction of these two processes implies a rapid increase in physiological performance up to a critical threshold, beyond which performance rapidly declines as chemical reactions cease to be catalyzed by the denaturing enzymes. Therefore thermal performance curves of enzymes are hump shaped and distinctly asymmetrical (Aquillette Jr, 2006; Tomlinson, 2019), which is an important trait conspicuously absent in published early models (e.g. Bradford, 2002). There is also variability in the breadth of these responses that has evolutionary and ecological value (Huey et al., 1989). Seeds of widely distributed flora are expected to have broad thermal tolerance ranges (eurythermy) to match the breadth of climatic conditions across their distributions, while range-restricted congenerics are expected to be thermally specialized (stenothermy; Debat and David, 2001; Ghalambor et al., 2007). In this manner, seed germination is consistent with general models of stenothermy and eurythermy (Seebacher and Franklin, 2005). However, there is a shortage of studies that have incorporated these well-established principles of chemical kinetics to quantify the impact of thermal stress on germination response in the literature. Further, there is a major shortage of research data on how a species distribution affects germination response to water stress. Given that rocky outcrop habitats comprise highly variable microclimates and that these landscapes can be very hot and dry for much of the year, especially in the lower rainfall regions of Western Australia, the optimal performance windows might reflect highly specific local adaptations and thus provide some insight concerning in situ recruitment processes (Byrne and Hopper, 2008; Tapper et al., 2014b). This study aimed to compare the germination responses of two range-restricted granite outcrop specialist species with those of two widely distributed co-occurring taxa to address the following two research questions: (i) Are seeds of non-dormant range-restricted species more sensitive to incubation temperature compared to common congeneric taxa? And (ii) Are seeds of non-dormant range-restricted species more sensitive to water stress compared to common congeneric taxa?

Materials and methods
Species selection and sourcing
We conducted this study using four readily germinable, non-dormant species of Eucalyptus endemic to southwest Western Australia. Species were selected to eliminate the potential confounding effect of seed dormancy on examining seed germination responses. We selected seeds of two range-restricted species native to either granite outcrop habitats (E. caesia Benth. subsp. caesia) or laterite ridges (E. ornata Crisp) and two widely distributed congeneric species (E. salmonophloia F.Muell. and E. salubris F.Muell.). Seeds were either freshly harvested from wild populations (E. caesia) or obtained from a commercial seed supplier (E. ornata, E. salmonophloia and E. salubris—Nindethana Seed Company, King River, Western Australia) with known collection locations and dates of collection (Table 1). Eucalyptus caesia Benth, subsp. caesia and E. ornata are range-restricted mallees that are gazetted as priority 3 and 4 respectively (W.A. Herbarium, 2018) so are of some conservation concern (Coates et al., 2001). Eucalyptus caesia subsp. caesia is distributed across 25 populations in the Avon Wheatbelt, Coolgardie and Mallee (Bezemer et al., 2019), whereas the range of E. ornata is limited to five populations in the Avon Wheatbelt and the Mallee IBRA (Interim Biogeographic Regionalisation for Australia) Regions (Thackway and Cresswell, 1997). In contrast, E. salmonophloia and E. salubris are common, widely distributed dominant mallees native to south west Western Australia (Yates et al., 1994). Their habitats are diverse and include undulating low hills, plains and slopes surrounding granite outcrops. The distributional range of E. salmonophloia and E. salubris extends from the relatively

| Species               | Collection location | Collection date | 1000-seed weight (mg) | X-ray fill (%; n = 100) | Viability (%; n = 20) (tetrazolium test) | Germinability (% of viable seeds; n = 25) |
|-----------------------|---------------------|-----------------|-----------------------|------------------------|------------------------------------------|------------------------------------------|
| Eucalyptus caesia subsp. caesia | Kuender, WA         | 01/2018         | 2552                  | 96                     | 90                                       | 93                                       |
| Eucalyptus ornata      | Kondinin, WA        | 11/2008         | 1843                  | 100                    | 100                                      | 100                                      |
| Eucalyptus salmonophloia| Kondinin, WA        | 09/2017         | 209                   | 93                     | 100                                      | 100                                      |
| Eucalyptus salubris    | Kondinin, WA        | 03/2016         | 574                   | 100                    | 90                                       | 100                                      |

*Interim Biogeographic Regionalisation for Australia (Thackway and Cresswell, 1997) **Initial germination success of filled seeds was assessed by incubating 25 seeds of each species on moist germination paper in Petri dishes under constant darkness at 10, 15, 20, 25, 30 and 35°C followed by daily scoring of germination rate.
mesic Mediterranean SWAFR (South West Australia Floristic Region) to the semi-arid IBRA regions such as Coolgardie (Yates et al., 1994). Seed accessions used in this experiment were collected in 2008 (E. ornata), 2016 (E. salubris), 2017 (E. salmonophloia) and 2018 (E. caesia) from locations within ~50 km of each other (Table 1). Seeds from all species were stored under cool, dry conditions until utilized in this study.

**Seed quality**

Prior to experimentation, seeds were stored in a controlled environment (15°C and 15% relative humidity) at the Biodiversity Conservation Centre, Kings Park, Western Australia. We used a vacuum aspirator (SELECTA BV Gravity Seed Separator, the Netherlands) to separate seeds from chaff. For each test species percentage seed fill was determined by X-ray analysis of 100 seeds (MX-20 digital X-Ray cabinet, Faxitron, USA). A seed containing a fully developed embryo and endosperm can be identified by uniform white/grey coloration (filled tissue), whereas the absence of these tissues indicates a lack of seed fill (Erickson et al., 2016).

