

Modelling seed germination response to temperature in *Eucalyptus* L'Her. (Myrtaceae) species in the context of global warming

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Abstract

Seed germination is vital for persistence of many plant species, and is linked to local environmental conditions. Small increases in temperature during this critical life history transition may threaten species by altering germination timing and success. Such changes in turn may influence population dynamics, community composition and the geographic distributions of species. In this investigation, a bi-directional temperature gradient plate was used to profile thermal constraints for germination in 26 common, threatened and geographically restricted *Eucalyptus* species (Myrtaceae) from southern Western Australia. These observed data were used to populate models to predict optimum germination responses (mean time to germination, germination timing and success) under current (1950–2000 averages) and future (2070 high greenhouse gas emission climate scenario) mean monthly minimum and maximum temperatures. Many species demonstrated wide physiological tolerance for high germination temperatures and an ability to germinate outside current and forecast future autumn–winter wet season temperatures, suggesting that climatic distribution is a poor proxy for thermal tolerance for *Eucalyptus* seed germination. Germination for some species is predicted to decline under forecast conditions, but the majority will maintain or improve germination particularly during the cooler winter months of the year. Although thermal tolerance may benefit persistence of many *Eucalyptus* species in southern Western Australia as warming becomes more severe, large rainfall declines are also forecast which may prove more detrimental to plant survival. Nonetheless, this framework has the potential to identify seed resilience to heat stress in an early life history phase and hence species vulnerability to one characteristic of forecast environmental change.

Keywords: *Eucalyptus*, global warming, mean time to germination, percentage germination, temperature gradient plate, Western Australia

Introduction

Eucalyptus L'Her. is the largest genus in the family Myrtaceae with over 800 species almost entirely restricted to Australia. It is the dominant tree in many vegetation communities and a genus with high commercial value for timber and wood pulp, in addition to being of great ecological importance as a provider of habitat and food for many faunal species. A high degree of regional endemism is evident within the species (Brooker, 2000).

Global warming is predicted to impact heavily on many of these *Eucalyptus* species, with suggestions that more than 40% of the realized climatic niches of most species from across the country have ranges of less than 2°C mean annual temperature, including 25% with less than 1°C (Hughes *et al.*, 1996). Most studies have based their findings on modelling of past and projected species distributions (Dalmaris *et al.*, 2015), or concentrated on the climate tolerances of mature plants (Butt *et al.*, 2013), yet few have taken into account the disparity between the fundamental and realized niche characteristics of species tolerances (Booth, 2016). Evidence exists to show that many *Eucalyptus* can grow successfully outside their natural distributions at warmer mean annual temperatures (Butt *et al.*, 2013; Booth, 2015; Booth *et al.*, 2015) and are highly plastic in traits related to maintaining fitness under novel conditions (Byrne *et al.*, 2013; McLean *et al.*, 2014). Adult plants may have the capacity to buffer extremes of climate through physiological resistance or phenotypic plasticity (for example, changes in water use efficiency and specific leaf area) (Nicotra *et al.*, 2010; Drake *et al.*, 2014) but it is thought that long-lived tree species are unlikely to track rapidly changing environments effectively due to slow

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response times to changing climates (Pearson and Dawson 2003; Butt *et al.*, 2013). The reproductive stages of a plant's life cycle often represent a major bottleneck for species persistence, and tolerance in this early developmental stage is critical for survival, more so than at the adult stage (Watkinson, 1997; Lloret *et al.*, 2004). Studying germination is therefore essential for understanding plant distributions across multiple scales.

Germination is a physiological process that cannot occur outside the range of temperatures for normal metabolic function. Seeds of many plant species generally germinate over a range of temperatures and within this range there will be an optimal temperature (or temperatures), below and above which germination is delayed or depressed, but not prevented (Mott and Groves, 1981). Understanding the ability of seeds to cope with changes to temperature signals involves research into seed sensitivity and tolerance to 'novel' conditions. Most Western Australian *Eucalyptus* species are thought to exhibit highly variable germination responses to environmental factors (Bell *et al.*, 1993; Bell *et al.*, 1995). In the Mediterranean-type climate (i.e. winter wet, summer dry) zone of southern Western Australia species are usually considered to have low-temperature optima for germination that coincides with period of highest moisture availability; although resprouting species appear to be less sensitive to temperature conditions for germination than obligate seeders in the region (Bell *et al.*, 1995). By comparison, species from south-eastern Australia are considered to be more influenced by habitat and geographical range, particularly at elevation (Battaglia, 1993; Battaglia, 1996; Mok *et al.*, 2012).

Changes to climate have the capacity to introduce new threats and speed up existing declines (Watson, 2016). In southern Western Australia, a changing climate is expected to impact heavily on the biota (Abbott and Le Maitre, 2010). By 2070 the region may experience mean annual temperature increases of up to 3°C and rainfall declines of 20–30% (Bates *et al.*, 2012). These warmer drier conditions will alter the environmental cues that seeds rely on for germination, probably causing shifts in timing of recruitment and its success (Walck *et al.*, 2011). Indeed, future climates may well exceed environmental tolerances of many species. These changes may have significant impacts on ecosystems in this already seasonally dry region, with cascading effects on species distributions, abundance, composition and health, including consequences for the persistence of dependent fauna.

