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Environmental effects on germination phenology of co-occurring eucalypts: implications for regeneration under climate change

Deepa S. Rawal · Sabine Kasel · Marie R. Keatley · Craig R. Nitschke

Abstract Germination is considered one of the important phenological stages that are influenced by environmental factors, with timing and abundance determining plant establishment and recruitment. This study investigates the influence of temperature, soil moisture and light on the germination phenology of six *Eucalyptus* species from two co-occurring groups of three species representing warm-dry and cool-moist sclerophyll forests. Data from germination experiments were used to calibrate the germination module of the mechanistic model TACA-GEM, to evaluate germination phenology under a range of climate change scenarios. With the exception of *E. polyanthemos*, the optimal niche for all species was characterised by cool-moist stratification, low light, cool temperatures and high soil moisture. Model results indicated that of the warm-dry species, *Eucalyptus microcarpa* exhibited greater germination and establishment under projected changes of warmer drier conditions than its co-occurring species *Eucalyptus polyanthemos* and *Eucalyptus tricarpa* which suggests that *E. microcarpa* could maintain its current distribution under a warmer and drier climate in southeastern Australia. Among the cool-moist species, *Eucalyptus radiata* was the only species that established under projected climate change of the 2080s but at such a low probability that its persistence compared to *Eucalyptus obliqua* and *Eucalyptus sieberi* cannot be posited. For all cool-moist species, germination did not benefit from the phenological shifts they displayed. This study successfully demonstrated environmental effects on germination phenology and how a shift in climate can influence the timing and success of recruitment.

Keywords *Eucalyptus* · Climate response · Establishment · Modelling · Recruitment

Introduction

There are three primary phenological stages in flowering plants: flowering and pollination, fruiting and seed dispersal (Primack 1987), and seedling growth and establishment (Wielgolaski 1974) which are interrelated and all are affected by external environmental and internal physiological factors (Primack 1987; Fenner 1998). These three phenological stages are considered a major component of plant fitness and can be used as an important tool for predicting tree distribution and abundance (Primack 1980; Chuine and Beaubien 2001; Morin and Chuine 2005). Among these stages, seed germination is one of the most important stages in the life cycle of plants (Cochrane et al. 2011). The germination stage marks the high-risk transition phase between seed and seedling (Meyer et al. 1997) in which environmental conditions (Baskin and Baskin 1998) are strong drivers affecting the abundance of germination and the probability of seedling survival (Meyer et al. 1990). Changes in the Earth's climate may have major impacts on plant regeneration (Walck et al. 2011; Mok et al. 2012), which highlights the need for understanding the link between germination and environmental cues to better inform management and restoration of species under climate variability and change (Khurana and Singh 2001; IPCC 2007).

Germination patterns are sensitive to environmental factors (Cavieres and Arroyo 2000), in particular to light, temperature and soil moisture conditions that may act independently or in

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varying combinations (Baskin and Baskin 1988; Bell and Williams 1997; Bell 1999; Ranieri et al. 2012). The role of moisture can be important; for example, the combined effect of temperature and moisture has been found to affect germination in some eucalypts with the optimum germination temperature coinciding with annual rainfall within the native range of the species (Bell 1999). In a study of 28 Australian species in the jarrah (*Eucalyptus marginata*) forests of Western Australia, germination was greater during the lower temperature (10–15 °C) and higher rainfall conditions in winter (Bell and Bellairs 1992). Germination of species from moist habitats, however, tends to be greater under higher temperatures reflective of a warmer season (Bell et al. 1993). For *Eucalyptus rudis*, which grows near rivers and coastal areas, germination peaks when temperatures reach 20–30 °C since soil moisture is available during summer. In contrast, for *Eucalyptus wandoo*, which inhabits slopes and inland river margins, germination was greater at temperatures of 15–20 °C due to limited soil moisture during the dry summer that inhibits germination (Bell et al. 1993). For *Eucalyptus oleosa*, an inland species which experiences limited soil moisture availability during most of the season, the optimum germination temperature was 10–20 °C (Bell et al. 1993).

Most *Eucalyptus* seeds exhibit high variability in their response to environmental factors and for some eucalypts like *Eucalyptus occidentalis* light may be the decisive factor in improving germination under different levels of osmotic stress (Zohar et al. 1975). However, no effect of light has been documented for *Eucalyptus obliqua* and *Eucalyptus baxteri* (Facelli and Ladd 1996). For *Eucalyptus camadulensis*, the requirement of light was reduced when temperature increased and chilling requirements were met (Grose and Zimmer 1958), while for the Western Australian species *Eucalyptus marginata*, light reduced germination (Bell et al. 1995). Moreover, for many Australian species, a combination of multiple environmental factors affects germination, including moisture availability, temperature and light (Bell and Williams 1997; Baskin and Baskin 1998).

In addition to environmental factors, germination response depends on life history traits, phylogeny, taxa and geographic distribution of the species (Grime et al. 1981; Karlsson and Milberg 2007). Optimal temperature and soil moisture requirements for germination may differ according to the restricted habitat and geographical range occupied by any particular species (Facelli and Ladd 1996; Bell 1999; Khurana and Singh 2001). For many eucalypts, the regeneration niche is closely associated with their geographic range (Battaglia 1996). For example, *Eucalyptus delegatensis* inhabits high altitudes (up to1500 m, Boland et al. 2006) often subject to cold winter frosts and snow, and its seed exhibit a high degree of dormancy requiring long periods of cool moist stratification

for germination while *Eucalyptus amygdalina*, which inhabits lowland forests with mild winters (0–750 m, Boland et al. 2006), does not have a physiological dormancy and can germinate under a wider range of moisture conditions (Battaglia 1996). Differences in the germination niches between these species suggest that species have different regeneration niches (Battaglia 1996). The phenological pattern of germination may be important for giving species a competitive advantage within their geographical range over other species as the timing of germination in response to environment variation is likely a bet-hedging strategy to maximise fitness (Kirschbaum 2000; Cochrane et al. 2011). Changes in the environment may shift the timing of germination however leading to lower germination rates as fitness is overcome which in turn could place species fecundity at risk and potentially facilitate changes in plant distribution (Meyer et al. 1990; Hobbie and Chapin 1998).

Climate change predictions for southeastern Australia suggest upwards of a 4.5 °C temperature increment with significant rise in drought events by the 2080s (CSIRO and BOM 2007). In relation to future climate, germination and establishment constraints due to increases in temperature and moisture limitation may make species susceptible to climate change. However, research on regeneration strategies that plants adopt under climate change is limited, although some models have been developed for assessing the impacts of climate change on plant regeneration and distribution (Mok et al. 2012). Reproduction and phenology however have not been considered explicitly in the majority of these distribution models, although it is clear that the species presence is dependent on survival and production of viable seeds in a given area (Chuine and Beaubien 2001). Statistical modelling approaches can relate the timing of phenological events to climatic factors but are limited in considering niche breadth conditions (Chuine et al. 2013). Mechanistic models, however, are based on cause-effect relationships between biological processes and some environmental factors (Morin et al. 2009; Chuine et al. 2013). Attributes such as growing degree hours/days, threshold temperatures (Snyder et al. 2013), including frost, drought (Shugart and Noble 1981), chilling hours, photoperiod, and the occurrence of freeze events in the spring are the base of phenological models (Chuine et al. 2013). Mechanistic models are required to incorporate all the aforementioned phenological attributes to enable the integration of the important biological processes that govern germination phenology.