For seeds that were filled, seed viability was also investigated using Tetrazolium staining (Lakon, 1949). Reduction of 2,3,5-triphenyltetrazolium chloride (C19H15N4Cl) by dehydrogenase enzymes present in live tissues produces an intense pink colour, indicating that a seed is metabolically active, and thus viable (Lakon, 1949; Jeremiah et al., 2002). Samples of 20 seeds per species were horizontally dissected and exposed to 1% tetrazolium for a period of 4 h at 25°C. We used stained seeds to calculate percentage viability of seed lots (Table 1).

**Temperature tolerance**

To assess the germination response of seeds to temperature, we placed eight replicates of 25 seeds for each species on moist (9 ml of distilled water per petri dish) 84 mm germination paper (Advantec, Dublin, CA, USA) in 90-mm plastic Petri dishes and incubated at 5, 10, 15, 20, 25, 30 and 35°C (1400 total seeds per species). These conditions encompass a broad range of the temperatures reported for the location of the test species for all seasons (Bureau of Meteorology, 2018; Fig. 1). Seeds were surface sterilized with 2% (w/v) calcium hypochlorite (Ca(OCl)2) under vacuum (~70 kPa) for 30 min and washed with sterile deionized water three times for several minutes per wash prior to plating. We conducted seed plating under sterile conditions in a laminar flow cabinet. Petri dishes were sealed with plastic wrap to prevent moisture loss during the incubation period. Petri dishes were also covered with aluminium foil to eliminate the potential confounding effect of light on germination (Bell, 1994; Ruiz-Talonia et al., 2018). The temperature inside the incubators was recorded once an hour using iButton data loggers (Maxim Integrated™, San Jose, USA) placed in the middle of each stack of eight petri dishes (see Supplementary Material). We scored germination as radicle emergence greater than 2 mm, and plates were scored four days a week for a period of 28 days.

**Water stress tolerance**

To test the effect of water stress on germination, we placed seeds in 90-mm plastic Petri dishes on seed germination papers as previously described infused with different concentrations of polyethylene glycol 8000 (PEG) solution (9 ml of PEG per petri dish) following Michel (1983). Plates were incubated at a constant favourable incubation temperature (20°C), determined from temperature tolerance experiments. We exposed eight replicates of 25 seeds for each species to water stress levels of 0, −0.10, −0.20, −0.40, −0.70, −1.00 and −1.50 MPa (1400 total seeds per species). Seeds were surface sterilized as previously described prior to plating, and Petri dishes were tightly sealed with plastic wrap and incubated in constant darkness with ibuttons (Maxim Integrated™, San Jose, CA, USA) placed on the middle of each Petri dish stack to measure incubation temperature as previously described. We scored germination as radicle emergence greater than 2 mm, and plates were scored four days a week for a period of 28 days.

**Statistical analysis**

**Germination modelling**

Traditional attempts to identify critical thresholds of seed germination utilize binominal logistic regression to linearize the relationship between treatments and germination response (Ashford et al., 1970; Bradford, 2002). We adapted a non-linear regression approach (Ritz and Streibig, 2008) that is not yet common in studies of seed biology to assess the effect of incubation temperature and water stress on germination response. The main advantage of the non-linear curve-fitting approach we have used is that it does not compress the natural variance structure of the data in the way that linearization does and only fits the number of parameters that define the model. Therefore, since the risk of overfitting to the data is...
substantially reduced, non-linear regression is more objective and parsimonious than generalized additive modelling (GAM) approaches (Tomlinson, 2019). First, we assessed the relationship describing the germination response over time for each experimental temperature using curvilinear log-logistic germination models (Lewandrowski et al., 2017; Tarszisz et al., 2017). The drc package (Ritz et al., 2012) was used to fit a three-parameter log-logistic function to germination data in the R statistical environment (R Core Team, 2013);

\[ \text{germination} = \frac{G_{\text{max}}}{1 + \frac{t_{50}}{b}} \]

where \( G_{\text{max}} \) is the upper limit for the germination rate, and the lower limit of germination rate is assumed to be 0 (Lewandrowski et al., 2017). The function also calculates a point around which the equation is symmetrical, \( t_{50} \), which is an estimate of the time required for 50% of the seeds (as a percentage of \( G_{\text{max}} \)) to germinate and \( b \) indicates the slope of the germination function at \( t_{50} \). First, we resolved a convergent common curve for the number of germinants over the number of seeds incubated for all species under all temperature regimes. By grouping this function by species and incubation temperature, unique values were fitted to the parameters of the function to produce several permutations of the basic model. We utilized the AICcmdaug package (Mazerolle, 2013) to assess the explanatory power of ‘species’ and ‘incubation temperature’ as factors contributing to variability in germination response (in terms of \( t_{50} \) and \( G_{\text{max}} \)) by comparing each permutation with the common curve using the Akaike information criterion (Burnham et al., 2002). The log-logistic model grouped into unique species and temperature categories was utilized to estimate \( t_{50} \) and \( G_{\text{max}} \) values for each replicate of all species incubated under different treatment regimes. We used model estimates for \( b \), \( G_{\text{max}} \) and \( t_{50} \) to calculate time (in days) to reach \( G_{\text{max}} \) for all replicates exposed to different treatment regimes.