The specific objectives of this study were (1) to identify the thermal thresholds for germination in a selection of common and threatened and geographically restricted (i.e. conservation-listed) *Eucalyptus* species from southern Western Australia; and (2) to use these

data to model the impact of forecast higher regional temperatures on the timing and probability of germination. This combined approach seeks to offer vulnerability predictions for *Eucalyptus* species in the light of regional global warming scenarios.

Materials and methods

Study species

Twenty-six *Eucalyptus* species with varying life history characteristics and conservation status were selected for investigation (Fig. 1; Table 1). Seeds were collected from the wild across southern Western Australia over a period of 10 years from a broad range of individuals and subsequently stored under genebank conditions (cool and dry) until use. *Eucalyptus* seeds are generally held in the canopy in woody capsules. The seeds are mostly small and lack any feature that aids seed dispersal (Boland *et al.*, 1980). Seed dormancy is not a common characteristic of Western Australian *Eucalyptus* species (Bell, 1999), unlike some cool climate species from higher elevations in eastern Australia that may be dormant on dispersal and require cold chilling to overcome dormancy (Battaglia, 1993; Battaglia, 1997; Close and Wilson, 2002; Mok *et al.*, 2012). Seed release occurs when capsules dry and valves open and can be accelerated by drought or fire. Mature, viable *Eucalyptus* seeds from the region generally germinate freely once the requisite conditions of temperature, moisture and substrate combine to stimulate germination.

Environmental variables

The average monthly minimum and maximum temperatures for each seed source site were used as proxies for thermal tolerance and were obtained from WorldClim, a set of global climate layers with a spatial resolution of approximately 1 km² (Hijmans *et al.*, 2005). The data for 'current' conditions were derived from 1950–2000 averages. Future projections for the same climate variables were downloaded from the downscaled Hadley Centre Global Environment Model version 2 (HadGEM2-ES; <http://www.metoffice.gov.uk/research/modelling-systems/unified-model/climate-models/hadgem2>) using a high greenhouse gas emission scenario (Representative Concentration Pathway, RCP85) for 2070. The RCP85 scenario reflects high energy demand and greenhouse gas emission without climate change policies (Moss *et al.*, 2010). This scenario is extreme but reflects a likely climate outcome given the current level of mitigation activity. The HadGEM2-ES model (Jones *et al.*, 2011) includes dynamic vegetation, ocean biology and atmospheric chemistry, and has previously been used in simulating

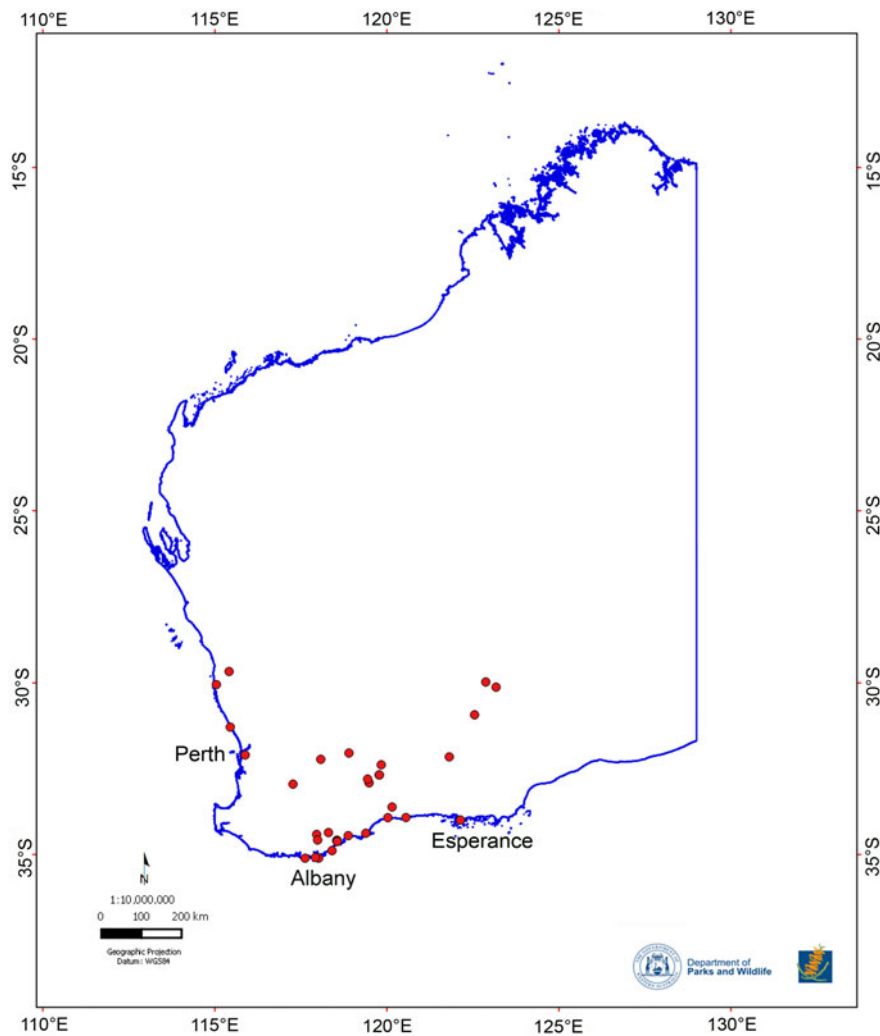


Figure 1. The location of 26 study species in the south west of Western Australia.

germination response in native species (Fernández-Pascual *et al.*, 2015; Cochrane, 2016), including use in the Fifth Assessment Report of the Intergovernmental Panel on Climate Change (IPPC, 2013).