Eucalyptus is the largest genus of the *Myrtaceae* family and the dominant tree species in Australia with botanical and commercial values (Boland et al. 2006). This study thus explores the effect of environmental variability on germination phenology of co-occurring eucalypts from two distinct

climatic regions of southeastern Australia. The study addresses three questions: (i) What are the key environmental factors that govern germination phenology? (ii) How does increased temperature and limited moisture conditions of climate change projected for this region alter timing and probability of germination? (iii) What does this mean for species persistence under climate change? The study incorporates an experimental component that attempts to define the germination niche by comparing the effect of light, temperature and soil moisture on germination timing and success. The outcomes of the seed ecology experiment are used to calibrate a mechanistic model to assess the impacts of climate change on regeneration. Using this combined approach, this study seeks to predict the possible vulnerability of selected *Eucalyptus* species in southeastern Australia to climate change.

Materials and method

Species selection

Six *Eucalyptus* species were selected from dry and wet sclerophyll forest types located in the temperate region of Victoria, southeastern Australia. The co-occurring *Symphyomyrtus* (subgenera) species *Eucalyptus microcarpa* (grey box), *Eucalyptus polyanthemos* (red box) and *Eucalyptus tricarpa* (red ironbark) represent the dry open 'box-ironbark' of the warm temperate region of Victoria (Newman 1961; Orscheg et al. 2011). Annual rainfall in this region ranges from 400 to 1000 mm, and the average monthly maximum/minimum temperature range from 23 to 33 °C/−1 to 7 °C, respectively (Boland et al. 2006). *Monocalyptus* (subgenera) species *E. obliqua* (messmate stringybark), *Eucalyptus radiata* (narrow-leaved peppermint) and *Eucalyptus sieberi* (silvertop ash) represent the wet sclerophyll forest. These forests are classified as 'ash forests' and occur within the wet and cool temperate regions of Australia which experience an annual rainfall range from 500 to 2400 mm and maximum/minimum temperature ranges of 19 to 30 °C/−4 to 8 °C, respectively. For the purpose of this study, the 'box-ironbark forest' species will be referred as warm-dry species and the 'ash forest' species as cool-moist species (Appendix 1). Seeds from two to four provenances (Prov) of each species were selected to represent different geographic locations and elevations (Appendix 1).

Experimental design

The seed germination experiment consists of a randomised factorial design and was conducted in controlled growth chambers (microdigital control, 'Axyos tech.'). Seeds were sown in 'Kwick' germination trays (100 cells) with a mixture of pine bark, mined sand, sieved coir peat, Saturaid 1500 g m^{-3} (wetting agent) and dolomite, and placed in the controlled growth chambers (Massawe et al. 2003). Seeds were carefully observed for epicotyl hook emergence each day for 42 days (Schütz and Rave 1999).

The experimental treatments included three soil moisture treatments (M), two light regimes (L), three temperatures (T) (Schütz and Rave 1999) and two stratification treatments (St) (Close and Wilson 2002) (detailed descriptions of treatments and abbreviations are provided in Table 1).

The soil sterilization, moisture measurement and water retention test follows the method provided by Fraser (2009) and Rey et al. (2005). The volumetric water content of the soil was estimated at both the soil's permanent wilting point and field capacity by oven drying the soil at 80 °C for 48 h. The dried soil was re-weighed for the estimation of dry weight and volume. The field capacity of the soil was found to be at volumetric water content close to 40 % and the permanent wilting point to be close to a volumetric water content of 5 % (Fraser 2009).

Soil moisture Soil was oven-dried (80 °C for 48 h) and moisture levels adjusted to high moisture 40 % (HM, 0.0 MPa), medium moisture 20 % (MM, −0.2 MPa) and low moisture 10 % (LM, −1.0 MPa) by adding the required amount of deionised water and mixed thoroughly. Soil moisture levels were maintained throughout the experiment by replacing water lost each day with deionised water according to difference in weight.

Humidity The air humidity level was maintained at the optimum level of 50–60 % for germination inside the growth chamber (Marsden et al. 1996).

Seed viability To achieve 100 % viability of seeds during the experiment (Schütz and Rave 1999), seed viability test was performed for five replicate samples (approximately 150 seeds) of known weight of seeds per provenance (see Table 1). Seed viability was assessed by tetrazolium tests following the method of Moore (1985) and Bell et al. (1995). As some of the eucalypts seeds have hard double-layered seed coats, seeds were moistened at room temperature in deionised water for 48 h (Moore 1985). The moistened seeds were completely immersed in tetrazolium solution for 24 h at room temperature covered by aluminium foil after rupturing with a dissecting blade for easier penetration of tetrazolium solution. Then, each individual seed was examined by opening the seed coat under the microscope and seeds with brightly red coloured endosperms were considered viable. Viability percentage was used to determine the weight of

Table 1 Experimental treatments, associated metrics and methods applied throughout the study

Treatments/metrics and abbreviations	Reference and description	Measurement technique, formula and/or units
Temperature treatments (T)	Schutz and Rave (1999)	
HT	Hot temperature treatment	36/22 °C (12/12 h)
MT	Mild temperature treatment	28/18 °C (12/12 h)
CT	Cool temperature treatment	20/14 °C (12/12 h)
Light treatment (L) (light level 280–295 lx)		
HL	High light	12/12-h light and darkness 280–295 lx
LL	Low light	1-h light (280–295 lx, for watering and counting germination), 23-h darkness
Soil moisture treatment (M)	Fraser (2009); Rey et al. (2005)	
HM	High moisture	40 % (0.0 MPa)
MM	Medium moisture	20 % (−0.2 MPa)
LM	Low moisture	10 % (−1 MPa)
Stratification (St)	Close and Wilson (2002)	
S	Stratified	Seeds were placed in a double layer of filter paper, moistened with deionised water, wrapped in aluminium foil, placed in zip lock bags and stored at 4±0.5 °C for 10 days
US	Unstratified	Seeds stored ≤4 °C
Germination metrics	Shahba and Qian (2008)	
FG	Final germination percentage	Total number of seed germinated×100 %
GDD	Growing degree days (Wielgolaski 1999; Green 2007)	$GDD=(T_{max}+T_{min})/2-T_{base}$ T_{max}=maximum temperature, T_{min}=minimum temperature, T_{base}=threshold temperature 5 °C
Seed weight used	Schutz and Rave (1999) (to achieve 100 % viability)	Initial seed weight (with 30 seeds)×1/proportion of viable seed×30 seeds

seed taken to represent the individual sample of approximately 30 viable seeds (see Table 1).