**Temperature tolerance**

The precision of curvilinear modelling is dependent upon assumptions related to the shape of the curve (Tomlinson, 2019). Although thermal performance generally shows an asymmetrical increase with a single peak (Angilletta Jr, 2006; Peterson et al., 2007), appropriate non-linear thermal performance functions are yet to be described for seeds (Yan et al., 2017). The thermal response of maximum rate of growth in plants;

\[ r_{\text{max}} = R_{\text{max}} \left( \frac{T_{\text{max}} - T}{T_{\text{max}} - T_{\text{opt}}} \right) \left( \frac{T}{T_{\text{opt}}} \right)^{T_{\text{opt}}/T_{\text{max}} - T_{\text{opt}}} \]

where \( r_{\text{max}} \) is the maximum germination rate at any temperature (\( T \)), \( T_{\text{opt}} \) is the optimum temperature for germination at the peak of the performance function, \( T_{\text{max}} \) is the limit of thermal tolerance, where germination ceases, and \( R_{\text{max}} \) is the asymptotic maximum germination rate at \( T_{\text{opt}} \). Henceforth, \( 1/t_{50} \) will be referred to as the thermal performance of maximum germination rate, \( r_{\text{max}} \), as a proxy for the speed of germination across a temperature gradient. The thermal performance of maximum germination rate at the optimum temperature is characterized as \( R_{\text{max}} \). A major advantage in this approach is that each parameter of the above equation can be directly translated into a factor that has biological meaning. Therefore, these parameters can be readily compared across taxa to gain insights into patterns of variability in germination response.

**Water stress tolerance**

In the same way that seed germination should be inhibited by the thermal performance of enzyme function at specific thermal thresholds, it should be impeded by reduced water availability as well, and \( t_{50} \) for seeds should escalate exponentially with increasing water stress up to a species specific threshold at which the low water potential of the external environment prevents imbibition (Bradford, 2002). A pattern of exponential increase in \( t_{50} \) in response to increasing water stress is consistent with previous studies on multiple taxa native to the SWAFR (Cochrane, 2018). Consequently, we selected an exponential function with the minimal number of parameters required to simulate the water stress response of non-dormant seeds to fit the \( t_{50} \) estimates for our water stress response data;

\[ t_{50} = g_0 + e^{k(x+wc)} \]

where \( t_{50} \) is the time required to reach 50% germination under any water stress level, \( g_0 \) is the base value of \( t_{50} \) prior to the beginning of its exponential increase, \( k \) is a scaling exponent and \( wc \) is the critical water stress level at which \( t_{50} \) begins to escalate exponentially.

**Unique parameterization**

We fitted the appropriate physiological functions (thermal performance or hydrological performance) to the log-logistic model estimates using the thermPerf package (Bruneaux, 2017) in the R statistical environment (R Core Team, 2013) to identify a global model. Subsequent to this we employed the nls function to fit unique values to the parameters of the performance function on the basis of species, following Ritz and Streibig, (2008) to parameterize unique values of \( R_{\text{max}} \), \( T_{\text{opt}} \), \( T_{\text{max}} \), \( g_0 \) and \( wc \) for each species in terms of \( t_{50} \) and \( G_{\text{max}} \).

**Results**

**Germination modelling**

The two range-restricted species displayed higher final germination percentages over a wider range of temperatures...
than the two broadly distributed taxa (Fig. 2a). Both *Eucalyptus caesia* subsp. *caesia* and *E. ornata* exhibited relatively constant high final germination percentages (>80%) from 10 to 30°C, while final germination percentages of *E. salmonophloia* and *E. salubris* decreased from 98% to < 76% at 25°C (Fig. 2a). For the two range-restricted taxa, the minimum final germination percentage was observed at 10°C, whereas for the two widely distributed taxa minimum final germination occurred at 5°C (Fig. 2a). For all taxa except *E. caesia* subsp. *caesia*, the maximum temperature at which...
was not observed below the highest stress level at which germination occurred was 35°C (Fig. 2a). Within the range of 15–25°C, estimated time to reach $G_{\text{max}}$ was ≤ 30 days for most replicates of the four species (Fig. 3a). For *E. caesia* subsp. *caesia* and *E. ornata*, deviation from favourable temperatures increased variability in $G_{\text{max}}$ and lengthened the time required to reach $G_{\text{max}}$ (Fig. 3a). However, for *E. salubris* the time to reach $G_{\text{max}}$ was relatively consistent across 10–30°C (Fig. 3a).

The range-restricted *E. caesia* subsp. *caesia* and *E. ornata* were more tolerant of water stress than the two widely distributed taxa, in terms of final germination percentage. The final germination percentage of the two range restricted taxa exceeded 90% even at −0.4 MPa (Fig. 2b). Conversely, the final germination of *E. salmonophloia* and *E. salubris* seeds decreased to <80% at −0.1 and −0.4 MPa, respectively (Fig. 2b). For *E. caesia* subsp. *caesia* and *E. salmonophloia*, the highest stress level at which germination occurred was −1 MPa, whereas for *E. ornata* and *E. salubris* germination was not observed below −0.7 MPa (Fig. 2b). For all tested species, estimates for time to reach $G_{\text{max}}$ and variability of these estimates increased with rising water stress (Fig. 3b).