Experimental design

A bi-directional temperature gradient plate (TGP) (GRD1, Grant Instruments, Cambridge, UK) was used to deliver 196 different temperature combinations (constant and alternating) simultaneously. The TGP method was chosen in order to detect thresholds more accurately than step-wise temperatures delivered by incubators, characterizing a fuller spectrum of responses to future temperature scenarios. Seeds were sown in 30 mm plastic Petri dishes on 0.75% w/v water agar. Seed numbers per dish ranged from 14 to 20 depending on species, and each species was exposed to 49 temperature combinations with a 12-h photoperiod for 6 weeks (four species per run of the TGP). There was no capacity to replicate

temperature conditions on the TGP but each species was represented by between *ca* 700–1000 seeds. Seed germination was checked three times weekly and germinated seeds showing a radicle at least double the length of the seed were removed. Percentage germination was calculated as the percentage of viable seeds that germinated within the incubation period. Seeds with a hard, white endosperm were considered potentially viable; empty seeds were removed from the original count. The experiments were carried out over a period of years from 2007 to 2016.

Data analysis

To define the response curves for each species and identify the optimal average and diurnally alternating temperature conditions for germination (percentage germination and mean time to germination), regression models were fitted to the empirical data from the TGP experiment as per Cochrane (2016). The proportion of seeds that germinated per dish and mean

Table 1. *Eucalyptus* species investigated in this study, their conservation status, fire response, seed weight and provenance

| Species | Conservation status | MAT (°C) | Latitude (S) | Longitude (E) | Fire response | 1000 seed weight (g) | Region |
|---|---------------------|----------|--------------|---------------|---------------|----------------------|------------|
| <i>E. aequiperta</i> | | 17.3 | 32.039 | 118.898 | Resprout | 0.24 | Wheat belt |
| <i>E. argutifolia</i> | DRF | 18.5 | 31.285 | 115.448 | Resprout | 0.98 | Coastal |
| <i>E. burdettiana</i> | DRF | 16.6 | 33.923 | 120.029 | Resprout | 4.41 | Coastal |
| <i>E. calcicola</i> subsp. <i>unita</i> | P4 | 15.1 | 35.105 | 117.623 | Resprout | 4.00 | Coastal |
| <i>E. conferruminata</i> | | 16.0 | 35.106 | 118.017 | Seeder | 2.22 | Coastal |
| <i>E. crispata</i> | DRF | 19.7 | 29.664 | 115.415 | Resprout | 0.56 | Inland |
| <i>E. erythrocorys</i> | | 19.4 | 30.046 | 115.045 | Resprout | 12.50 | Coastal |
| <i>E. georgei</i> subsp. <i>fulgida</i> | P4 | 16.7 | 32.382 | 119.837 | Seeder | 0.98 | Wheat belt |
| <i>E. insularis</i> | DRF | 15.9 | 33.992 | 122.139 | Resprout | 1.58 | Coastal |
| <i>E. jimberlanica</i> | P1 | 17.0 | 32.152 | 121.815 | Resprout | 1.09 | Inland |
| <i>E. kondininensis</i> | | 15.9 | 32.946 | 117.271 | Seeder | 0.42 | Wheat belt |
| <i>E. kruseana</i> | P4 | 17.7 | 30.927 | 122.550 | Resprout | 0.45 | Goldfields |
| <i>E. livida</i> | | 16.1 | 32.678 | 119.782 | Resprout | 0.49 | Wheat belt |
| <i>E. marginata</i> | | 18.4 | 32.097 | 115.882 | Resprout | 9.27 | Coastal |
| <i>E. megacornuta</i> | | 15.9 | 33.613 | 120.152 | Seeder | 2.61 | Coastal |
| <i>E. myriadena</i> | | 16.7 | 32.799 | 119.441 | Resprout | 0.16 | Wheat belt |
| <i>E. newbeyi</i> | P3 | 15.9 | 34.448 | 118.878 | Seeder | 1.43 | Coastal |
| <i>E. nigrifunda</i> | P4 | 19.0 | 29.970 | 122.874 | Seeder | 0.35 | Goldfields |
| <i>E. nutans</i> | DRF | 16.3 | 34.377 | 119.388 | Seeder | 0.44 | Coastal |
| <i>E. pimpiniana</i> | P3 | 18.6 | 30.120 | 123.174 | Resprout | 1.92 | Goldfields |
| <i>E. preissiana</i> subsp. <i>lobata</i> | P4 | 17.0 | 33.920 | 120.555 | Resprout | 3.83 | Coastal |
| <i>E. rugulata</i> | P4 | 16.1 | 32.678 | 119.777 | Seeder | 1.43 | Wheat belt |
| <i>E. spathulata</i> subsp. <i>salina</i> | P3 | 17.0 | 32.223 | 118.079 | Seeder | 0.56 | Wheat belt |
| <i>E. staeri</i> | | 15.5 | 34.884 | 118.407 | Resprout | 9.05 | Montane |
| <i>E. subtilis</i> | | 16.3 | 32.910 | 119.483 | Resprout | 0.28 | Wheat belt |
| <i>E. talyuberlup</i> | | 14.7 | 34.413 | 117.959 | Resprout | 2.72 | Montane |