Sample size For each provenance, each sample consisted of three replicates of 30 viable seeds. Across the treatments, the total number of seeds used per provenance was 3240 seeds that includes 3 temperature treatments (T), high, mild and cool (HT, MT, CT); ×2 light regimes (L), high and low (HL, LL); ×3 moisture treatments (M), high, medium and low (HM, MM, LM); ×2 stratification treatments (St), stratified and unstratified (S, US); ×30 seeds per provenance; and ×3 replicates (Table 1).

Germination metrics Final germination percentage and growing degree days (GDD) were calculated (Shahba and Qian 2008; Wielgolaski 1999; Green 2007; Table 1).

Statistical analysis

Treatment (T, M, L, St, Prov) effects on the success of species germination were tested using survival analysis following the method of Ranieri et al. (2012). Cox's proportional hazard analysis (Cox 1976) was performed to calculate the proportional hazard of environmental factors on germinated seeds using SPSS ver. 20 (2011). For the purpose of analysis,

germinated seeds were considered analogous to survival and seeds that did not germinate were considered equivalent to mortality (Ranieri et al. 2012). Moisture and temperature treatment where germination failed were not considered for this analysis.

To determine the optimum time and temperature needed for the highest germination percentage, germination percentage was analysed as a dependent variable of GDD accumulation using non-linear polynomial regression analysis (McDonald 2009).

TACA-GEM

The mechanistic model TACA-GEM (Mok et al. 2012), a variant of TACA (Nitschke and Hickey 2007; Nitschke and Innes 2008; Nitschke et al. 2012) was used to model the species specific germination responses to climate variability and change. The model primarily combines phenology with germination physiology which interacts with temperature, soil moisture and frost, and simulates the ability of a species to germinate and establish under different soil and climate conditions. This model also uses habitat niche conditions to determine establishment of a species after germination (Grubb 1977; Mok et al. 2012). Species-specific germination parameters (temperature and soil moisture) and non-linear

Table 2 Species-specific germination parameters and climatic origin taken for the calibration of TACA-GEM model

Parameter	Species					
	E. microcarpa	E. polyanthemos	E. tricarpa	E. obliqua	E. radiata	E. sieberi
Habitat						
Geographic origin for climate data	37° 41' S, 144° 44' E	37° 14' S, 144° 27' E	37° 56' S, 146° 43' E	37° 20' S, 145° 05' E	37° 44' S, 145° 27' E	37° 41' S, 148° 45' E
Soil texture[ac]	Loam	Loam	Loam	Loam	Loam	Loam
Seedfall Julian date (days)[bcd]	180	180	180	30	30	30
Rooting zone depth (m)[a]	0.10	0.10	0.10	0.10	0.10	0.10
Coarse fragment (%)[a]	0.10	0.10	0.10	0.30	0.30	0.30
Probalistic germination function thresholds of polynomial regression for germination based on GDD						
Minimum GDD threshold (days)	200	310	250	220	200	170
Maximum GDD threshold (days)	720	970	620	600	590	700
Minimum temperature (°C)	5	5	5	4	4	4
Maximum temperature (°C)	29	29	29	29	29	29
b_0	−0.4198	−5.0432	−0.0867	−0.1740	−0.3298	−0.3293
b_1	0.0028	0.0210	0.0005	0.0011	0.0023	0.0025
b_2	−3.1E−06	−1.6E−05	−6.0E−07	−1.4E−06	−2.9E−06	−2.9E−06
Other germination parameters						
Germination moisture threshold (MPa)	−1.0	−1.0	−1.0	−1.0	−1.0	−1.0
Physiological base temperature (°C)	10	8	6	5	7	7
Establishment parameters						
GDD minimum	811	811	811	784	613	1101
GDD maximum	4539	3764	2274	3640	3737	3640
Frost tolerance (0–1)	0.35	0.35	0.35	0.50	0.50	0.35
Frost season length (days)	50	50	70	100	100	70
Heat moisture index (dimensionless)	70.0	60.0	45.7	46.9	47.3	38.1
Turgor loss point (MPa)	−5.5	−5.0	−4.5	−3.65	−3.5	−4.0

GDD growing degree days

[a] Source: Mok et al. (2012)

[b] Source: http://avh.chah.org.au

[c] Source: Boland et al. (2006)

[d] Source: http://www.florabank.org.au

regression functions (GDD thresholds and GDD functions) identified from germination phenological experimental components were implemented into TACA-GEM (Table 2). As higher germination was found under CT (40 % moisture) for four out of six species, functions derived from the polynomial regression model were used to calibrate TACA-GEM. Moisture and upper temperature thresholds for TACA-GEM input were identified from the growth chamber germination experiment. Species-specific germination and establishment parameters used for calibration of TACA-GEM are described in Table 2. Detail of the model has been provided in Appendix 2.

Climate parameters and scenarios

For the present climate condition, 10 years of historical climate data for each selected geographic location were inputted into TACA-GEM. The climate data represented the nearest geographic origin for each of the co-occurring species within their respective warm-dry/cool-moist groups to provide an indication of the germination and establishment response across a range of environmental conditions that each species group occurs within (n=30 climate scenarios for each species). The historical climate data include both the minimum and maximum temperature, precipitation and solar radiation on a daily time step. Climate data from the years 1957–2002 (Silo climate data, Jeffrey et al. 2001) were selected using a rank and percentile test to select 10 years of data representing the 90th, 75th, 50th, 25th and 10th percentiles for mean annual temperature and precipitation. These 10 years were used as the present climate (PC) condition (Mok et al. 2012). The heat moisture index [mean annual temperature+10/(precipitation/1000)] (Wang et al. 2006) was also calculated and if a year was duplicated for mean annual temperature and precipitation an additional year that was not previously chosen was selected based on the heat moisture index. The projected climatic conditions for southeastern Australia were the same as used by Mok et al. (2012) where climate change scenarios were built using direct adjustment approach from the historical (present) climate data and global climate model (GCM) predictions. CSIRO OzClim web based database (CSIRO 2009) monthly outputs from four GCMs; CSIRO Mk 3.5, INM-CM 3.0, BCCR-BCM 2.0 and CCR MIROC-H and multiple SRES emission scenarios (A1F1, A1B and B1) were used to provide a range of future climate conditions. Based on the GCM-emission scenarios chosen, the range of future climate conditions for the 2020s was a temperature increase ranging from 0.4 to 1.25 °C with a 1–9 % decline in rainfall. For the 2050s, the temperature increase ranges from 0.75 to 2.75 °C with a 3–25 % decline in rainfall, and

for the 2080s, temperature increase ranges from 1.0 to 4.5 °C with a 4–40 % decline in rainfall (Mok et al. 2012). The projected climatic conditions were termed: 2020s, 2050s and 2080s. Based on the projected climatic conditions, TACA-GEM output provided a germination probability and establishment score ranging from 0 to 1, indicating failure (0.0) to 100 % success (1.0) in germination/establishment.