**Temperature tolerance**

The log-logistic curve incorporating both species and temperature regime was the best model to fit our thermal response data (AICc $= 25106.87$, $df = 76$, residual deviance $= 2.608$; Supplementary Material) indicating that both species and ‘incubation temperature’ were factors that contributed to variability in germination response (Supplementary Material). The log-logistic curve could not be fitted to the germination response data for 5 and 35°C since final germination percentages were very low (<31%) at these temperatures (Fig. 2a). The distribution of the $r_{\text{max}}$ values estimated by the log-logistic model for each species-by-temperature grouping across 10–30°C was hump shaped, increased exponentially with increasing temperature up to a peak, beyond which it decreased rapidly (Fig. 4a). The most parsimonious model resolved unique $r_{\text{max}}$, $T_{\text{opt}}$ and $T_{\text{max}}$ values defining the thermal performance of $r_{\text{max}}$ for each species (Equation 2, Fig. 4a). For *E. caesia* subsp. *caesia* and *E. ornata*, estimated $T_{\text{opt}}$ values were 25.4 ± 0.25 and 23.0 ± 0.37°C respectively, whereas for *E. salmonophloia* and *E. salubris* estimates for $T_{\text{opt}}$ were 17.7 ± 1.94 and 20.1 ± 0.97°C, respectively (Fig. 4a). The two widely distributed species had broader thermal tolerance ranges than the two range-restricted taxa, apparently reflecting a higher level of physiological plasticity (Fig. 4a). For all tested species, estimated $T_{\text{max}}$ was within the range of 30.5–32°C. A thermal performance function could not be resolved for the $G_{\text{max}}$ estimates of the log-logistic model (Fig. 3a) because they were highly conserved across all experimental temperatures.

**Water stress tolerance**

The best log-logistic function to fit our water stress response data was the permutation incorporating both species and water stress regime (the lowest AICc value $= 18869.09$, $df = 61$, residual deviance $= 6.503$; Supplementary Material). For *E. caesia* subsp. *caesia*, *E. ornata* and *E. salubris*, the $t_{50}$ values estimated by the log-logistic model were relatively constant up to a threshold water stress level, which was followed by an exponential rise in $t_{50}$ with increasing water stress (Fig. 4b). The exponential function fitted to the $t_{50}$ estimates resolved $g_0$, $k$ and $w_i$ estimates for the global model. However, the exponential model failed to resolve water stress response profiles on the basis of species. Therefore, unique values were fitted to the function parameters for each species-by-water stress regime separately (Fig. 4b). For *E. caesia* subsp. *caesia*, *E. ornata* and *E. salubris*, estimated $w_i$ values were $-0.266 \pm 0.098$, $-0.149 \pm 0.049$ and $-0.057 \pm 0.250$ MPa, respectively (Fig. 4b). However, this exponential model could not be fitted to the $t_{50}$ estimates for *E. salmonophloia* since final germination percentage declined to <10% for water stress regimes lower than −0.2 MPa (Figs. 2b and 4b). Furthermore, the exponential function could not be fitted to the $G_{\text{max}}$ estimates of the log-logistic model (Fig. 3b).

**Discussion**

The results of this study demonstrate that the thermal performance of the four selected taxa in terms of $t_{50}$ is hump-shaped, in accordance with established principles of thermal biology that germination response to temperature should resemble thermal performance curves of enzymes. The key elements captured by applying the Yan and Hunt (1999) model are the asymmetrical nature of the curves, and the ability to directly compare differences in the shape of these functions between different taxa. For example, our observations conform to the general models of stenoithermy and eurythermy in that the two range-restricted endemic taxa exhibited narrower thermal tolerance ranges than their co-occurring congenerics in terms of $t_{50}$. However, in terms of final germination percentage, the narrow-range endemics were more tolerant of thermal stress than the two widely distributed taxa. Our second hypothesis, that the range-restricted endemic taxa would be more sensitive to water stress, was not supported in terms of final germination percentage. However, it is not clear to what extent the four species differ in water stress tolerance in terms of $t_{50}$.

**Temperature and water stress tolerance**

The high seed viability that we observed is consistent with previous reports of high germination success in *Eucalyptus* species (a non dormant group; Baskin and Baskin, 2003) from across Australia when incubated under favourable thermal conditions (Bell et al., 1995; Ruiz-Talonía et al., 2018). According to the seed dormancy classification system proposed by Baskin and Baskin (2003), non-dormant species usually germinate within a period of 30 days under favourable environmental conditions. However, seed germination is a
physiological process that is limited to a temperature range suitable for normal metabolic activity (Bell et al., 1995, Jiménez-Alfaro et al., 2016). Bell et al. (1995) reported that in six species of Eucalyptus native to Western Australia, final germination percentage was highly variable, and Cochrane (2017) has reported that many Eucalyptus species native to southwestern Australia exhibit high plasticity to thermal stress in terms of final germination percentage. Our data did not provide strong support for these statements, in that, while temperature regimes beyond 10–20°C reduced mean

**Figure 3:** Estimates for maximum germination (G_max) and time to reach G_max predicted by a three-parameter log-logistic function for the germination responses of four Western Australian Eucalyptus species following incubation in constant darkness at varying temperature regimes (a) and water stress levels (b). Black dots depict G_max estimates and non-shaded dots represent time to reach G_max for each replicate of seeds following exposure to different treatment regimens. Eight replicates of 25 seeds were used for each treatment.
Figure 4: Thermal performance and water stress tolerance in four Western Australian Eucalyptus species in terms of time to reach 50% germination ($t_{50}$). (a) Dots represent $1/t_{50}$ estimates for each replicate of seeds after exposure to different temperature regimes and the smooth lines represent the permutations of the thermal performance curve fitted to the $1/t_{50}$ estimates ($r_{\text{max}}$) of each species. Coefficients for the permutations of the most parsimonious thermal performance function resolved on the basis of species are displayed. (b) Dots represent $t_{50}$ estimates for each replicate of seeds after exposure to different water stress regimes and the smooth lines represent the exponential models fitted to the $t_{50}$ estimates of each species. Coefficients for the most parsimonious water stress tolerance model fitted to the germination response data of each species are displayed. Eight replicates of 25 seeds were used for each treatment.