DRF denotes Declared Rare Flora gazetted under the Western Australian Wildlife Act 1950. Species listed as P1–P4 are data deficient and ranked in order of priority for survey and evaluation of conservation status so that consideration can be given to their declaration as threatened flora. MAT denotes mean annual temperature.

time to germination were the dependent variables in logistic and ordinary linear regression models, respectively. Each seed was treated as an independent unit in the logistic regression (i.e. it could either germinate or not). Percentage germination was calculated as the percentage of seeds that germinated within the incubation period and used in the ordinary linear regression models. Mean time to germination (MTG) was calculated for all temperatures (where germination occurred) using the equation: $MTG = (n \times d)/N$, where n = number of seeds germinated between scoring intervals; d = the incubation period in days at that time point; and N = total number of seeds germinated. In addition, time to the onset (lag) of first germination (T_0) was also calculated. Simple linear regression was used to examine the relationship between the mean of the diurnal temperatures that provided most rapid and complete germination (defined as mean T_{opt}) and T_0 and MTG. Simple linear regression was also used to investigate the relationship between the aforementioned germination parameters and latitude, and seed size.

Four alternative models were built to assess seed response to forecast warming for the region. The

temperature values for each cell of the gradient plate (diurnal temperatures, average of day/night temperatures, and amplitude of temperature range) were used as the independent variables to populate the models. Model 1 used the day and night temperatures within each cell as independent variables; model 2 used mean temperature in each cell as the independent variable; model 3 used the amplitude of temperature fluctuation in each cell; and model 4 used a combination of mean and amplitude of temperature. To allow for possible non-linear responses to temperature, the squares of temperature variables were included in each model. In all models a binary variable was included denoting the timing of light during the diurnal cycle (i.e. whether light coincided with the warm or cool part of the cycle) as one half of the cells on the temperature gradient plate had both day and night phases when temperature was higher (the latter being less meaningful, ecologically). Once the best-fitting of the four alternate models for germination was determined (based on the lowest deviance value), the optimal temperature conditions (day/night temperature cycle) for each species were estimated.

Finally, using the best-fitting models described above, the projected responses of each species under current and forecast monthly average minimum and maximum temperature conditions were estimated, thus providing a germination response for each month of the year. Statistical analysis was conducted in GenStat (16th edition, VSN International, Hemel Hempstead, UK); contour plots showing germination on the bi-directional temperature gradient plate were created using Origin 9.1 (OriginLab Corporation).

Results

Empirical data

The germination temperature profiles for many species revealed surprising tolerance to high diurnal temperatures for germination (Supplementary Fig. 1). Final cumulative germination was high (80–100%) under optimum mean temperature (mean T_{opt}) and ranged from 13 to 36°C (*E. georgei* and *E. erythrocorys*, respectively) (Table 2). This mean optimum temperature for germination was considered to be the mean of the diurnal temperatures under which highest germination occurred in the shortest possible time. The amplitude of the diurnal fluctuations ranged from 0°C (constant temperatures) to 23°C. Germination was retarded above and below the optimum temperatures but seeds could remain moist at sub- and supra-optimal temperatures for prolonged periods without losing viability.

First germination (T_0) occurred between 3 and 17 days (*E. erythrocorys* and *E. kondinensis*, respectively). The mean time to maximum germination at the optimum temperature (MTG at T_{opt}) ranged from 3 days (*E. erythrocorys*) to 23 days (*E. aequiperta* and *E. calcicola* subsp. *unita*). Seed size had no significant bearing on time to first germination, mean time to germination or temperature for optimum germination for the 26 study species (data not shown). There was, however, a strong negative relationship between mean T_{opt} for commencement of germination (T_0) ($P < 0.001$; $r^2 = 0.6889$) (Fig. 2A). This germination lag time was greatest at lower temperatures with germination commencement more rapid at warmer mean temperatures. There was likewise a negative relationship between mean T_{opt} and mean time to germination (MTG) ($P < 0.001$; $r^2 = 0.3881$), with time to complete germination more rapid at higher temperatures (Fig. 2B). There was no correlation between mean optimum temperature for germination and mean annual temperature or latitude of seed source sites (data not shown), suggesting a lack of adaptation to the local temperature environment for germination.