Results

Environmental effects and optimum germination conditions

Soil moisture was one of the most important predictors for germination as all the species favoured the HM treatment under MT and CT (Fig. 1). However, *E. sieberi* exhibited the capacity to germinate under the MM and CT conditions. Temperature had a significant effect (P≤0.001, Table 3) on seed germination across all species. No species germinated under HT, and germination under CT was generally greater than MT (Fig. 2). Under MT, warm-dry species displayed greater germination (1–10 %) than cool-moist species (<3 %).

LL and S conditions had a significant positive effect (0.05≤P≤0.001, Fig. 1) on the germination of all species except *E. polyanthemos* that required HL for germination and exhibited greater germination with US seed (Fig. 1). The significant interactions (P≤0.05, Table 3) of L×St and St×T on germination of *E. tricarpa*, *E. obliqua* and *E. sieberi* indicate that stratification and temperature reduces the importance of light.

Non-linear regression modelling identified significant relationships between GDD requirements and the initiation, optimality and cessation of germination. The resultant models show that within the warm-dry species group, *E. microcarpa* has a narrow germination window but greater germination success than the two other warm-dry species (Fig. 1). The most significant model for warm-dry species was found for *E. tricarpa* (LL, HM, S, r^2=0.85, P≤0.05). Among cool-moist species, *E. obliqua* exhibited the most significant model (LL, HM, US, r^2=0.88, P≤0.001) but lowest germination success (Fig. 1). *E. sieberi* was the only species to germinate under MM conditions (Fig. 1). Following stratification, the final germination percentage was greater for *E. microcarpa* (23.2 %) compared to the other warm-dry species, and for *E. sieberi* (22.3 %) compared to the other cool-moist species, irrespective of light conditions (Fig. 1). For *E. microcarpa*, however, there was no germination under the HL and US conditions (Fig. 1).

Prov had a significant effect (P≤0.05, Table 3) on the germination of all species with exception of

Fig. 1 Polynomial regression model prediction of germination percentage from the cool temperature treatment for the warm-dry (**a**–**c**) and cool-moist (**d**–**f**) eucalypt species. Significance level for r^2 is $P \leq 0.05$ (refer to Table 1 for abbreviations used)
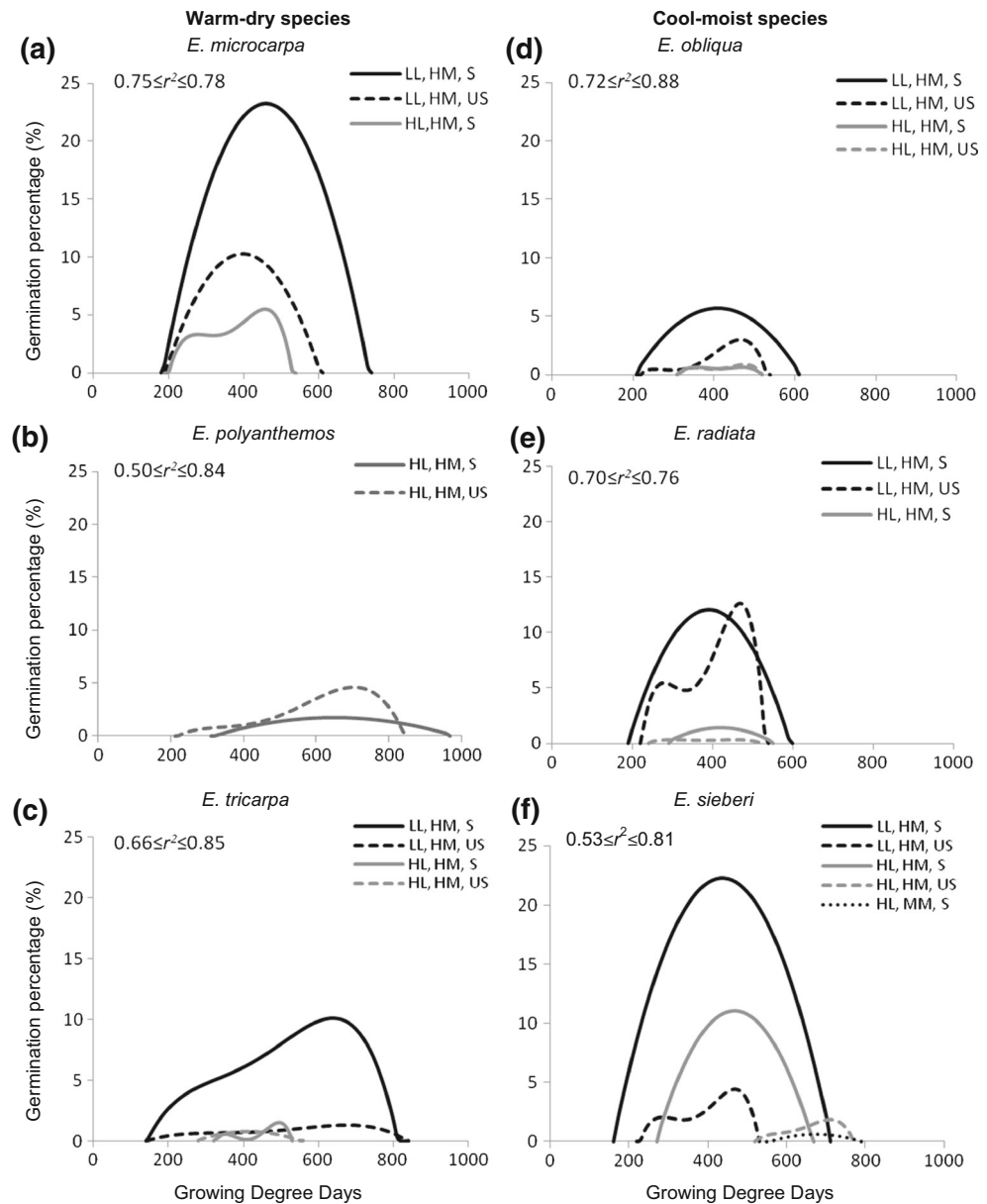
E. polyanthemos. For *E. obliqua*, greater germination was displayed by high elevation provenances under CT suggesting clinal variation (Eo3, Fig. 2, Appendix 1). Phenotypic plasticity was displayed by the provenances of *E. tricarpa* with the higher elevation provenance Et 2 exhibiting greater germination under MT than Et 1 (Fig. 2c). Significant interactions of M×Prov (*E. sieberi*) and Prov×T (*E. microcarpa*, *E. obliqua*, *E. radiata*, *E. sieberi*; Table 3) indicate that the germination niche varies in character (different optimum temperature and moisture regimes for germination) between the provenances of the species.