Final germination percentage in all species tested (Fig. 2a), more substantial influences could be seen on germination rate ($t_{30}$). Deviations from favourable temperature ranges for germination increased time to reach $G_{\text{max}}$ and variability in estimates for time to reach $G_{\text{max}}$ in all four taxa (Fig. 3a). It is possible that, at least insofar as understanding thermal constraints, maximum germination is a less informative functional trait (Saatkamp et al., 2019) than aspects of germination rate, and that, given a long enough window of opportunity, most non-dormant seeds will obtain high germination rates across a range of “sub-optimal” conditions, and it is the length of this window of opportunity that represents...
the selection pressure for thermal and drought tolerance in germination.

The $T_{\text{opt}}$ estimates for all four species were within a range of 17–26°C, and $T_{\text{max}}$ values were between 29 and 32 °C (Fig. 4a). Locations from which seeds for this study were collected are in a Mediterranean climate, characterized by hot dry summers and mild wet winters (Bell et al., 1993; Fig. 1). Consequently, it has been postulated that persistence of high soil moisture availability due to frequent rainfall events from late autumn through to early spring combined with low temperatures is likely to facilitate germination and seedling establishment of most local native species at this time of year (Bell et al., 1993). The $T_{\text{opt}}$ and $T_{\text{max}}$ estimates for the four taxa clearly reflect a preference for synchronizing germination between late autumn to early spring (Fig. 4a) and are consistent with previous reports that many *Eucalyptus* species from southwest Western Australia, including short-range endemic taxa, exhibit a low thermal optimum for germination (Bell, 1994; Bell et al., 1995). The coincidence of germination with periods of highest rainfall among species from Mediterranean climates is widely regarded as an adaptive mechanism for summer drought avoidance when conditions are far less favourable for supporting seedling growth and establishment (Luna et al., 2012; Clemente et al., 2017), and the data that we present here indicate that it can be parameterized according to the principles of thermal biology, at least insofar as rate-related germination traits are concerned.

Exposure to water stress reduced mean final germination percentages in all species tested in this study (Fig. 2b), consistent with previous studies of *Eucalyptus* species (Pearce et al., 1990), and the broader Western Australia flora (Cochrane, 2018; Turner et al., 2018).

**Patterns of distribution size and endemism**

In terms of final germination percentage, the two range-restricted endemic taxa were more tolerant of both thermal stress, represented by higher $T_{\text{opt}}$, and water stress, represented by lower $w_c$, compared to their widespread congeners (Fig. 2), but had narrower ranges of thermal tolerance in terms of $r_{\text{max}}$ (Fig. 4a). Of the four species, the broadly distributed *E. salmonophloia* and *E. salubris* were the most drought-sensitive, with critical thresholds at $-0.1$ and $-0.4$ MPa, respectively, compared to the critical threshold for *E. caesia* subsp. *caesia* and *E. ornata* at $-0.7$ MPa (Fig. 3b). We suggest that these adaptations to water stress relate to the below-ground environments that characterize the species’ preferred habitats: skeletal and shallow soils typical of rocky outcrops which retain water poorly, especially compared to the loamy soils that often surround these outcrops in Western Australia (Main, 1997; Mares, 1997). As well as generating extremely hot surface temperatures (Withers, 2000; Porembski and Barthlott, 2012), the water retention capacity of many habitats in outcrop environments is generally lower than the surrounding environment because the soils in these habitats are shallower compared to those of the surrounding matrix (Main, 1997; Mares, 1997). Furthermore, increased levels of evaporation due to high temperatures (especially in summer) can rapidly reduce the soil moisture availability of such microhabitats (Merritt et al., 2007) because outcrops are less shaded than the neighbouring vegetation matrix (Withers, 2000). In addition, summer rainfall events in southwest Western Australia are sporadic and therefore insufficient to increase and maintain soil water potential at levels favourable for seed germination and persistence of seedlings of most taxa (Cochrane, 2018). These elements of the physical environment conspire to limit the window of opportunity for germination on rocky outcrops, a constraint that we did not impose in our experimental germinations. Limitation of germination response to a narrow tolerance range in terms of $r_{\text{max}}$, combined with high drought tolerance in terms of $G_{\text{max}}$, and time to reach $G_{\text{max}}$ could be an adaptive strategy in range-restricted taxa such as *E. caesia* subsp. *caesia* and *E. ornata* to optimize recruitment success within a short period of opportunity in terms of high soil moisture availability following episodic rainfall events (Debat and David, 2001; Körner, 2003; Cochrane, 2018). *Eucalyptus salmonophloia* and *E. salubris* inhabit the relatively deep-soil environments surrounding granite outcrops (Yates et al., 1994). Low thermal and drought tolerance in terms of final germination percentage and the relatively low $r_{\text{max}}$ estimates that are consistent across a wide range of temperatures observed in *E. salmonophloia* and *E. salubris* may reflect a strategy for synchronizing seed germination with consistent rainfall during the cooler winter months under high and persistent soil moisture availability (Figs. 2 and 4a). Outside of the specific microhabitats of rocky outcrops, avoiding germination in summer is a strategy common among many species native to the deeper-soil environments surrounding granite outcrop habitats (Bell et al., 1993; Byrne and Hopper, 2008; Cochrane, 2017).