Modelling of future climate response

The strongest fit to the observed data for the majority of species integrated the mean and amplitude of temperature fluctuations (model 4), though model 1 (diurnal temperatures and their squares) was also a reasonable fit for many species. Models based on amplitude or average temperatures alone were not good predictors of seed response to higher temperature. Under current temperature conditions (1950–2000 averages) the best-fitting models predicted that optimum temperatures for most rapid and complete germination should occur between February and July depending on species, but in general, these models predicted that germination would be lower and slower than the observed data (Table 2). Under the high emission scenario for 2070 the models still predicted germination timing to be optimal between February and July, but a number of species would shift timing because of forecast temperature changes (see Supplementary Fig. 2). For *E. erythrocorys* optimum germination conditions were predicted to advance from February to March; *E. subtilis* and *E. livida* from March to April; *E. marginata* from April to May; *E. spathulata* from April to June; and *E. newbeyi* from June to July. In contrast, the models predict a reverse for *E. pimpiniana* with optimum germination timing retreating from April to March. Although temperatures are likely to be hotter in March compared with April, the forecast increase in summer rainfall in the locality of this species may indicate that soil will be sufficiently moist for germination to be successful during that time. Optimum germination timing for the remaining species is not predicted to shift. Mean times to germinate at T_{opt} will be reduced by several days for many species. Optimum diurnal temperatures for maximum germination plotted for each of the 26 species illustrate the shift in the germination temperature niche from observed to predicted current to forecast 2070 temperature conditions (Fig. 3). Generally, the models suggest that warmer temperatures will benefit some species and not others and conservation species will not be disproportionately impacted (Fig. 4).

The pattern of germination response over the course of a yearly cycle of diurnal temperatures is similar for many of the species: reasonably high to high levels of germination both under current and future predictions coupled with little to no change in mean time to germination (Supplementary Fig. 2). Exceptions such as *E. calcicola*, *E. livida*, *E. staeri*, *E. myriadena*, *E. nigrifunda* and *E. subtilis* show a preference for warmer months for germination but will also increase germination under the 2070 scenario. The models predict that *E. marginata* germination levels will decline in the warmer summer months but increase over winter in the future. The species predicted to be most likely to decline with warmer temperatures is *E. kondinensis*, a common resprouting species from the western part of the region. The contour

Table 2. Observed and modelled temperature conditions (diurnal and mean) and subsequent germination responses (percentage germination and mean time to germination, MTG) for 26 *Eucalyptus* species

| Species | Observed (TGP) | | | | | Predicted current (1950–2000) | | | | Modelled future (2070) | | | |
|---|--------------------------|-----------------------|--------------------|------|-----------------|----------------------------------|-------------------------------|------------------|-------------------------|-------------------------------|----------------------------|---------------|----------------------|
| | Diurnal temperature (°C) | Mean temperature (°C) | MTG T ₀ | days | Germination (%) | Current diurnal temperature (°C) | Current mean temperature (°C) | Current MTG days | Current germination (%) | 2070 diurnal temperature (°C) | 2070 mean temperature (°C) | 2070 MTG days | 2070 germination (%) |
| <i>E. aequiperta</i> | 17/12 | 14 | 14 | 23.4 | 80 | 17/6 | 17 | 28 | 38 | 20/8 | 14 | 25.6 | 36 |
| <i>E. argutifolia</i> | 18/15 | 16 | 11 | 15.7 | 100 | 19/10 | 14 | 27.8 | 74 | 21/12 | 17 | 25.4 | 72 |
| <i>E. burdettiana</i> | 25/22 | 24 | 7 | 7 | 100 | 28/15 | 28 | 15.4 | 89 | 30/17 | 24 | 15 | 90 |
| <i>E. calcicola</i> subsp. <i>unita</i> | 26/25 | 26 | 5 | 23.3 | 100 | 24/14 | 24 | 24 | 80 | 26/16 | 21 | 21 | 84 |
| <i>E. conferruminata</i> | 31/25 | 28 | 4 | 6.9 | 100 | 25/15 | 25 | 14.6 | 94 | 26/17 | 22 | 13.5 | 94 |
| <i>E. crispata</i> | 26/25 | 25 | 3 | 7.6 | 100 | 28/14 | 13 | 16.4 | 86 | 31/18 | 13 | 13.8 | 88 |
| <i>E. erythrocorys</i> | 41/31 | 36 | 3 | 3 | 100 | 33/18 | 33 | 6 | 99 | 34/20 | 27 | 5.7 | 99 |
| <i>E. georgei</i> subsp. <i>fulgida</i> | 15/11 | 13 | 14 | 19.6 | 100 | 15/5 | 15 | 31 | 63 | 19/8 | 14 | 30 | 57 |
| <i>E. insularis</i> | 22/12 | 17 | 14 | 18.4 | 100 | 18/8 | 18 | 23.6 | 85 | 19/10 | 15 | 22.7 | 85 |
| <i>E. jimberlanica</i> | 12/12 | 12 | 14 | 19.4 | 95 | 16/7 | 16 | 25.1 | 37 | 19/9 | 14 | 21.8 | 35 |
| <i>E. kondininensis</i> | 14/12 | 13 | 17 | 10.6 | 100 | 15/6 | 15 | 30.5 | 82 | 18/8 | 13 | 28 | 69 |
| <i>E. kruseana</i> | 20/17 | 19 | 10 | 12.6 | 100 | 16/6 | 16 | 28 | 78 | 20/8 | 14 | 24 | 76 |
| <i>E. livida</i> | 14/12 | 13 | 13 | 18.4 | 100 | 27/13 | 27 | 29.4 | 71 | 27/14 | 21 | 29.2 | 76 |
| <i>E. marginata</i> | 21/20 | 20 | 10 | 12.6 | 100 | 25/14 | 25 | 19.6 | 84 | 24/14 | 19 | 19.5 | 85 |
| <i>E. megacornuta</i> | 20/17 | 18 | 9 | 13.8 | 100 | 23/11 | 23 | 23.7 | 89 | 26/14 | 20 | 22.6 | 90 |
| <i>E. myriadena</i> | 41/23 | 32 | 6 | 11.8 | 100 | 31/15 | 31 | 22 | 63 | 34/18 | 26 | 20 | 69 |
| <i>E. newbeyi</i> | 23/19 | 21 | 7 | 12.4 | 100 | 17/7 | 17 | 23.8 | 90 | 19/9 | 14 | 22.2 | 88 |
| <i>E. nigrifunda</i> | 23/22 | 23 | 10 | 14.2 | 100 | 26/13 | 26 | 26 | 84 | 30/17 | 24 | 24.2 | 86 |
| <i>E. nutans</i> | 22/17 | 19 | 6 | 12.9 | 100 | 27/15 | 27 | 16.6 | 85 | 29/17 | 23 | 15.8 | 85 |
| <i>E. pimpiniana</i> | 23/15 | 19 | 8 | 10.1 | 100 | 26/13 | 26 | 20 | 88 | 24/12 | 18 | 20 | 89 |
| <i>E. preissiana</i> subsp. <i>lobata</i> | 23/20 | 21 | 11 | 11 | 100 | 28/16 | 22 | 19.3 | 90 | 29/18 | 24 | 18.1 | 91 |
| <i>E. rugulata</i> | 15/14 | 15 | 10 | 14.4 | 100 | 16/6 | 16 | 26 | 98 | 19/8 | 14 | 25 | 98 |
| <i>E. spathulata</i> subsp. <i>salina</i> | 25/20 | 23 | 6 | 8.7 | 100 | 24/11 | 24 | 18 | 81 | 23/11 | 17 | 20 | 83 |
| <i>E. staeri</i> | 18/17 | 18 | 14 | 17.2 | 90 | 25/15 | 25 | 29.8 | 72 | 26/17 | 22 | 28.9 | 73 |
| <i>E. subtilis</i> | 28/23 | 26 | 6 | 9.7 | 100 | 28/14 | 28 | 20 | 89 | 27/14 | 21 | 19 | 91 |
| <i>E. talyuberlup</i> | 22/19 | 21 | 6 | 9.5 | 100 | 21/10 | 21 | 17.1 | 91 | 24/13 | 19 | 14.9 | 91 |