Shift in germination timing, germination, and establishment

Warm-dry species Model results from TACA-GEM for *E. microcarpa* at the sites of the warm-dry species demonstrated significant change in germination timing from PC to 2080s that helped the species maintain its capacity to germinate and establish across the breadth of climatic conditions explored (Table 4). *E. polyanthemos* and *E. tricarpa* also exhibited significant change in germination timing which leads to slight increases in germination capacity; however, establishment success

Table 3 Cox's proportional hazard regression analysis on germination in the six study species indicating the significance of light, temperature, stratification, provenance and moisture on germination proportion

Treatment	Species					
	E.microcarpa	E.polyanthemos	E.tricarpa	E.obliqua	E.radiata	E.sieberi
Light (L)	***	***	***	***	***	***
Temperature (T)	***	***	***	***	***	***
Stratification (St)	***	***	***	*	ns	***
Provenance (Prov)	***	ns	**	***	***	***
Moisture (M)	NG	NG	NG	NG	NG	***
L×T	***	ns	**	ns	ns	ns
L×St	ns	ns	*	*	ns	**
L×M	NG	NG	NG	NG	NG	ns
L×Prov	*	ns	ns	ns	ns	ns
M×T	NG	NG	NG	NG	NG	**
St×T	ns	ns	***	*	ns	***
Prov×T	*	ns	ns	***	**	*
M×St	NG	NG	NG	NG	NG	ns
M×Prov	NG	NG	NG	NG	NG	**
Prov×St	ns	ns	ns	ns	ns	ns

NG indicates no germination at 20 % soil moisture regime. *$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$; *ns*, not significant

*$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$

ns not significant

declined and was modelled to completely fail by the 2080s.

Cool-moist species Model results from TACA-GEM demonstrated that despite significant changes in the timing of germinations, probability of germination and establishment declined from PC to the 2080s for all three species (Table 4). Probability of germination in *E. obliqua* was greater than for *E. radiata* and *E. sieberi* under each of the projected climatic conditions. However, establishment was limited for *E. obliqua* after 2020s while that for *E. sieberi* and *E. radiata* persisted into the 2050s and 2080s, respectively (Table 4). The results for this species group suggest that changes in germination timing will not maintain germination capacity or recruitment success as climate becomes warmer and drier (PC to 2080s).
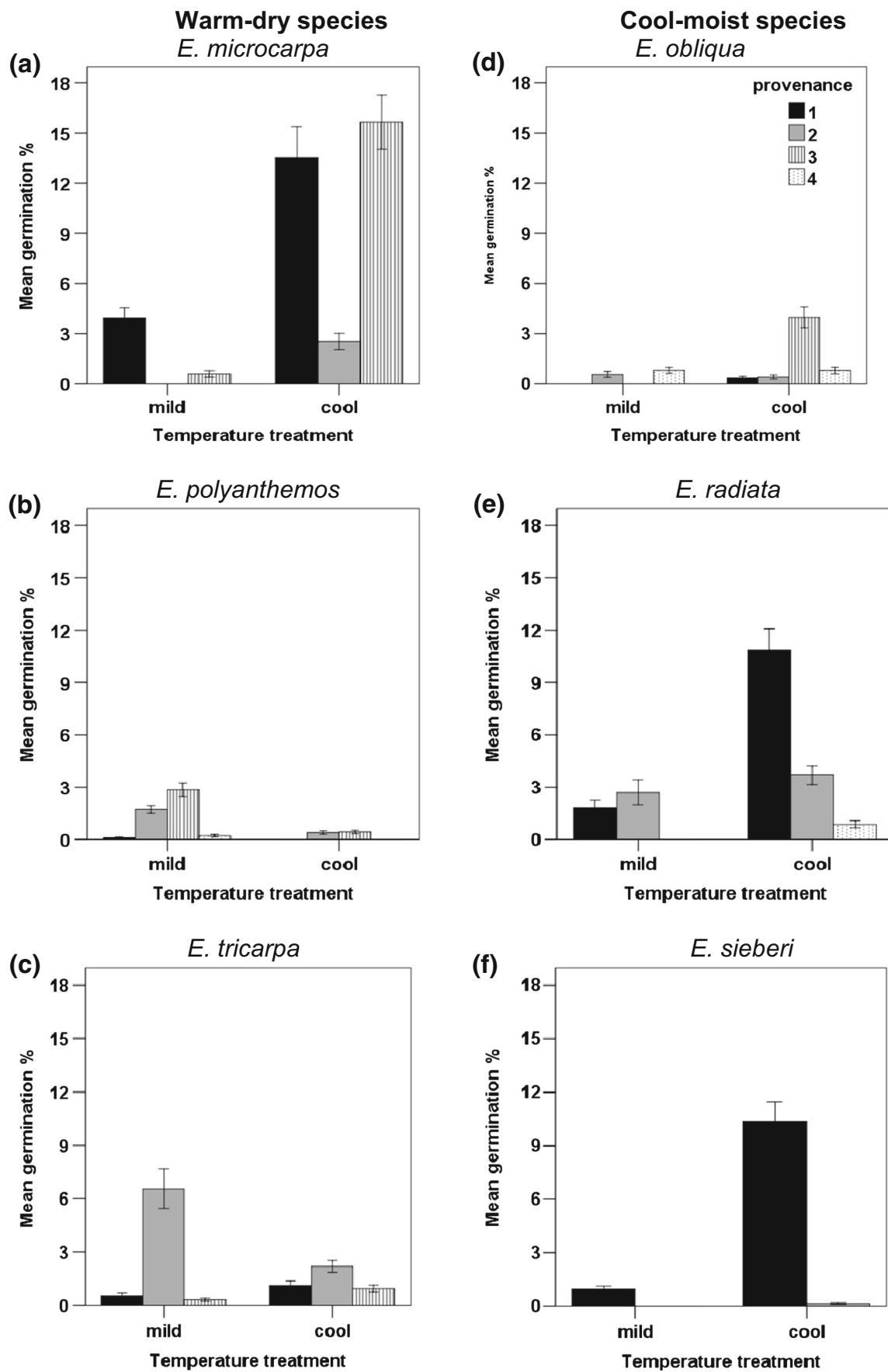
Discussion