Our findings are in line with previous reports that the optimum temperature range for germination of widespread *Eucalyptus* species (in terms of final germination percentage) reflects the soil water regime of the habitat of each species (Bell et al., 1993). Moreover, the results of our study are consistent with the findings of previous studies that seeds of range-restricted taxa that are limited by a narrow window of opportunity to germinate (in terms of soil moisture availability) exhibit high physiological plasticity for thermal and drought stress tolerance, whereas the germination response of broadly distributed congeners living in less restrictive habitats is less plastic (Graves et al., 1988; Giménez-Benavides et al., 2005; Giménez-Benavides et al., 2013). In this sense, the data reported in our study suggest that the seed germination traits of species from restricted distributions are consistent with general theories of stenothermic specialization in other taxa (Seebacher and Franklin, 2005).

**Limitations to interpretation**

The experimental approach employed in this study can be utilized to identify optimum conditions and critical thresholds
for germination in other species of threatened flora (Clemente et al., 2017). However, in order to get deeper insights into the role of temperature and water stress as drivers of rarity and endemism, the above hypotheses require testing at the level of populations and individuals and the responses of a wider range of species should be compared (Mooney et al., 1961; Felsenstein, 1985; Luna et al., 2012). Nevertheless, our findings are broadly consistent with results reported on the basis of larger numbers of species (Cochrane, 2017; Ruiz-Talonia et al., 2018).

While a phylogenetic perspective is critical in making comparative interpretations of this kind, it is also important to understand the trait in question. We characterized seed germination in terms of temperature at zero water stress, and water stress at optimal temperature, as have other authors faced with limited numbers of seed available from rare or range-restricted taxa (Turner et al., 2018). More correctly, seed germination responds to a dynamic hydro-thermal niche (Hardegree et al., 2015), where the two factors interact. Characterising this interactive response may be more informative in a comparative sense, both within and between species. While eucalypts are canonically non-dormant, it is also important to assess the role of seed dormancy in determining variability in germination responses of other floral groups to thermal stress and drought stress as over 70% of native species possess seeds with some form of seed dormancy (Merritt et al., 2007). Indeed, seed dormancy in most cases is also regulated by critical moisture and temperature thresholds working as another layer of environmental filters rendering seeds non-dormant in response to specific soil conditions (Baskin and Baskin, 2003; Turner et al., 2018).

Conclusions

We have established that in non-dormant taxa germination response to thermal stress is hump-shaped in terms of time to reach 50% germination (t50) and that at least some seed germination traits are consistent with broader theories of thermal biology. Water stress, however, caused an exponential increase in t50, and the theoretical bases of this remain to be clarified. The four species differed significantly in terms of thermal performance and the two range-restricted endemic taxa had narrower thermal tolerance ranges, implying adaptive stenothermy, than their widespread, eurythermic congeners. The two-short range-endemics exhibited higher lability to temperature and drought stress compared to the two widespread species in terms of final germination percentage. The insights gained in this study could be beneficial for identifying thresholds for temperature and water stress tolerance in seeds of other flora of conservation concern.

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Conflicts of interest

All authors acknowledge that regarding this manuscript there is no conflict of interest to declare.

Authors’ contributions

All authors conceived the ideas and designed methodology; R.P.V.G.S.W.R. collected the data; S.T. and R.P.V.G.S.W.R. analysed the data; all authors contributed to the interpretation of the results; R.P.V.G.S.W.R. and S.T. led the writing, and all authors contributed to writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

References

Anacker BL, Whittall JB, Goldberg EE, Harrison SP (2011) Origins and consequences of serpentine endemism in the California flora. Evolution 65: 365–376.

Angilletta MJ Jr (2006) Estimating and comparing thermal performance curves. J Therm Biol 31: 541–545.

Angilletta MJ Jr, Angilletta MJ (2009) Thermal Adaptation: A Theoretical and Empirical Synthesis. Oxford University Press, Oxford

Ashford JR, Sowden RR (1970) Multi-variate probit analysis. Biometrics 26: 535–546.

Baskin JM, Baskin CC (2003) Classification, biogeography, and phylogenetic relationships of seed dormancy. In RD Smith, JB Dickie, SH Linington, HW Pritchard, RJ Probert, eds, Seed Conservation: Turning Science into Practice. (eds rd smith, jb dickie, sh linington, hw pritchard, rj probert). The Royal Botanic Gardens, Kew, London

Bell DT (1994) Interaction of fire, temperature and light in the germination response of 16 species from the Eucalyptus marginata forest of South-Western Australia. Aust J Bot 42: 501–509.

Bell DT, Plummer JA, Taylor SK (1993) Seed germination ecology in southwestern Western Australia. Bot Rev 59: 24–73.