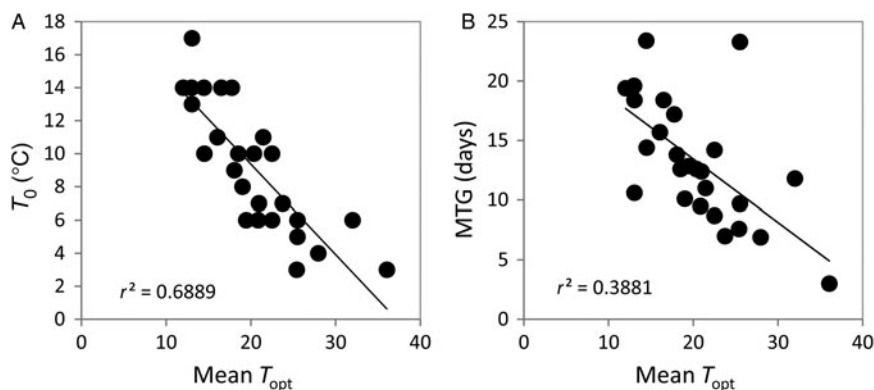


Figure 2. The relationship between mean optimum temperature for germination (mean T_{opt}) and (A) onset of germination (T_0); and (B) mean time to germination (MTG).

plot illustrating the observed TGP data combined with current and future germination response for each month of the year demonstrates a clear preference for the cool temperatures of autumn/winter for germination for this species (Supplementary Figs 1 and 2). Overall, another six species are predicted to suffer slight declines in level of germination (*E. aequiperta*, *E. argutifolia*, *E. georgei*, *E. jimberlanica*, *E. kruseana* and *E. newbeyi*), whilst seven species are predicted to have a slight increase in germination (*E. calcicola*, *E. crispata*, *E. livida*, *E. nigrifunda*, *E. myriadena*, *E. spathulata* and

E. subtilis) (Table 2). The remaining species are predicted to maintain similar germination levels in the future.

Discussion

Temperature plays a major role in determining when and where a seed will germinate (Probert, 2000) and seasonal temperature changes are an important determinant of the season of seedling establishment (Bell, 1999). Changes in the mean, as well as the frequency and severity of temperature extremes, can have a strong impact on the success of regeneration. This study used models to simulate a regeneration response by identifying major thermal constraints in germination in conjunction with 'real' data on germination thresholds for members of an important tree genus in southern Western Australia. In the main, the 26 *Eucalyptus* species investigated demonstrated wide thermal tolerances and very high temperature optima for germination, suggesting potential resilience to higher temperatures associated with anthropogenic climate change. Flexibility of temperature thresholds provides seed populations with a mechanism to adjust to forecast temperature increases. The models predicted that a number of species (*E. georgei*, *E. aequiperta* and *E. jimberlanica*) would suffer germination declines of 35% or more by 2070 relative to the observed data; however, the differences between the predicted current and future temperature conditions and the associated responses reveal only marginal germination declines. These anomalies are no doubt a result of the models used. The more worrying result is that predicted for *E. kondinensis* because of the large reduction in germination expected between predicted current and future conditions and the requirement for cooler conditions of autumn/winter for maximum germination.