Optimum regeneration niche

Light, temperature and moisture are the main environmental factors that govern the timing of germination (Baskin and Baskin 1988), and all of these factors affected germination

of all the study species to some extent. Species germination temperature niche reflects the temperature niche of its habitat origin. The warm-dry species inhabit warm dry sclerophyll forest with wider temperature niches than cool-moist species (Boland et al. 2006) which explains the greater germination success of warm-dry species under MT compared to the cool-moist species. Moreover, the germination response of cool-moist species like *E. obliqua* and *E. sieberi* with lower germination success under MT than *E. radiata* also shows that germination temperature niche correlates with habitat temperature range. All the species were sensitive to increasing temperature as germination was inhibited at HT (average 29 °C), lower germination occurred under MT (average 23 °C) but greater under CT (average 17 °C) for most of the species. Our finding is supported by Battaglia (1993) who found that temperatures of 25 °C and above impeded germination for *E. delegatensis* and optimum germination temperatures ranged between 15 and 20 °C. Similar effects have been found

Fig. 2 Mean germination percentage of species provenances under mild and cool temperature treatments for warm-dry (**a**–**c**) and cool-moist (**d**–**f**) species. There was no germination at high temperature across all species. Provenance numbers increase in order of increasing elevation. *Error bars* represent 95 % confidence intervals (refer to Appendix 1 for provenance details)

Warm-dry species

Cool-moist species

(a) *E. microcarpa*

(d) *E. obliqua*

(b) *E. polyanthemos*

(e) *E. radiata*

(c) *E. tricarpa*

(f) *E. sieberi*

Table 4 TACA-GEM model results of germination time (Julian days), germination and establishment probability score (0–1 indicating no to successful germination/establishment) for the six study species under present climate (PC) and projected climatic conditions of 2020s, 2050s and 2080s

	Warm-dry species			Cool-moist species		
	E. microcarpa	E. polyanthemos	E. tricarpa	E. obliqua	E. radiata	E. sieberi
Germination duration in Julian days (mean; std error)						
PC	233–296 (265; 1.3) a	254–300 (273; 1.6) a	241–348 (292; 3.0) a	94–283 (186; 4.2) a	87–297 (199; 3.3) a	91–237 (159; 3.8) a
2020s	223–283 (253; 1.3) b	240–267 (254; 1.3) b	231–365 (278; 3.3) b	44–276 (181; 3.3) a	42–286 (184; 3.7) b	41–365 (217; 3.1) b
2050s	214–275 (245; 1.3) c	229–276 (250; 1.0) b	221–361 (274; 3.2) bc	43–251 (146; 3.9) b	41–238 (137; 3.4) c	40–148 (90; 4.5) c
2080s	207–266 (235; 1.3) d	222–265 (243; 1.0) c	215–346 (265; 3.3) c	41–274 (150; 3.3) b	42–220 (133; 5.7) c	39–99 (63; 6.6) d
Germination probability score (std error)						
PC	0.49 (0.02)	0.56 (0.02)	0.23 (0.02)	0.80 (0.02)	0.76 (0.02)	0.72 (0.02)
2020s	0.41 (0.02)	0.53 (0.03)	0.25 (0.02)	0.73 (0.02)	0.69 (0.03)	0.50 (0.02)
2050s	0.46 (0.03)	0.58 (0.04)	0.31 (0.02)	0.66 (0.01)	0.55 (0.03)	0.35 (0.04)
2080s	0.45 (0.03)	0.60 (0.03)	0.30 (0.03)	0.38 (0.01)	0.17 (0.03)	0.16 (0.04)
Establishment probability score (std error)						
PC	0.45 (0.15)	0.16 (0.12)	0.43 (0.15)	0.52 (0.15)	0.61 (0.14)	0.64 (0.15)
2020s	0.31 (0.12)	0.01 (0.04)	0.03 (0.06)	0.08 (0.12)	0.48 (0.15)	0.29 (0.13)
2050s	0.43 (0.13)	0.00	0.00	0.01 (0.02)	0.20 (0.12)	0.05 (0.09)
2080s	0.42 (0.15)	0.00	0.00	0.00	0.01 (0.04)	0.00

Germination means followed by different letters indicate significant differences ($P \leq 0.05$) in germination days among the projected climatic conditions

for other *Eucalyptus* species with germination percentages found to be higher at temperatures of 15 °C (Bell et al. 1995). Greater germination found for *E. polyanthemos* than for other species under the mild temperature condition aligns with the germination temperature of 25 °C found for this species by Gunn (2001). However, Gunn (2001) found lower temperature optimum for the germination of *E. obliqua* (15 °C) and *E. radiata* (15 °C, 20 °C). In this study, the cool-moist species also displayed lower optimum germination temperature which is explained by the lower temperature ranges of their habitat niche and germination phenology reflecting their niche habitat condition.

In temperate forests, seasonal temperature change has been found to be an important predicator for regeneration (Baskin and Baskin 1988) but our study found equal importance of moisture with temperature for germination. Variability in the moisture requirements for germination exists between and within *Eucalyptus* species (Gibson and Bachelard 1989). Generally, germination does not occur under water potentials of less than −0.25 MPa for eucalypt species with greater germination typically occurring at 0.0 MPa (Lopez et al. 2000). This observation was supported by our study as germination declined as soil moisture fell below field capacity (0.0 MPa). In our study, *E. sieberi* was the only species to germinate under moisture conditions below field capacity (0.0 MPa, ~40 % soil moisture) which is surprising when

placed in the context of the warm-dry species studied, but supports the species' presence on warmer and drier sites within the cool and moist ash forests of Victoria (Layton and Parsons 1972). This response can also be explained by the study conducted by Gibson and Bachelard (1986) who found that *E. sieberi* can germinate at lower soil matric potentials than *E. pilularis* and *E. maculata*. *E. sieberi* seeds also display a 'stop and go' mechanism that allows germination to progress in wet periods that occur between dry periods (Gibson and Bachelard 1985). Typically, the seed coat in *Monocalyptus* is double layered with an inner integument that inhibits the passage of water (Boland et al. 1980; Gibson and Bachelard 1986); however, Bachelard (1985) found that the seed coat of *E. sieberi* may promote germination on drier soils as it allows for better hydraulic contact with the soil. These characteristics of *E. sieberi* likely explain the observed germination of this species.

The requirement for stratification can be considered an adaptive strategy, as cold moist stratification is generally fulfilled by winter condition and germination occurs in spring when the environment is suitable for seedling survival (Battaglia 1996; Close and Wilson 2002). The regeneration niche of some species is therefore governed by the presence of physiological dormancy that favours spring germination so that the seedlings avoid the occurrence of winter frosts (Battaglia 1996). Stratification is known to

enhance germination in eucalypts (Boland et al. 1980; Gunn 2001), particularly in the subgenus *Monocalyptus* (Davidson and Reid 1980). In our study, a positive effect of stratification was found for four of the species. *Eucalyptus radiata* did not exhibit a response to stratification while *E. polyanthemos* was negatively affected by stratification, suggesting that germination in *E. polyanthemos* may be limited by cool-moist conditions. The greater affinity towards germination following stratification for the other four species in our study suggests that these species benefit from cold moist stratification to maximise germination (Close and Wilson 2002). Stratification can also enhance seed germination in darkness (Baskin and Baskin 1988; Pons 2000); in our study, however, stratification enhanced germination under both full and low light conditions.