Bell DT, Rokich DP, McChesney CJ, Plummer JA (1995) Effects of temperature, light and gibberellic acid on the germination of seeds of 43 species native to Western Australia. J Veg Sci 6: 797–806.
Bezemer N, Krauss SL, Roberts DG, Hopper SD (2019) Conservation of old individual trees and small populations is integral to maintain species’ genetic diversity of a historically fragmented woody perennial. *Mol Ecol* 28: 3339–3357.

Bradford KJ (2002) Applications of hydrothermal time to quantifying and modeling seed germination and dormancy. *Weed Sci* 50: 248–260.

Bruneaux M (2017). Thermerperf: Model fitting for thermal performance curves: R package version 0.0.1, Ed

Burnham KP, Anderson DR (2002) Model selection and multimodel inference: A practical information-theoretic approach. Springer, New York.

Byrne M, Hopper SD (2008) Granite outcrops as ancient islands in old landscapes: evidence from the phyllogeography and population genetics of *Eucalyptus caesia* (Myrtaceae) in Western Australia. *Biol J Linn Soc* 93: 177–188.

Carter A, Bedini G, Müller J, Probert R (2013) Comparative seed dormancy and germination of eight annual species of ephemeral wetland vegetation in a Mediterranean climate. *Plant Ecol* 214: 339–349.

Clemente AS, Müller JV, Almeida E, Costa CA, Dias SL, Brehm JM, Rebelo R, Martins-Loução MA (2017) What can routine germination tests in seed banks tell us about the germination ecology of endemic and protected species? *Botany* 95: 673–684.

Coates DJ, Atkins KA (2001) Priority setting and the conservation of Western Australia’s diverse and highly endemic flora. *Biol Conserv* 97: 251–263.

Cochrane A (2017) Modelling seed germination response to temperature in *Eucalyptus L’Her.* (Myrtaceae) species in the context of global warming. *Seed Sci Res* 27: 99–109.

Cochrane A (2018) Salt and waterlogging stress impacts on seed germination and early seedling growth of selected endemic plant species from Western Australia. *Plant Ecol* 219: 633–647.

Cross AT, Barrett MD, Turner SR, Dixon KW, Merritt DJ (2018) Seed dormancy depth is partitioned more strongly among habitats than among species in tropical ephemerals. *Aust J Bot* 66: 230–242.

Cross AT, Turner SR, Merritt DJ, Niekerk A, Renton M, Dixon KW, Mucina L (2015a) Vegetation patterns and hydro-geological drivers of freshwater rock pool communities in the monsoon-tropical Kimberley region, Western Australia. *J Veg Sci* 26: 1184–1197.

Cross AT, Turner SR, Renton M, Baskin JM, Dixon KW, Merritt DJ (2015b) Seed dormancy and persistent sediment seed banks of ephemeral freshwater rock pools in the Australian monsoon tropics. *Ann Bot* 115: 847–859.

Debat V, David P (2001) Mapping phenotypes: canalization, plasticity and developmental stability. *Trends Ecol Evol* 16: 555–561.

Do Carmo FF, Jacobi CM (2016) Diversity and plant trait-soil relationships among rock outcrops in the Brazilian Atlantic rainforest. *Plant Soil* 403: 7–20.

Elliott CP, Lewandrowski W, Miller BP, Barrett M, Turner SR (2019) Identifying germination opportunities for threatened plant species in episodic ecosystems by linking germination profiles with historic rainfall events. *Aust J Bot* 67: 256–267.

Erickson TE, Merritt DJ (2016) Seed collection, cleaning, and storage procedures. In ‘Pilbara Seed Atlas and Field Guide: Plant Restoration in Australia’s Arid Northwest’. CSIRO Publishing, Melbourne, pp. 7–1.

Felsenstein J (1985) Phylogenies and the comparative method. *The American Naturalist* 125: 1–15.

Gates S (2016) *Chemical Kinetics.* Willford Press, Forest Hills, NY, USA.

Ghalambor CK, McKay JK, Carroll SP, Reznick DN (2007) Adaptive versus non-adaptive phenotypic plasticity and the potential for contemporary adaptation in new environments. *Funct Ecol* 21: 394–407.

Gibson N, Yates CJ, Dillon R (2010) Plant communities of the ironstone ranges of South Western Australia: hotspots for plant diversity and mineral deposits. *Biodivers Conserv* 19: 3951–3962.

Giménez-Benavides L, Escudero A, Pérez-García F (2005) Seed germination of high mountain Mediterranean species: altitudinal, interpopulation and interannual variability. *Ecol Res* 20: 433–444.

Giménez-Benavides L, Milla R (2013) Comparative germination ecology of two altitudinal vicariant *Saixifraga* species endemic to the north of Spain. *Plant Biol* 15: 593–600.

Graves J, Taylor K (1988) A comparative study of *Geum rivale* L. and *G. urbanum* L. to determine those factors controlling their altitudinal distribution. *New Phytol* 110: 391–397.

Hargreaves SP, Walters CT, Boehm AR, Ossoy PJ, Clark PE, Pierson FB (2015) Hydrothermal germination models: comparison of two data-fitting approaches with probit optimization. *Crop Sci* 55: 2276–2290.

Hopper S, Brown A, Marchant N (1997) Plants of Western Australian granite outcrops. *JR Soc West Aust* 80: 141–158.

Hopper SD, Gioia P (2004) The southwest Australian floristic region: distribution. *Aust J Bot* 66: 339–647.