Germination timing for some species shifted slightly when presented with warmer temperatures but most remained the same. Timing of germination

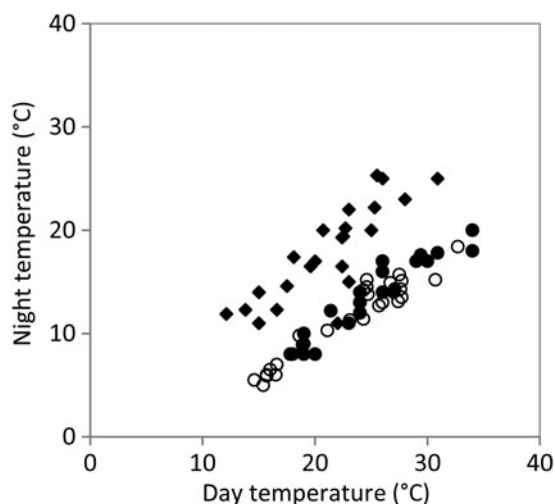


Figure 3. Relationship between TGP observed (\blacklozenge), predicted current (1950–2000) (o) and 2070 modelled (\bullet) diurnal temperatures for optimum germination. The observed data indicate a preference for germination under more-or-less constant temperatures, whereas optimum temperatures for germination predicted for current conditions (1950–2000) indicate species germinating better under slightly alternating temperature conditions with cooler nights. By 2070 conditions for optimal germination will shift again slightly to warmer day, and cooler night, temperatures overall but the coolest night temperatures will no longer exist.

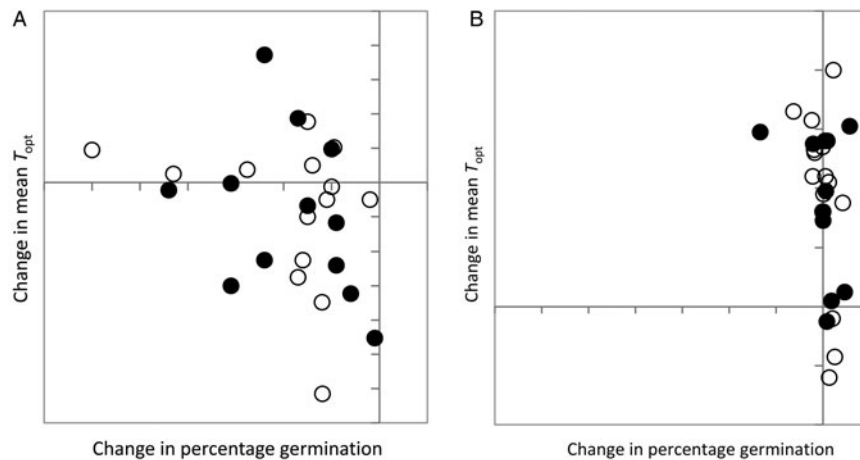


Figure 4. Predicted change in mean optimum temperature (T_{opt}) and percentage germination for 26 *Eucalyptus* species for 2070 with a high emission scenario (A) relative to observed TGP data; and (B) relative to predicted current (1950–2000) data. Common species (●); and conservation species (○). Note that the largest change in levels of germination across the 26 species occurs between observed and predicted current temperature conditions (A) and little change between predicted current and modelled 2070 percentage germination (B).

is closely related to seedling survival and establishment, and flexibility in germination times may provide an opportunity for alleviation or exacerbation of climate change impacts on regeneration: pushing germination closer to spring and summer dry can increase mortality in autumn/winter regenerating species in seasonally dry ecosystems and in summer rainfall regions and at higher elevations warmer temperatures may push germination later into autumn and winter, increasing the risk of frost mortality (Mok *et al.*, 2012; Rawal *et al.*, 2015). The possible consequences of shifts in germination timing may be a contraction of niches for species intolerant of warmer conditions and expansions of niches for those resilient to change.

Temperature conditions for germination of *Eucalyptus* species from southern Australia has previously been reported to be mostly around 15–25°C (Boland *et al.*, 1980). However, seed of some species has demonstrated tolerance to much higher temperatures. *Eucalyptus tetragona*, a species distributed predominantly south and east of Perth, Western Australia, has been observed to germinate at high levels between 10 and 35°C (Bellairs and Bell, 1990); *E. occidentalis* has demonstrated high levels of germination at 30°C (Zohar *et al.*, 1975) and *E. globulus* has exhibited fastest and most complete germination at 28°C, with germination only limited above 33°C (López *et al.*, 2000). Although seed might be able to germinate to high levels at high temperatures, speed of germination usually declines at temperatures above and below the optimum (Bellairs and Bell, 1990; Battaglia, 1993; López *et al.*, 2000). In this study the models predicted a slight increase in germination speed for most species at the optimum germination temperature as the region warms. Rapid germination may be beneficial for seedling establishment, providing

competitive advantage in a seasonally dry and nutrient-poor environment.