Species that do not require light for germination have an advantage under subcanopy conditions within the forest understory (Li et al. 1994) and in buried conditions (Bell et al. 1995). For some species, light can cause reductions in germination. For example, germination in *E. marginata*, *E. sideroxylon*, *E. camaldulensis* and *E. occidentalis* is reduced under low light levels (Grose and Zimmer 1957; Zohar et al. 1975; Bell et al. 1995). In our study, germination of *E. polyanthemos* was stimulated by light. The light response exhibited by *E. polyanthemos* may be considered as an adaptive trait that increases the species ability to germinate on open and disturbed soil surfaces (Bell et al. 1995). Our results suggest that *E. polyanthemos* may require canopy gaps to regenerate more successfully while *E. microcarpa* and *E. tricarpa* may be able to recruit with greater success in the understory.

Germination experimental results versus model simulations

Differences between experimental observations and model simulations were evident in the results which can be explained in two ways: (1) the application of data from the experimental work into a format that can be utilised in the TACA-GEM model and (2) the comparison of germination over a 42-day period on constant diurnal cycle versus a 365-day analysis with variable temperature regimes and moisture conditions. The parameterisation of observational data requires the proportion of germinants that germinate at a given GDD sum to be regressed against GDD. This creates a cumulative response function, standardised to between zero and one, which enables germination to occur at variable rates and at times of the year within temperature and moisture thresholds. The use of a 365-day period of variable temperature and moisture regimes allows for seeds to remain quiescent in periods that are unsuitable but

allow for uniform or periodic germination as conditions permit throughout the year. The model therefore integrates species germination response to temperature and moisture at daily scales with daily to monthly to seasonal patterns in climate.

Provenance effect, phenotypic plasticity and clinal variation

Provenance differences detected within the species suggest that limited provenances displayed phenotypic plasticity. However, *E. obliqua* (Eo 3) provenance exhibited stronger clinal variation for germination as provenances exhibited germination according to its distributional (altitudinal) range. Stronger clinal trends were also found for *E. delegatensis* in response to moisture which was related to the environment of the seed origin with seeds from the warmest zone exhibiting the weakest degree of dormancy while provenances from the coldest zone had the strongest dormancy (Battaglia 1993). Provenance variation for germination timing exhibiting clinal trends was also documented for *Campanulastrum americanum* in response to temperature difference (Prendeville et al. 2013). A lack of phenotypic plasticity but a stronger clinal trend shown by some provenances in germination traits may make these populations and species more vulnerable to climate change (Aitken et al. 2008; Cochrane et al. 2011; Aguilar-Kirigin and Naya 2013).

Cochrane et al. (2011) suggests that species may exhibit a wider regeneration niche than is reflected by their current distribution which may provide a greater degree of phenotypic plasticity which in turn will enable them to adapt to climatic warming. *E. microcarpa* and *E. radiata* exhibited a higher degree of plasticity for germination that may help these species persist under climate change. The response of *E. sieberi* found in this study suggests this species has a wider climatic niche than it currently occupies and that it has phenotypic plasticity within its regeneration niche. This characterisation was absent in the other species studied.

Climate change, germination timing shift, establishment and species potential risk

The timing of germination is closely connected with seedling survival and establishment (Meyer et al. 1990) which in turn may contribute to determine the competitive interactions of coexisting species (Rice and Dyer 2001). Our results indicate that fitness of some species may become disconnected from their germination phenology in the future and this may lead to some species gaining a competitive edge. For example, the warm-dry species were able to maintain or increase

germination under climate change; however, establishment consistently failed for all species except *E. microcarpa*. For *E. microcarpa*, the shift in germination phenology helped it maintain establishment success suggesting that this species may be able to adapt to climate change through changes in its recruitment phenology. Newell et al. (2009) also indicated that the distribution of *E. microcarpa* may not change significantly under future climate change compared to co-occurring species.

Although a shift in phenology was displayed by *E. polyanthemos* and *E. tricarpa*, the shift was only beneficial to their germination capacity not establishment as the latter declined with increasingly warmer and drier conditions imposed under the projected climate change. After germination, the primary recruitment threat to species in 'dry sclerophyll' forests is summer droughts (Pook et al. 1965). Despite the significant shift in the germination phenology for *E. polyanthemos* and *E. tricarpa*, these two species were unable to avoid spring and summer droughts which lead to recruitment failure in the model. Our results highlight that these two species are vulnerable to climate change as they may be unable to regenerate in the areas investigated in this study. Our outcomes for *E. tricarpa* are supported by Orscheg et al. (2011) who found that recruitment of *E. tricarpa* is restricted and climate change may have adverse effects on its germination. The rapid decline in establishment potential for *E. tricarpa* is also supported by Byrne et al. (2013) who found that the species can only perform well under a narrow range of conditions and Newell et al. (2009) who predicted that the distribution of *E. tricarpa* may decline under climate change and that the species may be lost from most of its current distribution.

The cool-moist species all exhibited significant earlier shift in germination towards autumn (PC to 2080s) with *E. sieberi* exhibiting the greatest shift. Compared to the warm-dry species the cool-moist species showed dramatic shifts to autumn dominated germination. This earlier shift however did not have a beneficial impact on the germination and establishment of these species. As was the case for *E. polyanthemos* and *E. tricarpa* our results indicate that the fitness associated with the timing of germination may become disconnected from germination phenology in the future. For the cool-moist species, the shift to autumn-based germination placed the species in interaction with frost which is one of the primary recruitment threats faced by species in the 'wet sclerophyll' forests of Victoria (Battaglia 1996; Layton and Parsons 1972; Woldendorp et al. 2008). The increased warming and drying under the projected climate change also affected establishment success as summer droughts pushed into autumn and spring thereby increasing the proportion of the year under drought stress which limited and then constrained recruitment in these species. Our study demonstrates that

changes in the timing of germination may benefit some species in their recruitment phase and provide opportunities for species to adapt to climate change through phenotypic plasticity. Our results also demonstrate that antagonistic effects may arise for some species due to shifts in their germination phenology. Some species may become maladapted in situ and will require evolution through selection and/or gene migration to facilitated adaptation (Rehfeldt et al. 2001; Aitken et al. 2008). This will require germination to be successful however as demonstrated this was not the case for five of the six species under the projected changes by the 2080s.