Huey RB, Kingsolver JG (1989) Evolution of thermal sensitivity of ectotherm performance. *Trends Ecol Evol* 4: 131–135.

Jacobi CM, Do Carmo FF, Vincent RC, Stehmann JR (2007) Plant communities on ironstone outcrops: a diverse and endangered Brazilian ecosystem. *Biodivers Conserv* 16: 2185–2200.

Jacobi CM, Fonseca do Carmo F (2008) The contribution of ironstone outcrops to plant diversity in the iron quadrangle, a threatened Brazilian landscape. *AMBO: A Journal of the Human Environment* 37: 324–326.

James JJ, Sheley RL, Erickson T, Rollins KS, Taylor MH, Dixon KW (2013) A systems approach to restoring degraded drylands. *J Appl Ecol* 50: 730–739.

Jeremiah TS, Mohler CL (2002) Evaluating seed viability by an unimbibed seed crush test in comparison with the tetrazolium test. *Weed Technol* 16: 781–786.
Jiménez-Alfaro B, Silveira FA, Fidelis A, Poschlod P, Commander LE (2016) Seed germination traits can contribute better to plant community ecology. J Veg Sci 27: 637–645.

Körner C (2003) Alpine Plant Life: Functional Plant Ecology of High Mountain Ecosystems. Springer-Verlag, Berlin Heidelberg.

Lakon G (1949) The topographical tetrazolium method for determining the germinating capacity of seeds. Plant Physiol 24: 389–394.

Lewandrowski W, Erickson TE, Dixon KW, Stevens JC (2017) Increasing the germination envelope under water stress improves seedling emergence in two dominant grass species across different pulse rainfall events. J Appl Ecol 54: 997–1007.

Liu F-H, Yu F-H, Liu W-S, Krüsi BQ, Cai X-H, Schneller JJ, Dong M (2007) Large clones on cliff faces: expanding by rhizomes through crevices. Ann Bot 100: 51–54.

Lloret F, Peñuelas J, Estiarte M (2004) Experimental evidence of reduced diversity of seedlings due to climate modification in a Mediterranean-type community. Glob Change Biol 10: 248–258.

Luna B, Pérez B, Torres I, Moreno J (2012) Effects of incubation temperature on seed germination of Mediterranean plants with different geographical distribution ranges. Journal of the Institute of Botany, Academy of Sciences of the Czech Republic 47: 17–27.

Main B (1997) Granite outcrops: a collective ecosystem. J R Soc West Aust 80: 113–122.

Mares M (1997) The geobiological interface: granite outcrops as a selective force in mammalian evolution. J R Soc West Aust 80: 131–139.

Mazerolle M (2013) Accmmodavg: model selection and multimodel inference based on (q) aicc. R package version 1: 32.

Merritt D, Turner S, Clarke K, Dixon D (2007) Seed dormancy and germination stimulation syndromes for Australian temperate species. Aust J Bot 55: 336–344.

Michel BE (1983) Evaluation of the water potentials of solutions of polyethylene glycol 8000 both in the absence and presence of other solutes. Plant Physiol 72: 66.

Mooney HA, Billings W (1961) Comparative physiological ecology of arctic and alpine populations of Oxyria digyna. Ecol Monogr 31: 1–29.

Pearce P, Van Der Moezel P, Bell D (1990) Seed germination under salinity stress in western australian species of eucalyptus. Seed Sci Technol 18: 113–118.

Peterson ME, Daniel RM, Danson MJ, Eisenthal R (2007) The dependence of enzyme activity on temperature: determination and validation of parameters. Biochem J 402: 331–337.

Porembski S, Barthlott W (2000) Granitic and gneissic outcrops (inselbergs) as centers of diversity for desiccation-tolerant vascular plants. Plant Ecol 151: 19–28.

Porembski S, Barthlott W (2012) Inselbergs: Biotic Diversity of Isolated Rock Outcrops in Tropical and Temperate Regions. Springer, Berlin.
ogy and effect of cold stratification on germination. Seed Sci Res 7: 47–58.

Wege J, Thiele K, Shepherd K, Butcher R, Macfarlane T, Coates D (2015) Strategic taxonomy in a biodiverse landscape: a novel approach to maximizing conservation outcomes for rare and poorly known flora. Biodivers Conserv 24: 17–32.

Western Australian Herbarium (1998) Florabase – the Western Australian Flora, Department of Parks and Wildlife. Perth, W.A.

Withers P (2000) Overview of granite outcrops in Western Australia. JR Soc West Aust 83: 103–108.

Wyatt R (1997) Reproductive ecology of granite outcrop plants from the south-eastern United States. JR Soc West Aust 80: 123–129.

Yan W, Hunt L (1999) An equation for modelling the temperature response of plants using only the cardinal temperatures. Ann Bot 84: 607–614.

Yates CJ, Hobbs RJ, Bell RW (1994) Landscape-scale disturbances and regeneration in semi-arid woodlands of southwestern Australia. Pac Conserv Biol 1: 214–221.

Yates CJ, Hopper SD, Brown A, Leeuwen S (2003) Impact of two wildfires on endemic granite outcrop vegetation in Western Australia. JVeg Sci 14: 185–194.

Yates CJ, Ladd PG (2004) Breeding system, pollination and demography in the rare granite endemic shrub Verticordia staminosa ssp. staminosa in south-west Western Australia. Aust Ecol 29: 189–200.