Climate is often considered a primary constraint on species distributions at a regional scale (Pearson and Dawson, 2003); however, this research has demonstrated that modelling climatic conditions within species geographic distributions has the potential to strongly underestimate the thermal tolerance of the germination niche. The overall pattern emerging from these results suggests broader temperature tolerance for most species than would be expected from distributions alone. This fact confounds the models somewhat and suggests that climatic distribution is a poor proxy for temperature tolerance during seed germination. In contrast, investigations in south east Australian *Eucalyptus* species suggested that the temperature germination niche tended to reflect the temperature niche within the temperature range in the habitat of species (Rawal *et al.*, 2015). However, very little is known of field timing of germination in these particular *Eucalyptus* species, and temperatures in the upper soil stratum can be much higher than that recorded for air, and have been known to frequently exceed 60–70°C (Hnatiuk and Hopkins, 1981). Under forecast warming scenarios, a 1°C increase in air temperature can result in an associated soil temperature increase of 1.5°C in some environments (Ooi *et al.*, 2012). Nonetheless, it seems unlikely that forecast temperatures will exceed the current environmental tolerances of seed of the southern Western Australian *Eucalyptus* species investigated. The ability to germinate at high temperatures suggests that seed of some species (for example *E. erythrocorys*) might recruit at any time of the year following heavy rain; species germinating with lower thermal optima (for example *E. kondinensis*) are more likely to be restricted to germinate during winter.

Using a single environmental variable to understand a complex biological process is problematic for a broader understanding of species response to multiple interacting climatic factors. Water uptake is an essential initial step towards germination (Bewley and Black, 1994) and cannot be discounted. A number of studies have considered the impact of moisture stress on germination in *Eucalyptus* species from south-eastern Australia (Edgar, 1977; Bachelard, 1985; Battaglia, 1993) with high sensitivity to moisture stress reported in *Eucalyptus globulus* (López *et al.*, 2000). Very little research has been conducted on moisture stress in species from south-western Australia, although germination in *E. occidentalis* was reported to be inhibited by low water potential (Zohar *et al.*, 1975). An average decline in mean annual rainfall of ca 34% is expected across the 26 seed source sites by 2070 given a high greenhouse gas emission scenario. Summer rainfall can occur but it is erratic and usually associated with localized thunderstorm activity, or rain-bearing low pressure systems associated with tropical cyclones (Gentili, 1972). While heat stress can be alleviated by moisture, moisture stress is exacerbated by high temperatures through the process of evapotranspiration. Soil moisture reductions combined with rising temperatures can lead to greater seedling mortality over dry summer months, particularly in seasonal ecosystems. These climatic changes represent potential tipping points that may lead to large spatial shifts in species regeneration niches (Mok *et al.*, 2012), with likely impacts on ecosystem composition and species abundance.

There was no evidence of local adaptation to thermal conditions and rare species were no less tolerant of higher temperatures than commonly occurring species. However, insufficient collections restricted the use of more robust statistical analyses, including answering questions related to phylogeny, biogeographic patterning and seed trait relationships and vulnerability. *Eucalyptus* is a large genus of more than 800 species and a more complete sampling of the genus would provide better predictive power. In addition, seed response to changing climates will vary across spatial and temporal scales (Cochrane *et al.*, 2015) and has been demonstrated for germination behaviour (Rawal *et al.*, 2015) and growth (Drake *et al.*, 2014) in several *Eucalyptus* species. Sampling seed from across multiple populations will increase the robustness of our understanding of the variability within a species. Seed traits are important for predictive based plant ecology and are an integral part of a plant's life cycle, but in the end, laboratory results must be translated into field situations.

Implications

The capacity of long-lived tree species to respond to warming climates is regionally important, and of

global concern, as trees dominate the carbon cycle. If capacity to germinate under high temperatures is a stable trait across many of the 800-plus known *Eucalyptus* species, then this tolerance may prove beneficial for the persistence of the genus as climates become more severe.

However, other ontogenetic stages of the life cycle may not demonstrate such wide thermal tolerances and successful germination does not necessarily guarantee successful establishment, with mortality during the first year of growth high (Rawal *et al.*, 2015). Climate change can impact on rates of disturbance, tree mortality and life history processes that determine the ability of a species to recover from disturbance. Drought and temperature stress have been implicated in canopy tree mortality for many tree species worldwide (Allen *et al.*, 2010), an increase in wildfire risk (Millar *et al.*, 2007), as well as significant climate-driven changes to seed production in the future (Etterson, 2004; Redmond *et al.*, 2012). The potential for reduced seed yield, coupled with possible declines in establishment levels as a result of delayed and shorter and drier wet seasons may still render populations of some species quite vulnerable despite the capacity to germinate under higher than forecast temperatures. Consequently, planning restoration and conservation projects in a changing climate without considering species capacity for regeneration may incur a high risk of failure. Investigations such as this one can provide managers with necessary information to identify species vulnerable at the germination stage and to adjust expectations for natural regeneration and targeted restoration by direct seeding. They can also be used to improve germination efficiency and seedling uniformity for nursery production, including support for translocation of conservation species in advance of shifting climates.

Supplementary material

To view supplementary material for this article, please visit <https://doi.org/10.1017/S0960258517000010>

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Conflicts of interest

None.

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