Conclusion

Our study was able to demonstrate that the germination phenology of the studied species may change under climate change. The phenological shifts that were modelled allowed many of the species to germinate under future climate conditions but did not increase or maintain their ability to establish which highlights that recruitment may decline under climate change for the majority of the species. The warm-dry species, *E. microcarpa*, exhibited a high degree of plasticity and was able to maintain high germination rates and its establishment success under all climate change scenarios. Future climate change may have a greater impact on the germination phenology and recruitment of the cool-moist species. Our study also highlights that successful germination does not guarantee recruitment success which may limit any benefits that arise from shifts in phenology under climatic change. Further, our results highlight that divergent responses are likely to occur between co-occurring species. The long-term implications of changes in recruitment dynamics on stand composition and on species distributions under climate change requires further investigation. However, species germination phenology will remain responsive to changes in temperature and moisture across these spatial scales and thus will be sensitive to climate change. The implications of this phenological shift on species adaptation to climate change is complex; however, our study was successful in providing some initial insights on how climate change may vary germination phenology which in turn may affect the ability of some species to regenerate in the future.

Appendix 1

Table 5 Description of selected eucalypts and seed source information

Species	Geographical range	Temperature range (°C)	Rainfall range (mm year^{-1})	Altitude range (m)	Provenance (seed lot number)	Altitude (m asl)	Geographical position
Warm-dry species							
E. microcarpa[bcd] (grey box)	24.75–37.5° S	1–7/25–33	400–760	40–800	Em 1 (Seeding Vic. 6540)	118	37° 41' S, 144° 44' E
					Em 2 (Seeding Vic. 9273)	249	37° 05' S, 143° 74' E
					Em 3 (CSIRO 16036)	280	37° 06' S, 143° 32' E
E. polyanthemos[bce] (red box)	32.5–38°S	−1–4/23–30	450–970	120–780	Ep 1 (CSIRO 17222)	120	37° 39' S, 147° 50' E
					Ep 2 (CSIRO 15342)	240	36° 51' S, 144° 24' E
					Ep 3 (CSIRO 15337)	420	36° 28' S, 146° 41' E
					Ep 4 (Greening Aus. MIS 950764)	509	37° 14' 52 S, 144° 27' 13 E
E. tricarpa[bcf] (red ironbark)	25–38.25°S	2–4/24–28	550–1000	20–360	Et 1 (CSIRO 20450)	0	37° 56' S, 146° 43' E
					Et 2 (Seeding Vic. 2506)	120	36° 45' S, 144° 21' E
					Et 3 (Seeding Vic. 4414)	300	36° 43' S, 144° 25' E
Cool-moist species							
E. obliqua[abc] (messmate stringybark)	28–43.5°S	−4–8/19–29	500–2400	0–750	Eo 1 (CSIRO 15914)	194	37° 20' S, 145° 05' E
					Eo 2 (CSIRO 15901)	270	38° 51' S, 143° 30' E
					Eo 3 (Seeding Vic. 1578)	560	37° 47' S, 144° 31' S
					Eo 4 (CSIRO 15902)	616	37° 26' S, 144° 12' E
E. radiata[bce] (narrow leaved peppermint)	28–39°S	−4–2/23–30	650–1100	50–1200	Er 1 (Seeding Vic. 1715)	170	38° 30' S, 143° 41' E
					Er 2 (Greening Aus. 970312)	174	37° 44' S, 145° 27' E
					Er 3 (Seeding Vic. 3263)	380	37° 48' S, 143° 54' E
E. sieberi[bc] (silvertop ash)	33–42°S	−2–5/22–27	700–1400	0–100	Es 1	140	37° 41' S, 148° 45' E
					Es 2 (CSIRO 20043)	800	34° 20' S, 150° 12' E

[a] Source: Australian Forest Profiles (2002)

[b] Source: Boland et al. (2006)

[c] Source: www.ersa.edu.au

[d] Source: www.environment.gov.au

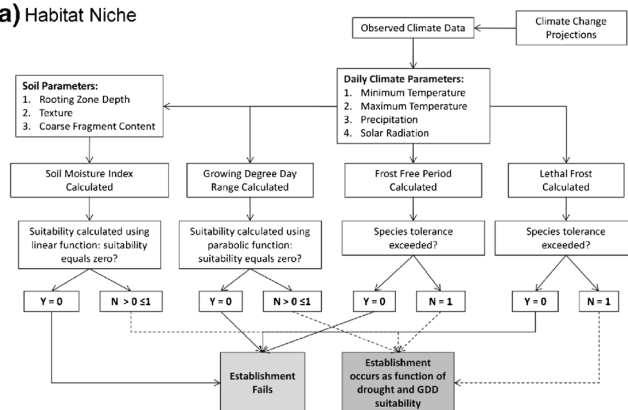[e] Source: www.metrotrees.com.au
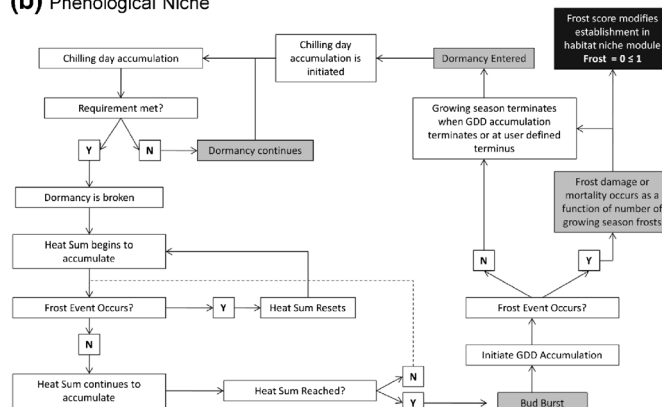
[f] Source: www.florabank.org.au/

Appendix 2

Conceptual framework for TACA-GEM. Three modules centred around niche elements (sensu Grubb 1977) are used to define the regeneration niche for a species and determine both germination and establishment potential from both single and multiple climate years. The three niche modules are characterised as follows: (a) habitat niche, (b) phenology

niche, and (c) germination niche. The phenological niche module relates to the timing of growth of established plants and the effect of frost on establishment and germination success. The germination niche module includes the phenological processes of germination and interacts with the habitat module to determine establishment. The user can evaluate a species' response to varying climate years/scenarios and soil types at both the germination and establishment phases.
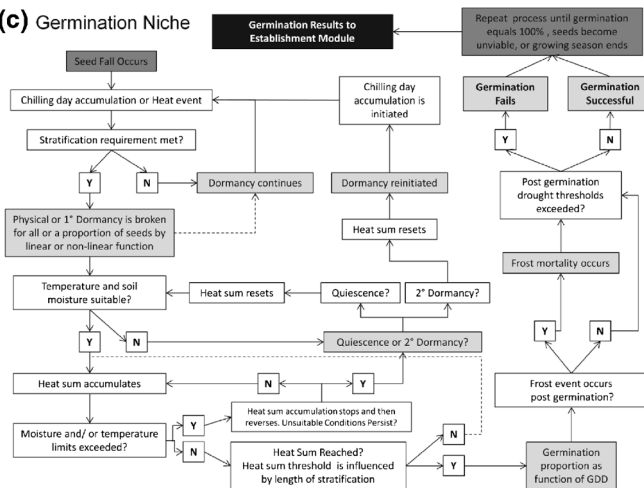
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